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(54)【発明の名称】シグナル－１／シグナル－２二官能性ペプチド阻害剤

(57)【要約】
一般式ＡＢを有する、新規ペプチド配列であって、式中、ＡおよびＢは各々、アミノ酸残基鎖を示し、そして前記Ａ鎖は抗原提示細胞上の主要組織適合複合体と結合可能であり、そして前記Ｂ鎖は抗原提示細胞上のシグナル－２受容体と結合可能である、前記ペプチド配列。該ペプチド配列の好ましい型は、Ａ鎖およびＢ鎖の間に配置されるＹ鎖をさらに含む。さらに、好ましい型は、シグナル１部分と少なくとも約１０％の配列相同性を有するか、またはシグナル－１部分のペプチド擬似体であるＡ鎖を含み、前記Ｂ鎖は、シグナル－２受容体部分と少なくとも１０％の配列相同性を有するか、前記Ｂ鎖は、シグナル－２受容体部分と少なくとも１０％の配列相同性を有するか、またはシグナル－２受容体部分のペプチド擬似体であり、そしてＸ鎖は、少なくとも１のアミノ酸残基を有するか、またはアミノ酸残基のペプチド擬似体である。好適に、新規ペプチド配列は、１型免疫反応から２型免疫反応に、または２型免疫反応から１型免疫反応にシフトすることが可能である。

【特許請求の範囲】
【請求項１】
第一の部分および第二の部分を含んでなるペプチドであって、前記第一の部分が、ＴＣＲエピトープ由来のペプチドと少なくとも約１０％の配列相同性を有する配列を含み、前記第二の部分が、シグナル－２部分由来のペプチドと少なくとも約１０％の配列相同性を有する配列を含む、前記ペプチド。
【請求項２】
連結部分をさらに含んでなる、請求項１のペプチド。
【請求項３】
前記連結部分が少なくとも１のアミノ酸残基を含んでなる、請求項２のペプチド。
【請求項４】
前記アミノ酸が柔軟な非基質アミノ酸である、請求項３のペプチド。
【請求項５】
前記連結部分がアミノ酸残基の配列を含んでなり、前記配列が親水性アミノ酸と交互に非基質アミノ酸を含んでなる、請求項２のペプチド。
【請求項６】
前記連結部分が一般式（Ａ，Ｂ）$_x$を有する、請求項２のペプチドであって、式中、ＡおよびＢはアミノ酸残基であり、そして前記Ａアミノ酸残基は、独立して、それぞれ、アミノカプロン酸、アミノヘキサン酸、アミノドデカン酸、およびβ－アラニンからなる群より選択され、そして前記Ｂアミノ酸残基はグリシンであり、そしてＸは１～１００の範囲である、前記ペプチド。
【請求項７】
前記Ａアミノ酸残基がアミノカプロン酸である、請求項６のペプチド。
【請求項８】
前記第一の部分が、配列番号１～２５からなる群より選択される配列と、少なくとも約１０％の配列相同性を有する配列を含む、請求項１のペプチド。
【請求項９】
配列番号１～２５からなる群より選択される配列と、少なくとも約５０％の配列相同性を有する、請求項８のペプチド。
【請求項１０】
配列番号１～２５からなる群より選択される配列と、少なくとも約９５％の配列相同性を有する、請求項９のペプチド。
【請求項１１】
前記第一の部分が、配列番号１～２５からなる群より選択されるペプチドのペプチド擬似体（ｐｅｐｔｉｄｏｍｉｍｅｔｉｃ）を含んでなる、請求項１のペプチド。
【請求項１２】
前記第一の部分が、配列番号１～２５からなる群より選択されるペプチドの誘導体を含んでなる、請求項１のペプチド。
【請求項１３】
前記ＴＣＲエピトープが既知の疾患状態と相関する、請求項１のペプチド。
【請求項１４】
前記第一の部分が、抗原提示細胞上の主要組織適合複合体と結合可能である、請求項１のペプチド。
【請求項１５】
前記ペプチド：ＭＨＣ複合体が、Ｔ細胞上のＴ細胞受容体と結合可能である、請求項１４のペプチド。
【請求項１６】
前記第二の部分が、配列番号３０～４１からなる群より選択される配列と、少なくとも約１０％の配列相同性を有する配列を含む、請求項１のペプチド。
【請求項１７】

配列番号３０～４１からなる群より選択される配列と、少なくとも約５０％の配列相同性を有する、請求項１６のペプチド。
【請求項１８】
配列番号３０～４１からなる群より選択される配列と、少なくとも約９５％の配列相同性を有する、請求項１７のペプチド。
【請求項１９】
前記第二の部分が、配列番号３０～４１からなる群より選択されるペプチドのペプチド擬似体を含んでなる、請求項１のペプチド。
【請求項２０】
前記第二の部分が、配列番号３０～４１からなる群より選択されるペプチドの誘導体を含んでなる、請求項１のペプチド。
【請求項２１】
前記第二の部分が、抗原提示細胞上のシグナル－２リガンドと結合可能である、請求項１のペプチド。
【請求項２２】
Ｔ細胞において、二次シグナルを開始可能である、請求項２１のペプチド。
【請求項２３】
免疫反応を、１型が優性である反応から２型が優性である反応に変更可能である、請求項１のペプチド。
【請求項２４】
免疫反応を、２型が優性である反応から１型が優性である反応に変更可能である、請求項１のペプチド。
【請求項２５】
前記第二の部分が特定の型の免疫反応と関連する、請求項１のペプチド。
【請求項２６】
前記連結部分が、前記第一の部分および前記第二の部分の間に配置されている、請求項２のペプチド。
【請求項２７】
１つの連続配列として合成可能である、請求項１のペプチド。
【請求項２８】
下記のステップを含む、免疫反応を変更する方法：
（ａ）抗原提示細胞をペプチドと接触させるステップ、ここで前記ペプチドは、前記抗原提示細胞上の主要組織適合複合体と結合してペプチド：ＭＨＣ複合体を形成する第一の部分と、前記抗原提示細胞上の二次シグナル部分に結合する第二の部分とを含んでなり；
（ｂ）前記ペプチド：ＭＨＣ複合体の形成に反応して、Ｔ細胞においてＴ細胞受容体の結合を引き起こすステップ；
（ｃ）前記二次シグナル部分への前記第二の部分の結合に反応して、Ｔ細胞において二次シグナルを遮断または改変するステップ；および
（ｄ）前記第一および第二のシグナルが、前記ペプチド：ＭＨＣ複合体と一般的に関連する免疫反応から外れた免疫反応を生じるステップ。
【請求項２９】
前記免疫反応が１型免疫反応から外れて２型免疫反応となる、請求項２８の方法。
【請求項３０】
前記免疫反応が２型免疫反応から外れて１型免疫反応となる、請求項２８の方法。
【請求項３１】
前記第一の部分が、シグナル－１部分由来のペプチドと少なくとも１０％の配列相同性を有する、請求項２８の方法。
【請求項３２】
前記第二の部分が、シグナル－２受容体部分由来のペプチドと少なくとも１０％の配列相同性を有する、請求項２８の方法。

【請求項３３】
一般式ＡＢを有するペプチドであって、式中、前記ＡおよびＢは各々、アミノ酸残基の鎖を示し、そして前記Ａ鎖は少なくとも約５アミノ酸残基を含んでなり、そしてシグナル－１部分と少なくとも約１０％の配列相同性を有し、そして前記Ｂ鎖は少なくとも約４アミノ酸残基を含んでなり、そしてシグナル－２受容体部分由来のペプチドと少なくとも約１０％の配列相同性を有する、前記ペプチド。
【請求項３４】
少なくとも約１のアミノ酸残基を含んでなるアミノ酸残基のＸ鎖をさらに含んでなる、請求項３４のペプチド。
【請求項３５】
前記Ｘ鎖が前記Ａ鎖および前記Ｂ鎖の間に配置されている、請求項３４のペプチド。
【請求項３６】
１つの連続配列として合成可能である、請求項３３のペプチド。
【請求項３７】
前記Ｘ鎖が、一般式（Ｙ，Ｚ）$_t$を有する、可動性の非基質リンカーを含んでなる、請求項３４のペプチドであって、式中、ＹおよびＺはアミノ酸残基であり、そして前記Ｙアミノ酸残基は、独立して、それぞれ、非基質アミノ酸からなる群より選択され、そして前記Ｚアミノ酸残基が、独立して、それぞれ、親水性アミノ酸からなる群より選択され、そしてＴが１～１００の範囲である、前記ペプチド。
【請求項３８】
前記非基質アミノ酸が、アミノカプロン酸、アミノヘキサン酸、アミノドデカン酸、およびβ－アラニンからなる群より選択される、請求項３７のペプチド。
【請求項３９】
前記非基質アミノ酸残基がアミノカプロン酸である、請求項３８のペプチド。
【請求項４０】
前記親水性アミノ酸がグリシンである、請求項３７のペプチド。
【請求項４１】
前記Ａ鎖が、配列番号１～２５からなる群より選択される配列と、少なくとも約１０％の配列相同性を有する配列を含む、請求項３３のペプチド。
【請求項４２】
前記Ａ鎖が、配列番号１～２５からなる群より選択される配列と、少なくとも５０％の配列相同性を有する、請求項４１のペプチド。
【請求項４３】
前記Ａ鎖が、配列番号１～２５からなる群より選択される配列と、少なくとも約９５％の配列相同性を有する、請求項４２のペプチド。
【請求項４４】
前記Ａ鎖が、配列番号１～２５からなる群より選択されるペプチドのペプチド擬似体を含んでなる、請求項３３のペプチド。
【請求項４５】
前記Ａ鎖が、配列番号１～２５からなる群より選択されるペプチドの誘導体を含んでなる、請求項３３のペプチド。
【請求項４６】
前記シグナル－１部分が、既知の健康状態と関連する、請求項３３のペプチド。
【請求項４７】
抗原提示細胞上の主要組織適合複合体と結合して、ペプチド：ＭＨＣ複合体を形成可能である、請求項３３のペプチド。
【請求項４８】
前記ペプチド：ＭＨＣ複合体がＴ細胞と結合可能である、請求項４７のペプチド。
【請求項４９】
前記Ｂ鎖が、配列番号３０～４１からなる群より選択される配列と、少なくとも約１０％

の配列相同性を有する配列を含む、請求項３３のペプチド。
【請求項５０】
前記Ｂ鎖が、配列番号３０～４１からなる群より選択される配列と、少なくとも約５０％の配列相同性を有する、請求項４９のペプチド。
【請求項５１】
前記Ｂ鎖が、配列番号３０～４１からなる群より選択される配列と、少なくとも約９５％の配列相同性を有する、請求項５０のペプチド。
【請求項５２】
前記Ｂ鎖が、配列番号３０～４１からなる群より選択されるペプチドのペプチド擬似体を含んでなる、請求項３３のペプチド。
【請求項５３】
前記Ｂ鎖が、配列番号３０～４１からなる群より選択されるペプチドの誘導体を含んでなる、請求項３３のペプチド。
【請求項５４】
抗原提示細胞上のシグナル－２リガンドと結合可能である、請求項３３のペプチド。
【請求項５５】
前記シグナル－２部分との結合に際して、Ｔ細胞への二次シグナルを遮断または改変可能である、請求項５４のペプチド。
【請求項５６】
免疫反応を、１型が優性である反応から２型が優性である反応に変更可能である、請求項３３のペプチド。
【請求項５７】
免疫反応を、２型が優性である反応から１型が優性である反応に変更可能である、請求項３３のペプチド。
【請求項５８】
細胞仲介免疫反応を体液性免疫反応にシフトすることが可能である、請求項３３のペプチド。
【請求項５９】
体液性免疫反応を細胞仲介反応にシフトすることが可能である、請求項３３のペプチド。
【請求項６０】
下記のステップを含む、免疫反応を調節するペプチドを調製する方法：
シグナル－１部分由来の配列と少なくとも約１０％の配列相同性を有する、第一のペプチド配列を選択するステップ；
シグナル－２受容体部分由来の配列と少なくとも約１０％の配列相同性を有する、第二のペプチド配列を選択するステップ；および
前記第一のペプチド配列と前記第二のペプチド配列とを含む連続ペプチド鎖として、前記ペプチドを合成するステップ。
【請求項６１】
少なくとも１のアミノ酸残基を含んでなる第三のペプチド配列を選択するステップをさらに含んでなる、請求項６０の方法。
【請求項６２】
前記第一のペプチド配列が、既知の健康状態と関連する、請求項６０の方法。
【請求項６３】
前記第一のペプチド配列が、抗原提示細胞上の主要組織適合複合体と結合して、ペプチド：ＭＨＣ複合体を形成可能である、請求項６０の方法。
【請求項６４】
前記第二のペプチド配列が、抗原提示細胞上のシグナル－２リガンドと結合可能である、請求項６０の方法。
【請求項６５】
抗原提示細胞上の主要組織適合複合体と前記ペプチドを接触させるステップをさらに含ん

でなる、請求項６０の方法。
【請求項６６】
抗原提示細胞上のシグナル－２リガンドと前記ペプチドを接触させるステップをさらに含んでなる、請求項６０の方法。
【請求項６７】
下記を含むペプチド：
Ｔ細胞において、一次シグナルを開始可能な第一のペプチド配列；および
Ｔ細胞において、二次シグナルを開始可能な第二のペプチド配列。
【請求項６８】
少なくとも１のアミノ酸残基を含んでなる連結ペプチド配列をさらに含んでなる、請求項６７のペプチド。
【請求項６９】
前記第一のペプチド配列がシグナル－１部分由来である、請求項６７のペプチド。
【請求項７０】
前記第二のペプチド配列がシグナル－２受容体部分由来である、請求項６７のペプチド。
【請求項７１】
前記第一のペプチド配列が、配列番号１～２５からなる群より選択されるペプチドと、少なくとも約１０％の配列相同性を有する、請求項６７のペプチド。
【請求項７２】
前記第二のペプチド配列が、配列番号３０～４１からなる群より選択されるペプチドと、少なくとも約１０％の配列相同性を有する、請求項６７のペプチド。
【請求項７３】
前記連結ペプチド配列が一般式（Ｙ，Ｚ）$_t$を有する、請求項６８のペプチドであって、式中、ＹおよびＺはアミノ酸残基であり、そしてＴは１～１００の範囲である、前記ペプチド。
【請求項７４】
前記Ｙアミノ酸残基が、独立して、それぞれ、アミノカプロン酸、アミノヘキサン酸、アミノドデカン酸、およびβ－アラニンからなる群より選択される、請求項７３のペプチド。
【請求項７５】
前記Ｚアミノ酸残基が、独立して、それぞれ、親水性アミノ酸残基からなる群より選択される、請求項７３のペプチド。
【請求項７６】
前記親水性アミノ酸残基がグリシンである、請求項７５のペプチド。
【請求項７７】
前記Ｙアミノ酸残基がアミノカプロン酸である、請求項７３のペプチド。
【請求項７８】
下記を含む、ＢＰＩを構築する方法：
（ａ）既知の健康状態を選択するステップ；
（ｂ）前記健康状態に特異的なＴＣＲエピトープを同定するステップ；および
（ｃ）前記ＴＣＲエピトープをＢＰＩに取り込むステップ。
【請求項７９】
シグナル－２受容体部分を選択し、そして前記シグナル－２受容体部分を前記ＢＰＩに取り込むステップをさらに含んでなる、請求項７８の方法。
【請求項８０】
前記ＴＣＲエピトープを前記シグナル－２受容体部分にコンジュゲート化させるステップをさらに含んでなる、請求項７９の方法。
【請求項８１】
前記コンジュゲート化ステップが、前記ＴＣＲエピトープと前記シグナル－２受容体部分との間にリンカーを配置することを含む、請求項８０の方法。

【請求項８２】
前記リンカーが少なくとも１のアミノ酸残基を含んでなる、請求項８１の方法。
【請求項８３】
前記リンカーが一般式（Ｙ，Ｚ）$_t$を有する、請求項８１の方法であって、式中、ＹおよびＺはアミノ酸残基であり、そしてＴは１～１００の範囲である、前記方法。
【請求項８４】
前記Ｙアミノ酸残基が、独立して、それぞれ、アミノカプロン酸、アミノヘキサン酸、アミノドデカン酸、およびβ－アラニンからなる群より選択される、請求項８３の方法。
【請求項８５】
前記Ｚアミノ酸残基が、独立して、それぞれ、親水性アミノ酸残基からなる群より選択される、請求項８３の方法。
【請求項８６】
前記親水性アミノ酸残基がグリシンである、請求項８５の方法。
【請求項８７】
前記ＴＣＲエピトープが、抗原提示細胞上の主要組織適合複合体と結合して、ペプチド：ＭＨＣ複合体を形成可能である、請求項７８の方法。
【請求項８８】
前記シグナル－２受容体部分が、抗原提示細胞上のシグナル－２リガンドと結合可能である、請求項７９の方法。
【請求項８９】
前記ＴＣＲエピトープが、配列番号１～２５から選択される配列と、少なくとも約１０％の配列相同性を有する、請求項７８の方法。
【請求項９０】
前記シグナル－２受容体部分が、配列番号３０～４１から選択される配列と、少なくとも約１０％の配列相同性を有する、請求項７９の方法。
【請求項９１】
下記の組み合わせ：
抗原提示細胞上の主要組織適合複合体と結合可能な第一のペプチド配列；および
抗原提示細胞上のシグナル－２リガンドと結合可能な第二のペプチド配列。
【請求項９２】
前記第一のペプチド配列と前記第二のペプチド配列との間に配置されたリンカーをさらに含む、請求項９１の組み合わせ。
【請求項９３】
前記第一のペプチド配列がシグナル－１部分由来である、請求項９１の組み合わせ。
【請求項９４】
前記第二のペプチド配列がシグナル－２受容体部分由来である、請求項９１の組み合わせ。
【請求項９５】
前記第一のペプチド配列が、配列番号１～２５からなる群より選択されるペプチドと、少なくとも約１０％の配列相同性を有する、請求項９１の組み合わせ。
【請求項９６】
前記第二のペプチド配列が、配列番号３０～４１からなる群より選択されるペプチドと、少なくとも約１０％の配列相同性を有する、請求項９１の組み合わせ。
【請求項９７】
前記リンカーが少なくとも１のアミノ酸残基を含んでなる、請求項９２の組み合わせ。
【請求項９８】
前記リンカーが一般式（Ｙ，Ｚ）$_t$を有する、請求項９２の組み合わせであって、式中、ＹおよびＺはアミノ酸残基であり、そしてＴは１～１００の範囲である、前記組み合わせ。
【請求項９９】

前記Ｙアミノ酸残基が、独立して、それぞれ、アミノカプロン酸、アミノヘキサン酸、アミノドデカン酸、およびβ－アラニンからなる群より選択される、請求項９８の組み合わせ。
【請求項１００】
前記Ｙアミノ酸残基がアミノカプロン酸である、請求項９９の組み合わせ。
【請求項１０１】
前記Ｚアミノ酸残基が、独立して、それぞれ、親水性アミノ酸残基からなる群より選択される、請求項９８の組み合わせ。
【請求項１０２】
前記親水性アミノ酸残基がグリシンである、請求項１０１の組み合わせ。
【請求項１０３】
配列番号４２～４６からなる群より選択されるペプチドと、少なくとも約１０％の配列相同性を有するペプチド。
【請求項１０４】
配列番号４２～４６からなる群より選択されるペプチドと、少なくとも約５０％の配列相同性を有する、請求項１０３のペプチド。
【請求項１０５】
配列番号４２～４６からなる群より選択されるペプチドと、少なくとも約９５％の配列相同性を有する、請求項１０４のペプチド。
【請求項１０６】
配列番号４２～４６からなる群より選択されるペプチドの誘導体。
【請求項１０７】
配列番号４２～４６からなる群より選択されるペプチドのペプチド擬似体。
【請求項１０８】
配列番号４２～４６からなる群より選択されるペプチドのペプチド模倣体（ｐｅｐｔｉｄｅ　ｍｉｍｉｃ）。
【発明の詳細な説明】
【０００１】
（配列表）
印刷した配列表が本出願に付随し、そしてまた、フロッピー（Ｒ）ディスクおよびＣＤＲＯＭ上、コンピュータ読み取り可能ＡＳＣＩＩファイルの形でも、同一の内容で提出している。
【０００２】
（発明の背景）
発明の分野
本発明は、Ｔ細胞による抗原提示細胞表面上のペプチド：ＭＨＣ複合体の認識によって開始される免疫反応に関する。本発明はまた、シグナル－２部分の、抗原提示細胞表面上の相補タンパク質への結合によって開始される免疫反応にも関する。より具体的には、本発明は、Ｔ細胞によるペプチド：ＭＨＣの認識によって、そしてシグナル－２部分のその相補タンパク質への結合によって開始される免疫反応に関する。さらにより具体的には、本発明は、この結合に反応して、特定の個体が生成する典型的な免疫反応の変更に関する。より具体的には、本発明は、１型から２型、または２型から１型に、所定の免疫反応を変更するかまたはシフトするため、ペプチド：ＭＨＣ複合体のペプチド部分由来のペプチドを、好ましいシグナル－２部分にコンジュゲート化することに関する。これは、サプレッサーＴ細胞を含む、特定の表現型の制御Ｔ細胞を含むことが可能である。
【０００３】
先行技術の説明
自己免疫疾患は、自己抗原に対するＴ細胞の活性化に特徴付けられる。これらのＴ細胞はその後、これらの抗原を提示する細胞を破壊する。例えば、インスリン依存性糖尿病（ＩＤＤＭ、Ｉ型糖尿病とも呼ばれる）は、膵臓のインスリン産生細胞に対するＴ細胞の活性

化およびそれに続くこれらのＴ細胞による破壊に特徴付けられる。自己免疫反応に関連する疾患および異常は、主要組織適合複合体（ＭＨＣ）クラスＩＩ分子と呼ばれる細胞表面タンパク質の特定のサブタイプ（対立遺伝子）と強く関連する。ＭＨＣ分子は、感染性病原体由来のタンパク質、アレルゲン、および自己タンパク質の断片（ペプチド）に結合し、そしてこのＭＨＣ：ペプチド複合体は、Ｔ細胞がその受容体（Ｔ細胞受容体、またはＴＣＲと呼ばれる）で認識する構造である。ＭＨＣ：ペプチド複合体は、抗原提示細胞（ＡＰＣ）と呼ばれる免疫系の他の細胞（すなわちＢ細胞、樹状細胞およびマクロファージ）の表面上に提示される。免疫反応が後に続くためには、免疫系の主要な制御細胞である未分化Ｔ細胞は、外来（ｆｏｒｅｉｇｎ）侵入物の小さい分解産物（ペプチド）を提示されなければならない。この提示は、ＡＰＣ表面上で起こる。その後、Ｔ細胞はＡＰＣと相互作用しなければならず、そしてこの相互作用は、Ｔ細胞が分裂し、そして分化して、直接または間接的に、同一のまたは非常に類似のＭＨＣ：ペプチド複合体を提示する細胞を攻撃する分子を産生するのを刺激する。ＭＨＣ分子をコードする遺伝子は、種内で非常に多様であり、そして異なるＭＨＣ対立遺伝子が、他のものよりもいくつかのペプチドに結合するのを好むことがよく知られている。他の遺伝的および環境的要因と共に、異なるＭＨＣ対立遺伝子の存在は、なぜ種のあるメンバーは例えば自己免疫疾患、アレルギー、喘息、さらには特定の感染性疾患等の状態を呈するのに、他のメンバーは同じ物質に影響を受けないか、または免疫であるままなのかを説明するのに役立つ。他の相違は、ペプチド：ＭＨＣ複合体と異なる細胞表面タンパク質もまた、Ｔ細胞上の特定の受容体に結合しなければならないため、生じる。Ｔ細胞およびＡＰＣ膜の境界面で、これらの他のタンパク質：タンパク質対は、シグナル－２として知られる同時刺激シグナルを提供し、このシグナルは、ＭＨＣ：ペプチド複合体のＴＣＲ認識によって生じるシグナル（シグナル－１として知られる）と共に、免疫反応を開始する。

【０００４】

免疫反応を特徴付ける段階は、２つのシグナルの結果としての、$CD4^+$ Ｔ細胞の１型ヘルパーＴ細胞（T_H1細胞）または２型ヘルパーＴ細胞（T_H2細胞）への分化である。T_H細胞のこれらの２つのサブタイプおよびこれらが選択的に活性化する細胞制御ネットワークは、ヒトの健康状態および疾患状態の公知の相関物である。T_H1細胞への分化は、主に細胞仲介免疫を生じ、一方、T_H2細胞への分化は、主に体液性免疫を生じる。これらの免疫型は各々、異なる種類の侵入に対して、体を防御するのを補助する。１型免疫は、細菌などの細胞内病原体に対して体を防御するが、器官特異的自己免疫疾患にも関与する。２型免疫は、細胞外寄生虫に対しての保護に重要であるが、アレルギー性反応にもまた関連する。T_H1細胞の発展は、マクロファージおよび樹状細胞として知られる免疫細胞に産生される、インターロイキン－１２と呼ばれるサイトカインによる。インターロイキン－１２は、未処置（ｎａｉｖｅ）Ｔ細胞（$CD4^+$ Ｔ細胞）を誘導または刺激して、インターフェロン－γ（ＩＦＮ－γ）およびインターロイキン－２（ＩＬ－２）を産生する。これらの２つのサイトカイン（ＩＬ－２およびＩＦＮ－γ）は、細胞傷害性Ｔリンパ球（ＣＴＬ）のクローン性増殖、マクロファージ活性化、および感作細胞の補体溶解を仲介するＩｇＧアイソタイプへのクラススイッチに関与する。T_H1免疫反応への拘束は、インターロイキン－１２（ＩＬ－１２）受容体の発現を上方制御するが、T_H2細胞の発展を阻害する、ＩＦＮ－γの存在によって増進される。T_H2免疫は、未処置Ｔ細胞によるインターロイキン－４（ＩＬ－４）産生から生じる。ＩＬ－４はT_H2発展、並びにそれに続く、インターロイキン－４（ＩＬ－４）、－５（ＩＬ－５）、－１０（ＩＬ－１０）、および－１３（ＩＬ－１３）の産生を誘導する。ＩＬ－４はまた、発展中の細胞上のＩＬ－１２受容体発現を下方制御するよう作用して、それによってT_H1発展を阻害し、そして未分化Ｔ細胞がT_H2細胞発展に拘束されるのを補助する。さらに、ＩＬ－４およびＩＬ－５は、Ｂ細胞を活性化し、そして中和抗体（マウスではＩｇＧ１）および即時型過敏の開始因子であるＩｇＥに切り換えることが知られる。

【０００５】

これらの免疫経路いずれかを活性化するため、Ｔ細胞を完全に活性化するのに、２シグナ

ルの機構が必要である。シグナル－１（Ｓ－１）は、Ｔ細胞抗原受容体（ＴＣＲ）が抗原提示細胞（ＡＰＣ）表面上のペプチド：ＭＨＣ－ＩＩ複合体を認識した際に生じる。この最初のシグナルは、Ｔ細胞受容体を通過して、そしてキナーゼおよびホスファターゼが仲介するチロシンリン酸化／脱リン酸化事象のカスケードを開始し、そしてＣａ$^{++}$流束、活性化Ｔ細胞の核因子（ＮＦ－ＡＴ）およびＮＦκＢ転写因子の活性化を導く。これらの因子は、Ｔ細胞の核に進入し、そしてエフェクター機能に関与する遺伝子のプロモーターに結合する。シグナル－２（Ｓ－２）は、シグナル－２受容体が、ＡＰＣ表面上のリガンドに結合することから生じる。シグナル－２受容体にはＣＤ２８およびそのリガンドＢ７と共にＬＦＡ－１およびそのリガンドＩＣＡＭ－１が含まれる。シグナル－２受容体およびそのリガンドが、Ｔ細胞およびＡＰＣ受容体膜間の境界面で複合体を形成すると、一連のシグナル伝達事象が起こる。これらの事象には、ＧＴＰアーゼ活性を持つアダプタータンパク質を活性化する、グアニンヌクレオチド交換因子の活性化と、セリン／スレオニンリン酸化／脱リン酸化とが含まれる。これらのシグナル伝達事象は、別個の組の転写因子を活性化する。ＣＤ２８：Ｂ７複合体を通じて搬送されるシグナルは、ＩＣＡＭ－１：ＬＦＡ－１複合体から搬送されるものとは異なり、特にＣＤ４$^{+}$　Ｔ細胞のＴ$_{H}$１エフェクター集団対Ｔ$_{H}$２エフェクター集団への分化に関しては異なる。

主な結合がＬＦＡ－１およびＩＣＡＭ－１の間で起こる場合、ＣＤ４$^{+}$　Ｔ細胞分化は、Ｔ$_{H}$１細胞を支持し、該細胞はＩＬ－２およびＩＦＮγを豊富に産生し、これらは遅延型過敏（ＤＴＨ）、細胞内病原体に対する免疫、およびいくつかの自己免疫疾患を含む、炎症性免疫反応の傑出した開始因子である。主な結合がＣＤ２８およびＢ７の間で起こる場合、ＣＤ４$^{+}$　Ｔ細胞はＴ$_{H}$２細胞に分化する。Ｔ$_{H}$１細胞と対照的に、Ｔ$_{H}$２細胞は、ＩＬ－２またはＩＦＮγサイトカインを豊富に産生しないが、その代わり、アレルギーおよび喘息などの即時型過敏の仲介因子、すなわち、ＩＬ－４、ＩＬ－５、ＩＬ－１０、およびＩＬ－１３を放出する。したがって、二次シグナルを提供する複合体の相対的な寄与を操作する能力は、与えられた自己組織抗原に対して引き出される免疫反応の種類に対して、重大な影響を有する。

【０００６】

ＴＣＲおよびＡＰＣ間の会合は、免疫学的シナプスと呼ばれるＴＣＲおよびＡＰＣ間の特殊な結合部または境界面で起こる。免疫シナプスを、図１に模式的に示す。この免疫シナプスは、Ｔ細胞およびＡＰＣ間の境界面で組み立てられる活性化分子の編成された構造と定義可能である。神経系のシナプス同様、免疫シナプスは、細胞膜間の緊密な会合である。免疫反応が後に続くためには、免疫系の主要な制御細胞である未分化Ｔ細胞は、外来侵入物の小さい分解産物（ペプチド）を提示されなければならない。不活性化Ｔ細胞において、ＴＣＲおよび接着分子は、Ｔ細胞膜上で、無作為に分散している。免疫学的シナプスの形成は、Ｔ細胞が潜在的な抗原性リガンドを区別するのを可能にする活性でそして動的な機構である。免疫学的シナプスは、接着分子の輪に取り巻かれたＴ細胞受容体の中央クラスターからなる。免疫シナプスの安定な形成は、内部にＴＣＲそして外部にＬＦＡ－１を有するドーナツ様構造を形成するため、ＬＦＡ－１などの接着分子およびペプチド認識受容体（ＴＣＲ）を必要とする。活性化中、ＴＣＲおよびＬＦＡ－１分子は、ドーナツ様構造形成中に、Ｔ細胞脂質二重層内で互いにすれ違う（このプロセスは転位置（ｔｒａｎｓｌｏｃａｔｉｏｎ）と呼ばれる）。これらの分子が、免疫シナプス内で転位置しない場合、Ｔ細胞シグナルは完全には受け取られず、そしてＴ細胞内で遺伝子活性の異なるプログラムが後に続く可能性がある。このことは、Ｔヘルパー細胞が、ＩＦＮγ放出を導くであろう遺伝子プログラム（Ｔ$_{H}$１細胞および１型免疫）から、最終的にＩＬ－４産生を活性化するプログラム（すなわちＴ$_{H}$２細胞および２型免疫）に外れる場合には、特に、免疫反応に劇的な影響を与える可能性がある。

【０００７】

さらに詳細には、Ｔ$_{H}$１優性を導く経路を活性化するには、ＴＣＲは、ペプチド：ＭＨＣ－ＩＩ複合体を認識し、そしてシグナル－１をＴ細胞に送る。さらに、ＬＦＡ－１がＩＣＡＭ－１に結合し、そしてこれらの分子は、ペプチド：ＭＨＣ－ＩＩ複合体と共に転位置

して、最終段階の免疫シナプスを形成する。これが、非拘束T_H細胞によるＣＤ４０リガンド（ＣＤ１５４）の有効な発現を導く。ＣＤ４０（抗原提示細胞上に発現される）とリガンドとの相互作用は、炎症性サイトカインＩＬ－１２のＮＦκＢ上方制御を生じる。その後、ＩＬ－１２は未分化T_H細胞上の受容体に結合し、そして転写制御因子、Ｓｔａｔ４およびＴｂｅｔの上方制御を含む、T_H１プログラムを開始する。これは、自己抗原（例えばグルタミン酸デカルボキシラーゼ、ＧＡＤ６５）に対するT_H１優性を導き、これはＴＣＲ：ペプチド：ＭＨＣ－ＩＩ複合体のＧＡＤ６５ペプチド構成要素に開始された。T_H２優性を導く経路に関しては、ＴＣＲは、同一のペプチド：ＭＨＣ－ＩＩ複合体を認識でき、それによってシグナル－１を送る。しかし、この場合、シグナル－１の強度がより弱くおよび／またはシグナル－２部分との間の結合が変わったりまたはブロックされていると、最終段階免疫シナプスが変更された形となることになる。同様に、シグナル－１のこのより弱い強度またはＬＦＡ－１二次シグナルの別個の関与が、こうした優性T_H２分化という異なる結果を導く。例えば、変更された免疫シナプスは、ＣＤ４０リガンドが発現されずしたがってＩＬ－１２がＡＰＣに放出されないことを指示可能である。この経路を図２に模式的に示す。ここでは、ＩＬ－４は集積しており、それによってＴ細胞内のＳｔａｔ６およびＧＡＴＡ－３の上方制御したがってT_H２パターンの分化への拘束が導かれる。

【０００８】

現代の応用免疫学の主要なゴールは、T_H１優性免疫（例えば自己免疫疾患および移植拒絶で見られるようなもの）からこれらの同一の組織抗原に対するT_H２反応へのスイッチを可能にすることである。他の症例では、弱いT_H２免疫をT_H１優性に置き換えて、強いＴ細胞増殖および細胞傷害性Ｔ細胞（ＣＴＬ）の有効な生成を導くことが非常に有用であろう。これらの症例には、Ｃ型肝炎およびＡＩＤＳのような慢性ウイルス疾病が含まれる可能性があり；そして黒色腫のような特定の癌が含まれる可能性がある。したがって、当該技術分野で必要なのは、異なるヒト疾患状態または健康状態と戦うために望ましいように、２型免疫を１型免疫に置き換え可能であるか、または１型免疫を２型免疫に置き換え可能であるような、これらの免疫反応の調節因子である。

【０００９】

（発明の概要）

本発明は、先行技術に見られた問題を解決し、そして最先端の明確な進歩をもたらす。簡潔には、本発明は、一端にシグナル－１部分の一部を、そして他方の端にシグナル－２部分の一部を含むペプチドを含む。これらの二端は、互いに直接連結されているか、または柔軟な非基質リンカーを介して連結されていることが可能である。ペプチド部分の直接のまたはリンカーを介した連続ペプチド鎖へのこのコンジュゲート化は、二官能性ペプチド阻害剤（ＢＰＩ）と称する新規種類の免疫療法ペプチドをもたらす。これらのＢＰＩは、Ｔ細胞活性化の２シグナルの機構に基づき、そしてＴ細胞活性化を変更するため、シグナル－１およびシグナル－２部分を連結する。言い換えると、本発明は、Ｔ細胞の特定の疾患関連集団のみが、本発明の産物に標的とされるような、非常に特定された方式で、Ｔ細胞およびそれに続く免疫を調節する方法を提供する。したがって、本発明は、損なわれていない（ｉｎｔａｃｔ）免疫系に必要な構成要素が、その名目上の（ｎｏｍｉｎａｌ）防御方式で作動するままにしておく。

【００１０】

より詳細には、本発明は、目的のＴＣＲエピトープ（シグナル－１部分）を一端に、そしてシグナル－２を生じるタンパク質：タンパク質相互作用由来のペプチド（シグナル－２部分）を有するペプチド配列を構築することを記載する。これらの２つのペプチド配列は、シグナル－１部分をシグナル－２部分にカップリングする柔軟なリンカーを介して連結可能であるし、または直接共に連結可能である。いくつかの場合、２つのペプチド配列間の連結は、各部分由来の隣接残基を含むことが可能である。シグナル－２部分とカップリングしたシグナル－１部分の組み合わせがＢＰＩを構成する。したがって、目的のＴＣＲエピトープをひとたび同定し、そして望ましい免疫反応（１型または２型）を決定したら

、本発明にしたがったＢＰＩを生成可能である。

【００１１】

上述のように、免疫反応の重要な段階は、１型ヘルパーＴ細胞（T_H１細胞）または２型ヘルパーＴ細胞（T_H２細胞）いずれかへのＣＤ４$^+$　Ｔ細胞の分化である。T_H１細胞への分化は、主に細胞仲介免疫を生じ、一方、T_H２細胞への分化は、主に体液性免疫を生じる。これらの免疫種は各々、異なる種類の侵入に対して体を防御するのを助ける。T_H１細胞は、細菌などの細胞内病原体に対して体を防御し、そしてまた器官特異的自己免疫疾患にも関与する。T_H２細胞は、細胞外寄生虫に対しての防御と共にアレルギー性反応に重要である。T_H１細胞の発生は、免疫細胞（マクロファージおよび樹状細胞として知られる）が産生するインターロイキン－１２と呼ばれるサイトカインによって駆動される。インターロイキン－１２は、未処置Ｔ細胞を誘導または刺激して、インターフェロン－γ（ＩＦＮ－γ）およびインターロイキン－２（ＩＬ－２）を産生させる。これらの２つのサイトカイン（ＩＬ－２およびＩＦＮ－γ）は、細胞傷害性Ｔリンパ球（ＣＴＬ）のクローン性増殖、マクロファージ活性化、および感作細胞の補体溶解を仲介するＩｇＧアイソタイプへのクラススイッチなどの古典的細胞仲介機能に関与する。T_H１免疫反応への拘束は、ＩＦＮ－γ（インターロイキン－１２（ＩＬ－１２）受容体の発現を上方制御するがT_H２細胞への発展を阻害する）の存在によって増進される。この経路を図３に模式的に示す。

【００１２】

T_H２免疫は、未処置Ｔ細胞によるインターロイキン－４（ＩＬ－４）産生から生じる。ＩＬ－４は、T_H出現を、そしてそれに続いて、転写制御因子Ｓｔａｔ６の活性化を通じて、インターロイキン－４（ＩＬ－４）、５（ＩＬ－５）および１３（ＩＬ－１３）の産生を誘導する。ＩＬ－４はまた、発展中の細胞上のＩＬ－１２受容体発現を下方制御するよう作動して、それによってT_H１への発展を阻害し、そして未分化Ｔ細胞がT_H２細胞への発展に拘束されるのを助ける。さらに、ＩＬ－４およびＩＬ－５は、Ｂ細胞を活性化し、そして中和抗体（マウスではＩｇＧ１）および即時型過敏の開始因子であるＩｇＥに切り換えることが知られる。このプロセスは図２に模式的に提示される。

【００１３】

上述のように、T_H細胞を完全に活性化するのに、２シグナルの機構が必要である。シグナル－１は、Ｔ細胞抗原受容体（ＴＣＲ）が抗原提示細胞（ＡＰＣ）表面上のペプチド：ＭＨＣ－ＩＩ複合体を認識するかまたは該複合体に結合する際に生じる。この最初のシグナルは、Ｔ細胞受容体を通じて伝達され、そしてキナーゼおよびホスファターゼが仲介する、チロシンリン酸化／脱リン酸化事象のカスケードを開始し、そしてＣａ$^{++}$流束、ＮＦ－ＡＴおよびＮＦκＢ転写因子の活性化を導く。これらの因子は、Ｔ細胞の核に進入し、そしてエフェクター機能に関与する遺伝子のプロモーターに結合する。シグナル－２は、Ｔ細胞上のシグナル－２受容体が、ＡＰＣ上のタンパク質リガンドに結合することから生じる。シグナル－２受容体にはＣＤ２８およびそのリガンドＢ７と共にＬＦＡ－１およびそのリガンドＩＣＡＭ－１が含まれる。シグナル－２受容体およびそのリガンドが、Ｔ細胞の細胞膜とＡＰＣ膜との間の境界面で複合体を形成すると、一連のシグナル伝達事象が起こり、これらの事象には、セリン／スレオニンリン酸化／脱リン酸化と共に、ＧＴＰアーゼ活性を持つアダプタータンパク質を活性化する、グアニンヌクレオチド交換因子の作動が含まれる。これらのシグナル伝達事象は、別個の組の転写因子を活性化する。ＣＤ２８：Ｂ７複合体を通じて搬送されるシグナルは、ＩＣＡＭ－１：ＬＦＡ－１複合体から搬送されるものとは異なり、特にT_H２エフェクター集団と対比して、T_H１へのＣＤ４$^+$　Ｔ細胞の分化に関しては異なる。このシグナル伝達は、本明細書において、図４として模式的に提示する。主な結合がＬＦＡ－１およびＩＣＡＭ－１の間で起こる場合、ＣＤ４$^+$　Ｔ細胞は、T_H１細胞に分化する。T_H１分化状態のＣＤ４$^+$　Ｔ細胞はＩＬ－２およびＩＦＮγを豊富に産生し、この２つのサイトカインは遅延型過敏（ＤＴＨ）、細胞内病原体に対する免疫、およびいくつかの自己免疫疾患などの、炎症性免疫反応の傑出した開始因子である。主な結合がＣＤ２８およびＢ７の間で起こる（すなわちＬＦＡ－１：ＩＣＡ

Ｍ－１シグナル伝達が減少する）場合、ＣＤ４$^{+}$　Ｔ細胞はT_H２細胞に分化する。T_H１細胞と対照的に、T_H２細胞は、ＩＬ－２およびＩＦＮγサイトカインを産生しないが、その代わり、アレルギーおよび喘息などの即時型過敏の仲介因子、すなわち、ＩＬ－４、ＩＬ－５、ＩＬ－１０、およびＩＬ－１３を放出する。したがって、シグナル－２を提供する複合体の相対的な寄与を操作する能力は、自己組織抗原に対して惹起される免疫反応の種類に重大な影響を及ぼす。

【００１４】

ＴＣＲおよびＡＰＣ間の会合は、免疫学的シナプス（図１に示す）と呼ばれる特別な結合部で起こる。免疫反応が進行するため、未分化T_H細胞は、ＡＰＣ表面上に外来侵入物のペプチドを提示されなければならない。不活性化Ｔ細胞において、ＴＣＲおよび接着分子は、Ｔ細胞膜上に無作為に分散している。免疫学的シナプスの形成は、Ｔ細胞が潜在的な抗原性リガンドを区別するのを可能にする、活性でそして動的な機構である。免疫学的シナプスは、接着分子の輪に取り巻かれたＴ細胞受容体の中央クラスターからなる。この配置を図１に模式的に示す。この図では、ＴＣＲ：ペプチド：ＭＨＣ－ＩＩ複合体は、黒い円の中心であり、これは、シグナル－２受容体およびシグナル－２リガンドを構成するタンパク質：タンパク質対を示す。免疫シナプスの安定な形成は、内部にペプチド認識受容体（ＴＣＲ）そして外部にＬＦＡ－１などの接着分子を有するドーナツ様構造を形成するため、ＬＦＡ－１等およびＴＣＲを必要とする。活性化中、ＴＣＲおよびＬＦＡ－１分子は、実際にＴ細胞脂質二重層内で互いを通り越して転位置する。これらの分子が免疫シナプス内で転位置しない場合、Ｔ細胞シグナルは完全には受け取られず、そしてＴ細胞内で遺伝子活性の異なるプログラムが起こる可能性がある。これは、Ｔヘルパー細胞（T_H）が、T_H１免疫反応を導く遺伝子プログラムから、T_H２免疫反応を活性化するプログラムに外れることを引き起こす場合は特に、免疫反応に劇的な影響を与える可能性がある。図２に示すように、ＢＰＩ機構の解釈によって、ＢＰＩがＭＨＣ－ＩＩおよび二次シグナルリガンド両方に結合することが示唆される。これは、ＭＨＣ－ＩＩ：ペプチドおよびＩＣＡＭ－１分子を有効に束縛し、それによって免疫シナプス形成の転位置ステップを妨げる。

【００１５】

本発明の１つの側面において、既知のＴＣＲエピトープをＢＰＩの第一のペプチド部分として用いる。この方式では、免疫原としての能力のある最小ペプチド配列を利用する。これらの最小ペプチド配列（例えば抗原性ペプチド）は、目的の免疫反応（すなわち自己免疫疾患、感染性疾患、アレルギー、癌など）に関与するＴＣＲと効果的に結合する。すでに多くの既知の興味深いＴＣＲエピトープ（シグナル－１部分）があり、そしてその配列は文献に定義されてきている。いくつかの代表的なシグナル－１部分のリストの一部を表１に示す。シグナル－１部分は何千もありえるから、このリストはいかなる意味でも包括的ではない。

【００１６】

本発明の別の側面において、ＢＰＩの第一の部分が合成可能であるように、目的のＴＣＲエピトープを同定する。この側面では、これらの優性ＴＣＲエピトープは、先行技術によってそう決定されてきており、そして配列は文献中に開示されている。あるＴ細胞反応の焦点となるペプチド（例えば糖尿病関連抗原ＧＡＤ６５に対する反応）は、大部分のエフェクターＴ細胞が抗原のこの部分に反応し、そして他の部分に反応しないという事実によって同定される。マウスモデル系において、動物を全タンパク質抗原で免疫する。次に、抗原が免疫系をプライミングした後、Ｔ細胞を除去する。これらのＴ細胞は、抗原の短い重複ペプチドと共に、別個に培養中に入れる。反応の大部分は、単一のペプチドに対するものであろうし、そしてこれは優性ＴＣＲエピトープである。ヒトにおいて、Ｔ細胞をまず、患者からクローニングする。これらのクローニングしたＴ細胞を、重複するペプチド（関与する抗原の個々の部分、例えばＨＩＶ－１、ｐ２４（配列番号８）に相当する）と共に、別個に培養中に入れる。再び、大部分のＴ細胞クローンが反応するペプチドは、優性ＴＣＲエピトープである。前述の内容は、Ｓｃｈｏｕｎｔｚら，　ＭＨＣ遺伝子型は、

リガンド密度の容量を調節して、ｉｎ ｖｉｖｏでＴヘルパー（Ｔｈ）－１／Ｔｈ－２プライミングを切り換える，１５７ Ｔｈｅ Ｊｏｕｒｎａｌ ｏｆ Ｉｍｍｕｎｏｌｏｇｙ ３８９３－３９０１（１９９６）に記載され、この文献の解説および内容は、本明細書に援用される。

【００１７】

本発明の別の側面において、シグナル－２受容体由来のペプチドを用いて、Ｔ細胞上の名目上の受容体およびＡＰＣ表面上の相補リガンド間の相互作用を変更する。表３は、いくつかの既知のシグナル－２受容体部分の代表的なリストを含む。もちろん、このリストは代表であり、そしてすべてを含んではいないため、一般の当業者は、該表に列挙されていない他のシグナル－２部分を同定可能であろう。

【００１８】

本発明の別の側面は、生じる免疫反応を変更するため、ＴＣＲエピトープ（すなわちシグナル－１部分）をシグナル－２受容体ペプチド模倣体（ｐｅｐｔｉｄｅ ｍｉｍｉｃ）（すなわちシグナル－２部分）に連結することである。この連結は、シグナル－１部分およびシグナル－２部分間に直接にまたは隣接残基を介して存在できる。あるいは、この連結は、シグナル－１部分およびシグナル－２部分間に配置されるリンカーを介して行うことも可能である。リンカーは、天然存在アミノ酸または化学合成アミノ酸を含む、いかなるアミノ酸であることも可能である。好ましくは、プロテアーゼ攻撃に耐性であるため、非基質アミノ酸を使用するであろう。さらにより好ましくは、リンカーは、小アミノ酸または親水性アミノ酸と交互に非基質アミノ酸を含んでなるであろう。さらにより好ましくは、リンカーは、リンカーの各端に隣接するシグナル－１部分およびシグナル－２部分と共に、１つの連続配列として合成可能である。さらにより好ましくは、リンカーは、一般式$(A, B)_x$を有し、式中、ＡおよびＢはアミノ酸残基であり、そしてＡアミノ酸残基は、独立して、それぞれ、アミノカプロン酸、アミノヘキサン酸、アミノドデカン酸、およびβ－アラニンからなる群より選択され、そしてＢアミノ酸残基は小または親水性アミノ酸である。この式において、Ｘが１～１００の範囲であることが可能である。特に代表的なＢ残基はグリシンである。この態様において、リンカーは、潜在的に、グリシン残基（Ｇ）と交互にアミノカプロン酸（Ａｃ）、アミノヘキサン酸（Ａｈｘ）、アミノドデカン酸（Ａｄｏ）、およびβ－アラニン（βＡ）を有することが可能である（例えばＡｃ－Ｇ－Ａｈｘ－Ｇ－Ａｄｏ－Ｇ－βＡ）。リンカーを構築するのに用いる残基の選択は、リンカーの望ましい長さとともに静電妨害に関する考慮に基づくことができる。１つの好ましいリンカーは、交互に現れるＡｃ残基およびＧ残基を含んでなる。このリンカーは、選択する他アミノ酸残基（Ａｈｘ、Ａｄｏ、βＡ）を含むことによって、長くすることもまたは短くすることも可能である。いくつかの代表的なリンカーが、配列番号２６～２９として表２に含まれる。

【００１９】

およそ10^9の異なるＴＣＲが、大量の感染性病原体に対する防御免疫の原因となり、そしてこのＴＣＲは、自己免疫疾患において、自己に背く可能性があるＴＣＲのレパートリーを含有する。さらに、ＴＣＲはまた、潜在的な腫瘍抗原および環境中の無数のアレルギー性物質にも特異的である。所定のＢＰＩのＴＣＲエピトープを変化させることによって、我々はＢＰＩの免疫調節能力を、選択したＴＣＲ群に向ける。言い換えると、ＢＰＩに取り込むＴＣＲエピトープの選択は、非常に特異的な様式で、特定のヒト疾患に関与するＴ細胞を標的とする。例えば、ＧＡＤ６５エピトープをＢＰＩに取り込むと、１型糖尿病の誘導に関与する自己攻撃Ｔ細胞が標的とされる。特定のＴＣＲのこうした標的化によって、感染性病原体または癌に対する免疫に必要なＴ細胞が顕著な影響を及ぼされることはないだろう。したがって、ＢＰＩは、Ｔ細胞免疫を１つの抗原に特異的に調節する一方、感染性病原体および癌発症に対する防御免疫に必要なＴ細胞レパートリーには影響を与えないでおく可能性をもたらす。

【００２０】

上述のように、本発明のシグナル－１部分は、好ましくは、ＴＣＲエピトープに由来し、

そして既知の代表的なエピトープのリストを表１に示し、これらの既知のエピトープは、配列番号１～２５として示す。ＴＣＲエピトープの誘導体（ｄｅｒｉｖａｔｉｖｅ）を用いてＢＰＩを構築する場合、好ましくは、選択するＴＣＲエピトープは、既知の健康状態または疾患状態と相関するであろう。ＢＰＩを構築するのに、表１に示す代表的なペプチドの１つを用いる際、該ペプチドが、配列番号１～２５からなる群より選択される配列と、少なくとも約１０％の配列相同性を有する配列を含むことが好ましい。より好ましくは、ペプチドは、配列番号１～２５からなる群より選択される配列と、少なくとも３０％の配列相同性を有するであろう。より好ましくは、ペプチドは、配列番号１～２５からなる群より選択される配列と、少なくとも５０％の配列相同性を有するであろう。さらにより好ましくは、ペプチドは、配列番号１～２５からなる群より選択される配列と、少なくとも７０％の配列相同性を有するであろう。最も好ましくは、ペプチドは、配列番号１～２５からなる群より選択される配列と、少なくとも約９５％の配列相同性を有するであろう。もちろん、ＴＣＲエピトープ由来のペプチドと同様の機能を有する部分を構築するための、ペプチド擬似体（ｐｅｐｔｉｄｏｍｉｍｅｔｉｃ）の使用もまた、当該技術分野にはよく知られている。これに関連して、Ｆａｌｃｉｏｎｉら，　自己免疫疾患関連クラスＩＩ主要組織適合分子による抗原提示を阻害する、ペプチド擬似体化合物，　１７　Ｎａｔｕｒｅ　Ｂｉｏｔｅｃｈｎｏｌｏｇｙ，　５６２－５６７（１９９９）の解説が本明細書に援用される。したがって、ＢＰＩのこのシグナル－１部分ポーションのすべてまたは一部を、こうしたペプチド擬似体に含むことが可能である。好ましくは、ペプチド擬似体は、配列番号１～２５からなる群より選択されるペプチドの擬似体であろう。あるいは、シグナル－１部分は、配列番号１～２５からなる群より選択されるＴＣＲエピトープまたはペプチドの誘導体であろう。いずれにしても、ＢＰＩのこの第一の部分（または一次シグナルを開始するのに関与する部分）が抗原提示細胞（ＡＰＣ）上の主要組織適合複合体（ＭＨＣ）と結合可能であることが望ましい。さらに、この生じたペプチド：ＭＨＣ複合体が、重要なＴＣＲと結合し、そしてＴ細胞へのいくつかの型のシグナルを開始可能であることが好ましい。

【００２１】

上述のように、リンカーの、シグナル－１部分と反対側に用いるペプチドは、好ましくは、シグナル－２受容体由来である。ＢＰＩのこの第二の部分は、直接またはリンカーを介して、第一の部分に連結される。好ましい型において、第二の部分は、配列番号３０～４１からなる群より選択される配列と、少なくとも約１０％の配列相同性を有する配列を含む。より好ましくは、第二の部分のペプチドは、配列番号３０～４１からなる群より選択される配列と、少なくとも約３０％の配列相同性を有する。さらにより好ましくは、第二の部分のペプチドは、配列番号３０～４１からなる群より選択される配列と、少なくとも約５０％の配列相同性を有する。さらにより好ましくは、第二の部分のペプチドは、配列番号３０～４１からなる群より選択される配列と、少なくとも約７０％の配列相同性を有する。最も好ましくは、第二の部分のペプチドは、配列番号３０～４１からなる群より選択される配列と、少なくとも約９５％の配列相同性を有する配列を含む。第一の部分の場合のように、第二の部分のアミノ酸残基のすべてまたはいくつかの代わりに、ペプチド擬似体が使用可能である。好ましい型において、第二の部分のペプチド擬似体は、配列番号３０～４１からなる群より選択されるペプチドの模倣体であろう。あるいは、ＢＰＩの第二の部分は、配列番号３０～４１からなる群より選択されるペプチドの誘導体を含んでなるであろう。第一の部分同様、第二の部分が、抗原提示細胞上の相補リガンド（例えばシグナル－２リガンド）と結合可能であることが好ましい。例えば、ＬＦＡ－１由来のペプチドを、ＢＰＩのこのシグナル－２部分として用いる場合、ＡＰＣ表面上のＩＣＡＭ－１に結合するべきである。さらに、ＡＰＣ上のシグナル－２リガンドとのこの結合が、この部分の親受容体（Ｔ細胞上）のこの同一のＡＰＣリガンドへの結合を阻害するかまたは改変することが好ましい。

【００２２】

上に説明するように、免疫反応はシグナル２つの機構を伴い、そして本発明の目的は、所

定の免疫反応を、例えば１型免疫から２型免疫に、または２型免疫から１型免疫に変更することである。免疫反応表現型のこの変更またはシフトは、本発明にしたがったＢＰＩによって達成される。いくつかの場合、ＢＰＩが、免疫反応を、T_H１優性または細胞溶解免疫反応から、T_H２優性反応に変更することが好ましく；そして、他の場合、ＢＰＩが、免疫反応を、T_H２優性反応から、T_H１または細胞溶解優性反応に変更することが好ましい。ある場合、ＢＰＩは、非常に特異的なＴ細胞表現型、例えばペプチド特異的サプレッサーＴ細胞の活性化を介して作動する可能性がある。抗原がT_H１反応に向かう系を刺激する名目上の状況と対照的に（図２に示す）、ＧＡＤ　６５－ＣＤ１１ａＢＰＩに類似したＢＰＩが免疫シナプスに導入される際に生じる反応は非常に異なり、反応を１型から２型にシフトするよう作動する。この状況を図２に模式的に示す。この方式で、シグナル－１部分、柔軟な非基質リンカー、およびシグナル－２部分を含んでなるＢＰＩを形成し、そして免疫シナプスに導入する。ＴＣＲがＡＰＣ上のペプチド：ＭＨＣ複合体を認識し、そして一次シグナルを開始する。しかし、ＢＰＩの第二の部分（シグナル－２部分）が、ＬＦＡ－１／ＩＣＡＭ－１（または他のＢＰＩ：ＣＴＬＡ－４／Ｂ７、またはＣＤ４０Ｌ／ＣＤ４０、またはＦａｓＬ：Ｆａｓ）間で典型的なシグナル－２相互作用が生じるのを遮断し、そして中央クラスターへのＴＣＲの転位置を遮断する。ＬＦＡ－１／ＩＣＡＭ－１またはＣＴＬＡ－４／Ｂ７相互作用が、特定のＢＰＩ構築物に標的とされるのかどうかに応じて、おそらくＭＨＣ－ＩＩ：ペプチド複合体を二次シグナルリガンドに束縛することによって、シグナルは、異なる分化方向に改変されるであろう。例えば、ＢＰＩのシグナル－２ペプチド部分がＬＦＡ－１由来である場合、このことはＣＤ４０リガンド発現の減少、したがってＩＬ－１２放出を欠如させるように助けるであろう。対照的に、最初のＴ細胞活性化中に放出されるＩＬ－４は、シナプス周囲に、より高いレベルで集積するであろう。ＩＬ－４のこの集積は、未処置Ｔ細胞におけるＳｔａｔ６およびＧＡＴＡ－３上方制御を導き、そして最終的に、２型パターンへの拘束を導く。あるいは、ＢＰＩのシグナル－２部分のペプチド部分がＣＴＬＡ－４由来である場合、ＣＴＬＡ－４およびＣＤ－２８のＢ７リガンドへの通常の結合が影響を受け、そしてしたがってより多くのＣＤ４０リガンドが発現され（すなわち高親和性ＬＦＡ－１：ＩＣＡＭ－１のより大きい役割が、Ｂ７受容体を遮断することによって決定づけられる）；したがって、ＩＬ－１２の放出が増加する。インターロイキン－１２は、未処置Ｔ細胞がより多くのＩＦＮ－γおよびＩＬ－２を産生するよう誘導または刺激し、こうして１型免疫に向かう正のフィードバックをもたらす。これらの２つのサイトカイン（ＩＬ－２およびＩＦＮ－γ）は、細胞傷害性Ｔリンパ球（ＣＴＬ）のクローン性増殖、マクロファージ活性化、および感作細胞の補体溶解を仲介するＩｇＧアイソタイプへのクラススイッチなどの古典的細胞仲介機能に関与する。こうした反応は、ヒトウイルス疾患に対する防御免疫の顕著な特徴である。これはまた、ＴＣＲエピトープをＦａｓの受容体に連結するのにも作動するであろう。Ｆａｓ：ＦａｓＬ相互作用はアポトーシスを支配するため、アポトーシス事象を遮断することによって、特定のＴＣＲを持つ細胞の頻度を増加させることもできるであろう。これは、ＨＩＶ、ＨＰＶ、ＨＣＶ、および癌に対するＢＰＩ設計に重要であろう。

【００２３】

したがって、本発明の重要な側面は、特定のＴＣＲエピトープをシグナル－２受容体ペプチド模倣体に束縛すると、これらの受容体のみを持つＴ細胞および／またはこれらのペプチド特異的サブセットに間接的に結び付けられるＴ細胞集団が関与するＴ細胞分化の改変が導かれることである。所定の免疫優性ペプチド抗原に対するＴ細胞反応を遮断するかまたは改変する能力は、免疫病理学的状態に対する非常に正確な処置を提供するであろう。現在の免疫療法の主な欠点は、広い特異性範囲のＴ細胞が影響を受けるため、宿主を感染および癌により罹りやすいままにすることである。本発明のＢＰＩは、所望のＴ細胞集団およびこれらの初期Ｔ細胞に依存して起こる反応だけを遮断および／または改変するはずである。また、ＢＰＩは、所望のエフェクター機能へと活性化させるために、特定のＴＣＲを有する集団を標的とするであろう。

【００２４】

本発明の別の側面において、Ｔ細胞－ＡＰＣ相互作用によって生じるシグナルの相対強度は、最終的な免疫反応が１型または２型反応であるかどうかに影響を及ぼす。これに関連して、Ｍｕｒｒａｙ，　ＭＨＣはどのようにT_H1／T_H2免疫を選択するか，　１９　Ｉｍｍｕｎｏｌｏｇｙ　Ｔｏｄａｙ　１５７－１６３（１９９８）の解説が本明細書に援用される。

【００２５】

本発明の別の側面において、ＡＰＣ上のＭＨＣおよびシグナル－２リガンドに結合可能なペプチドとＡＰＣとを接触させ、そしてＴ細胞に伝達されるシグナルの改変を引き起こすことによって、免疫反応を変更する。こうして、免疫反応は、免疫原ペプチドやその対応する抗原（すなわち感染性病原体、自己タンパク質、またはアレルゲン）と一般的に関連する免疫反応から外れていく。

【００２６】

本発明の別の側面において、一般式ＡＸＢを有するペプチドを提供する。Ａ、Ｘ、およびＢは、アミノ酸残基の鎖を表し、Ａ鎖は、少なくとも約５残基を、そしてＴＣＲエピトープと少なくとも約１０％の配列相同性を有し、Ｂ鎖は、少なくとも４残基を、そしてシグナル－２部分由来のペプチドと少なくとも約１０％の配列相同性を有し、そしてＸ鎖はリンカーである。リンカーは、天然存在アミノ酸または化学合成アミノ酸を含む、いかなるアミノ酸であってもよい。さらに、Ｘ鎖は少なくとも１残基を有する。Ｘを伴わず、ＡをＢに直接連結することもまた可能であるが、ＡＰＣ表面上のＭＨＣ－ＩＩおよび二次シグナルリガンド間の距離を補うため、ある程度の大きさのリンカーが好ましい。上述のように、好ましくは、プロテアーゼ攻撃に耐性であるため、非基質アミノ酸を用いるであろう。さらにより好ましくは、リンカーは、小アミノ酸または親水性アミノ酸と交互に非基質アミノ酸を含んでなるであろう。さらにより好ましくは、リンカーは、リンカーの各端に隣接するシグナル－１部分およびシグナル－２部分と共に、１つの連続配列として合成可能である。さらにより好ましくは、リンカーは、一般式$(A，B)_x$を有し、式中、ＡおよびＢはアミノ酸残基であり、そしてＡアミノ酸残基は、独立して、それぞれ、アミノカプロン酸、アミノヘキサン酸、アミノドデカン酸、およびβ－アラニンからなる群より選択され、そしてＢアミノ酸残基は小アミノ酸または親水性アミノ酸である。この式において、Ｘが１～１００の範囲であることが可能である。特に代表的なＢ残基はグリシンである。この態様において、リンカーは、潜在的に、グリシン残基（Ｇ）と交互にアミノカプロン酸（Ａｃ）、アミノヘキサン酸（Ａｈｘ）、アミノドデカン酸（Ａｄｏ）、およびβ－アラニン（βＡ）を有することが可能である（例えばＡｃ－Ｇ－Ａｈｘ－Ｇ－Ａｄｏ－Ｇ－βＡ）。リンカーを構築するのに用いる残基の選択は、リンカーの所望の長さとともに静電妨害に関する考慮、疎水性、電荷などに基づくことが可能である。１つの好ましいリンカーは、交互に現れるＡｃおよびＧ残基を含んでなる。このリンカーは、他のアミノ酸残基（Ａｈｘ、Ａｄｏ、βＡ）を選択して含むことによって、長くすることもまたは短くすることも可能である。いくつかの代表的なリンカーが、配列番号２６～２９として表２に含まれる。さらに、Ｘ鎖は、Ａ鎖およびＢ鎖の間に配置され、そして全ペプチドは、１つの連続配列として合成可能である。いくつかの好ましい配列は、配列番号１～２５のいずれか１つと少なくとも約１０％の配列相同性を有するＡ鎖、配列番号２６～２９のいずれか１つと少なくとも約２％の配列相同性を有するＸ鎖、および配列番号３０～４１のいずれか１つと少なくとも約１０％の配列相同性を有するＢ鎖、を有するであろう。ペプチドは、１型反応から２型反応へ、またはその逆のシフトを行うことが可能であることが好ましい。もちろん、ペプチド擬似体を合成して、リンカーを含む、ＢＰＩのいかなる部分も模倣することが可能である。好ましくは、Ａ鎖はＡＰＣ上のＭＨＣに結合して、ペプチド：ＭＨＣ複合体を形成する。この複合体は、決定的なＴ細胞集団上にあるＴＣＲに結合可能である。さらにより好ましくは、Ｂ鎖は、ペプチド：ＭＨＣ複合体の形成と同時点で、ＡＰＣ上のシグナル－２リガンドに結合可能である。このＡＰＣへの結合の組み合わせは、Ｔ細胞に搬送されるシグナルを改変可能であるはずである。一次シグナルおよび二次シグナルの組み合わせは、Ｔ細胞を完全に活性化可能であり、そしてＡ鎖に用いるペプ

チドおよびＢ鎖に用いるペプチドを選択することによって、免疫反応を通常の進行から外れさせることができる。正常に活性化された、T_H１細胞の上方制御を導く１型反応の場合には、この反応を、T_H２細胞の上方制御を導く２型反応を生じるよう改変可能である。正常に活性化された、T_H２細胞の上方制御を導く２型反応の場合には、この反応を、T_H１細胞の上方制御を導く１型反応を生じるよう、改変可能である。Ａ鎖は、その配列と通常関連する健康状態に基づいて、選択可能である（例えば表４を参照されたい）。

【００２７】

本発明の別の側面において、免疫反応を調節するペプチドを調製する方法を提供する。この方法は、ＴＣＲエピトープ由来の配列と少なくとも約１０％の配列相同性を有する第一のペプチド配列を選択し、シグナル－２受容体部分由来の配列と少なくとも約１０％の配列相同性を有する第二のペプチド配列を選択し、柔軟な非基質リンカーである第三のペプチド配列を選択し、そしてこれらのペプチドを連続ペプチド鎖として合成するステップを含んでなる。好ましくは、リンカーは、ＴＣＲエピトープ由来のペプチドと一端で隣接し、そしてシグナル－２部分由来のペプチドと他方の端で隣接する。好ましくは、第一のペプチド配列は、既知の健康状態と関連し、そしてＡＰＣ上のＭＨＣと結合可能であるべきである。同様に、第二のペプチド配列が、ＡＰＣ上のシグナル－２リガンド部分と結合可能であることが好ましい。この方法は、ＴＣＲと名目上のペプチド免疫原を接触させ、それによって第一のペプチド配列をＭＨＣに結合させ、そして第二のペプチド配列をシグナル－２リガンドに結合させ、それによって、一次シグナルリガンドおよび改変／遮断された二次シグナルリガンド（これらのシグナルリガンドは所望の免疫反応を活性化する強力なものである）を所持するＡＰＣを生じるステップをさらに含んでなることが可能である。

【００２８】

ＢＰＩ設計に固有なのは抗原特異的部分であるが、この抗原特異的部分は、所定のＴ細胞集団が活性化されてそれに対して反応し（すなわちＴＣＲエピトープ）、最終的に防御またはある場合は病的免疫反応を生じる免疫反応のカスケードを導く。これらのエピトープは、抗原提示細胞（ＡＰＣ）表面上の主要組織適合複合体（ＭＨＣ）分子に結合した際、Ｔ細胞活性化の２シグナルの機構のシグナル－１を提供する。したがって、重要な考慮は、ＭＨＣ分子に対する所定のペプチドの親和性である。マウスおよびヒトにおいて、この親和性は、ビオチン化ペプチドを、Ｔ細胞にペプチド：ＭＨＣ複合体を提示する細胞に結合させることによって、直接試験した。可能性のあるペプチドエピトープに対するＴ細胞クローンを生成し、Ｔ細胞機能を特異的に刺激するある反応の免疫優性ＴＣＲへの結合に関して、ＥＬＩＳＰＯＴアッセイによってｉｎ　ｖｉｔｒｏで試験した。ＴＣＲに実際に接触するペプチド残基の変更は、ＢＰＩ開発の一部であるため、ＭＨＣ分子に結合するエピトープの既知の結晶構造が利用可能であることもまた、好ましい。これによって、特定のアミノ酸置換または擬似体が、発展中のＴ細胞が遭遇する実際の構造にどのように影響を与えるかを正確に三次元予測することが可能になる。しかし、既知の結晶構造なしでも、他のペプチド：ＭＨＣ構造の入手可能な座標に基づき、仮説的なペプチド：ＭＨＣ構造の形状を予測することが可能である。この予測可能性は、部分的には、確立されたペプチド結合モチーフに負うものであり、これが、エピトープのどの残基が、与えられたＭＨＣ対立遺伝子の特定の結合ポケットに、最も容易に適合するかの予測を可能にする。これらの予測は、コラーゲンペプチドに結合する２つの異なる対立遺伝子（Ｉ－A^sおよびＩ－A^b）結合を用いて達成される（Ｉ－Ａ分子の各多型位をこれらの位で既知のアミノ酸で置換した）。全体の構造は、異なるペプチドに結合する参照対立遺伝子Ｉ－A^kの公表された座標に基づいた。したがって、特定の疾患関連エピトープの３Ｄ構造が知られていない場合でも、同様の対立遺伝子置換およびモデリングアプローチによって、ＴＣＲがペプチドに接触する場所を予測する構造をもたらすことができる。これらのＴＣＲ接触位を同定する（または少なくとも予測する）ことが重要である。ＴＣＲ接触位に対するある種の改変は、免疫の経過を決定可能なT_H１またはT_H２型へのＴ細胞の機能的分化を変化可能であることがよく知られている（Ｍｕｒｒａｙら，　主要組織適合複合体（ＭＨＣ）クラ

スＩＩ分子は、T_H1またはT_H2反応において、同一の免疫原ペプチドの反対側にＴＣＲ特異性を向ける（未公表原稿、２０００）；およびＭｕｒｒａｙ；１９ Ｉｍｍｕｎｏｌｏｇｙ Ｔｏｄａｙ １５７－１６３（１９９８）、この文献の解説は本明細書に援用される）。

【００２９】

特に、ペプチドが生存ＡＰＣ上のＭＨＣ分子に結合することが確認された。簡潔には、脾臓細胞密度勾配分画（マウス由来）、またはＰＢＬ、またはＡＰＣ株（ヒト由来）を、増加する濃度の個々のビオチン化ペプチドと共に、丸底９６ウェルプレート中で、３７℃、５％CO_2で１６時間インキュベーションした。ＡＰＣへのＢＰＩの結合後、アビジン－ＦＩＴＣを、細胞と氷上で３０分間インキュベーションし、その後、ビオチン化抗アビジンと1時間インキュベーションし、その後、再びアビジン－ＦＩＴＣとインキュベーションした。ＢＰＩ力価測定（ｔｉｔｒａｔｉｏｎ）には、滅菌０．５％ＢＳＡ－ＰＢＳ中の０．１－１００μＭへ増加する濃度のビオチン化誘導体を、上述のように、ＡＰＣと１６時間インキュベーションした。図５に示すように、予測されるとおり、ＧＡＤ６５ ＢＰＩはＮＯＤ ＡＰＣに優先的に結合する。ＬＦＡ－１部分またはＧＡＤ６５部分は、糖尿病系統のＡＰＣへ対するこのような増加した結合を示さなかった。三色解析は、商業的に入手可能な、ＡＰＣの既知の表面マーカーに対するＣｙ－クロムまたはＰＥ－コンジュゲート化抗体を用いた。結合ペプチドのアビジン－ＦＩＴＣおよびビオチン化抗アビジン検出は、Ｍｕｒｒａｙら，２４ Ｅｕｒ．Ｊ．Ｉｍｍｕｎｏｌ．２３３７－２３４４（１９９４）；Ｍｕｒｒａｙ；１９ Ｉｍｍｕｎｏｌｏｇｙ Ｔｏｄａｙ １５７－１６３；およびＳｃｈｏｕｎｔｚら，１５７ Ｔｈｅ Ｊｏｕｒｎａｌ ｏｆ Ｉｍｍｕｎｏｌｏｇｙ ３８９３－３９０１（１９９６）に先に記載されるとおりであった。この方法のわずかな変更を用いて、生存ＡＰＣ表面上で直接にＭＨＣおよびＩＣＡＭ分子の同時キャッピングおよびペプチド阻害（すなわちペプチド親和性）を試験した。まず、ＭＨＣまたはＩＣＡＭ分子に対するモノクローナル抗体（ｍＡｂ）はＢＰＩ結合を遮断し、したがって、ＢＰＩがこれらの分子に結合することが示された（図６）。非標識ペプチドを競合アッセイで用いて、ＡＰＣに対するＢＰＩの相対親和性を得て、そしてＢＰＩがＡＰＣ上のＭＨＣおよびＩＣＡＭ分子を架橋する能力は、ＩＣＡＭを抗ＭＨＣ ｍＡｂで同時キャッピングすることによって調べた。ここでは、細胞をＢＰＩとインキュベーションした。次に、抗ＭＨＣ ｍＡｂを添加した。３７℃で３０分間処理した後、細胞を氷上に移し、そしてＰＥ標識抗ＩＣＡＭで染色した（図７）。

【００３０】

次に、ＢＰＩで後に使用するための、ＴＣＲエピトープを決定するため、Ｔ細胞クローンを生成した。これらの実験は、先に記載する方法（Ｍｕｒｒａｙら，２４ Ｅｕｒ．Ｊ．Ｉｍｍｕｎｏｌ．２３３７－２３４４（１９９４）；Ｍｕｒｒａｙ；１９ Ｉｍｍｕｎｏｌｏｇｙ Ｔｏｄａｙ １５７－１６３；およびＳｃｈｏｕｎｔｚら，１５７ Ｔｈｅ Ｊｏｕｒｎａｌ ｏｆ Ｉｍｍｕｎｏｌｏｇｙ ３８９３－３９０１（１９９６））を用いて、予測されるＴＣＲエピトープに対して免疫した、ヒトまたはマウス由来のＣＤ４＋またはＣＤ８＋ Ｔ細胞クローンを利用した。これらのクローンは、照射組織適合リンパ球、ペプチド、および組換えＩＬ２で隔週、再刺激することによって維持した。所定のＴＣＲエピトープが、サイトカイン合成の活性化に有効であるか決定するため、ＥＬＩＳＰＯＴアッセイを用いた。もちろん、他のサイトカインアッセイもまた使用可能である。解析のためには、予測されるＴＣＲ接触位で置換したＢＰＩを用いて、これらのＢＰＩ変異体のうちどれが、上で解析するような、増殖、および個々のクローンからのサイトカイン放出の阻害に最も有効であるかを決定するであろう。分子モデリングによってもたらされた構造において、予測される位を、異なるアミノ酸または擬似体でスキャンして、ＴＣＲとの相互作用を改変するであろう。

【００３１】

次に、分子動力学／エネルギー最小化（ＭＤＥＭ）を用いて、ペプチドコンホメーションを研究するであろう。ＭＤシミュレーションを結晶データと組み合わせて用いて、ＢＰＩ

の溶液コンホメーションを予測するであろう。ＭＤＥＭシミュレーションは、柔軟度、並びにＢＰＩのＭＨＣ－ＩＩおよびＩＣＡＭ－１への結合中のコンホメーション変化を予測可能である。これらのモデル研究によってまず予測されるように、両受容体への同時結合を助けるため、シグナル－１部分および／またはシグナル－２部分にアミノ酸置換を導入することも可能である。Ｉ－A^{g7}：ＢＰＩ：Ｄ１構造（図８を参照されたい）は、疾患関連ペプチド：ＭＨＣ構造（Ｉ－A^{g7}：ＧＡＤ６５ペプチド）の結晶構造座標を用いる例である。この図では、ＩｎＳｉｇｈｔＩＩ（ＭＳＩ／Ｂｉｏｓｙｍ）を用いて、Ｓｉｌｉｃｏｎ Ｇｒａｐｈｉｃ Ｏｃｔａｎｅワークステーション上でドッキング研究を行った。ＬＦＡ－１－ＩＣＡＭ－１構造は、既知の回折座標から取り、そしてＩ－A^{g7}：ＧＡＤ６５ペプチドは既知の座標由来であった。もちろん、マウスおよびヒトの多様なＭＨＣ分子に結合するため、類似の方法を用いて、表１、２、３および４に列挙するＢＰＩをモデリングすることが可能である。

【００３２】

ＢＰＩプロセスの第二の段階は、Ｔ細胞の機能的分化に関与する、確立された二次シグナル受容体分子のペプチド模倣体の選択である。これらの構造を決定するため、結晶構造およびその生理学的リガンドに結合する二次シグナル受容体の入手可能なモデルを用いて、リガンドと接触する受容体の領域を予測するであろう。このアプローチを用いて

【００３３】

【化１】

(EIAPVFVLLE)-(AcGAcGAc)-(ITDGEATDSG)

GAD65(208-217)　リンカー　LFA-1(237-247)

【００３４】

と示すことが可能なＥＧＡＤ－ＢＰＩを設計した。

これらの相互作用はまた、二次シグナル分子に部位特異的変化を作成して、そしてこうした突然変異体分子がなお、問題のリガンドに結合するかどうか決定することによって、経験的に決定可能である。しかし、これらの研究の多くは、ＬＦＡ－１、ＣＤ２８／ＣＴＬＡ４、ＣＤ４０Ｌ、およびＣＤ９５Ｌを含む、既知の二次シグナル受容体を用いて行われてきた。分子モデリング研究を上述のように用いて、これらの二次シグナル部分とそのリガンドの相互作用を別個に、そして該部分がＢＰＩ構造の一部である場合、モデリングするであろう。

【００３５】

ＭＨＣおよび二次シグナルリガンドに有効に結合して、そしてｉｎ ｖｉｔｒｏでＴ細胞機能を改変する、候補ＢＰＩ部分をひとたび同定したら、これらの部分を、相対的に短い合成ペプチドの反対側で合成し、多様な長さの非タンパク質分解基質リンカーによってこれらを連結した。原型的なＥＧＡＤ－ＢＰＩに記載されるように、シグナル－１部分（すなわち疾患関連ＴＣＲエピトープ）は、この柔軟なリンカーによって、シグナル－２受容体模倣ペプチド（すなわちT_H1／T_H2分化に関与すると考えられる二次シグナル受容体）に連結した。これらの合成ペプチドは、ペプチド合成の従来法によって生成した。いくつかの場合、ＢＰＩは、単離ＭＨＣおよび二次シグナルリガンドへの結合に関して試験し、そしてＮＭＲ、分子モデリングおよび結晶学を用いて、その正確な３Ｄ構造を決定する。最後に、与えられたＢＰＩがｉｎ ｖｉｖｏで生物学的に活性であるかどうかを決定した。マウスをＢＰＩで処置し、そして免疫細胞を単離し、そしてＥＬＩＳＰＯＴによってサイトカイン産生に関して試験した（図９および１０を参照されたい）。もちろん、他のサイトカインアッセイが一般の当業者によく知られているであろうし、そしてＥＬＩＳＰＯＴの代わりに使用可能である。ＮＯＤ．Ｓｃｉｄモデルと同様のｉｎ ｖｉｖｏモデル（すなわちヒトＳｃｉｄ、トランスジェニックノックアウト系統など）を、ＢＰＩ有効性のより厳密な試験として用いた（調べる各疾患に必要な変更を行ったが）。例えば、ＨＩＶ－１ ｐ２４ ＴＣＲエピトープの場合、養子移植実験に、ヒト－Ｓｃｉｄマウスを用

いる。この実験においては、患者由来のＴ細胞をまず、従来法によってクローニングし、そしてｉｎ　ｖｉｔｒｏで、ＢＰＩで刺激するであろう。次にこれらの細胞をヒト－Ｓｃｉｄマウスに移植し、そしてＮＯＤ．Ｓｃｉｄ養子移植実験においてＥＧＡＤ－ＢＰＩのように解析するであろう。これらの実験の結果は、図１１～１３に示す。

【００３６】

ＢＰＩを合成するため、クロロトリチル樹脂上のＦｍｏｃ化学反応を用いた。保護アミノ酸は、８倍過剰で１時間、二重カップリングした。樹脂をＤＭＦとＭｅＯＨで洗浄し、そして試薬Ｒ：ＴＦＡ、ＥＤＴＡ、チオアニソール、アニソール中で切断した。溶液中にペプチドを含有するＴＦＡ混合物は、エーテル中で沈殿させ、そして徹底的に洗浄した。ペプチドの調製用ＨＰＬＣは、０．１％ＴＦＡ中の０－８０％アセトニトリルの勾配によって達成した。多様な分画の凍結乾燥およびＶｏｙａｇｅｒ質量分析計（ＰｅｒＳｅｐｔｉｖｅ、カリフォルニア州フォスターシティー）を用いたＭＡＬＤＩ－ＴＯＦによる確認によって、ＴＦＡ塩として、合成ペプチドを得た。上述のようなモデリング、結晶学および結合についての研究を用いて、予測されるＢＰＩ複合体構造を作り出した。

【００３７】

糖尿病ＢＰＩをｉｎ　ｖｉｖｏで試験する別の例において、１５００万の疾患関連リンパ球（すなわち患者Ｔ細胞、または疾患過程に関連するＴ細胞集団）を、（ｉｎ　ｖｉｖｏまたはｉｎ　ｖｉｔｒｏで）ＢＰＩ化合物を投与したＴ細胞を含みまたは含まずに注入し、そして組換えＩＬ－２中、２４時間増殖させた。いくつかの実験では、養子移植の前に、ＣＤ１５４、ＣＤ２５、ＣＤ６２Ｌ、ＣＤ１５２などに対するｍＡｂおよび磁気粒子を用いてＴ細胞の特定のサブセットを枯渇させるであろう。ＢＰＩの個々の部分で処理したＴ細胞（マウスまたはヒト由来）が、生理食塩水のみで処置したマウス由来のＣＤ４＋細胞と共に、陰性対照として用いられるであろう（図９）。自発的糖尿病をブロックするため、５つの群の１０匹のメスＮＯＤマウス（１２週齢）を用いて、標準的糖計測装置（ＡｃｃｕＣｈｅｋ－ｃｏｍｐｌｅｔｅ、Ｒｏｃｈｅ　Ｄｉａｇｎｏｓｔｉｃｓ）を用いて、非糖尿病性血液グルコースレベルをモニターした。各マウスを標識し、そして実験の経過中、毎週、それぞれの血液グルコースレベルをモニターした。この５群には、（ａ）８週齢でＢＰＩの静脈内（ｉ．ｖ．）注射（１００μｇ、１００μｌ内毒素不含生理食塩水／注射液中）、（ｂ）同用量のＧＡＤ６５（２０８－２１７）エピトープのみ、（ｃ）同用量のＣＤ１１ａ（２３７－２４７）ペプチドのみ、または（ｄ）生理食塩水のみ、のいずれかを投与した。他の系に関しては、異なるシグナル－１ペプチド、シグナル－２ペプチド、ＢＰＩを伴う同様の処置群を用いるであろう。マウスは、問題の特定のＢＰＩに応じて、適切な感染性病原体または抗原での攻撃によって試験されるであろう。

【００３８】

免疫組織学によって疾患過程を評価するため（例えば図１２Ａ～Ｄを参照されたい）、脾臓、膵臓、または他の標的器官（例えばＭＢＰペプチドＢＰＩに関してはＣＮＳ、またはＲＳＶペプチドＢＰＩに関しては肺）を、各群の安楽死させたマウスから除去し、そして中性緩衝ホルマリン中で固定しパラフィン中に包埋することによって組織像用に調製するか、またはＯ．Ｃ．Ｔ．培地中で瞬間凍結した。炎症をスコア化するため、各マウスから最小５切片を用いて、与えられたＢＰＩのブロッキング効果を評価した。細胞浸潤上の標準的Ｔ細胞マーカーを性質決定するため、多様な細胞表面抗原に対するビオチン化ｍＡｂをクリオスタット切片とそれぞれインキュベーションし（２時間）、その後、アビジン－アルカリホスファターゼ（Ｖｅｃｔｏｒ　ｌａｂｏｒａｔｏｒｉｅｓ）とインキュベーションするであろう。あるいは、細胞サブセットは、Ｍｕｒｒａｙら，２４　Ｅｕｒ．Ｊ．Ｉｍｍｕｎｏｌ．２３３７－２３４４（１９９４）；Ｍｕｒｒａｙ；１９　Ｉｍｍｕｎｏｌｏｇｙ　Ｔｏｄａｙ　１５７－１６３；およびＳｃｈｏｕｎｔｚら，１５７　Ｔｈｅ　Ｊｏｕｒｎａｌ　ｏｆ　Ｉｍｍｕｎｏｌｏｇｙ　３８９３－３９０１（１９９６）に記載されるような標準的フローサイトメトリー法によって表現型決定されるであろう。最後に、スチューデントｔ－検定またはＡＮＯＶＡを用いて、群および個々のマウス間に観察される統計学的有意差を推定するであろう。

【００３９】
リンカーを介して共に連結されるシグナル－１部分およびシグナル－２受容体部分からなる、組み立てられたＢＰＩのいくつかの代表を、配列番号４２～４６として、表４に示す。これらの代表的なＢＰＩは、１型から２型反応に、そしてその逆に、特定の免疫反応をシフトするよう作動可能である。都合よいことには、他の抗原性ペプチドに対する他の免疫反応は、好ましくは影響を受けないであろう。
【００４０】
（好ましい態様の詳細な説明）
本出願において、以下の定義が適用されるであろう：「配列同一性」は、当該技術分野に知られるように、２以上のポリペプチド配列または２以上のポリヌクレオチド配列、すなわち参照配列と与えられた配列（参照配列と比較しようとする配列）との関係を指す。配列同一性は、こうした一連の配列間のマッチによって決定されるような、最高の度合いの配列類似性を生じるように配列を最適に並列した後、参照配列に所定の配列を比較することによって決定する。こうした並列に際して、配列同一性は、位対位に基づいて確かめ、例えば、特定の位で、ヌクレオチドまたはアミノ酸残基が同一であるならば、配列はその位で「同一」である。その後、こうした位同一性の総数を、参照配列におけるヌクレオチドまたは残基の総数で割って、％配列同一性を得る。配列同一性は、限定されるわけではないが、Ｃｏｍｐｕｔａｔｉｏｎａｌ Ｍｏｌｅｃｕｌａｒ Ｂｉｏｌｏｇｙ，Ｌｅｓｋ，Ａ．Ｎ．監修，Ｏｘｆｏｒｄ Ｕｎｉｖｅｒｓｉｔｙ Ｐｒｅｓｓ，ニューヨーク（１９８８），Ｂｉｏｃｏｍｐｕｔｉｎｇ：Ｉｎｆｏｒｍａｔｉｃｓ ａｎｄ Ｇｅｎｏｍｅ Ｐｒｏｊｅｃｔｓ，Ｓｍｉｔｈ，Ｄ．Ｗ．監修，Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ，ニューヨーク（１９９３）；Ｃｏｍｐｕｔｅｒ Ａｎａｌｙｓｉｓ ｏｆ Ｓｅｑｕｅｎｃｅｓ Ｄａｔａ，Ｐａｒｔ Ｉ，Ｇｒｉｆｆｉｎ，Ａ．Ｍ．およびＧｒｉｆｆｉｎ，Ｈ．Ｇ．監修，Ｈｕｍａｎａ Ｐｒｅｓｓ，ニュージャージー（１９９４）；Ｓｅｑｕｅｎｃｅ Ａｎａｌｙｓｉｓ ｉｎ Ｍｏｌｅｃｕｌａｒ Ｂｉｏｌｏｇｙ，ｖｏｎ Ｈｅｉｎｇｅ，Ｇ．，Ａｃａｄｅｍｉｃ Ｐｒｅｓｓ（１９８７）；Ｓｅｑｕｅｎｃｅ Ａｎａｌｙｓｉｓ Ｐｒｉｍｅｒ，Ｇｒｉｂｓｋｏｖ，Ｍ．ら監修，Ｍ．Ｓｔｏｃｋｔｏｎ Ｐｒｅｓｓ，ニューヨーク（１９９１）；およびＣａｒｉｌｌｏ，Ｈ．ら，Ａｐｐｌｉｅｄ Ｍａｔｈ．，４８：１０７３（１９８８）に記載されるものなどを含む、既知の方法によって、容易に計算可能であり、これらの文献の解説は、本明細書に援用される。配列同一性を決定するのに好ましい方法を設計して、試験する配列間の最大マッチを得る。配列同一性を決定する方法は、与えられた配列間の配列同一性を決定する、公共に入手可能なコンピュータプログラムに体系化されている。こうしたプログラムの例には、限定されるわけではないが、ＧＣＧプログラムパッケージ（Ｄｅｖｅｒｅｕｘ，Ｊ．ら，Ｎｕｃｌｅｉｃ Ａｃｉｄｓ Ｒｅｓｅａｒｃｈ，１２（１）：３８７（１９８４））、ＢＬＡＳＴＰ、ＢＬＡＳＴＮおよびＦＡＳＴＡ（Ａｌｔｓｃｈｕｌ，Ｓ．Ｆ．ら，Ｊ．Ｍｏｌｅｃ．Ｂｉｏｌ．，２１５：４０３－４１０（１９９０））が含まれる。ＢＬＡＳＴＸプログラムはＮＣＢＩおよび他の供給源から公共に入手可能である（ＢＬＡＳＴマニュアル，Ａｌｔｓｃｈｕｌ，Ｓ．ら，ＮＣＶＩ ＮＬＭ ＮＩＨ Ｂｅｔｈｅｓｄａ，ＭＤ ２０８９４，Ａｌｔｓｃｈｕｌ，Ｓ．Ｆ．ら，Ｊ．Ｍｏｌｅｃ．Ｂｉｏｌ．，２１５：４０３－４１０（１９９０），これらの文献の解説は、本明細書に援用される）。これらのプログラムは、与えられた配列および参照配列間に最高レベルの配列同一性を生じるため、デフォルトギャップ加重を用いて、配列を最適に並列する。例えば、参照ヌクレオチド配列に、少なくとも例えば９５％の「配列同一性」を有するヌクレオチド配列を有するポリヌクレオチドによっては、与えられたポリヌクレオチド配列のヌクレオチド配列が、参照ヌクレオチド配列の各１００ヌクレオチドあたり５までの点突然変異を含むことが可能であること以外は、参照配列と同一であることを意図する。言い換えると、参照ヌクレオチド配列に比較して、少なくとも９５％の同一性を有するヌクレオチド配列を有するポリヌクレオチドにおいて、参照配列中の５％までのヌクレオチドが、欠失しているかまたは別の

ヌクレオチドで置換されていることが可能であり、あるいは、参照配列の総ヌクレオチドの５％までの数のヌクレオチドが、参照配列に挿入されていることが可能である。参照配列のこれらの突然変異は、参照ヌクレオチド配列の５'または３'末端位で、あるいはこれらの末端位の間のどこかで、参照配列のヌクレオチドの間に個々に分散しているかまたは参照配列内の１以上の隣接基で、生じることが可能である。同様に、参照アミノ酸配列に、少なくとも、例えば９５％の配列同一性を有する所定のアミノ酸配列を有するポリペプチドによって、このポリペプチドの所定のアミノ酸配列が、参照アミノ酸配列の各１００アミノ酸あたり５アミノ酸までの改変を含むことが可能であること以外は、参照配列に同一であることを意図する。言い換えると、参照アミノ酸配列に比較して、少なくとも９５％の配列同一性を有する所定のポリペプチド配列を得るには、参照配列中の５％までのアミノ酸残基が、欠失しているかまたは別のアミノ酸で置換されていることが可能であり、あるいは、参照配列のアミノ酸残基総数の５％までの数のアミノ酸が、参照配列に挿入されていることが可能である。参照配列のこれらの改変は、参照アミノ酸配列のアミノまたはカルボキシ末端位で、あるいはこれらの末端位の間のどこかで、参照配列の残基の間に個々に分散しているか、または参照配列内の１以上の隣接基で、生じることが可能である。同一でない残基位は、保存的アミノ酸置換によって異なっていることが好ましい。しかし、保存的置換は、配列同一性を決定する際のマッチには含まれない。

【００４１】

同様に、「配列相同性」もまた、本願において、２つの配列の関連性を決定する方法を指す。配列相同性を決定するため、２以上の配列を上述のように最適に並列し、そして必要な場合、ギャップを導入する。しかし、「配列同一性」と対照的に、保存的アミノ酸置換は、配列相同性を決定する際のマッチに数える。言い換えると、参照配列と９５％の配列相同性を有するポリペプチドまたはポリヌクレオチドを得るには、参照配列中のアミノ酸残基またはヌクレオチドの９５％は、別のアミノ酸またはヌクレオチドとマッチするか、またはそれらとの保存的置換を含んでならなければならず、あるいは、参照配列において、保存的置換を含まない、総アミノ酸残基または総ヌクレオチドの５％までの数のアミノ酸またはヌクレオチドが、参照配列に挿入されることが可能である。

【００４２】

「保存的置換」は、アミノ酸残基またはヌクレオチドの置換であって、全体の機能性が顕著に変化しないように、同様の特徴または特性（サイズ、荷電、疎水性など）を有する別のアミノ酸残基またはヌクレオチドでの、アミノ酸残基またはヌクレオチドの置換を指す。

【００４３】

「単離」は、「人の手によって」天然状態から改変されること、すなわち天然に生じる場合、その元来の環境から変化したかまたは除去されているか、あるいは両方であることを意味する。例えば、生存生物に天然に存在するポリヌクレオチドまたはポリペプチドは、「単離」されていないが、該用語を本明細書において使用する際、天然状態の同時存在物質から分離されている、同じポリヌクレオチドまたはポリペプチドは、「単離」されている。最後に、本明細書に明らかには援用されていない、本明細書に引用する参考文献および解説はすべて、本明細書に援用される。

【００４４】

配列番号１～４６のいずれか１つと少なくとも約１０％の配列同一性を有する配列を含むかまたは有し、かつＡＰＣへの同様の結合特性または２つのペプチド配列間の連結特性を示す配列が、本発明の範囲内である。好ましくは、こうした配列は、配列番号１～４６のいずれか１つと、少なくとも約３０％の配列同一性、さらにより好ましくは、少なくとも約５０％の配列同一性、さらにより好ましくは、少なくとも約７０％の配列同一性、そして最も好ましくは、少なくとも約９５％の配列同一性を有するであろう。あるいは、配列番号１～４６のいずれか１つと少なくとも約１０％の配列相同性を有する配列を含むかまたは有し、かつＡＰＣへの同様の結合特性または２つの隣接ペプチド配列間の連結特性を示す配列が、本発明に含まれる。より好ましくは、こうした配列は、配列番号１～４６の

いずれか１つと、少なくとも約３０％の配列相同性、さらにより好ましくは、少なくとも約５０％の配列相同性、さらにより好ましくは、少なくとも約７０％の配列相同性、そして最も好ましくは、少なくとも約９５％の配列相同性を有するであろう。さらに、突然変異事象、一連の突然変異事象、または化学的誘導体化により、配列番号１～４６のいずれか１つと異なってはいるが、なお、望ましい特性を示す配列もまた、本発明に含まれる。こうした突然変異事象または誘導体化には、限定されるわけではないが、点突然変異、欠失、挿入、再編成、ペプチド擬似体、および他の化学的修飾が含まれる。

【００４５】

「リンカー」は、天然存在アミノ酸または化学合成アミノ酸を含む、いずれかのアミノ酸と定義する。好ましくは、「リンカー」は、タンパク質分解性分解に耐性であるアミノ酸残基の柔軟な非基質配列であり、シグナル－１部分をシグナル－２部分にコンジュゲート化および／またはカップリングするのに使用可能である。

【００４６】

「シグナル－１部分」は、ペプチドエピトープ、すなわち、重要なＴＣＲが結合する、抗原のペプチド部分および／またはこれらの抗原性ペプチドの擬似体と定義する。

「シグナル－２部分」または「シグナル－２受容体部分」は、ＡＰＣ上の相補リガンドに結合するおよび／または受容体がＡＰＣ上のその相補リガンドに結合するのに影響を及ぼす、二次シグナル受容体のペプチド部分と定義する。これは、目的の受容体／リガンド構造のペプチド模倣体および擬似体を含むことが可能である。

【００４７】

「シグナル－２リガンド」は、受容体部分および／またはシグナル－２受容体部分が顕著な親和性を有しそして結合する、ＡＰＣ上のシグナル－２受容体部分の相補タンパク質である。

【００４８】

本明細書において、「誘導体」は、ペプチドに関しては、アミノ酸付加、欠失、交換、置換、および／または修飾によって生じる変化；組換えおよび／またはＤＮＡシャッフリングによって生じる突然変異体；および部分的に、ペプチドの塩、溶媒和物、および他の化学合成／修飾型であって単離天然ペプチドの活性を保持するものを指す。

【００４９】

ＢＰＩは、標準法によって、Ｆｍｏｃアミノ酸化学反応を使用する、ロボット多重ペプチド合成装置による自動化ペプチド合成を用いて生成した。Ｗａｎｇ樹脂（ｐ－ベンジルオキシベンジルアルコールポリスチレン）を固体支持体として用いた。ペプチドは、逆相ＨＰＬＣおよびエレクトロスプレー質量分析によって性質決定した。メリフィールドペプチド合成と呼ばれるこの合成は、ペプチド鎖を長くしながら支持体構造に付着させるように、固体支持体上で行う伝統的有機化学反応を利用する。ペプチドは、ＴＦＡを用いて樹脂から切断し、そして逆相ＨＰＬＣによって精製し、そして質量分析によって解析するであろう。あるいは、これらの反応は、より多い量のペプチドが望まれる場合、溶液中で行うことが可能である。もちろん、本発明のペプチドは、当該技術分野によく知られたいくつかの技術によって合成するかまたは調製することが可能である。例えば、本明細書に完全に援用される、Ｃｒｅｉｇｈｔｏｎ，１９８３，Ｐｒｏｔｅｉｎｓ：Ｓｔｒｕｃｔｕｒｅｓ ａｎｄ Ｍｏｌｅｃｕｌａｒ Ｐｒｉｎｃｉｐｌｅｓ，Ｗ．Ｈ．Ｆｒｅｅｍａｎ ａｎｄ Ｃｏ．，ニューヨークを参照されたい。例えば、短いペプチドは、固体支持体上で、または溶液中で、合成可能である。より長いペプチドは、組換えＤＮＡ技術を用いて作成可能である。ここで、一般的な当業者に公知の技術にしたがって、本発明のペプチドをコードするヌクレオチド配列を合成、および／またはクローニングし、そして発現させることが可能である。例えば、Ｓａｍｂｒｏｏｋら，１９８９，Ｍｏｌｅｃｕｌａｒ Ｃｌｏｎｉｎｇ，Ａ ｉｓ Ｌａｂｏｒａｔｏｒｙ Ｍａｎｕａｌ，Ｖｏｌｓ．１－３，Ｃｏｌｄ Ｓｐｒｉｎｇ Ｈａｒｂｏｒ Ｐｒｅｓｓ，ニューヨークを参照されたい。

【００５０】

あるいは、本発明のペプチドは、ペプチドのアミノ酸残基を連結する１以上の結合が非ペプチド結合であるように、合成可能である。これらの代替の非ペプチド結合は、当業者に公知の反応を利用することによって形成可能であり、そして限定されるわけではないが、いくつかのみを挙げると、アミノ、エステル、ヒドラジド、セミカルバジド、およびアゾ結合を含むことが可能である。本発明のさらに別の態様において、上述の配列を含んでなるペプチドは、例えば、ペプチドの安定性、生物学的利用能、および／または阻害活性を増進させるように、アミノおよび／またはカルボキシ末端に存在するさらなる化学基を含んで合成することが可能である。例えば、カルボベンゾキシル、ダンシル、またはｔ－ブチルオキシカルボニル基などの疎水性基をペプチドのアミノ末端に付加することが可能である。同様に、アセチル基または９－フルオレニルメトキシ－カルボニル基をペプチドのアミノ末端に置くことが可能である。さらに、疎水性基、ｔ－ブチルオキシカルボニル、またはアミド基をペプチドのカルボキシ末端に付加することが可能である。

【００５１】

あらかじめ形成されたペプチドが商業的なペプチド合成実験室から容易に購入可能であるため、これらを購入することによって、２５アミノ酸以下を有するペプチドの別の代替供給源が提供される。後の合成スキームにおいて、ペプチド擬似体化合物を、ペプチド部分の代わりに合成し、そして同じ化学反応によって連結することが可能である。ペプチド擬似体の設計は、確立された技術であり、そしてペプチドの重要なアミノ酸の既知の相関物を、先に公表された方法によって、合成可能である。さらに、当該技術分野によく知られているように、本明細書に詳細に記載する好ましいペプチドと同じ調節特性を有するペプチド擬似体を開発可能である。これらのペプチド擬似体は、常用的な当該技術分野の技術以上のものを必要とすることなく作り出せるため、こうしたペプチド擬似体は、本出願に含まれる。特に、これらのペプチド擬似体の側鎖は本明細書の好ましいペプチドの側鎖と構造が非常に類似であろうが、そのペプチド主鎖は非常に異なるかまたはまったく似ていなくてもよい。ｉｎ　ｖｉｖｏでの分解に対する耐性またはより大きいコンホメーション安定性が望ましい場合、本発明のペプチドは、いかなる公知の方法によって、環化することも可能である。１つのこうした方法は、ＮおよびＣ末端にペニシラミン（Ｐｅｎ）およびシステイン（Ｃｙｓ）残基を付加して、ＰｅｎおよびＣｙｓ残基間のジスルフィド結合を介した環状ペプチドを形成する。この環状ペプチドの形成は、ペプチドコンホメーションを制限して、コンホメーション安定性を生じ、それによって、その直鎖対応物よりも、細胞表面受容体に対する、より優れた選択性を提供する。

【００５２】

シグナル－１部分およびシグナル－２部分間に渡るＢＰＩの部分をリンカーと称する。上述のように、リンカーは、ＢＰＩを形成するのに必須ではない。しかし、リンカーを用いる場合、リンカーは、いかなる天然存在アミノ酸または化学合成アミノ酸であることも可能である。好ましくは、リンカーは、プロテアーゼ攻撃を妨げるのを補助する非基質アミノ酸残基鎖である。特に好ましいリンカーは、非天然アミノ酸であるアミノカプロン酸（Ａｃ）、およびアミノ酸グリシン（Ｇ）の反復鎖である（例えばＡｃ－Ｇ－Ａｃ－Ｇ－Ａｃ）。リンカーにもっと短い長さが必要である場合、１以上のＡｃ残基をベーターアラニン残基（βＡｌａ）で置き換えることが可能である。リンカーにもっと長い鎖が必要である場合、１以上のＡｃ残基をアミノ－ドデカン酸残基（Ａｄｏｄ）で置き換えることが可能である。当該技術分野によく知られているように、これらのリンカーアミノ酸のペプチド擬似体もまた、合成してＢＰＩ構造に挿入することが可能である。

【００５３】

以下の実施例は、本発明の好ましい態様を示す。しかし、これらの実施例は、例示のためにのみ提供するものであり、実施例中のいずれも本発明の全体の範囲に対する限定とみなしてはならないことを理解すべきである。

【００５４】

（実施例１）

本実施例は、ＢＰＩを作り出す方法を記載する。

材料および方法：
ペプチド合成は、クロロトリチル樹脂上のＦｍｏｃを介した。保護アミノ酸は、８倍過剰で１時間、二重カップリングした。樹脂を、ジメチルホルムアミド（ＤＭＦ）とメタノール（ＭｅＯＨ）で洗浄し、そして試薬Ｒ：トリフルオロ酢酸（ＴＦＡ）、エチレンジアミン四酢酸（ＥＤＴＡ）、チオアニソール、アニソール中で切断した。溶液中にペプチドを含有するＴＦＡ混合物は、エーテル中で沈殿させ、そして徹底的に洗浄した。ペプチドの調製用ＨＰＬＣは、０．１％ＴＦＡ中の０～８０％アセトニトリルの勾配によって達成した。多様な分画の凍結乾燥および質量分析による確認によって、ＴＦＡ塩として、合成ペプチドを得た。モデリング、結晶学および結合についての研究は上述のとおりである。
【００５５】
結果：
本実施例で作り出したペプチドを表１に示し、配列番号１～４６としても列挙する。これらのペプチドには、シグナル－１部分、シグナル－２部分およびこれら２つの部分間の非基質リンカーが含まれる。ＢＰＩを作り出すために、上述のペプチド合成を用いて、いかなるシグナル－１部分を、いかなるシグナル－２部分に、いかなるリンカーを介して、連結することも可能である。言い換えると、ＢＰＩは、シグナル－１ペプチド配列、続いてリンカー配列、続いてシグナル－２ペプチド配列を含んでなる１つの連続ペプチド鎖として作り出される。さらに、いくつかの代表的なＢＰＩを、後に実験で使用するためにつくった。これらのＢＰＩは、本明細書において、表４に含まれる。しかし、これらのＢＰＩは代表（表１－４に列挙される各ＢＰＩ部分も同様）であり、そしてまったく包括的でないことに注目することが重要である。
【００５６】
表１．シグナル－１ペプチド
【００５７】
【表１】

配列番号	配列	名称、供給源	生物	健康障害
1	EIAPVFVLLE	GAD65 (208-217)	ヒト (Homo sapiens)	1型糖尿病
2	EIAPVFVLLE	GAD67 (217-226)	マウス (Mus musculus)	1型糖尿病
3	QYMRADQAAGGLR	コラーゲンII (1168-1180)	ヒト	慢性関節リウマチ
4	RVVINKDTTIII	エルシニアHSP (322-333)	腸炎エルシニア (Yersinia enterocolitica)	反応性関節炎
5	ENPVVHFFKNIVTPR	ミエリンBP (84-98)	ヒト	多発性硬化症
6	GYKVLVLNPSVAAT	HCV, NS3 (1248-61)	C型肝炎ウイルス	肝炎
7	GSDTITLPCRIKQFINMWQE	HIV, gp120 (410-429)	HIV-1	AIDS
8	PIVQNLQGQMVHQAISPRTL	HIV, p24 (133-152)	HIV-1	AIDS
9	STPESANL	SIV, Tat (28-35)	サル免疫不全ウイルス	サルAIDS
10	AICKRIPNKKPGKKT	RSV, G (183-197)	呼吸器合胞体ウイルス	喘息
11	VYRDGNPYA	HPV 16, E6 (60-68)	ヒトパピローマウイルス (HPV)	子宮頸癌
12	DRAHYNI	HPV 16, E7 (48-54)	HPV	子宮頸癌
13	YMLDLQPETT	HPV 16, E7(11-20)	HPV	子宮頸癌
14	ASDLRTIQQLLMGTV	HPV 33, E7 (73-87)	HPV	子宮頸癌
15	AELYHFLLKYRAR	MAGE (3114-3126)	ヒト	黒色腫
16	LLKYRAREPVTKAE	MAGE (3120-3133	ヒト	黒色腫
17	EQVAQYKALPVVLENA	Fel d 1 (22-37)	ネコ (Felis domesticus)	ネコアレルギー
18	KALPVVLENARILKNCV	Fel d 1(28-44)	ネコ	ネコアレルギー
19	LVPCAWAGNVCGEKRAYCCS	Amb a 5 (1-20)	アンブロシア・アルテミシーフディア (Ambrosia artenisiifdia)	ブタクサアレルギー
20	PIGKYCVCYDSKAICNKNCT	Amb t 5 (21-40)	アンブロシア・トリフィダ (Ambrosia tridida)	ブタクサアレルギー
21	KSMKVTVAFNQFGPN	Cryj 1 (211-225)	スギ (Cryptomeria japonica)	スギアレルギー
22	IDIFASKNFHLQKNTIGTG	Cryj 2 (182-200)	スギ	スギアレルギー
23	YFVGKMYFNLIDTKCYK	ホスホリパーゼ2 (81-97)	セイヨウミツバチ (Apis mellifera)	ハチアレルギー
24	ASEQETADATPEKEEPTAAP	Hev b 5 (37-56)	パラゴムノキ (Hevia brasiliensis)	ラテックス
25	FGISNYCQIYPPNANKI	Der p 1 (111-127)	ヤケヒョウダニ (Dermatophagoides pteronyssinus)	チリダニ

【００５８】

表２．リンカー

【００５９】

【表２】

配列番号	配列
26	Ac-G-Ac-G-Ac
27	Ac-G-βAla-G-Ac
28	Ac-G-Adod-G-Ac
29	Ahx-G-Ahx-G-Ahx

【００６０】

表３．シグナル－２ペプチド

【００６１】

【表３】

配列番号	配列	供給源	生物	免疫シフト
30	ITDGEATDSG	CD11a (237-247)	ヒト	１型→２型
31	TDGEATDSGN	CD11a (238-248)	ヒト	１型→２型
32	ASPGKATEVR	CTLA4 (24-33)	ヒト	２型→１型
33	SPSHNTDEVR	CTLA4 (24-33)	マウス	２型→１型
34	KVELMYPPPYYL	CTLA4 (93-104)	ヒト	２型→１型
35	KVELMYPPPYFV	CTLA4 (93-104)	マウス	２型→１型
36	ITDGEATDSG	CD11a (237-247)	マウス	１型→２型
37	KGYYTMSNNLVTL	CD154 (CD40L) (93-104)	ヒト	１型→２型
38	KGYYTMSNNLVTL	CD154 (CD40L) (93-104)	マウス	１型→２型
39	YMRNSKYRAGGAYGPG	Ｆａｓリガンド (CD95L) (143-155)	ヒト	２型→１型
40	YMRNSKYRAGGAYGPG	Ｆａｓリガンド (CD95L) (143-155)	マウス	２型→１型
41	TDGEATDSGN	CD11a (238-248)	マウス	１型→２型

【００６２】

表４．いくつかの代表的なＢＰＩ

【００６３】

【表４】

ＴＣＲエピトープ	リンカー	シグナルー２ 受容体部分	完全ＢＰＩ配列	配列 番号	効果
(GAD65) 糖尿病： ELAPVFVLLE	AcGAcGAc	(CDlla) ITDGEATDSG	ElAPVFVLLEAcGAcGAclTDGEATD SG	42	１型→２型
(tat) AIDS: STPESANL	AcGAdodGAc	(Ｆａｓ－リガンド) YMRNSKYRAGGAYGPG	STPESANLAcGAdodGAc YMRNSKYRAGGAYGPG	43	↑CTL
(p24) PIVQNLQGQMVHQAISPRTL (コラーゲンＩＩ) 慢性関節リウマチ： QYMRADQAAGGLR	AcGAcGAc	(CTLA4) KVELMYPPPYFV	PIVQNLQGQMVHQAISPRTLAcGAc GAcKVELMYPPPYFV	44	↑１型
	AcGAhxGAc	(CD4OL) KGYYTMSNNLVTL	QYMRADQAAGGLRAcGAhxGAcKG YYTMSNNLVTL	45	１型→２型
(HPVE7) 子宮頚癌 YMLDLQPETT	AcGAcGAc	(Ｆａｓ－リガンド) YMRNSKYRAGGAYGPG	YMLDLQPETTAcGAcGAcYMRNSK YRAGGAYGPG	46	↑CTL

【００６４】

考察：

リンカー、シグナルー１部分、およびシグナルー２部分をひとたび選択したら、上述の方法を用いて、完全なＢＰＩが合成可能である。本発明と組み合わせて使用可能な、潜在的なシグナルー１部分は数千ある。これらの部分は各々、別個の免疫学的反応または疾患状態と関連する可能性がある。エピトープ構造および配列をひとたび決定したら、適切なリ

ンカーを選択し、そしてＢＰＩの他の部分、すなわち二次シグナル部分を選択することが可能である。これらの配列すべてを決定したら、ペプチド配列自体、ペプチド擬似体、または２つの組み合わせを用いて、ＢＰＩを設計することが可能である。適切なペプチド擬似体の構築は、その内容および解説が本明細書に援用される、Ｆａｌｃｉｏｎｉら，１７ Ｎａｔｕｒｅ Ｂｉｏｔｅｃｈｎｏｌｏｇｙ，５６２－５６７（１９９９）に詳述される。

【００６５】

図８は、本発明の方法によって産生した、ＣＤ１１ａ（２３７－２４７）二次シグナル部分に連結したＴＣＲエピトープ、ＧＡＤ６５（２０８－２１７）の構造を例示する。これは、Ｉ－A^{g7}の溝およびＩＣＡＭ－１のＤ１ドメインに結合することが示される。Ｉ－A^{g7}：ＧＡＤ６５ペプチド構造をモデリングするため、ＩｎＳｉｇｈｔＩＩソフトウェア（ＭＳＩ／Ｂｉｏｓｙｍ）を用いて、Ｓｉｌｉｃｏｎ Ｇｒａｐｈｉｃ Ｏｃａｔａｎｅワークステーション上でドッキング研究を行った。ＬＦＡ－１ペプチド：ＩＣＡＭ－１ドメイン構造は、その解説および開示が本明細書に援用される、Ｅｄｗａｒｄｓ，Ｃ．Ｐ．ら，Ｊ．Ｂｉｏｌ．Ｃｈｅｍ．２７３：２８９３７（１９９８）のドッキングモデルに基づく。Ｉ－A^{g7}のアルファ炭素リボンはピンクで示し；ＩＣＡＭ－１のＤ１は明るい青で示し；ＢＰＩは、原子によって、炭素は緑、酸素は赤、そして窒素は青で示す。この構造は、ＧＡＤ６５（２０８－２１７）－［Ａｃ－Ｇ－Ａｃ－Ｇ－Ａｃ］－ＣＤ１１ａ（２３７－２４７）と命名可能である。好都合には、リンカーの長さは、シグナル－１部分とシグナル－２部分との間を最適な長さにつなぐため、必要に応じてまたは得られた実験データいずれかに示されるように、変更可能である。

【００６６】

これらの構造は、模式的に（図２および３）、および構造モデル（図８）に示す予備的機構によって例示する。用いたリンカーは配列－［Ａｃ－Ｇ－Ａｃ－Ｇ－Ａｃ］－を有する。リンカーを長くするため、アミノドデカン酸の代わりに１以上のアミノカプロン酸（Ａｃ）残基が使用可能である。リンカーを短くするため、アミノカプロン酸の代替物としてベーター２アラニンが使用可能である。

【００６７】

もちろん、一般の当業者の１人が、ＢＰＩと類似の活性を有するであろういかなる数のペプチド擬似体または誘導体を産生することも可能であり、そしてこうした修飾は、上により詳細に記載されるように、本発明に含まれる。

【００６８】

（実施例２）

本実施例は、ビオチン化ＢＰＩを用いて、非標識ペプチドまたは生存ＡＰＣ上のＭＨＣ－ＩＩおよびＩＣＡＭ－１に対するモノクローナル抗体によるＢＰＩ結合の競合的阻害に関して試験し、そして生存ＡＰＣに対する抗原性ペプチド結合を確める。さらに、ＭＨＣ－ＩＩまたはＩＣＡＭ－１に対するモノクローナル抗体が、糖尿病ＢＰＩ（ＧＡＤ６５（２０８－２１７）－［Ａｃ－Ｇ－Ａｃ－Ｇ－Ａｃ］－ＣＤ１１ａ（２３７－２４７））（以後、ＥＧＡＤ－ＢＰＩと称する）のＮＯＤ脾臓細胞への結合を有効に遮断することを示した。

【００６９】

材料および方法：

ビオチン化ＢＰＩを得るため、合成したＥＧＡＤ ＢＰＩを、Ｍｕｒｒａｙら，２４ Ｅｕｒ．Ｊ．Ｉｍｍｕｎｏｌ．２３３７－２３４４（１９９４）に記載されるように、ＮＨＳ－ビオチンでビオチン化した。正常（非免疫）ＮＯＤ、ＢＡＬＢ／ｃおよび他のＭＨＣコンジェニック系統由来の脾臓細胞密度勾配分画を、増加する濃度の個々のビオチン化ペプチドと共に、丸底９６ウェルプレート中、３７℃、５％CO_2で１６時間インキュベーションした。ＢＰＩのＡＰＣへの結合後、アビジン－ＦＩＴＣを、氷上で細胞と３０分間インキュベーションし、その後、ビオチン化抗アビジンと１時間、その後、再びアビジン－ＦＩＴＣとインキュベーションした。ＢＰＩ力価測定のため、滅菌０．５％Ｂ

ＳＡ－ＰＢＳ中の０．１－１００μＭへ増加する濃度のビオチン化誘導体を、上述のように、ＡＰＣと１６時間インキュベーションした。三色解析は、Ｃｙ－クロムまたはＰＥ－コンジュゲート化抗Ｂ２２０（ｍＡｂ　ＲＡ３－６Ｂ２（ＣＤ４５Ｒ　Ｂ細胞マーカー））、抗ＭＨＣクラスＩＩ（ＫＨ７４または１０－３．６２ｍＡｂ）、または抗ＩＣＡＭ－１（３Ｅ２　ｍＡｂ）を用いた；（すべてＰｈａｒＭｉｎｇｅｎ、カリフォルニア州サンディエゴから購入）。結合ペプチドは、前方／側方散乱解析によってゲート処理された生存細胞上で、アビジン－ＦＩＴＣ／ビオチン化抗アビジン／アビジン－ＦＩＴＣで検出した。対照はビオチン化ペプチド以外のすべての検出試薬を含有した；各ヒストグラムに関して、ＦＡＣＳｃａｎ（Ｂｅｃｔｏｎ－Ｄｉｃｋｉｎｓｏｎ）フローサイトメーターで、２０，０００の事象を解析した。

【００７０】

非標識ペプチドまたは生存ＡＰＣ上のＭＨＣ－ＩＩおよびＩＣＡＭ－１に対するモノクローナル抗体でのＢＰＩ結合の競合的阻害に関して試験するため、単離したばかりの新鮮脾臓細胞分画を、あらかじめビオチン化しておいたＢＰＩとインキュベーションした。しかし、実験のこの部分に関しては、実験ウェルは、多様な非標識ペプチド（例えば抗原性ペプチドまたはＬＦＡ－１ペプチド）および／またはモノクローナル抗体（例えば抗ＭＨＣ－ＩＩまたは抗ＩＣＡＭ－１　ｍＡｂ）阻害剤を含有した。磁気粒子にコンジュゲート化したモノクローナル抗体を用いたネガティブ選択法を用いて、Ｂ細胞、マクロファージまたは樹状細胞に関して脾臓細胞分画を濃縮すると共に、これらの異なる集団へのＢＰＩ結合の相違を調べた。これらの方法は、Ｓｃｈｏｕｎｔｚら，１５７ Ｔｈｅ Ｊｏｕｒｎａｌ ｏｆ Ｉｍｍｕｎｏｌｏｇｙ ３８９３－３９０１（１９９６）に詳述され、該文献の解説および内容は、本明細書、上記に援用される。

【００７１】

生存ＡＰＣへの抗原性ペプチド結合を確かめるため、最初のＥＧＡＤ－ＢＰＩを、ＮＯＤ（Ｉ－Ａ^{g7}）ＡＰＣへの選択的結合に関してスクリーニングし、そして生存ＡＰＣを用いたフローサイトメトリー法によって、ＭＨＣ－ＩＩまたはＩＣＡＭ－１いずれかに対するモノクローナル抗体を用いた同時結合に関してアッセイした（Ｍｕｒｒａｙら，２４ Ｅｕｒ．Ｊ．Ｉｍｍｕｎｏｌ．２３３７－２３４４（１９９４）；Ｍｕｒｒａｙ；１９ Ｉｍｍｕｎｏｌｏｇｙ Ｔｏｄａｙ １５７－１６３；およびＳｃｈｏｕｎｔｚら，１５７ Ｔｈｅ Ｊｏｕｒｎａｌ ｏｆ Ｉｍｍｕｎｏｌｏｇｙ ３８９３－３９０１（１９９６））。このアッセイでは、増加する濃度のビオチン化－ＢＰＩ、－ＣＤ１１ａ（２３７－２４７）もしくは－ＧＡＤ６５（２０８－２１７）ペプチドを、各近交系由来の脾臓細胞と一晩インキュベーションした。結合ペプチドは、ビオチン化抗アビジン試薬の使用によって、アビジン－ＦＩＴＣ蛍光の増幅、その後、二周期目のアビジン－ＦＩＴＣ結合で検出した。ビオチン化ペプチドは、５０μＭのペプチド濃度で、１０^{6}生存細胞とインキュベーションした。結合ペプチドは、アビジン－ＦＩＴＣ／ビオチン化抗アビジン－ＦＩＴＣで検出した。解析のため、前方／側方散乱ゲートを生存リンパ球上にセットし、そしてこのゲートで、２０，０００の事象を収集した。バックグラウンド蛍光（検出試薬のみ）を、５０μＭバイオ－ペプチドヒストグラム（ペプチド－蛍光強度＝ＦＬ１）と共に、図５ａ～５ｃの各パネルに示す。陽性細胞（Ｍ２）の割合は、ＣＥＬＬＱｕｅｓｔ^{TM}プログラム（Ｂｅｃｔｏｎ－Ｄｉｃｋｉｎｓｏｎ）によって決定し、そしてＭ２集団の中央値チャネル蛍光（ＭＣＦ）と共に、各パネルに示す（すべてのデータは、Ａｐｐｌｅ　Ｇ３コンピュータ上で作動させたＣＥＬＬＱｕｅｓｔ^{TM}プログラムの直接出力である）。

【００７２】

ＭＨＣ－ＩＩまたはＩＣＡＭ－１に対するモノクローナル抗体が、ＮＯＤ脾臓細胞への糖尿病ＢＰＩ（ＥＧＡＤ－ＢＰＩ）の結合を有効に遮断することを明らかにするため、図５ａ～５ｃに用いたのと同一のアッセイを用いた。しかし、精製１０－３．６２（抗ＭＨＣ－ＩＩ）または３Ｅ２（抗ＩＣＡＭ－１）いずれかを脾臓細胞とビオチン化ＢＰＩの一晩インキュベーションに含ませた。両抗体は、ＰｈａｒＭｉｎｇｅｎから購入し、そして５

μｇ／ｍｌ最終濃度で用いた。モノクローナル抗体をまったく添加しない対照も、試験した。解析は、生存リンパ球に関して、前方／側方散乱ドットプロット上でゲート処理し、そして各ヒストグラムに関して、２０，０００事象を解析した。この例の結果を図６ａ～６ｃに示す。

【００７３】

結果および考察：

図５ａ～５ｃに提供するデータに例示するように、ＮＯＤ脾臓細胞は、ＢＡＬＢ／ｃ、Ａ．ＳＷ、またはＡ．ＢＹからまったく同じに精製した脾臓細胞より高い密度で、糖尿病ＢＰＩ（ＥＧＡＤ－ＢＰＩ）に結合する。先のデータは、リンパ球分離培地（ＬＳＭ）密度勾配遠心分離によって単離したこれらの脾臓細胞調製において、Ｂ細胞が主な抗原性ペプチド結合細胞であることを示した（Ｍｕｒｒａｙら，２４ Ｅｕｒ．Ｊ．Ｉｍｍｕｎｏｌ．２３３７－２３４４（１９９４）；Ｍｕｒｒａｙ；１９ Ｉｍｍｕｎｏｌｏｇｙ Ｔｏｄａｙ １５７－１６３；およびＳｃｈｏｕｎｔｚら，１５７ Ｔｈｅ Ｊｏｕｒｎａｌ ｏｆ Ｉｍｍｕｎｏｌｏｇｙ ３８９３－３９０１（１９９６））。高密度結合細胞の割合には、３０％（ＮＯＤ）から６％（Ａ．ＢＹ）までの顕著な相違があった。対照的に、シグナル－１（ＧＡＤ６５ペプチド）部分およびシグナル－２（ＣＤ１１ａペプチド）部分が別々では、優先的にＮＯＤ ＡＰＣに結合しなかった。これを支持するデータを図５ｂおよび５ｃに示す。図５ｂは、図５ａに示すものと同一の脾臓細胞調製への、ビオチン化ＬＦＡ－１（ＣＤ１１ａ ２３７－２４７）ペプチドの直接結合を例示する。このシグナル－２部分がすべての系統の脾臓細胞に同様に結合したことに注目されたい。図５ｃは、図５ａおよび５ｂに示すのと同一の脾臓細胞調製への、ビオチン化ＧＡＤ６５（２０８－２１７）ペプチドの直接結合を例示する。これらのデータは、その各解説が本明細書に援用される、Ｃｏｒｐｅｒら，Ｉ－Ａ^{g7}および自己免疫糖尿病間の関連を解読するための構造的フレームワーク，２８８ Ｓｃｉｅｎｃｅ ５０５－５１１（２０００）、およびＤｅｓｓｅｎら，ヒトコラーゲンＩＩ由来のペプチドと複合体化したＨＬＡ－ＤＲ４（ＤＲＡ*０１０１、ＤＲＢ*０４０１）のＸ線結晶構造，７ Ｉｍｍｕｎｉｔｙ ４７３－４８１（１９９７）に論じられるように、ＢＰＩを操作して、特定のＭＨＣペプチド結合モチーフに適合させることが可能であることを示す。

【００７４】

さらに、図６ａ～６ｃに例示するように、他の研究は、ＭＨＣ－ＩＩおよびＩＣＡＭ－１に対するモノクローナル抗体が、ＮＯＤ脾臓細胞へのペプチド結合を遮断することを示した。これらのデータは、糖尿病ＢＰＩが、ＡＰＣ表面上の両受容体に結合することを示す。したがって、ＭＨＣ－ＩＩまたはＩＣＡＭ－１に対するモノクローナル抗体は、糖尿病ＢＰＩ（ＥＧＡＤ－ＢＰＩ）のＮＯＤ脾臓細胞への結合を有効に遮断する。事実上、ＢＰＩの予測される二官能性の性質は、これらの結果によって明らかにされ、そしてＢＰＩがＡＰＣ表面上で、ＭＨＣ－ＩＩをＩＣＡＭ－１に連結するであろうことが示唆される。この機構は、同時キャッピング実験によって、さらに明らかにされた。

【００７５】

（実施例３）

本実施例は、同時キャッピング実験を利用して、ＢＰＩがＭＨＣ－ＩＩおよびＩＣＡＭ－１分子へ同時結合することを明らかにする。

【００７６】

材料および方法：

ＢＰＩがＭＨＣ－ＩＩおよびＩＣＡＭ－１分子に同時結合する、さらなる裏付けを、ビオチン化ｍＡｂ１０－３．６２およびストレプトアビジンを用いてＢＰＩの存在下または非存在下でＭＨＣ－ＩＩをキャッピングする同時キャッピング実験で観察した。ＢＰＩペプチドが、ＡＰＣ表面上のＭＨＣ－ＩＩおよびＩＣＡＭ－１分子を連結する能力を試験するため、我々は、同時キャッピング実験（もともとはモノクローナル抗体に関して記載されたもの）を変更して行った。簡潔には、あらかじめ所定のＢＰＩ変異体または生理食塩水の静脈内（ｉ．ｖ．）注射によって処置したＮＯＤマウスから新たに単離したＡＰＣと、

ＭＨＣ－ＩＩに対するビオチン化モノクローナル抗体（１０－３．６２）とをインキュベーションする。その後、抗体結合細胞をストレプトアビジンとインキュベーションして（３７℃ｘ１５分間）、ＡＰＣ表面上のＭＨＣ－ＩＩ分子をキャッピングする。細胞を氷上に移し、そしてＩＣＡＭ－１に対する蛍光（ＰＥ）モノクローナル抗体（３Ｅ２）で標識した。細胞を蒔き、そして所定のＢＰＩがＩＣＡＭ－１をＭＨＣ－ＩＩキャップに連結する証拠を、標準的蛍光顕微鏡法および画像解析によって観察する。１つの実験において、１６時間前にＥＧＡＤ－ＢＰＩ（ｉ．ｖ．）で処置したマウス由来のＴ枯渇脾臓細胞は、バイオ－１０－３．６２／ストレプトアビジンの存在下、ＭＨＣ－ＩＩとＩＣＡＭ－１の同時キャッピングを示した。他の実験において、１６時間前に生理食塩水のみで処置したマウス由来のＴ枯渇脾臓細胞は、同時キャッピングを示さなかった。これらの実験の結果は、図７に示す。

【００７７】

結果：

図７の上部パネルは、１６時間前にＥＧＡＤ－ＢＰＩ（ｉ．ｖ．）で処置したマウス由来のＴ枯渇脾臓細胞の結果を例示し、ここで、ＩＣＡＭ－１は、バイオ－１０－３．６２／ストレプトアビジンの存在下、ＭＨＣ－ＩＩと同時キャッピングした。図７の下部パネルは、生理食塩水のみで処置したマウスの結果を例示し、ここでは同時キャッピングは示されない。これは、ＩＣＡＭ－１の残りがＢ細胞膜上に分散したままであることによって立証される。示されるように、１６時間前に４０ナノモルのＥＧＡＤ－ＢＰＩまたは生理食塩水（ＰＢＳ）のみいずれかのｉ．ｖ．注射によって処置したＮＯＤマウスから単離したＢ細胞は、氷上のＰＥ標識化３Ｅ２（抗ＩＣＡＭ－１）で染色することによって測定するように、ＩＣＡＭ－１発現の２つの別個のパターンを示した。ＢＰＩ処置マウス由来の細胞上では、ＩＣＡＭ－１は、ＭＨＣ－ＩＩ分子と同時キャッピングされているようである（上部パネルのＩＣＡＭ－１赤色蛍光の単一の濃縮された点に注目されたい）。対照的に、ＩＣＡＭ－１の名目上の分散した密度は、ＰＢＳ処置ＮＯＤマウスから単離した細胞上に観察される（下部パネルの全表面の赤色蛍光染色に注目されたい）。

【００７８】

したがって、ＢＰＩが生存ＡＰＣ表面上のＭＨＣ－ＩＩおよびＩＣＡＭ－１構造に同時に結合する能力を有し、そしてしたがってT_H1／T_H2分化に必要な経路を伴うシグナル改変を提供する可能性があることが明らかなようである。T_H1／T_H2免疫逸脱に対するＢＰＩの影響を直接調べるため、ＥＧＡＤ－ＢＰＩを注射したマウス由来のＴ細胞を、サイトカイン解析によって調べた。

【００７９】

（実施例４）

本実施例は、ＥＬＩＳＰＯＴを用いて、ＢＰＩ注射によって改変されるような、T_H1／T_H2頻度を決定した。

【００８０】

材料および方法：

３～５のＮＯＤマウスの群を、尾の付け根で、ＣＦＡ中のＧＡＤ６５ペプチド（４０ナノモル／マウス）を用いて皮下（ｓ．ｃ．）免疫した。ＥＧＡＤ－ＢＰＩ、その単一のＴＣＲエピトープ（シグナル－１部分）、またはそのＣＤ１１ａペプチド（シグナル－２部分）いずれかを、それぞれ異なる群にｉ．ｖ．（すべて４０ナノモル／マウス）で投与した。６～８日後、もう一度同一物の４０ナノモル注射を各マウスに行い、そして翌日、ｓ．ｃ．注射部位の領域リンパ節を、培養のため単一細胞懸濁物にした。同一の一次培養を９６時間インキュベーションし；その後、生存Ｔ細胞を密度勾配遠心分離によって回収した。これらの細胞１００万を、ＰＢＳ中、１０μｇ／ｍｌの濃度の、マウスＩＦＮγ（クローンＲ４－６Ａ２）またはマウスＩＬ－４（クローンＢＶＤ４－１Ｄ１１）いずれかに対するｍＡｂであらかじめコーティングした（５０μｌ／ウェル）ニトロセルロース底９６ウェルプレート（Ｍｉｌｌｉｔｉｔｅｒ－ＨＡ、Ｍｉｌｌｉｐｏｒｅ、マサチューセッツ州ベッドフォード）中で合わせた。３つ組培養の群を、コンカナバリン－Ａ（２μｇ／ｍ

ｌ）あるいは２０Ｕ／ｍｌ組換えＩＬ２（Ｒ＆Ｄ　Ｓｙｓｍｅｍｓ）を加えたシグナル－１ペプチド部分のいずれかとインキュベーションした。３７℃および５％CO_2で７２－９６時間培養した後、プレートをＰＢＳ－０．０５％Ｔｗｅｅｎ－２０で３回洗浄した。適切なウェルに、１μｇ／ｍｌの濃度で、ビオチン化抗ＩＦＮγ（クローンＸＭＧ１．２）またはビオチン化抗ＩＬ－４（クローンＢＶＤ６－２４Ｇ２）を添加して、そして室温で１時間インキュベーションする。陽性対照ウェルには、正常Ｔ細胞の代わりに、既知のT_H1またはT_H2クローンを入れる。ＩＬ１２、ＩＬ１０、およびＩＬ２に対するｍＡｂ対もまた入手可能であり、そしてこれを用いて、同一アッセイにおいてこれらのサイトカインに関して試験するであろう。すべてのｍＡｂおよび組換え対照は、ＰｈａｒＭｉｎｇｅｎ（カリフォルニア州サンディエゴ）から購入する。最後に、プレートをＰＢＳ－Ｔｗｅｅｎで３回洗浄し、そしてその後、１：２０００希釈のストレプトアビジン・アルカリホスファターゼ（Ｊａｃｋｓｏｎ　ＩｍｍｕｎｏＲｅｓｅｒｃｈ、ペンシルバニア州ウェストグローブ）１００μｌに１時間曝露し、そして前と同様に洗浄した。サイトカイン産生細胞は、ＢＣＩＰ／ＮＢＴ基質キット（ＢｉｏＲａｄ　Ｌａｂｓ、カリフォルニア州リッチモンド）を用いて膜を現像し、その後、デジタルカメラおよびＮＩＨ画像ソフトウェアと連結した標準的立体顕微鏡を用いて、画像捕捉し、そして解析することによって数えた（Ｍｕｒｒａｙら，　２４　Ｅｕｒ．　Ｊ．　Ｉｍｍｕｎｏｌ．　２３３７－２３４４（１９９４）；Ｍｕｒｒａｙ；１９　Ｉｍｍｕｎｏｌｏｇｙ　Ｔｏｄａｙ　１５７－１６３；およびＳｃｈｏｕｎｔｚら，　１５７　Ｔｈｅ　Ｊｏｕｒｎａｌ　ｏｆ　Ｉｍｍｕｎｏｌｏｇｙ　３８９３－３９０１（１９９６））。先に記載する方法によって、ＧＡＤ６５（２０８－２１７）ペプチドで免疫したＮＯＤマウス由来のＣＤ４＋　Ｔ細胞クローンを、別個の実験において、同一アッセイで使用することが可能である。クローンは、Ｍｕｒｒａｙら，　２４　Ｅｕｒ．　Ｊ．　Ｉｍｍｕｎｏｌ．　２３３７－２３４４（１９９４）に記載する方法を用いて生成し、該文献の解説は本明細書に援用される。これらのクローンは、照射ＮＯＤリンパ球、ＧＡＤペプチド、および組換えＩＬ－２で、隔週で再刺激することによって維持されるであろう。予測されるＴＣＲ接触位で置換したＢＰＩは、増殖および個々のクローンからのサイトカイン放出の阻害（上記のように解析される）において、これらのＢＰＩ変異体のいずれが最も有効であるかを決定するさらなる解析のために用いられるであろう。システインを除くすべてのアミノ酸でスキャンされるであろう、予測される位は、アミノ酸２０８、２１３、および２１６である。これらの残基は、最近解明された、Ｉ－A^{g7}に結合するＧＡＤ６５ペプチドの結晶構造において、ＴＣＲに面している（Ｃｏｒｐｅｒら，　２８８　Ｓｃｉｅｎｃｅ　５０５－５１１（２０００）および図８を参照されたい）。

【００８１】

結果：

重要なことに、本実施例は、与えられたＢＰＩの機能的免疫反応を調節する能力を示す。このＢＰＩで処置したマウスが豊富なＩＬ－４を産生し、一方、対照マウスがこのサイトカインを産生しないことがわかる（図９ａおよび９ｃに例示する）。ＩＬ－４は、２型免疫の代表的なサイトカインであるため、本実施例は、ＢＰＩが、優性１型免疫からT_H2分化および２型反応に切り換える能力を有することを示す。さらに、我々は、このｉｎ　ｖｉｖｏアッセイ系を発展させて、与えられたＢＰＩの免疫制御効果の比較的迅速な検査を提供した。T_H1／T_H2調節が、本実施例におけるように確認されたら、その後、研究は、以下に記載するような養子移植を用いた、ＢＰＩ効果のより厳密な試験へと移ることが可能である。

【００８２】

図９ａおよび９ｃに示すように、ＩＬ－４産生は、Ｔ細胞がｉｎ　ｖｉｔｒｏで分裂促進因子を用いて刺激したＢＰＩ処置動物由来である場合、およそ１０倍増加する。ＩＦＮ－γ産生もまた増加するが、より少ない度合いである（図９ｂおよび９ｄを参照されたい）。

【００８３】

（実施例５）
本実施例は、ＢＰＩがＮＯＤマウスにおいて膵臓島のリンパ球浸潤を阻害する能力を試験した。リンパ球浸潤は、膵島炎および１型糖尿病発症の顕著な特徴である。
【００８４】
材料および方法：
これらの研究は、免疫優性ＧＡＤ６５　ＴＣＲエピトープ（ＥＧＡＤ－ＢＰＩ）を含有するＢＰＩがｉｎ　ｖｉｖｏで生物学的に活性であり、そして膵臓炎症の発症を阻害することを確認するのが目的であった。３匹の正常血糖のＮＯＤオス（８週齢）の群を、実施例４に記載するように、ＣＦＡ中のＧＡＤ６５（２０８－２１７）ペプチドで免疫した。対照群にはＰＢＳを投与し、そして別個の実験群には、ＥＧＡＤ－ＢＰＩ、ＧＡＤペプチドのみ（すなわちＴ細胞受容体エピトープ）、またはＣＤ１１ａペプチドのみ（すなわち二次シグナル受容体部分）いずれかを、実施例４に記載するような２回の静脈内注射によって、投与した。第１０日、膵臓を除去して１０％ＰＢＳ緩衝ホルマリンに入れ、パラフィンに包埋し、そしてその全体が本明細書に援用される、Ｙｏｏｎら，　β細胞におけるＧＡＤ発現または抑制による、ＮＯＤマウスにおける自己免疫糖尿病の調節，　２８４　Ｓｃｉｅｎｃｅ　１１８３－１１８７（１９９９）に先に記載されるように、単核細胞浸潤に関して、５ミクロン連続切片を組織学的に検査した。
【００８５】
結果：
この実験の結果は、図１１および１２に示す。図１１は、この解析の累積的データを示し、ここで、島浸潤の重症度をスコア化し、そして調べた島の割合としてプロットした。５より多い組織切片で、各群から１００を超える島を、３人の独立の観察者によって解析した。図１１に示すように、単核細胞浸潤（膵島炎）に対するＥＧＡＤ－ＢＰＩ処置の明らかな阻害効果があった。ＢＰＩ処置動物の９５％を超える島が損なわれておらず、そして浸潤を示さなかった（すなわち等級０の島）。他の群はすべて、疾患のこの初期の段階であってさえ、膵島炎の何らかの徴候を示した。特に、ＧＡＤペプチド処置動物は、最大の膵島炎を示した（等級０の島は６２％に減少し、そして３７．５％の島は等級２以上にスコア付けされた）。これを、ＣＤ１１ａペプチド処置群の６６．７％の正常な島およびＰＢＳ処置動物の７１．４％の正常な島に比較した。したがって、ＰＢＳ対照と比較すると、ＥＧＡＤ－ＢＰＩ処置は、膵島炎の８４％の阻害を提供した［％島@等級１－４（ＰＢＳ　Ｒｘ）から％島@等級１－４（ＥＧＡＤ－ＢＰＩ　Ｒｘ）を減じ、％島@等級１－４（ＰＢＳ　Ｒｘ）で除して１００を乗じたものとして計算］。実験の各群の代表的な島を、ヘマトキシリンおよびエオジンで染色したものとして、以下、図１２ａ～１２ｄに示す。図１２ｂおよび１２ｄでは、単一のシグナル－１部分またはシグナル－２部分で処置した群で重度のリンパ球浸潤が観察されたが、一方、ＥＧＡＤ－ＢＰＩ処置マウスの島は、損なわれていないものが優勢であったことに注目されたい（図１２ｃを参照されたい）。総合すると、これらのデータは、これらのシグナル－１ペプチド部分およびシグナル－２ペプチド部分を含有する糖尿病ＢＰＩでの処置が、１型糖尿病のこの動物モデルでは、膵臓島へのリンパ球の浸潤を有意に阻害することを強く示す。したがって、ＢＰＩは、膵臓自己抗原に対する自己寛容の通常の瓦解に関与する機構を介して作動する、と予測される。これらのデータは、ＢＰＩが免疫逸脱を通じて作動し、膵臓抗原に対する自己免疫反応を遮断可能であることを示す。この理論をさらに試験するため、我々は、ＥＧＡＤ－ＢＰＩの存在下でプライミングしたＴ細胞を、糖尿病発症が遺伝的にプログラミングされたＮＯＤ．Ｓｃｉｄマウスに移植した。
【００８６】
（実施例６）
本実施例は、免疫学的に再構成したＮＯＤ．Ｓｃｉｄマウスの、よく説明されている、損なわれていない免疫系において、糖尿病発症のＢＰＩ遮断を試験して、糖尿病進行を研究した。
【００８７】

材料および方法：

ＮＯＤマウスモデルにおいて、Ｔ細胞主導型の糖尿病の進行をブロックするための既知の免疫優性ペプチドＧＡＤ６５に対するT_H1／T_H2反応の調節に関して活性であることが証明されたＢＰＩを試験した。ＮＯＤ．Ｓｃｉｄ養子移植モデル（ＣＤ２５枯渇ＮＯＤ脾臓細胞が、養子移植後２～４週間程度の早期に糖尿病を誘導することが観察されている）をこの目的に用いた。ＮＯＤ．Ｓｃｉｄ養子移植は、その内容が本明細書に援用される、Ｓｏｌｏｍｏｎら，「自己免疫糖尿病を調節するＣＤ４$^+$　ＣＤ２５免疫制御Ｔ細胞の恒常性に、Ｂ７／ＣＤ２８同時刺激が必須である」　１２　Ｉｍｍｕｎｉｔｙ　４３１－４４０（２０００）に記載するプロトコルを変更して行った。８週齢（非糖尿病）メスのＮＯＤ脾臓細胞を用いて、精製モノクローナル抗体（ｍＡｂ　７Ｄ４、ＰｈａｒＭｉｎｇｅｎ）、その後、低毒性（ｌｏｗ－ｔｏｘ）ウサギ補体（Ｃｅｄａｒｌａｎｅ）での処理によって、ＣＤ２５－／ＣＴＬＡ４－枯渇集団を濃縮した（ＰＥ標識ＵＣ１０－４Ｆ１０－１１　ｍＡｂでのフロー解析による、ＣＴＬＡ４＋細胞の８０％枯渇；未提示）。これらの誘導細胞（１５ｘ１０^6／マウス）を、ＥＧＡＤ－ＢＰＩで処置したマウスかあるいはＥＧＡＤ－ＢＰＩの代わりにＰＢＳを用いたこと以外は同一に処置したマウスのいずれか由来の３ｘ１０^6　ＣＤ４＋　Ｔ細胞と共に、６週齢ＮＯＤ．Ｓｃｉｄメス（Ｊａｃｋｓｏｎ　Ｌａｂｓ）に注射した（ｉ．ｖ．）。ｉｎ　ｖｉｔｒｏクローン増殖は、ＥＬＩＳＰＯＴ実験に関して記載するように、組換えＩＬ－２（Ｒ＆Ｄ　ｓｙｓｔｅｍｓ）またはＣｏｎＡいずれかを用いた。動物および細胞の操作はすべて、ラミナフローフード中で行い、そして動物は、オートクレーブした餌および水を与えて、ラミナフローバリアの後ろのマイクロアイソレーターケージ中でずっと飼育した。いくつかの実験は、養子移植前に、ＣＤ１５４、ＣＤ２５、ＣＤ６２Ｌ、ＣＤ１５２などに対するｍＡｂおよび磁気粒子を用いて、ＣＤ４＋細胞の特定のサブセットを枯渇させるであろう。ＢＰＩの個々の部分で処置したマウス由来のＣＤ４＋細胞は、生理食塩水のみで処置したマウス由来のＣＤ４＋細胞と共に、陰性対照として使用可能である。所定のＢＰＩで処置した、マッチしたＮＯＤマウスから濃縮したＣＤ４＋Ｔ細胞の同時移植は、ＢＰＩ処置により糖尿病の開始を遅延させることが可能な制御Ｔ細胞が導かれることを示す。さらに、表１に列挙する他の疾患を、この同じ方式で（すなわち制御Ｔ細胞の養子移植によって）試験することが可能である。

【００８８】

結果：

重要なことに、我々は、現在、２つの実験群間で、高血糖症および糖尿病の発症に、明らかな相違があることを見出した。図１３でわかるように、養子移植７週後、ビヒクル（ＰＢＳ）を投与したマウスの８０％が、高血糖症および糖尿病を発症した。対照的に、ＥＧＡＤ－ＢＰＩで処置したマウスのわずか４０％しか、高血糖症と糖尿病への進行とを示さなかった。したがって、これらのデータは、ＢＰＩ処置に仲介されるものとして、糖尿病進行のブロックを示す。ＢＰＩ構造へのさらなる変更は、このモデルにおいて、そして１型糖尿病の治療において、その有効性を増進させる可能性がある。これらのデータは、糖尿病発症を抑制可能であるＴ細胞が、ＢＰＩの存在下に生成され、そしてｉｎ　ｖｉｖｏで作動して、ＮＯＤ．Ｓｃｉｄマウスの損なわれていない系内で糖尿病進行を阻害したことを示す。したがって、同様の制御Ｔ細胞が、他の疾患関連抗原のＴＣＲエピトープを含有するＢＰＩによって活性化されると予期されるであろう。例えば、コラーゲン－ＩＩペプチドエピトープは、慢性関節リウマチに関与するサプレッサーＴ細胞を惹起することが可能である。さらに、T_H1集団を拡大するであろう制御Ｔ細胞が、ＣＴＬＡ４二次シグナル部分を含有するＢＰＩによって生成される可能性があり、そしてこれらは、ＨＩＶ１感染または他の慢性ウイルス疾患などの疾患に使用可能である。ＮＯＤモデルは、ヒト疾患の有意な代表と認識されているため、この実施例から臨床試験への推定は容易なはずである（例えばＡｔｋｉｎｓｏｎおよびＬｅｉｔｅｒ，　１型糖尿病のＮＯＤマウスモデル：これ以上よくならない？　５　Ｎａｔｕｒｅ　Ｍｅｄｉｃｉｎｅ，　６０１－６０４（１９９９）を参照されたい）。

【００８９】
（実施例７）
ここで、他の自己免疫疾患のＢＰＩの予測される例を、簡潔に詳述する。具体的には、コラーゲン誘導関節炎（ＣＩＡ）およびミエリン塩基性タンパク質誘導実験アレルギー性脳脊髄炎（ＥＡＥ）の免疫優性ＴＣＲエピトープを含有するＢＰＩが論じられるだろう。また、ＣＤ４０シグナルを遮断すると、ＩＬ１２産生が減少すると予測されるため、ＣＤ４０Ｌペプチド模倣体は、T_H2免疫を助けると予測される（Ｒｕｅｄｌら，　抗原用量は、ＣＤ４０リガンドの制御によって、Ｔヘルパーサブセット発展を決定する，　３０　Ｅｕｒ．　Ｊ．　Ｉｍｍｕｎｏｌ．　２０５６－２０６４（２０００））。したがって、これらの自己免疫モデルにおいて、そしてＮＯＤモデルにおいて、適切なＴＣＲエピトープをＣＤ４０Ｌペプチド模倣体と共に、糖尿病阻害で用いるＣＤ１１ａ模倣体に連結することによって、T_H2免疫を助けることを試みるであろう。このループペプチドにおける突然変異は、ＣＤ４０Ｌの結合および機能に影響を及ぼすことが示された。
【００９０】
材料および方法：
異なる交差反応性免疫優性エピトープ、異なるマウス系統、および解析する組織を使用する、いくつかの自己免疫モデルがある。これらの種類のモデルは、ＥＧＡＤ－ＢＰＩを試験するのに我々が設計した糖尿病発展の短期モデルに非常に似ている。ＣＩＡに関しては：Ｍｕｒｒａｙら，　２４　Ｅｕｒ．　Ｊ．　Ｉｍｍｕｎｏｌ．　２３３７－２３４４（１９９４）に記載するようなＣＦＡ注射プロトコルにおいて、単一ペプチドによって疾患を誘導するであろう。ＮＯＤ系と同様、これらのマウスには、ＢＰＩ、ＰＢＳ、または単一ペプチド部分いずれかを投与するであろう。最小免疫優性コラーゲンＩＩエピトープは上に列挙され、そしてＢＰＩへの変更は、この複合体のｘ線構造に基づくであろう（Ｄｅｓｓｅｎら，　７　Ｉｍｍｕｎｉｔｙ　４７３－４８１（１９９７））。ＥＡＥに関しては、疾患は、Ｍｕｒｒａｙら，　２４　Ｅｕｒ．　Ｊ．　Ｉｍｍｕｎｏｌ．　２３３７－２３４４（１９９４）の方法によって誘導する。この方法はまた、我々のＮＯＤ系において記載するのと似た方法によって、ＣＦＡ中の単一ペプチド（ミエリン塩基性タンパク質（ＭＢＰ）ペプチド、８５－１０１）注射を用いる。
【００９１】
結果：
コラーゲンおよびＭＢＰで構築したＢＰＩは、これらの抗原に対する免疫をT_H2優性パターンに調節する際に有効であろうと予期される。これらの疾患は、主なT_H1免疫病理（上記参考文献を参照されたい）を伴うと考えられるため、こうしたスイッチは、これらのマウスの疾患を表面上遅延させるかまたは治癒させるであろう（Ｍｕｒｒａｙら，　２４　Ｅｕｒ．　Ｊ．　Ｉｍｍｕｎｏｌ．　２３３７－２３４４（１９９４）；Ｍｕｒｒａｙ；１９　Ｉｍｍｕｎｏｌｏｇｙ　Ｔｏｄａｙ　１５７－１６３；およびＳｃｈｏｕｎｔｚら，　１５７　Ｔｈｅ　Ｊｏｕｒｎａｌ　ｏｆ　Ｉｍｍｕｎｏｌｏｇｙ　３８９３－３９０１（１９９６））。ＮＯＤマウスにおけるように、こうした結果は、これらの種類の免疫反応が臨床的に観察される、慢性関節リウマチおよび多発性硬化症のヒト疾患において使用するための類似の化合物の開発を標的とするであろう。最も重要なことには、ＢＰＩは、自己免疫Ｔ細胞反応を遮断する能力を提供しつつ、感染性病原体および癌発症に対する宿主免疫を維持する。
【００９２】
（実施例８）
本実施例は、感染性疾患および特定の癌の予測されるＢＰＩを記載する。具体的には、特定のヒト病原体の免疫優性ＴＣＲエピトープを含有する所定のＢＰＩを試験する一般的なプロトコルを、ＨＩＶ－１　ｐ２４エピトープの例を用いて記載するであろう（Ｈａｒｃｏｕｒｔら，　ＨＩＶ－１変異は、ＣＤ４　Ｔリンパ球認識を減少させる，　１８８　Ｊ．　Ｅｘｐ．　Ｍｅｄ．，　１７８５－１７９３（１９９８））（配列番号８）。
【００９３】

材料および方法：
先の実験例と対照的に、これらのＢＰＩは、主に、ヒト患者由来の長期Ｔ細胞クローンに対する影響によって試験するであろう。簡潔には、末梢血単核細胞（ＰＢＭＣ）を患者および対照全血から調製し、そして磁気粒子を用いたネガティブ選択プロトコルによって、ＣＤ８＋細胞を除去する。これらの細胞は、１ｍｌ中、４ｘ１０^{6}細胞で、組織培地中、２０μＭのｐ２４ペプチドの存在下、６日間増殖させ、そして密度勾配遠心分離によって芽球を単離する。これらの細胞の二次培養は、組換えヒトＩＬ－２（２０Ｕ／ｍｌ）を含有し、そして最大１０～１４日間続ける。組織適合ドナー由来の照射ＡＰＣと共にｐ２４ペプチドを含む再培養過程およびＩＬ－２中でのＴ細胞芽球の増殖を反復することによって、株を増殖させた。クローンは、照射ＡＰＣ、ペプチドおよびＩＬ－２（５０Ｕ／ｍｌ）を含有する新鮮なプレートにおいて、限界希釈クローニングを行うことによって、調製した（Ｍｕｒｒａｙら，２４ Ｅｕｒ．Ｊ．Ｉｍｍｕｎｏｌ．２３３７－２３４４（１９９４）；Ｍｕｒｒａｙ；１９ Ｉｍｍｕｎｏｌｏｇｙ Ｔｏｄａｙ １５７－１６３；およびＳｃｈｏｕｎｔｚら，１５７ Ｔｈｅ Ｊｏｕｒｎａｌ ｏｆ Ｉｍｍｕｎｏｌｏｇｙ ３８９３－３９０１（１９９６））。Ｓｃｈｏｕｎｔｚら，ペプチド配列における非自己位の自己置換によって明らかになった、ペプチド抗原の正確な自己相関物のユニークなＴ細胞アンタゴニスト特性，１６８ Ｃｅｌｌｕｌａｒ Ｉｍｍｕｎｏｌｏｇｙ １９３－２００（１９９６）に記載される、我々の詳細な方法によって、ヒトＴ細胞のこれらの樹立株の増殖およびサイトカイン放出をＢＰＩが阻害する能力に関して試験するであろう。ここで、我々は、重要なＴＣＲ接触残基でアミノ酸置換によって変化したペプチドが、クローン化されたＴ細胞の増殖およびサイトカイン放出を改変することを示す。与えられたＢＰＩが、ｐ２４ペプチド特異的クローンへのサイトカイン放出を、顕著に阻害するかまたは改変するならば、このことにより、このＢＰＩはより厳密なｉｎ ｖｉｖｏ試験の対象となるだろう。マウスモデルを用いて、感染性疾患に関する候補ＢＰＩを試験することが可能である。これらの研究は、ＧＡＤ６５ ＢＰＩに関して記載するように、ＧＡＤ６５ ＴＣＲエピトープペプチドに対する感染性病原体の免疫優性ペプチドの置換を伴って、行うであろう。これらのｉｎ ｖｉｖｏ研究における肯定的な結果は、生存病原体を使った動物研究を具体化するであろう。ヒト組織がＳｃｉｄマウスに養子移植される、Ｓｃｉｄ－ヒトマウスモデルを用いて、ＨＩＶ（Ｊｅｎｋｉｎｓら，Ｂｌｏｏｄ（１９９８）８：２６７２）、Ｃ型肝炎ウイルス（ＨＣＶ）（Ｂｒｏｎｏｗｉｃｋｉ，Ｊ．ら，Ｈｅｐａｔｏｌｏｇｙ（１９９８）２８：２１１）、ヒトパピローマウイルス（ＨＰＶ）（Ｔｅｗａｒｉら，Ｇｙｎｅｃｏｌ．Ｏｎｃｏｌ．（２０００）７７：１３７）、および呼吸器合胞体ウイルス（ＲＳＶ）（Ｎａｄａｌら，Ｃｌｉｎ．Ｅｘｐ．Ｉｍｍｕｎｏｌ．８５：３５８）感染に対する防御免疫を調べることが可能である。

【００９４】

結果：
二次シグナル部分として、ＣＤ２８／ＣＴＬＡ４ペプチドまたはＦａｓリガンドペプチドを取り込むと、T_H1サブセットの方に免疫がシフトし、そしてＣＴＬ反応が誘導または増進されるであろうと仮定される（Ｐａｄｒｉｄ，Ｐ．ら，Ａｍ．Ｊ．Ｒｅｓｐｉｒ．Ｃｅｌｌ．Ｍｏｌ．Ｂｉｏｌ．（１９９８）１８：４５３）。ＣＴＬＡ４は、Ｔ細胞活性化のネガティブ制御因子であり、そしてＣＤ２８を通じたシグナル伝達は、T_H2分化と結び付けられてきている（Ｒｕｌｉｆｓｏｎ，Ｉ．ら，Ｊ．Ｉｍｍｕｎｏｌ．（１９９７）１５８：６５８）。ＬＦＡ－１シグナルがT_H2分化に阻害性であり、そしてＣＤ２８シグナル伝達がT_H2分化を助ける証拠を考慮し、この選択的分化パラダイムを用いて、ＢＰＩ設計をさらに変更して、これらの研究を進めるであろう。同様に、証拠は、ＣＤ９５（Ｆａｓとも呼ばれる）とＣＤ９５リガンド（ＦａｓＬ）との相互作用がT_H1分化を助けることを示唆する（Ｃｈａｔｔｅｒｇｏｏｎら，Ｎａｔｕｒｅ Ｂｉｏｔｅｃｈ．（２０００）１８：９７４）。ＦａｓＬのＹ２１８ループ由来のＦａｓＬ（Ｔ細胞：ＡＰＣ境界面でＦａｓと相互作用すると予測される）のペプチド模倣体を

持つＢＰＩは、T_H１活性化と慢性T_H２－優性疾患に対するＣＴＬ反応とを助けるのに使用されるだろう。

【００９５】

（実施例９）

ここで、我々は、記載されるアレルゲン配列（予測されるＣＤ２８／ＣＴＬＡ４ペプチド模倣体および／または予測されるＦａｓＬペプチド模倣体に合成的に連結されている）のＴＣＲエピトープを含有する、いくつかの可能性のあるＢＰＩを記載する。

【００９６】

材料および方法：

これらの研究は再び、ヒトクローン化Ｔ細胞株、およびこれらの公知のアレルギー剤に対するアレルギーの動物モデルを用いた解析の組み合わせを用いるであろう。アレルギーは、ヒトの非常に多数の集団に影響を及ぼし、多くのアレルギーおよび喘息の概算は、そうでなければ健康な個体の１０％に達する。我々は、先に記載した７～１０日プロトコルを用いて、表に記載するアレルゲンペプチドに対する免疫反応のＢＰＩ調節を調べるであろう。先に記載されるように、このプロトコルは、第１日、ＣＦＡ中のアレルゲンペプチドの注射、その後、第１日および第６、７、８または９日のＢＰＩの注射を伴う。第７、８、９または１０日、領域リンパ節を培養し、そしてＥＬＩＳＰＯＴによってT_H１対T_H２サイトカインを比較する（詳細な方法に関しては実施例４を参照されたい）。所定のアレルゲンの候補ＢＰＩが、この比較的短期の実験によって同定されたら、アトピー患者からクローニングした株を用いて、ＣＤ４　Ｔ細胞反応の改変に関して、これらのＢＰＩを試験するであろう。簡潔には、ＣＤ４　Ｔ細胞クローンは、実施例８に記載するように、患者末梢血リンパ球から樹立するであろう。先に記載するようなＥＬＩＳＰＯＴを用いて、所定のＢＰＩが、これらの細胞によるサイトカイン放出を阻害または改変する能力を試験するであろう。最後に、所定のＢＰＩの検査を、ヒトＭＨＣ（ＨＬＡ）トランスジェニックマウスにおいてこれらの特定のアレルゲンを用いて、同一に記載される方法を用いる臨床試験に移すであろう（Ｓｖｅｔｌａｎａ，　Ｐ．ら，　Ｊ．　Ｉｍｍｕｎｏｌ．（１９９８）１６１：２０３２）。これらのマウスは、ヒトＭＨＣ分子を有する利点を提供し、そしてしたがって、ＢＰＩ結合は、このモデルにおいてヒトアレルギー状態と非常に類似であろう。所定のＢＰＩが、天然アレルゲンに対して、これらのマウスにおいて、T_H２活性化を遮断するならば、ヒトＳｃｉｄマウスでの養子移植研究（実施例８で用いるのと類似）を、ヒト臨床試験の前置きとして用いることが可能である。

【００９７】

結果：

ＬＦＡ－１シグナル伝達を優先するＣＴＬＡ４／ＣＤ２８経路を遮断すると、ＢＰＩの存在下、これらのアレルゲンに対するT_H１分化が助けられるであろう。また、ＦａｓＬ部分を持つＢＰＩは、１型免疫を助けるはずである。注射によって与えられるアレルゲンでアトピー患者を「脱感作」するのは長く続けられている実務であり、そして脱感作は、特定のアレルゲンに対するT_H１反応へのシフトを介して作動すると考えられる（Ｈｏｌｔら，　Ｎａｔｕｒｅ（１９９９）４０２：６７６０　ｓｕｐｐｌ：Ｂ１２－１７）。したがって、慢性T_H２関連感染性疾患の症例におけるように、我々の作業仮説は、この結果を達成するため、ＬＦＡ－１シグナル伝達がT_H１を促進する能力と共に、ＣＴＬＡ４／ＣＤ２８分子を介してT_H２シグナル伝達を阻害する可能性を用いることである。また、ＡＰＣ内のＦａｓおよび抗原の二重発現がT_H１免疫およびＣＴＬ誘導を助長することが示されているため、Ｆａｓペプチド模倣体に連結したアレルゲンエピトープの影響もまた調べるであろう（Ｃｈａｔｔｅｒｇｏｏｎ，　Ｍ．ら，　Ｎａｔ．　Ｂｉｏｔｅｃｈ．（２０００）１８：９７４）。アレルギーおよび喘息において、アレルゲンエピトープを提示するＡＰＣの細胞死を誘導し、それによってＲＳＶ誘起小児期および老年期細気管支炎を含む、T_H２型制御細胞の活性化を制限することが可能である可能性がある。

【図面の簡単な説明】

【００９８】

本特許のファイルは、少なくとも１つのカラーで作成した図を含有する。カラー図（類）を含むこの特許のコピーは、要望し、そして必要な料金を支払うと、特許商標庁（Ｐａｔｅｎｔ　ａｎｄ　Ｔｒａｄｅｍａｒｋ　Ｏｆｆｉｃｅ）に提供されるであろう。

【図１】図１は、Ｔ細胞およびＡＰＣ間の免疫シナプスの模式的提示であり、ＴＣＲ：ペプチド：ＭＨＣ位のドーナツ構造を例示する。

【図２】図２は、代表的なＢＰＩがどのようにT_H1優性免疫反応を導く分化を遮断し、そして免疫をT_H2優性免疫反応にシフトするか、模式的に提示する。

【図３】図３は、免疫シナプス内の細胞表面タンパク質間の相互作用を通じた、１型免疫反応の名目上の活性化の模式的提示である。

【図４】図４は、Ｔ細胞活性化の２シグナルの機構の、単純化した模式的提示である。

【図５－ａ】図５ａは、異なるマウス系統への代表的ＢＰＩの結合を比較する、フローサイトメトリー解析の結果を示すグラフである。

【図５－ｂ】図５ｂは、異なるマウス系統への代表的ＢＰＩ部分の結合を比較する、フローサイトメトリー解析の結果を示すグラフである。

【図５－ｃ】図５ｃは、異なるマウス系統への代表的ＢＰＩ部分の結合を比較する、フローサイトメトリー解析の結果を示すグラフである。

【図６－ａ】図６ａは、ＭＨＣ－ＩＩまたはＩＣＡＭ－１に対する抗体を含まない、マウス系統のＡＰＣに結合する代表的ＢＰＩ結合のフローサイトメトリー解析の結果を例示するグラフである。

【図６－ｂ】図６ｂは、ＩＣＡＭ－１に対する抗体が存在した場合の、マウス系統のＡＰＣに結合する代表的ＢＰＩ結合のフローサイトメトリー解析の結果を例示するグラフである。

【図６－ｃ】図６ｃは、ＭＨＣ－ＩＩに対する抗体が存在した場合の、マウス系統のＡＰＣに結合する代表的ＢＰＩ結合のフローサイトメトリー解析の結果を例示するグラフである。

【図７】図７は、ＭＨＣ－ＩＩに対する抗体で同時キャッピングすることによって、ＮＯＤ　ＡＰＣ上のＭＨＣ－ＩＩおよびＩＣＡＭ－１構造に同時に結合する代表的ＢＰＩの蛍光顕微鏡解析の結果を示すカラー写真であり、上部パネルは、代表的なＢＰＩで処置したマウスＡＰＣ由来であり、そして下部パネルは、生理食塩水ビヒクルのみで処置している。

【図８】図８は、ＮＯＤマウスのＭＨＣ－ＩＩ（Ｉ－A^{g7}）およびＩＣＡＭ－１のＤ１ドメインへの代表的ＢＰＩ結合の分子モデルのカラー写真であり、ＭＨＣ－ＩＩはピンクで表し、ＩＣＡＭ－１は明るい青色であり、ＢＰＩは原子によって、炭素は緑、酸素は赤、そして窒素は青で示す。

【図９－ａ】図９ａは、ＥＧＡＤ－ＢＰＩまたは生理食塩水対照で処置したＮＯＤマウスから採取したＴ細胞による、ＩＬ－４サイトカイン放出のＥＬＩＳＰＯＴ解析を示すグラフである。

【図９－ｂ】図９ｂは、ＥＧＡＤ－ＢＰＩまたは生理食塩水対照で処置したＮＯＤマウスから採取したＴ細胞による、ＩＦＮ－γサイトカイン放出のＥＬＩＳＰＯＴ解析を示すグラフである。

【図９－ｃ】図９ｃは、ＡＧＡＤ－ＢＰＩまたは生理食塩水対照で処置したＮＯＤマウスから採取したＴ細胞による、ＩＬ－４サイトカイン放出のＥＬＩＳＰＯＴ解析を示すグラフである。

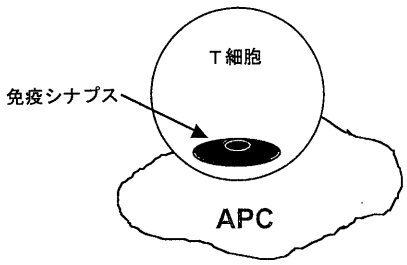
【図９－ｄ】図９ｄは、ＡＧＡＤ－ＢＰＩまたは生理食塩水対照で処置したＮＯＤマウスから採取したＴ細胞による、ＩＦＮ－γサイトカイン放出のＥＬＩＳＰＯＴ解析を示すグラフである。

【図１０】図１０は、図９ａ～９ｄのグラフに用いたＥＬＩＳＰＯＴ解析の生データの代表的な写真である。

【図１１】図１１は、ＥＧＡＤ－ＢＰＩ、ＥＧＡＤ－ＢＰＩの別個の部分、そして生理食塩水による、膵島炎の阻害の示標としての、島浸潤の重症度のグラフである。

【図１２】図１２ａは、生理食塩水対照で処置したＮＯＤマウスにおける、単核細胞による膵臓浸潤の組織学的解析の代表的なカラー写真である。
図１２ｂは、ＧＡＤペプチドで処置したＮＯＤマウスにおける、単核細胞による膵臓浸潤の組織学的解析の代表的なカラー写真である。
図１２ｃは、ＥＧＡＤ－ＢＰＩで処置したＮＯＤマウスにおける、単核細胞による膵臓浸潤の組織学的解析の代表的なカラー写真である。
図１２ｄは、ＣＤ１１ａペプチドで処置したＮＯＤマウスにおける、単核細胞による膵臓浸潤の組織学的解析の代表的なカラー写真である。
【図１３】図１３は、ＣＤ２５ネガティブ糖尿病誘導細胞と共に、ＥＧＡＤ－ＢＰＩまたは生理食塩水いずれかを注射したＮＯＤマウス由来のＴ細胞を投与したＮＯＤ．Ｓｃｉｄマウスにおける血液グルコースレベルの１０週間のモニター結果を例示するグラフである。

【図１】



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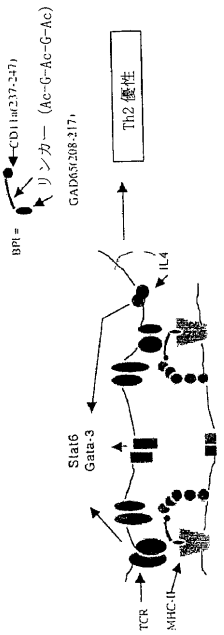

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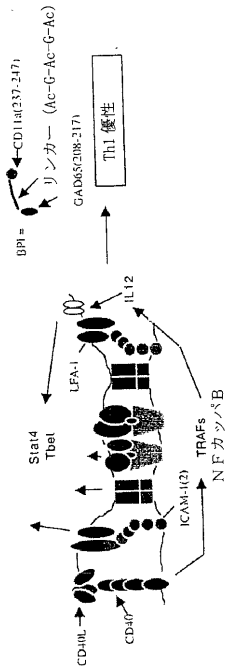

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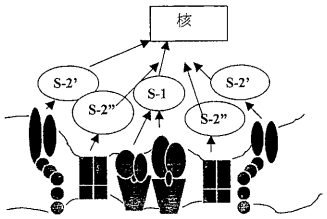

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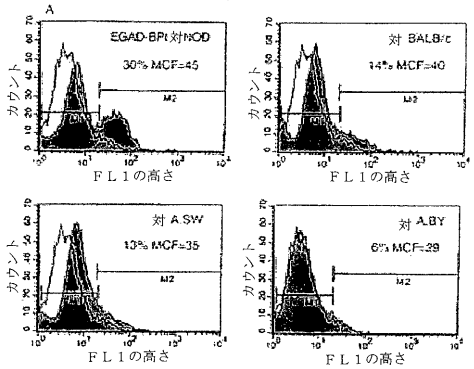

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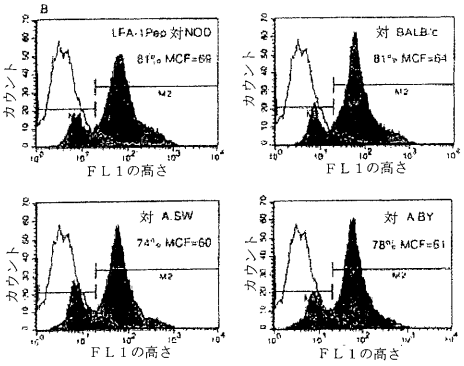
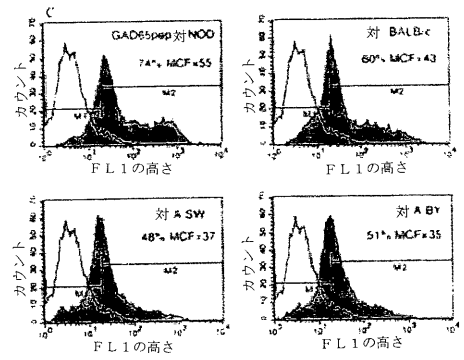
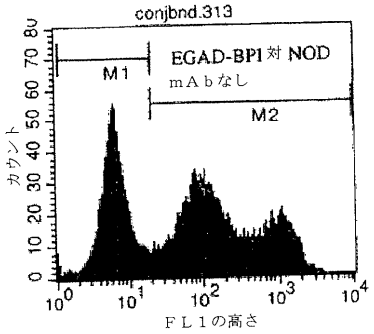


【図５－ｃ】



【図６－ａ】



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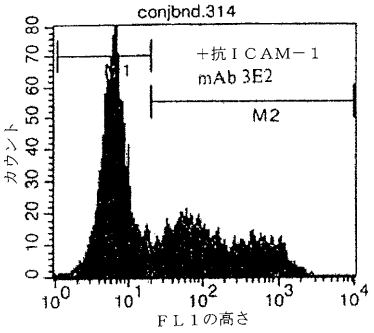

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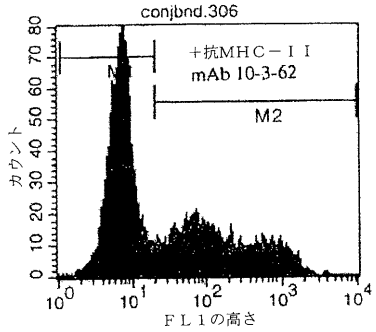
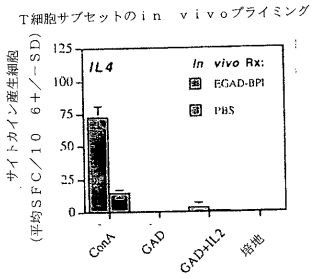


【図９－ａ】



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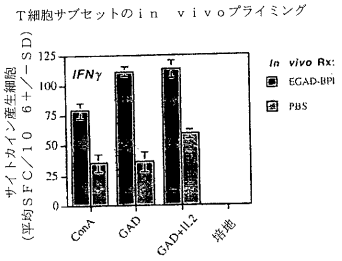

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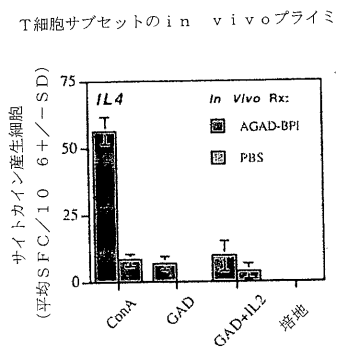

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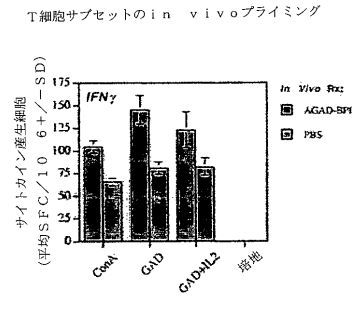

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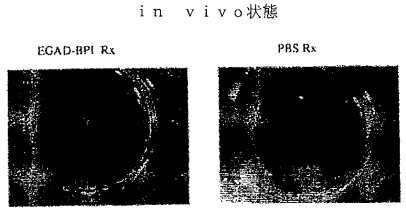

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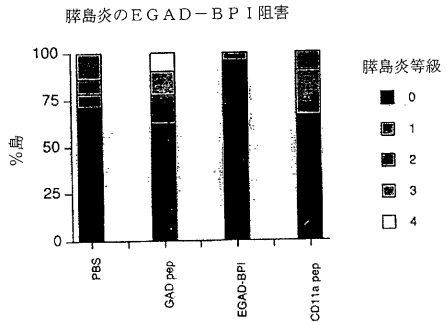

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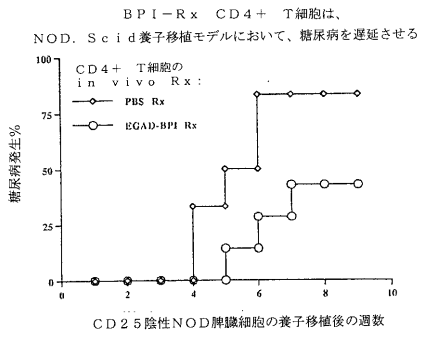

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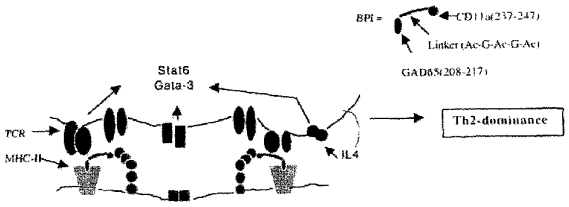
[Continued on next page]

(54) **Title:** SIGNAL-1/SIGNAL-2 BIFUNCTIONAL PEPTIDE INHIBITORS



(57) **Abstract:** A novel peptide sequence having the general formula AB wherein each of A and B represent a chain of amino acid residues and wherein said A chain is capable of binding with a mojor histocompatibility comples on an antigen presenting cell, and wherein said B chain is capable of binding wiht a Signal-2 receptor on an antigen presenting cell. Preferred forms of the peptide sequence further include and Y chain positioned intermediate the A chain and the B chain. Moreover, preferred forms include and A chain which has at least about 10% sequence homology with a Signal-1 moiety, or is a peptidomimetic of a Signal-1 moiety, said B chain has at least 10% sequence homology with a Signal-2 receptor moiety, said B chain has at least 10% sequence homology with a Signal-2 receptor moiety, or is a peptidomimetic of a Signal-2 receptpr moiety, and wherein the X chain has at least one amino acid residue, or is a peptidomimetic of that amino acid residue. Advantageously, the movel peptide sequence is capable of shifting a type-1 immune response to a type-2 immune response or from a type-2 immune response to a type-1 immune response.

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— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

SIGNAL-1/SIGNAL-2 BIFUNCTIONAL PEPTIDE INHIBITORS

SEQUENCE LISTING

A printed Sequence Listing accompanies this application, and has also been submitted with identical contents in the form of a computer-readable ASCII file on a floppy diskette and a CDROM.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention concerns immune responses initiated by the recognition of a peptide:MHC complex on the surface of antigen presenting cells by T-cells. The present invention also concerns immune responses initiated by the binding of a Signal-2 moiety to its complement protein on the surface of an antigen presenting cell. More particularly, the present invention concerns the immune responses initiated by the recognition of the peptide:MHC by the T-cell and by the binding of a Signal-2 moiety to its complement protein. Still more particularly, the present invention concerns the modification of the typical immune response generated by a particular individual in response to this binding. Most particularly, the present invention concerns the conjugation of peptides derived from the peptide portion of the peptide:MHC complex to the preferred Signal-2 moiety in order to modify or shift a given immune response from type-1 to type-2 or from type-2 to type-1. This may include specific phenotypes of regulatory T-cells including suppressor T-cells.

Description of the Prior Art

Autoimmune diseases are characterized by the activation of T-cells against self-antigens. These T-cells then destroy cells presenting these antigens. For example, insulin-dependent diabetes mellitus (IDDM, also called Type-I diabetes) is characterized by the activation of T-cells against the insulin-producing cells of the pancreas and their subsequent destruction by these T-cells. The diseases and conditions associated with autoimmune responses are strongly associated with specific subtypes (alleles) of cell surface proteins

called major histocompatability complex (MHC) class II molecules. MHC molecules bind fragments (peptides) of proteins from infectious agents, allergens, and self proteins, and this MHC:peptide complex is the structure that T-cells recognize with their receptor (called the T-cell receptor, or TCR). The MHC:peptide complex is displayed on the surfaces of other cells of the immune system (i.e., B cells, dendritic cells and macrophages) which are called antigen presenting cells (APC). In order for an immune response to ensue, the major regulatory cell of the immune system, the undifferentiated T-cell, must be presented with small breakdown products (peptides) of the foreign invader. This presentation occurs on the surface of the APC. The T-cell must then interact with the APC, and this interaction stimulates the T-cell to divide and differentiate to produce molecules that attack, either directly or indirectly, cells displaying the same or highly similar MHC:peptide complex. It is well known that the genes that encode the MHC molecules are extremely variable within the species, and the different MHC alleles prefer to bind some peptides over others. Along with other genetic and environmental factors, the existence of different MHC alleles helps to explain why some members of a species develop conditions such as autoimmune diseases, allergies, asthma, and even certain infectious diseases, while others remain seemingly unaffected, or immune, to the same substances. Other differences arise because cell surface proteins distinct from the peptide:MHC complex must also bind to specific receptors on the T-cell. These other protein:protein pairs at the interface of the T-cell and APC membranes provide a costimulatory signal, known as Signal-2 which, along with the signal generated by the TCR recognition of the MHC:peptide complex (known as Signal-1), initiates an immune response.

A defining stage of the immune response is the differentiation of $CD4^+$ T-cells into either type-1 helper T-cells (T_H1 cells) or type-2 helper T-cells (T_H2 cells) as a result of the two signals. These two subtypes of T_H cells and the regulatory network of cells that they selectively activate are well-known correlates of human health conditions and disease states. Differentiation into T_H1 cells results in predominantly cell-mediated immunity while differentiation into T_H2 cells results in predominantly humoral immunity. Each of these immunity types helps to protect the body against different types of invasion. Type-1 immunity protects the body against intracellular pathogens such as bacteria, but is also implicated in organ-specific autoimmune diseases. Type-2 immunity is important for

protection against extracellular parasites, but is associated with allergic reactions as well. Development of T_H1 cells is driven by a cytokine called interleukin-12, which is produced by immune cells known as macrophages and dendritic cells. Interleukin-12 induces or stimulates the naive T-cell ($CD4^+$ T-cells) to produce interferon-γ (IFN-γ) and interleukin-2 (IL-2). These two cytokines (IL-2 and IFN-γ) are involved in classic cell-mediated functions such as clonal expansion of cytotoxic T-lymphocytes (CTLs), macrophage activation, and class switching to IgG isotypes that mediate complement lysis of sensitized cells. Commitment to a T_H1 immune response is enhanced by the presence of IFN-γ which up-regulates expression of the interleukin-12 (IL-12) receptor while inhibiting the development of T_H2 cells. T_H2 immunity results from the production of interleukin-4 (IL-4) by the naive T-cell. IL-4 induces T_H2 development and the subsequent production of interleukins-4 (IL-4), -5 (IL-5), -10 (IL-10), and -13 (IL-13). IL-4 also operates to down-regulate expression of the IL-12 receptor on developing cells, thereby inhibiting T_H1 development and helping undifferentiated T-cells to commit to T_H2 cell development. Additionally, IL-4 and IL-5 are known to activate B cells and switch to neutralizing antibody (IgG1 in the mouse) and IgE, the initiator of immediate hypersensitivity.

In order for either of these immune pathways to be activated, a two-signal mechanism is required to fully activate the T-cell. Signal-1 (S-1) occurs when the T-cell antigen receptor (TCR) recognizes the peptide:MHC-II complex on the surface of an antigen presenting cell (APC). This first signal passes through the T-cell receptor and initiates a cascade of tyrosine phosphorylation/dephosphorylation events mediated by kinases and phosphatases and leads to the activation of Ca^{++} flux, nuclear factor of activated T cells (NF-AT) and NFκB transcription factors. These factors enter the nucleus of the T-cell and bind to promoters of genes responsible for effector functions. Signal-2 (S-2) arises from the binding of Signal-2 receptors to their ligands on the surface of an APC. Signal-2 receptors include CD28 and its ligand B7 as well as LFA-1 and its ligand ICAM-1. When a Signal-2 receptor and its ligand form a complex at the interface between the T-cell and APC receptor membranes, a series of signaling events occur. These events include serine/threonine phosphorylation/dephosphory-lation and activation of guanine nucleotide exchange factors that activate adapter proteins with GTPase activity. These signaling events activate a separate set of transcription factors. The signal delivered through the CD28:B7 complex is

distinct from that delivered from the ICAM-1:LFA-1 complex, particularly with respect to the differentiation of $CD4^+$ T-cells into T_H1 versus T_H2 effector populations. When the predominant binding occurs between LFA-1 and ICAM-1, the $CD4^+$ T-cell differentiation favors T_H1 cells which are abundant producers of IL-2 and IFNγ, the preeminent initiators of inflammatory immune responses including delayed-type hypersensitivity (DTH), immunity to intracellular pathogens, and several autoimmune diseases. When the predominant binding occurs between CD28 and B7, the $CD4^+$ T-cells differentiate into T_H2 cells. In contrast to T_H1 cells, T_H2 cells do not produce abundant IL-2 or IFNγ cytokines, but instead release the mediators of immediate-type hypersensitivity such as allergy and asthma, i.e., IL-4, IL-5, IL-10, and IL-13. Thus, the ability to manipulate the relative contribution of the complex providing the second signal has a profound effect on the type of immune response that is elicited against a given self-tissue antigen.

The associations between the TCR and APC occur at a specialized junction or interface between the TCR and the APC called the immunological synapse. An immune synapse is depicted schematically in Fig. 1. This immune synapse can be defined as the organized structure of activation molecules that assemble at the interface between the T-cell and the APC. Like a synapse in the nervous system, the immune synapse is a close association between cellular membranes. In order for an immune response to ensue, the major regulatory cell of the immune system, the undifferentiated T-cell must be presented with small breakdown products (peptides) of the foreign invader. In an unactivated T-cell, TCR and adhesion molecules are dispersed randomly on the T-cell membrane. The formation of the immunological synapse is an active and dynamic mechanism that allows T-cells to distinguish potential antigenic ligands. The immunological synapse consists of a central cluster of T-cell receptors surrounded by a ring of adhesion molecules. The stable formation of the immune synapse requires adhesion molecules such as LFA-1 and the peptide-recognition receptor (TCR) to form a doughnut-like structure with the TCR on the inside and LFA-1 on the outside. During activation, the TCR and LFA-1 molecules pass by each other within the T-cell lipid bilayer during the formation of the doughnut-like structure (this process is called translocation). If these molecules do not translocate within the immune synapse then the T-cell signal is not fully received and a different program of gene activity may ensue within the T-cell. This can drastically effect the immune response,

especially if the T helper cell deviates from a gene program that would lead to IFNγ release (T_H1 cells and type-1 immunity) to a program that ultimately activates IL-4 production (i.e., T_H2 cells and type-2 immunity).

In more detail, to activate the pathway leading to T_H1 dominance, the TCR recognizes the peptide:MHC-II complex and sends Signal-1 to the T-cell. Additionally, LFA-1 binds to ICAM-1, and these molecules, along with the peptide:MHC-II complex, translocate to form the end-stage immune synapse. This leads to the effective expression of the CD40 ligand (CD154) by the uncommited T_H cell. CD40 interaction (expressed on the antigen presenting cell) with its ligand generates NFκB up-regulation of the inflammatory cytokine, IL-12. IL-12 then binds to its receptor on the undifferentiated T_H cell and initiates the T_H1 program, including the up-regulation of the transcription regulators, Stat4 and Tbet. This leads to T_H1 dominance against the autoantigen (e.g., glutamic acid decarboxylase, GAD65), which was initiated by the GAD65 peptide component of the TCR:peptide:MHC-II complex. For the pathway leading to T_H2 dominance, the TCR can recognize the same peptide:MHC-II complex, thereby sending Signal-1. However, in this case, a weaker strength of Signal-1 and/or altered or blocked binding between Signal-2 moieties leads to an altered form of the end-stage immune synapse. Likely, this lower strength of Signal-1 or distinct participation of the LFA-1 second signal leads to this different result, i.e., dominant T_H2 differentiation. For example, the altered immune synapse can dictate that the CD40 ligand is not expressed and IL-12 is therefore not released by the APC. This pathway is schematically represented in Fig. 2. Here, IL-4 appears to accumulate, thereby leading to the up-regulation of Stat6 and GATA-3 within the T-cell and hence commitment to a T_H2 pattern of differentiation.

A major goal of modern applied immunology is to be able to switch from T_H1-dominant immunity (e.g., as seen in autoimmune diseases and transplant rejection) to T_H2 responses against these same tissue antigens. In other cases, it would be extremely valuable to replace weak T_H2 immunity with T_H1 dominance leading to strong T-cell proliferation and the effective generation of cytotoxic T-cells (CTL). These cases may include chronic viral illnesses, like hepatitis-C and AIDS; and could include certain cancers like melanoma. Accordingly, what is needed in the art is modifiers of these immune responses so that type-2 immunity can be replaced with type-1 immunity or type-1 immunity can be replaced with

type-2 immunity, as desired in order to combat different human disease states or health conditions.

SUMMARY OF THE INVENTION

The present invention solves the problems found in the prior art and provides a distinct advance in the state of the art. Briefly, the present invention embraces a peptide which includes a portion of a Signal-1 moiety at one end and a portion of a Signal-2 moiety at the other end. These two ends can be directly connected to each other or connected via a flexible, non-substrate linker. This conjugation of the peptide portions directly and via a linker into a continuous peptide chain produces a new class of immunotherapeutic peptides termed bifunctional peptide inhibitors (BPI). These BPI are based upon the two signal mechanism of T-cell activation and link Signal-1 and Signal-2 moieties in order to alter T-cell activation. In other words, the present invention provides a method of modulating T-cells and subsequent immunity in a very specified manner such that only specific disease-associated populations of these cells are targeted by the products of the present invention. Thus, the present invention leaves necessary components of the intact immune system to operate in their nominal protective manner.

In more detail, the present invention describes constructing a peptide sequence having a TCR epitope of interest (a Signal-1 moiety) at one end and a peptide derived from the protein:protein interaction (the Signal-2 moiety) which generates Signal-2. These two peptide sequences can be connected via a flexible linker which couples the Signal-1 moiety to the Signal-2 moiety or can be directly linked together. In some cases, the linkage between the two peptides sequences may include flanking residues from each portion. The combination of the Signal-1 moiety coupled with the Signal-2 moiety constitutes a BPI. Accordingly, once a TCR epitope of interest is identified and the desired immune response (type-1 or type-2) determined, a BPI according to the present invention, can be generated.

As noted above, an important stage of the immune response is the differentiation of $CD4^+$ T-cells into either type-1 helper T-cells (T_H1 cells) or type-2 helper T-cells (T_H2 cells). Differentiation into T_H1 cells results in predominantly cell-mediated immunity while differentiation into T_H2 cells results in predominantly humoral immunity. Each of these immunity types help to protect the body against different types of invasion. T_H1 cells protect the body against intracellular pathogens such as bacteria, and are also implicated in organ-

specific autoimmune diseases. T_H2 cells are important for protection against extracellular parasites as well as allergic reactions. Development of T_H1 cells is driven by a cytokine called interleukin-12, which is produced by immune cells known as macrophages and dendritic cells. Interleukin-12 induces or stimulates the naive T-cell to produce interferon-γ (IFN-γ) and interleukin-2 (IL-2). These two cytokines (IL-2 and IFN-γ) are involved in classic cell-mediated functions such as clonal expansion of cytotoxic T-lymphocytes (CTLs), macrophage activation, and class switching to IgG isotypes that mediate complement lysis of sensitized cells. Commitment to a T_H1 immune response is enhanced by the presence of IFN-γ which up-regulates expression of the interleukin-12 (IL-12) receptor while inhibiting the development of T_H2 cells. This pathway is shown schematically in Fig. 3.

T_H2 immunity results from the production of interleukin-4 (IL-4) by the naive T-cell. IL-4 induces T_H2 development and the subsequent production of interleukins 4 (IL-4), 5 (IL-5) and 13 (IL-13), through activation of the transcription regulator Stat6. IL-4 also operates to down-regulate expression of the IL-12 receptor on developing cells, thereby inhibiting T_H1 development and helping undifferentiated T-cells to commit to T_H2 cell development. Additionally, IL-4 and IL-5 are known to activate B cells and switch to neutralizing antibody (IgG1 in the mouse) and IgE, the initiator of immediate hypersensitivity. Again, a schematic representation of this process is depicted in Fig. 2.

As noted above, a two-signal mechanism is required to fully activate the T_H cell. Signal-1 occurs when the T-cell antigen receptor (TCR) recognizes or engages the peptide:MHC-II complex on the surface of an antigen presenting cell (APC). This first signal is transmitted through the T-cell receptor and initiates a cascade of tyrosine phosphorylation/dephosphorylation events mediated by kinases and phosphatases and leads to the activation of Ca^{++} flux, NF-AT and NFκB transcription factors. These factors enter the nucleus of the T-cell and bind to promoters of genes responsible for effector functions. Signal-2 arises from the binding of a Signal-2 receptor on the T-cell to its protein ligand on the APC. Signal-2 receptors include CD28 and its ligand B7 as well as LFA-1 and its ligand ICAM-1. When a Signal-2 receptor and its ligand form a complex at the interface between the T-cell and APC membranes, a series of signaling events occurs including serine/threonine phosphorylation/dephosphorylation along with actuation of guanine nucleotide exchange factors that activate adapter proteins with GTPase activity. These

signaling events activate a separate set of transcription factors. The signal delivered through the CD28:B7 complex is distinct from that delivered from the ICAM-1:LFA-1 complex, particularly with respect to the differentiation of $CD4^+$ T-cells into T_H1 versus T_H2 effector populations. A schematic representation of this signaling is provided herein as Fig. 4. When the predominant binding occurs between LFA-1 and ICAM-1, the $CD4^+$ T-cells differentiate into T_H1 cells. The $CD4^+$ T-cells of the T_H1 differentiation state are abundant producers of IL-2 and IFNγ, two cytokines that are the preeminent initiators of inflammatory immune responses, such as delayed-type hypersensitivity (DTH), immunity to intracellular pathogens, and several autoimmune diseases. When the predominant binding occurs between CD28 and B7 (i.e., decreased LFA-1:ICAM-1 signaling), the $CD4^+$ T-cells differentiate into T_H2 cells. In contrast to T_H1 cells, T_H2 cells do not produce IL-2 and IFNγ cytokines, but instead release the mediators of immediate-type hypersensitivity such as allergy and asthma, i.e., IL-4, IL-5, IL-10, and IL-13. Thus, the ability to manipulate the relative contribution of the complex providing Signal-2 has a profound effect on the type of immune response that is elicited against a given self-tissue antigen.

The associations between the TCR and APC occur at a specialized junction called the immunological synapse (shown in Fig. 1). In order for the immune response to proceed, the undifferentiated T_H cell, must be presented with peptides of the foreign invader on the surface of the APC. In an unactivated T-cell, TCR and adhesion molecules are dispersed randomly on the T-cell membrane. The formation of the immunological synapse is an active and dynamic mechanism that allows T-cells to distinguish potential antigenic ligands. The immunological synapse consists of a central cluster of T-cell receptors surrounded by a ring of adhesion molecules. This arrangement is depicted schematically in Fig. 1. In this figure, the TCR:peptide:MHC-II complex is in the center of the dark circle which represents the protein:protein pair constituting the Signal-2 receptor and the Signal-2 ligand. The stable formation of the immune synapse requires adhesion molecules such as LFA-1 and the peptide-recognition receptor (TCR) to form a doughnut-like structure with the TCR on the inside and LFA-1 on the outside. During activation, the TCR and LFA-1 molecules actually translocate past one another within the T-cell lipid bilayer. If these molecules do not translocate within the immune synapse then the T-cell signal is not fully received and a different program of gene activity may occur within the T-cell. This can drastically effect

the immune response, especially if this causes the T helper cell (T_H) to deviate from a gene program leading to a T_H1 immune response to a program that activates a T_H2 immune response. As shown in Fig. 2, an interpretation of the BPI mechanism suggests that BPI bind to both the MHC-II and second signal ligands. This effectively tethers the MHC-II:peptide and ICAM-1 moecules thereby preventing the translocation step of immune synapse formation.

In one aspect of the present invention, known TCR epitopes are used as the first peptide portion of the BPI. In this manner, minimal peptide sequences that are potent immunogens are utilized. These minimal peptide sequences (e.g. antigenic peptides) effectively engage the TCR involved in immune responses of interest (i.e. autoimmune diseases, infectious diseases, allergies, cancers, etc). There are already many known TCR epitopes of interest (Signal-1 moieties) and their sequences have been defined in the literature. A partial list of some representative Signal-1 moieties is provided in Table 1. This list is by no means exhaustive as there are potentially thousands of Signal-1 moieties.

In another aspect of the present invention, TCR epitopes of interest are identified so that the first portion of the BPI can be synthesized. In this aspect, these dominant TCR epitopes have been so determined by previous art and the sequences are available in the literature. The peptide to which a given T-cell response is focused upon, (e.g., the response against the diabetes-associated antigen GAD65) is identified by the fact that most effector T-cells respond to this portion of the antigen and not other portions. In mouse model systems, animals are immunized with the whole protein antigen. Next, T-cells are removed after the antigen has primed the immune system. These T-cells are placed separately in cultures with short overlapping peptides of the antigen. Most of the response will be to a single peptide and this is the dominant TCR epitope. In humans, T-cells are first cloned from patients. These cloned T-cells are placed separately in cultures with overlapping peptides (again, representing individual portions of the antigen involved, e.g., HIV-1, p24 (SEQ ID No. 8)). Again, the peptide to which most T-cell clones respond is the dominant TCR epitope. The foregoing is described by Schountz et al., *MHC Genotype Controls the Capacity of Ligand Density to Switch T Helper (Th)-1/Th-2 Priming In Vivo*, 157 The Journal of Immunology 3893-3901 (1996), the teachings and content of which are hereby incorporated by reference herein.

In another aspect of the present invention, peptides derived from Signal-2 receptors are used to alter interactions between the nominal receptors on T-cells and their complementary ligands on the APC surface. Table 3 includes a representative list of some known Signal-2 receptor moieties. Of course, those of ordinary skill in the art will be able to identify other Signal-2 moieties not listed therein, as this list is representative and not all-inclusive.

Another aspect of the present invention is the linking of the TCR epitope (i.e. the Signal-1 moiety) to a Signal-2 receptor peptide mimic (i.e., the Signal-2 moiety) in order to modify the resultant immune response. This linkage can be between the Signal-1 moiety and the Signal-2 moiety directly, or through flanking residues. Alternatively, this linking can be done via a linker which is positioned between the Signal-1 moiety and the Signal-2 moiety. The linker could be any amino acid including naturally occurring or chemically synthesized amino acids. Preferably, non-substrate amino acids will be used due to their resistance to protease attack. Still more preferably, the linker will comprise a non-substrate amino acid alternating with a small or hydrophilic amino acid. Even more preferably, the linker is synthesizable as one continuous sequence along with the Signal-1 and Signal-2 moieties, which flank the linker at each respective end. Still more preferably, the linker has the general formula $(A,B)_X$, wherein A and B are amino acid residues, and the A amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine, and the B amino acid residue is a small or hydrophilic amino acid. In this formula, X can range from 1 to 100. A particularly representative B residue is glycine. In this embodiment, a linker could potentially have aminocaproic acid (Ac), aminohexanoic acid (Ahx), aminododecanoic acid (Ado), and β-alanine (βA) alternating with glycine residues (G) (e.g., Ac-G-Ahx-G-Ado-G-βA). The choice of the residues used to construct the linker can be based upon the desired length of the linker as well as steric hindrance considerations. One preferred linker comprises alternating Ac and G residues. This linker can be lengthened or shortened by the inclusion of the other amino acid residue choices (Ahx, Ado, βA). Some representative linkers are included in Table 2 as SEQ ID Nos. 26-29.

Approximately 10^9 different TCR account for protective immunity to the universe of infectious agents and contain the repertoire of TCR that may turn against self in

autoimmune diseases. Moreover, the TCR are also specific for potential tumor antigens and the myriad of allergenic substances in the environment. By changing the TCR epitope of a given BPI we direct the immunomodulating capacity of the BPI to a select group of TCR. In other words, the selection of a TCR epitope to incorporate into the BPI targets T-cells that are involved in a particular human disease in a highly specific fashion. For example, incorporating the GAD65 epitope into a BPI targets autoaggressive T-cells involved in the induction of type-1 diabetes. This targeting to specific TCR allows that T-cells necessary for immunity to infectious agents or cancers will not be significantly compromised. Thus, BPI offer the possibility to specifically modulate T-cell immunity to one antigen while leaving intact the T-cell repertoire necessary for protective immunity to infectious agents and developing cancers.

As noted above, the Signal-1 moieties of the present invention are preferably derived from TCR epitopes and a list of representative known epitopes is provided in Table 1 wherein these known epitopes are presented as SEQ ID Nos. 1-25. When a derivative of a TCR epitope is used to construct the BPI, preferably, the TCR epitope selected will be correlated with a known health condition or disease state. When using one of the representative peptides shown in Table 1 to construct the BPI, it is preferred that the peptides include a sequence having at least about 10% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. More preferably, the peptide will have at least 30% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. More preferably, the peptide will have at least 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. Even more preferably, the peptide will have at least 70% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. Most preferably, the peptide will have at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. Of course, it is also well known in the art to use peptidomimetics to construct moieties having similar functions as the peptides derived from the TCR epitopes. In this respect, the teachings of Falcioni et al. in *Peptidomimetic Compounds That Inhibit Antigen Presentation by Autoimmune Disease-Associated Class II Major Histocompatability Molecules*, 17 Nature Biotechnology, 562-567 (1999), are incorporated by reference herein. Accordingly, all or part of this Signal-1 moiety portion of the BPI can include such

peptidomimetics. Preferably, the peptidomimetic will be a mimetic of a peptide selected from the group consisting of SEQ ID Nos. 1-25. Alternatively, the Signal-1 moiety will be a derivative of a TCR epitope or a peptide selected from the group consisting of SEQ ID Nos. 1-25. At any rate, it is desired that this first portion of the BPI (or the portion responsible for initiating the first signal) be capable of binding with a major histocompatability complex (MHC) on an antigen presenting cell (APC). Furthermore, it is preferred that this resulting peptide:MHC complex be capable of engaging important TCR and initiating some form of the signal to the T-cell.

As noted above, the peptides used on the side of the linker opposite the Signal-1 moiety are preferably derived from Signal-2 receptors. This second portion of the BPI is connected to the first portion either directly or via the linker. In preferred forms, the second portion includes a sequence having at least about 10% sequence homology with a sequence selected from a group consisting of SEQ ID Nos. 30-41. More preferably, the second portion peptide has at least about 30% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41. Still more preferably, the second portion peptide has at least about 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41. Even more preferably, the second portion peptide has at least about 70% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41. Most preferably, the second portion peptide includes a sequence having at least about 95% sequence homology with a sequence selected from the group of SEQ ID Nos. 30-41. As with the first portion, peptidomimetics can be used in place of all or some of the amino acid residues of the second portion. In preferred forms the peptidomimetic of the second portion will be a mimic of a peptide selected from the group consisting of SEQ ID Nos. 30-41. Alternatively, the second portion of the BPI will comprise a derivative of a peptide selected from the group consisting of SEQ ID Nos. 30-41. Similar to the first portion, it is preferred that the second portion be capable of binding with a complementary ligand (e.g. the Signal-2 ligand) on an antigen presenting cell. For example, when a peptide derived from LFA-1 is used as this Signal-2 moiety of the BPI, it should bind to ICAM-1 on the surface of the APC. Additionally, it is preferred that this binding with the Signal-2 ligand on the APC inhibits or alters the binding of the moiety's parent receptor (on the T-cell) to this same APC ligand.

As explained above, the immune response involves a two signal mechanism and the purpose of the present invention is to modify a given immune response, e.g., from type-1 immunity to type-2 immunity or from type-2 immunity to type-1 immunity. This modification or shifting of immune response phenotype is brought about by BPI according to the present invention. It is preferred in some cases for the BPI to modify an immune response from a T_H1 dominated or cytolytic immune response to a T_H2 dominated response; and, in other cases, it is preferred for the BPI to modify an immune response from a T_H2 dominated response to a T_H1 or cytolytic dominated response. In some cases, BPI may operate via the activation of very specific T-cell phenotypes, e.g., peptide-specific suppressor T-cells. In contrast to the nominal situation where an antigen stimulates the system toward a T_H1 response (depicted in Fig. 2), the response generated when a BPI similar to the GAD 65-CD11a BPI is introduced into the immune synapse is quite different and operates to shift the response from type-1 to type-2. This situation is depicted schematically in Fig. 2. In this manner, a BPI comprising a Signal-1 moiety, a flexible, non-substrate linker, and a Signal-2 moiety is formed and introduced into the immune synapse. The TCR recognizes the peptide:MHC complex on the APC and initiates the first signal. However, the second portion of the BPI (the Signal-2 moiety) blocks the typical Signal-2 interaction occurring between LFA-1/ICAM-1, (or for other BPI:CTLA-4/B7, or CD40L/CD40, or FasL:Fas) and the translocation of the TCR into the central cluster. Depending on whether the LFA-1/ICAM-1 or CTLA-4/B7 interaction is targeted by the specific BPI construction, perhaps by tethering the MHC-II:peptide complex to the second signal ligand, the signal will be altered in a different direction of differentiation. For example, when the Signal-2 peptide portion of the BPI is derived from LFA-1, this would favor a decrease in CD40-ligand expression and hence, a lack of IL-12 release. By contrast, IL-4 released during the initial T-cell activation will accumulate to higher levels surrounding the synapse. This accumulation of IL-4 leads to Stat6 and GATA-3 up-regulation in the naive T-cell and ultimately to commitment to a type-2 pattern. Alternatively, when the Signal-2 moiety peptide portion of the BPI is derived from CTLA-4, the normal binding of CTLA-4 and CD-28 to B7 ligands is affected and thus more CD40 ligand is expressed (i.e., a greater role for high affinity LFA-1:ICAM-1 is dictated by blocking the B7 receptors); hence, the release of IL-12 increases. Interleukin-12 induces or stimulates the naive T-cell to produce more IFN-γ

and IL-2, thus providing a positive feedback toward type-1 immunity. These two cytokines (IL-2 and IFN-γ) are involved in classic cell-mediated functions such as clonal expansion of cytotoxic T-lymphocytes (CTLs), macrophage activation, and class switching to IgG isotypes that mediate complement lysis of sensitized cells. Such responses are hallmarks of protective immunity against human viral diseases. It will also operated to link TCR epitopes to the receptor for Fas. Since Fas:FasL interaction governs apoptosis, it will be possible to increase the frequency of specific TCR-bearing cells by blocking the apoptotic event. This will be important for BPI design against HIV, HPV, HCV, and cancers.

Thus, an important aspect of the present invention is that tethering a specific TCR epitope to a Signal-2 receptor peptide mimic leads to alteration of T-cell differentiation involving T-cells bearing only these receptors and/or T-cell populations indirectly linked to these peptide specific subsets. The ability to block or alter T-cell responses to a given immunodominant peptide antigen would offer extremely precise treatments for immunopathological conditions. A major drawback to current immunotherapies is that broad specificities of T-cells are affected leaving the host more susceptible to infections and cancers. The BPI of the present invention should block and/or alter only the desired T-cell population and subsequent responses that depend on these initial T-cells. Also, BPI will target a specific TCR-bearing population for activation toward a desired effector function.

In another aspect of this invention, the relative strength of signal generated by the T-cell-APC interaction has an affect on whether the ultimate immune response is a type-1 or a type-2 response. In this regard, the teachings of Murray in *How the MHC Selects T_H1/T_H2 Immunity*, 19 Immunology Today 157-163 (1998) are hereby incorporated by reference.

In another aspect of the present invention, an immune response is modified by contacting an APC with a peptide capable of binding to an MHC and to a Signal-2 ligand on the APC and causing an altered signal to be transmitted to the T-cell. Thus, the immune response is deviated from the immune response generally associated with the immunogenic peptide and its corresponding antigen (i.e., infectious agent, self protein, or allergen).

In another aspect of the present invention, a peptide having the general formula AXB is provided. The A, X, and B represent a chain of amino acid residues wherein the A chain has at least about five residues and at least about 10% sequence homology with a TCR

epitope, the B chain has at least four residues and at least about 10% sequence homology with a peptide derived from a Signal-2 moiety, and the X chain is a linker. The linker could be any amino acid including naturally occurring or chemically synthesized amino acids. Additionally, the X chain has at least one residue. It is possible to link A to B directly without X as well, although a linker of some size is preferred in order to span the distance between the MHC-II and second signal ligands on the APC surface. As noted above, preferably, non-substrate amino acids will be used due to their resistance to protease attack. Still more preferably, the linker will comprise a non-substrate amino acid alternating with a small or hydrophilic amino acid. Even more preferably, the linker is synthesizable as one continuous sequence along with the Signal-1 and Signal-2 moieties, which flank the linker at each respective end. Still more preferably, the linker has the general formula $(A,B)_X$, wherein A and B are amino acid residues, and the A amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine, and the B amino acid residue is a small or hydrophilic amino acid. In this formula, X can range from 1 to 100. A particularly representative B residue is glycine. In this embodiment, a linker could potentially have aminocaproic acid (Ac), aminohexanoic acid (Ahx), aminododecanoic acid (Ado), and β-alanine (βA) alternating with glycine residues (G) (e.g., Ac-G-Ahx-G-Ado-G-βA). The choice of the residues used to construct the linker can be based upon the desired length of the linker as well as steric hindrance considerations, hydrophobicity, charge, etc. One preferred linker comprises alternating Ac and G residues. This linker can be lengthened or shortened by the inclusion of the other amino acid residue choices (Ahx, Ado, βA). Some representative linkers are included in Table 2 as SEQ ID Nos. 26-29. Additionally, the X chain is positioned between the A chain and the B chain and the entire peptide can be synthesized as one continuous sequence. Some preferred sequences will have an A chain having at least about 10% sequence homology with any one of SEQ ID Nos. 1-25, an X chain having at least about 2% sequence homology with any one of SEQ ID Nos. 26-29, and a B chain having at least about 10% sequence homology with any one of SEQ ID Nos. 30-41. Preferably, the peptide is capable of shifting a type-1 response to a type-2 response, or vice versa. Of course, peptidomimetics may be synthesized to mimic any part of the BPI, including the linker. Preferably, the A chain binds to the MHC on an APC to form a

peptide:MHC complex. This complex is capable of engaging the TCR on critical T-cell populations. Still more preferably, the B chain is capable of binding to a Signal-2 ligand on the APC at the same time as the formation of the peptide:MHC complex. This combined binding to the APC should be capable of altering the signal delivered to the T-cell. The combination of the first signal and the second signal are capable of fully activating a T-cell and by selecting the peptide used for the A chain and the peptide used for the B chain, the immune response can be deviated from its normal progression. In the case of a normally activated type-1 response leading to the up-regulation of T_H1 cells, the response can be altered to give a type-2 response leading to the up-regulation of T_H2 cells. In the case of a normally activated type-2 response leading to the up-regulation of T_H2 cells, the response can be altered to give a type-1 response leading to the up-regulation of T_H1 cells. Again, the A chain can be chosen based on the health condition normally associated with the sequence (for example, see Table 4).

In another aspect of the present invention, a method is provided for preparing a peptide for modulating immune responses. This method comprises the steps of selecting a first peptide sequence which has at least about 10% sequence homology with a sequence derived from a TCR epitope, selecting a second peptide sequence which has at least about 10% sequence homology with a sequence derived from a Signal-2 receptor moiety, selecting a third peptide sequence which is a flexible, non-substrate linker, and synthesizing the peptides as a continuous peptide chain. Preferably, the linker is flanked on one end with the peptide derived from the TCR epitope and flanked on the other end with the peptide derived from the Signal-2 moiety. Preferably, the first peptide sequence should be associated with a known health condition and be capable of binding with an MHC on an APC. Similarly, it is preferred that the second peptide sequence be capable of binding with a Signal-2 ligand moiety on the APC. The method can further comprise the step of contacting the nominal peptide immunogen with the TCR, thereby binding the first peptide sequence to the MHC and the second peptide sequence to the Signal-2 ligand, thereby generating APC bearing potent first and altered/blocked second signal ligands which activate a desired immune response.

Inherent in the BPI design is the antigen-specific moiety that a given T-cell population is activated to respond against (i.e., the TCR epitope), ultimately leading to the

cascade of immune reactions that generate protective or in some cases pathologic immune responses. These epitopes when bound to major histocompatibility complex (MHC) molecules on the antigen presenting cell (APC) surface provide the Signal-1 of the two-signal mechanism of T-cell activation. Thus, an important consideration is the affinity of a given peptide for MHC molecules. This affinity was directly tested by the binding of biotinylated peptide to the cells that present the peptide:MHC complex to the T-cells in mouse and man. T-cell clones to possible peptide epitopes were generated and tested for binding to the immunodominant TCR of a response and specifically stimulate T-cell functions in vitro by the ELISPOT assay. Because modifications in the peptide residues that actually contact the TCR are part of the BPI development, it is also preferable that known crystallographic structures of the epitope bound to MHC molecules are available. This allows for precise three-dimensional predictions of how a particular amino acid substitution or mimetic will affect the actual structure encountered by the developing T-cells. However, in the absence of known crystal structures, it is possible to predict the shape of a hypothetical peptide:MHC structure based upon the available coordinates of other peptide:MHC structures. This predictability is partially due to established peptide binding motifs, that allow for prediction of which residues of the epitope fit most readily into the particular binding pockets of a given MHC allele. These predictions have been accomplished using two different alleles ($I\text{-}A^s$ and $I\text{-}A^b$) binding a collagen peptide wherein each polymorphic position of the I-A molecules were substituted with the known amino acids at these positions. The overall structure was based upon the published coordinates of a reference allele $I\text{-}A^k$ binding a different peptide. Thus, in cases where the 3D structure of a particular disease-associated epitope is not known, a structure for predicting where the TCR contacts the peptide by a similar allelic-substitution and modeling approach can still be generated. It is important to identify (or at least predict) these TCR contact positions. It is well-known that certain alterations to TCR-contact positions can change the functional differentiation of T-cells into the T_H1 or T_H2 types that can determine the course of immunity (see Murray, et al., Major Histocompatibility Complex (MHC) Class II Molecules Direct TCR-Specificity for Opposite Ends of the Same Immunogenic Peptide in T_H1 or T_H2 responses (unpublished manuscript, 2000); and Murray; 19 *Immunology Today* 157-163 (1998), the teachings of which are hereby incorporated by reference.

Specifically, it was verified that the peptide binds to MHC molecules on live APC. Briefly, spleen cell density-gradient fractions (from mice), or PBL, or APC lines (from humans) were incubated in round bottom 96-well plates with increasing concentrations of individual biotinylated peptides at 37°C, 5% CO_2 for 16 hours. Following binding of the BPI to the APC, Avidin-FITC was incubated with the cells on ice for 30 minutes, followed by biotinylated anti-Avidin for 1 hour, then again with Avidin-FITC. For BPI titrations, increasing concentrations (0.1-100 μM) of the biotinylated derivatives in sterile 0.5% BSA-PBS were incubated with the APC for 16 hours as above. As shown in Fig. 5, the GAD 65 BPI binds preferentially to NOD APC as predicted. The LFA-1 moiety or the GAD 65 moiety did not display this increased binding to the diabetes strain's APC. Three-color analyses used Cy-Chrome or PE-conjugated antibodies to known surface markers of APC that were commercially available. Avidin-FITC and biotinylated anti-Avidin detection of the bound peptide was as previously described in Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996). Slight modifications of this method were used to test peptide inhibition (i.e., peptide affinity) and co-capping to the MHC and ICAM molecules directly on the live APC surface. First, monoclonal antibodies (mAb) against MHC or ICAM molecules blocked BPI binding, therefore showing that the BPI binds to these molecules (Fig. 6). Unlabeled peptide was used in a competition assay to derive the relative affinity of the BPI for the APC and the ability of the BPI to crosslink the MHC and ICAM molecules on the APC was examined by cocapping ICAM with an anti-MHC mAb. Here, cells were incubated with the BPI. Next, an anti-MHC mAb was added. After 30 minutes at 37°C, the cells were transferred to ice and stained with PE-labeled anti-ICAM (Fig. 7).

Next, T-cell clones were generated for determination of TCR epitopes for later use in BPI. These experiments utilized CD4+ or CD8+ T-cell clones from humans or mice immunized against predicted TCR epitopes using previously described methods (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). These clones were maintained by biweekly restimulation with irradiated histocompatible lymphocytes, the peptide, and recombinant IL2. To determine if a given TCR-epitope is effective for the activation to cytokine synthesis, an ELISPOT assay was used. Of course, other cytokine

assays could also be used. For analysis, BPI that have been substituted at predicted TCR-contact positions will be used to determine which of these BPI variants are most effective in the inhibition of proliferation and cytokine release from individual clones as analyzed above. Predicted positions will be scanned with different amino acids or mimetics to alter the interaction with the TCR in the structures generated by molecular modeling.

Next, Molecular Dynamics/Energy Minimizations (MDEM) will be used to study the peptide conformations. MD simulations will be used in conjunction with crystallographic data to predict the solution conformations of the BPI. MDEM simulations can predict flexibility and conformational changes during binding of BPI to MHC-II and ICAM-1. Amino acid substitutions can also be introduced into the Signal-1 and/or Signal-2 moieties in order to favor simultaneous binding to both receptors, as first predicted by these model studies. The I-A^{g7}:BPI:D1 structure (see Fig. 8) is an example of using the crystal structure coordinates of a disease-associated peptide:MHC structure (I-A^{g7}:GAD65 peptide). For this figure, docking studies were performed on a Silicon Graphic Octane workstation using InSight II (MSI/Biosym). The LFA-1-ICAM-1 structure was taken from known diffraction coordinates and the I-A^{g7}:GAD65 peptide was from known coordinates. Of course, analogous methods can be used to model the BPI listed in Tables 1, 2, 3 and 4 for binding to the various MHC molecules of mouse and man.

The second stage in the BPI process is selection of peptide mimics of established second signal receptor molecules involved in the functional differentiation of T-cells. To determine these structures, the crystallographic structures and available models of the second signal receptors bound to their physiological ligands will be used to predict the regions of the receptors that make contact with the ligand. This approach was used to design the EGAD-BPI which can be depicted as

(EIAPVFVLLE)-(AcGAcGAc)-(ITDGEATDSG)
GAD65(208-217) linker LFA-1(237-247)

These interactions can also be determined empirically by making site-directed changes in the second signal molecules and determining whether such mutant molecules still bind to the ligand in question. However, many of these studies have been performed with

known second signal receptors, including LFA-1, CD28/CTLA4, CD40L, and CD95L. Molecular modeling studies will be used as above to model the interactions of these second signal moieties with their ligands separately and when the moiety is part of the BPI structure.

Once candidate BPI moieties that bind effectively to the MHC and second signal ligands and that alter T-cell functions in vitro were identified, these moieties were synthesized at opposite ends of relatively short synthetic peptides, connecting them by a non-proteolytic substrate linker of variable length. As described by the prototypical EGAD-BPI, the Signal-1 moiety (i.e., the disease-associated TCR epitope) was linked by this flexible linker to the Signal-2 receptor mimic peptide (i.e., a second signal receptor thought to be involved in T_H1/T_H2 differentiation. These synthetic peptides were generated by conventional methods of peptide synthesis. In some cases, BPI are tested for binding to isolated MHC and second signal ligands, and NMR, molecular modeling and crystallography are used to determine their exact 3D structures. Finally, it was determined whether a given BPI was biologically active *in vivo*. Mice were treated with the BPI and immune cells isolated and tested for cytokine production by ELISPOT (see Figs. 9 & 10). Of course other cytokine assays will be well known to those of ordinary skill in the art and can be used in place of ELISPOT. *In vivo* models similar to the NOD.Scid model (i.e., human Scid, transgenic knockout strains, etc.) were used, with modifications necessary for each disease being examined, as a more stringent test of BPI efficacy. For example, in the case of the HIV-1 p24 TCR-epitope, human-Scid mice are used for the adoptive transfer experiments. In this experiment, T-cells from patients will first be cloned by conventional methods and stimulated with the BPI *in vitro*. Next, these cells will be transferred into the human-Scid mice and analyzed as with the EGAD-BPI in the NOD.Scid adoptive transfer experiment. Results from these experiments are given in Figs. 11-13.

To synthesize the BPI, Fmoc chemistry on chlorotrityl resins was used. Protected amino acids were double coupled at 8-fold excess for 1 hour. Resins were DMF and MeOH washed and cleaved in Reagent R: TFA, EDTA, Thioanisole, Anisole. The TFA mixture containing the peptide in solution was precipitated in ether and washed extensively. Preparative HPLC of peptides was accomplished by a gradient of 0-80% acetonitrile in 0.1% TFA. Lyophilization of the various fractions and verification by MALDI-TOF using a Voyager mass spec (PerSeptive, Foster City, CA) yielded the synthetic peptide as a TFA salt.

Modeling, crystallography and binding studies, as described above, were used to generate the predicted BPI complex structure.

In another example which tested the diabetes BPI *in vivo*, 15 million disease-linked lymphocytes (i.e., patient T-cells, or T-cell populations linked to the disease process) were injected with or without T-cells that received the BPI compound (in vivo or in vitro) and were expanded for 24 hours in recombinant IL-2. Some experiments will deplete specific subsets of the T-cells using mAb to CD154, CD25, CD62L, CD152, etc. and magnetic particles prior to adoptive transfer. T-cells (from mice or humans) treated with the individual moieties of the BPI will be used as negative controls along with CD4+ cells from mice treated with saline alone (Fig. 9). For blocking spontaneous diabetes, five groups of ten female NOD mice (12 weeks of age) were used and monitored for nondiabetic blood glucose levels with a standard glucometer (AccuChek-complete, Roche Diagnostic). Each mouse was labeled and individually monitored for blood glucose levels weekly for the course of the experiment. The five groups received either (a) intravenous (i.v.) injection of the BPI (100 μg in 100 μl endotoxin-free saline/injection) at 8 weeks of age, (b) same dose GAD65 (208-217) epitope alone, (c) same dose CD11a (237-247) peptide alone, or (d) saline alone. For other systems, similar treatment groups involving the different Signal-1 and Signal-2 peptides and BPI will be used. Mice will be tested by challenging with the appropriate infectious agent or antigen depending upon the particular BPI in question.

To evaluate the disease process by immunohistology (*see*, e.g., Fig. 12A-D), spleen, pancreas, or other target organs, e.g., the CNS for the MBP peptide BPI, or lung for the RSV peptide BPI, were removed from euthanized mice of each group and prepared for histology by fixing in neutral buffered formalin and embedding in paraffin, or snap frozen in O.C.T. medium. For scoring inflammation, minimums of five sections from each mouse were used to assess the blocking affect of a given BPI. For characterization of standard T-cell markers on cellular infiltrates, biotinylated mAb to various cell surface antigens will be incubated individually with the Cryostat sections (2 hours), followed by avidin-alkaline phosphatase (Vector laboratories). Alternatively, cell subsets will be phenotyped by standard flow cytometry methods as described in Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996). Finally, the students t-test or ANOVA will be used to

estimate the statistical significance of differences observed between groups and individual mice.

A few representative assembled BPI consisting of a Signal-1 moiety and a Signal-2 receptor moiety joined together via a linker are provided in Table 4 as SEQ ID Nos. 42-46. These representative BPI are operable for shifting specific immune responses from a type-1 to a type-2 response and vice-versa. Advantageously, other immune responses to other antigenic peptides will be preferably unaffected.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1 is a schematic representation of an immune synapse between a T cell and an APC illustrating the doughnut structure of the TCR:peptide:MHC location;

Fig. 2 is a schematic representation of how a representative BPI blocks differentiation leading to a T_H1 dominated immune response and shifts immunity to T_H2 dominated immune response;

Fig. 3 is a schematic representation of nominal activation of a type-1 immune response through the interactions between cell surface proteins within the immune synapse;

Fig. 4 is a simplified schematic representation of the two signal mechanism of T-cell activation;

Fig. 5a is a graph representing the results of a flow cytometry analysis comparing the binding of a representative BPI to different mouse strains;

Fig. 5b is a graph representing the results of a flow cytometry analysis comparing the binding of a representative BPI portion to different mouse strains;

Fig. 5c is a graph representing the results of a flow cytometry analysis comparing the binding of a representative BPI portion to different mouse strains;

Fig. 6a is a graph illustrating the results of a flow cytometry analysis of a representative BPI binding to the APC of a mouse strain, without antibodies to MHC-II or ICAM-1;

Fig. 6b is a graph illustrating the results of a flow cytometry analysis of a representative BPI binding to the APC of a mouse strain, when antibodies to ICAM-1 are present;

Fig. 6c is a graph illustrating the results of a flow cytometry analysis of a representative BPI binding to the APC of a mouse strain, when antibodies to MHC-II are present;

Fig. 7 is a color photograph representing the results of a fluorescent microscopy analysis of a representative BPI simultaneously binding to MHC-II and ICAM-1 structures on the NOD APC by co-capping with antibodies to MHC-II, the top panels are from mice APC treated with a representative BPI and the bottom panels are treated with just the saline vehicle;

Fig. 8 is a color photograph of the molecular model of a representative BPI binding to the NOD mouse's MHC-II ($I\text{-}A^{g7}$) and the D1 domain of ICAM-1, MHC-II is shown in pink, ICAM-1 is in light blue, the BPI is shown by atom with the carbon in green, oxygen in red, and nitrogen in blue;

Fig. 9a is a graph representing the ELISPOT analysis of IL-4 cytokine release by T-cells taken from NOD mice treated with the EGAD-BPI or the saline control;

Fig. 9b is a graph representing the ELISPOT analysis of IFN-γ cytokine release by T-cells taken from NOD mice treated with the EGAD-BPI or the saline control;

Fig. 9c is a graph representing the ELISPOT analysis of IL-4 cytokine release by T-cells taken from NOD mice treated with the AGAD-BPI or the saline control;

Fig. 9d is a graph representing the ELISPOT analysis of IFN-γ cytokine release by T-cells taken from NOD mice treated with the AGAD-BPI or the saline control;

Fig. 10 are representative photographs of the raw data of the ELISPOT analysis used for the graphs in Figs. 9a-9d;

Fig. 11 is a graph of the severity of islet infiltration as an indicator of the inhibition of insulitis by the EGAD-BPI, separate portions of the EGAD-BPI, and saline;

Fig. 12a is a representative color photograph of the histological analysis of pancreata infiltration by mononuclear cells in NOD mice treated with the saline control;

Fig. 12b is a representative color photograph of the histological analysis of pancreata infiltration by mononuclear cells in NOD mice treated with the GAD peptide;

Fig. 12c is a representative color photograph of the histological analysis of pancreata infiltration by mononuclear cells in NOD mice treated with the EGAD-BPI;

Fig. 12d is a representative color photograph of the histological analysis of pancreata infiltration by mononuclear cells in NOD mice treated with the CD11a peptide; and

Fig. 13 is a graph illustrating the results of a 10 week monitoring of blood glucose levels in NOD.Scid mice which received CD25-negative diabetes-inducer cells together with T-cells from NOD mice injected with either the EGAD-BPI or saline.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

As used herein, the following definitions will apply: "Sequence Identity" as it is known in the art refers to a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, namely a reference sequence and a given sequence to be compared with the reference sequence. Sequence identity is determined by comparing the given sequence to the reference sequence after the sequences have been optimally aligned to produce the highest degree of sequence similarity, as determined by the match between strings of such sequences. Upon such alignment, sequence identity is ascertained on a position-by-position basis, e.g., the sequences are "identical" at a particular position if at that position, the nucleotides or amino acid residues are identical. The total number of such position identities is then divided by the total number of nucleotides or residues in the reference sequence to give % sequence identity. Sequence identity can be readily calculated by known methods, including but not limited to, those described in Computational Molecular Biology, Lesk, A. N., ed., Oxford University Press, New York (1988), Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York (1993); Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H. G., eds., Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology, von Heinge, G., Academic Press (1987); Sequence Analysis Primer, Gribskov, M. et al., eds., M. Stockton Press, New York (1991); and Carillo, H., et al. Applied Math., 48:1073 (1988), the teachings of which are incorporated herein by reference. Preferred methods to determine the sequence identity are designed to give the largest match between the sequences tested. Methods to determine sequence identity are codified in publicly available computer programs which determine sequence identity between given sequences. Examples of such

programs include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research, 12(1):387 (1984)), BLASTP, BLASTN and FASTA (Altschul, S. F. et al., J. Molec. Biol., 215:403-410 (1990). The BLASTX program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al., NCVI NLM NIH Bethesda, MD 20894, Altschul, S. F. et al., J. Molec. Biol., 215:403-410 (1990), the teachings of which are incorporated herein by reference). These programs optimally align sequences using default gap weights in order to produce the highest level of sequence identity between the given and reference sequences. As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "sequence identity" to a reference nucleotide sequence, it is intended that the nucleotide sequence of the given polynucleotide is identical to the reference sequence except that the given polynucleotide sequence may include up to 5 point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, in a polynucleotide having a nucleotide sequence having at least 95% identity relative to the reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Analogously, by a polypeptide having a given amino acid sequence having at least, for example, 95% sequence identity to a reference amino acid sequence, it is intended that the given amino acid sequence of the polypeptide is identical to the reference sequence except that the given polypeptide sequence may include up to 5 amino acid alterations per each 100 amino acids of the reference amino acid sequence. In other words, to obtain a given polypeptide sequence having at least 95% sequence identity with a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total number of amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or the carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal

positions, interspersed either individually among residues in the reference sequence or in the one or more contiguous groups within the reference sequence. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. However, conservative substitutions are not included as a match when determining sequence identity.

Similarly, "sequence homology", as used herein, also refers to a method of determining the relatedness of two sequences. To determine sequence homology, two or more sequences are optimally aligned as described above, and gaps are introduced if necessary. However, in contrast to "sequence identity", conservative amino acid substitutions are counted as a match when determining sequence homology. In other words, to obtain a polypeptide or polynucleotide having 95% sequence homology with a reference sequence, 95% of the amino acid residues or nucleotides in the reference sequence must match or comprise a conservative substitution with another amino acid or nucleotide, or a number of amino acids or nucleotides up to 5% of the total amino acid residues or nucleotides, not including conservative substitutions, in the reference sequence may be inserted into the reference sequence.

A "conservative substitution" refers to the substitution of an amino acid residue or nucleotide with another amino acid residue or nucleotide having similar characteristics or properties including size, charge, hydrophobicity, etc., such that the overall functionality does not change significantly.

"Isolated" means altered "by the hand of man" from its natural state., i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Finally, all references and teachings cited herein which have not been expressly incorporated by reference are hereby incorporated by reference.

Sequences including or having a sequence which has at least about 10% sequence identity with any one of SEQ ID Nos. 1-46 and which exhibit similar binding properties to APC or linking properties between two peptide sequences are within the scope of the present invention. Preferably, such sequences will have at least about 30% sequence identity with any one of SEQ ID Nos. 1-46, still more preferably at least about 50% sequence identity,

even more preferably, at least about 70% sequence identity, and most preferably at least about 95% sequence identity. Alternatively, sequences including or having a sequence which has at least about 10% sequence homology with any one of SEQ ID Nos. 1-46 and which exhibit similar binding properties to APC or linking properties between the two adjacent peptide sequences are embraced in the present invention. More preferably, such sequences will have at least about 30% sequence homology with any one of SEQ ID Nos. 1-46, still more preferably at least about 50% sequence homology, even more preferably at least about 70% sequence homology, and most preferably at least about 95% sequence homology. Additionally, sequences which differ from any one of SEQ ID Nos. 1-46 due to a mutation event, a series of mutation events, or chemical derivatization but which still exhibit desired properties are also embraced in the present invention. Such mutation events or derivatizations include but are not limited to point mutations, deletions, insertions, rearrangements, peptidomimetics, and other chemical modifications.

A "linker" is defined as any amino acid including naturally occurring or chemically synthesized amino acids. Preferably, a "linker" is a flexible, non-substrate sequence of amino acid residues resistant to proteolytic degradation which can be used to conjugate and/or couple a Signal-1 moiety to a Signal-2 moiety.

A "Signal-1 moiety" is defined as a peptide epitope, i.e., the peptide portion of an antigen and/or mimetics of these antigenic peptides to which important TCRs bind.

A "Signal-2 moiety" or a "Signal-2 receptor moiety" is defined as a peptide portion of a second signal receptor known to bind to and/or affect binding of the receptor to its complimentary ligand on the APC. This can include peptide mimics and mimetics of the receptor/ligand structure of interest.

A "Signal-2 ligand" is the complementary protein of the Signal-2 receptor moiety on the APC to which the receptor portion and/or the Signal-2 receptor moiety has significant affinity and binds.

As used herein "derivative" with respect to peptides refers to changes produced by amino acid addition, deletion, replacement, substitution, and/or modification; mutants produced by recombinant and/or DNA shuffling; and salts, solvates, and other chemically synthesized/modified forms of the peptide that retain in part the activity of the isolated native peptide.

BPI were generated using automated peptide synthesis by a robotic multiple peptide synthesizer employing Fmoc amino acid chemistry by standard methods. Wang resin (*p*-benzyloxybenzyl alcohol polystyrene) was used as the solid support. Peptides were characterized by reversed-phase HPLC and electrospray mass-spectrometry. This synthesis, referred to as Merrifield peptide synthesis, utilizes traditional organic chemical reactions carried out on a solid material so that the peptide chain is lengthened while attached to the support structure. The peptides will be cleaved from the resin using TFA, and purified by reverse-phase HPLC and analyzed by mass spectroscopy. Alternatively, these reactions can be carried out in solution when larger amounts of the peptides are desired. Of course, the peptides of the invention may be synthesized or prepared by a number of techniques which are well known in the art. See, for example, Creighton, 1983, Proteins: Structures and Molecular Principles, W. H. Freeman and Co., New York, which is incorporated herein by reference in its entirety. Short peptides, for example, can be synthesized on a solid support or in solution. Longer peptides may be made using recombinant DNA techniques. Here, the nucleotide sequences encoding the peptides of the invention may be synthesized, and/or cloned, and expressed according to techniques well known to those of ordinary skill in the art. See, for example, Sambrook, et al., 1989, Molecular Cloning, A is Laboratory Manual, Vols. 1-3, Cold Spring Harbor Press, New York.

Alternatively, the peptides of the invention may be synthesized such that one or more of the bonds which link the amino acid residues of the peptides are non-peptide bonds. These alternative non-peptide bonds may be formed by utilizing reactions well known to those in the art, and may include, but are not limited to amino, ester, hydrazide, semicarbazide, and azo bonds, to name but a few. In yet another embodiment of the invention, peptides comprising the sequences described above may be synthesized with additional chemical groups present at their amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptides is enhanced. For example, hydrophobic groups such as carbobenzoxyl, dansyl, or t-butyloxycarbonyl groups, may be added to the peptides' amino termini. Likewise, an acetyl group or a 9-fluorenylmethoxy-carbonyl group may be placed at the peptides' amino termini. Additionally, the hydrophobic group, t-butyloxycarbonyl, or an amido group may be added to the peptides' carboxy termini.

Purchasing preformed peptides provides another alternative source of peptides having 25 amino acids or less as these are easily purchased from commercial peptide synthesis laboratories. In later synthesis schemes, peptide mimetic compounds may be synthesized in place of the peptide moieties and linked by the same chemistry. The design of peptidomimetics is an established technique and known correlates of key amino acids of the peptide can be synthesized by previously published methods. Furthermore, as it is well known in the art, peptidomimetics may be developed which have the same modulation properties as the preferred peptides detailed herein. As these peptidomimetics require no more than routine skill in the art to produce, such peptidomimetics are embraced within the present application. Notably, the side chains of these peptidomimetics will be very similar in structure to the side chains of the preferred peptides herein, however, their peptide backbone may be very different or even entirely dissimilar. If resistance to degradation *in vivo* or greater conformational stability were desired, the peptides of the present invention could be cyclized by any well known method. One such method adds Penicillamine (Pen) and cysteine (Cys) residues to the N- and C-termini to form cyclic peptides via a disulfide bond between the Pen and Cys residues. The formation of this cyclic peptide restricts the peptide conformation to produce a conformational stability, thereby providing better selectivity for cell surface receptors than its linear counterpart.

The portion of the BPI which spans between the Signal-1 moiety and the Signal-2 moiety is referred to as a linker. As noted above, the linker is not essential in forming a BPI. However, when a linker is used, the linker can be any naturally occurring or chemically synthesized amino acid. Preferably, the linker is a non-substrate amino acid residue chain which helps to prevent protease attack. A particularly preferred linker is a repeating chain of the non-natural amino acid, aminocaproic acid (Ac), and the amino acid glycine (G) (e.g. Ac-G-Ac-G-Ac). If a shorter length was needed for the linker, beta-alanine residues (βAla) could be substituted for one or more of the Ac residues. If a longer chain was needed for the linker, amino-dodecanoic acid residues (Adod) could be substituted for one or more of the Ac residues. As is well known in the art, peptide mimetics of these linker amino acids may also be synthesized and inserted into the BPI structure.

The following examples set forth preferred embodiments of the present invention. It is to be understood, however, that these examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

EXAMPLE 1

This example describes the methods used to generate the BPI.

Materials and Methods:

Synthesis of peptides was via Fmoc on chlorotrity resins. Protected amino acids were double coupled at 8-fold excess for one hour. Resins were dimethylforamide (DMF) and methanol (MeOH) washed and cleaved in Reagent R: trifluorolacetic acid (TFA), ethylene diamine tetraacetic acid (EDTA), Thioanisole, Anisole. The TFA mixture containing the peptide in solution is precipitated in ether and washed extensively. Preparative HPLC of peptides was accomplished by a gradient of 0-80% acetonitrile in 0.1% TFA. Lyophilization of the various fractions and verification by mass spectroscopy yielded the synthetic peptides as a TFA salt. Modeling, crystallography and binding studies are as described above.

Results:

The peptides produced in this example are provided in Table 1 and are also listed as SEQ ID Nos. 1- 46. These peptides include the Signal-1 moiety, the Signal-2 moiety and the non-substrate linker between the two moieties. To produce the BPI, any Signal-1 moiety could be linked with any Signal-2 moiety via any linker using the peptide synthesis described above. In other words, the BPI are generated as one continuous peptide chain comprising a Signal-1 peptide sequence followed by a linker sequence followed by a Signal-2 peptide sequence. Additionally, some representative BPI were generated for later use in the experiments. These BPI are included herein in Table 4. However, it is important to note that these BPI are representative (as are each of the BPI portions listed in Tables 1-4) and not all inclusive.

TABLE 1. Signal-1 Peptides

SEQ ID No.	Sequence	Name, Source	Organism	Health Condition
1	EIAPVFVLLE	GAD65 (208-217)	Homo sapiens	type-1 diabetes
2	EIAPVFVLLE	GAD67 (217-226)	Mus musculus	type-1 diabetes
3	QYMRADQAAGGLR	Collagen II (1168-1180)	Homo sapiens	rheumatoid arthritis
4	RVVINKDTTIII	Yersinia HSP (322-333)	Yersinia enterocolitica	reactive arthritis
5	ENPVVHFFKNIVTPR	Myelin BP (84-98)	Homo sapiens	multiple sclerosis
6	GYKVLVLNPSVAAT	HCV, NS3 (1248-61)	Hepatitis C virus	hepatitis
7	GSDITITLPCRIKQFINMWQE	HIV, gp120 (410-429)	HIV-1	AIDS
8	PIVQNLQGQMVHQAISPRTL	HIV, p24 (133-152)	HIV-1	AIDS
9	STPESANL	SIV, Tat (28-35)	Simian immunodeficiency virus	simian AIDS
10	AICKRIPNKKPGKKT	RSV, G (183-197)	Respiratory syncytial virus	asthma
11	VYRDGNPYA	HPV 16, E6 (60-68)	Human papillomavirus (HPV)	cervical cancer
12	DRAHYNI	HPV 16, E7 (48-54)	HPV	cervical cancer
13	YMLDLQPETT	HPV 16, E7(11-20)	HPV	cervical cancer
14	ASDLRTIQQLLMGTV	HPV 33, E7 (73-87)	HPV	cervical cancer
15	AELYHFLLKYRAR	MAGE (3114-3126)	Homo sapiens	melanoma

16	LLKYRAREPVTKAE	MAGE (3120-3133	Homo sapiens	melanoma
17	EQVAQYKALPVVLENA	Fel d 1 (22-37)	Felis domesticus	cat allergy
18	KALPVVLENARILKNCV	Fel d 1(28-44)	Felis domesticus	cat allergy
19	LVPCAWAGNVCGEKRAYCCS	Amb a 5 (1-20)	Ambrosia artenisiifdia	ragweed allergy
20	PIGKYCVCYDSKAICNKNCT	Amb t 5 (21-40)	Ambrosia trifida	ragweed allergy
21	KSMKVTVAFNQFGPN	Cry j 1 (211-225)	Cryptomeria japonica	cedar allergy
22	IDIFASKNFHLQKNTIGTG	Cry j 2 (182-200)	Cryptomeria japonica	cedar allergy
23	YFVGKMYFNLIDTKCYK	Phospholypase 2 (81-97)	Apis mellifera	bee allergy
24	ASEQETADATPEKEEPTAAP	Hev b 5 (37-56)	Hevia brasiliensis	latex
25	FGISNYCQIYPPNAXKI	Der p 1 (111-127)	Dermatophagoides pteronyssinus	dust mites

TABLE 2. Linkers

SEQ ID No.	Sequence
26	Ac-G-Ac-G-Ac
27	Ac-G-βAla-G-Ac
28	Ac-G-Adod-G-Ac
29	Ahx-G-Ahx-G-Ahx

TABLE 3. Signal-2 Peptides

SEQ ID No.	Sequence	Source	Organism	Shift in Immunity
30	ITDGEATDSG	CD11a (237-247)	Homo sapiens	type-1→type-2
31	TDGEATDSGN	CD11a (238-248)	Homo sapiens	type-1→type-2
32	ASPGKATEVR	CTLA4 (24-33)	Homo sapiens	type-2→type-1
33	SPSHNTDEVR	CTLA4 (24-33)	Mus musculus	type-2→type-1
34	KVELMYPPPYYL	CTLA4 (93-104)	Homo sapiens	type-2→type-1
35	KVELMYPPPYFV	CTLA4 (93-104)	Mus musculus	type-2→type-1
36	ITDGEATDSG	CD11a (237-247)	Mus musculus	type-1→type-2
37	KGYYTMSNNLVTL	CD154 (CD40L) (93-104)	Homo sapiens	type-1→type-2
38	KGYYTMSNNLVTL	CD154 (CD40L) (93-104)	Mus musculus	type-1→type-2
39	YMRNSKYRAGGAYGPG	Fas-ligand (CD95L) (143-155)	Homo sapiens	type-2→type-1
40	YMRNSKYRAGGAYGPG	Fas-ligand (CD95L) (143-155)	Mus musculus	type-2→type-1
41	TDGEATDSGN	CD11a (238-248)	Mus musculus	type-1→type-2

Table 4. Some Representative BPI

TCR Epitope	Linker	Signal-2 Receptor Moiety	Complete BPI Sequence	SEQ ID No.	Effect
(GAD65) Diabetes: EIAPVFVLLE	AcGAcGAc	(CD1la) ITDGEATDSG	EIAPVFVLLEAcGAcITDGEATD SG	42	type-1 - type-2
(tat) AIDS: STPESANL	AcGAdodGAc	(Fas-ligand) YMRNSKYRAGGAYGPG	STPESANLAcGAdodGAc YMRNSKYRAGGAYGPG	43	↑ CTL
(p24) PIVQNLQGQMVHQAISPRTL	AcGAcGAc	(CTLA4) KVELMYPPPYFV	PIVQNLQGQMVHQAISPRTLAcGAc GAcKVELMYPPPYFV	44	↑ type-1
(collagen-II) Rheumatoid Arthritis: QYMRADQAAGGLR	AcGAhxGAc	(CD40L) KGYYTMSNNLVTL	QYMRADQAAGGLRAcGAhxGAcKG YYTMSNNLVTL	45	type-1 - type-2
(HPVE7) Cervical Cancer: YMLDLQPETT	AcGAcGAc	(Fas-ligand) YMRNSKYRAGGAYGPG	YMLDLQPETTAcGAcGAcYMRNSK YRAGGAYGPG	46	↑ CTL

Discussion:

Once the linker, the Signal-1 moiety, and the Signal-2 moiety are chosen, the entire BPI can be synthesized using the above-described methods. There are thousands of potential Signal-1 moieties which could be used in connection with the present invention. Each of these moieties may be associated with a distinct immunological response or disease state. Once the epitope structure and sequence are determined, an appropriate linker could be selected and the other portion of the BPI, i.e., the second signal moiety, can be chosen. Once all of these sequences have been determined, BPI can be designed using the peptide sequences themselves, peptidomimetics, or combinations of the two. Construction of appropriate peptidomimetics is detailed by Falcioni, et al, 17 *Nature Biotechnology*, 562-567 (1999), the content and teachings of which are hereby incorporated by reference herein.

Fig. 8 illustrates the structure of the GAD65 (208-217), TCR epitope linked to the CD11a (237-247) second signal moiety produced by the present methods. It is shown bound to the groove of I-A^{g7} and the D1 domain of ICAM-1. For modeling the I-A^{g7}:GAD65 peptide structure, docking studies were performed on a Silicon Graphic Ocatane work station using InSight II software (MSI/Biosym). The LFA-1 peptide:ICAM-1 domain structure is based on the docking model of Edwards, C.P. et al. *J. Biol. Chem.* 273:28937 (1998), the teachings and disclosure of which is incorporated by reference herein. The alpha carbon ribbon of I-A^{g7} is shown in pink; D1 of ICAM-1 is in light-blue; the BPI is shown by atom, carbon in green, oxygen in red, and nitrogen in blue. This structure can be denominated as GAD65 (208-217) - [Ac-G-Ac-G-Ac] - CD11a (237-247). Advantageously, the length of the linker may be modified as needed or as indicated by any experimental data obtained in order to span between the Signal-1 and Signal-2 moieties at an optimum length.

These structures are illustrated by the preliminary mechanism depicted schematically (Figs. 2 and 3), and the structural model (Fig. 8). The linker used has the sequence –[Ac-G-Ac-G-Ac]-. To lengthen the linker, one or more aminocaproic acid (Ac) resides can be substituted with aminododecanoic acid. To shorten the linker, beta-2 alanine can be used as a substitute for aminocaproic acid.

Of course, it is possible that one of ordinary skill in the art could produce any number of peptidomimetics or derivatives which would have similar activity to the BPI, and such modifications are encompassed by the present invention as described in more detail above.

EXAMPLE 2

This example uses biotinylated BPI to test for competitive inhibition of BPI binding by unlabeled peptides or monoclonal antibodies to MHC-II and ICAM-1 on live APC, and to verify antigenic peptide binding to live APC. Additionally, it was shown that monoclonal antibodies to MHC-II or ICAM-1 effectively block binding of the diabetes BPI (GAD65 (208-217) - [Ac-G-Ac-G-Ac] - CD11a (237-247)) (hereinafter referred to as EGAD-BPI) to NOD spleenocytes.

Materials and Methods:

To obtain biotinylated BPI, the synthesized EGAD BPI was biotinylated with NHS-Biotin as described in Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994). Spleen cell density-gradient fractions from normal (unimmunized) NOD, BALB/c and other MHC congenic strains were incubated in round bottom 96-well plates with increasing concentrations of individual biotinylated peptides at 37°C, 5% CO_2 for 16 hours. Following binding of the BPI to the APC, Avidin-FITC was incubated with the cells on ice for 30 minutes, followed by biotinylated anti-Avidin for 1 hour, then again with Avidin-FITC. For BPI titrations, increasing concentrations (0.1-100 μM) of the biotinylated derivatives in sterile 0.5% BSA-PBS were incubated with the APC for 16 hours as above. Three-color analyses used Cy-Chrome or PE-conjugated anti-B220 (mAb RA3-6B2 (CD45R B-cell marker), anti-MHC class II (KH74 or 10-3.62 mAb), or anti-ICAM-1 (3E2 mAb); (all purchased from PharMingen, San Diego, CA). Bound peptide was detected with avidin-FITC/biotinylated anti-avidin/avidin-FITC on live cells gated by forward/side scatter analysis. Controls contained all detecting reagents in absence of the biotinylated peptide; 20,000 events were analyzed for each histogram with a FACScan (Becton-Dickinson) flow cytometer.

To test for competitive inhibition of BPI binding with unlabeled peptides or monoclonal antibodies to MHC-II and ICAM-1 on live APC, freshly isolated fractions of spleen cells were incubated with the previously biotinylated BPI. However, for this portion of the experiment, the experimental wells contained various unlabeled peptides (e.g., antigenic peptides or LFA-1 peptides), and/or monoclonal antibody (e.g., anti-MHC-II or anti-ICAM-1 mAb) inhibitors. Negative selection methods with monoclonal antibodies

conjugated to magnetic particles were used to enrich the spleen cell fractions for B cells, macrophages, or dendritic cells as well as to examine differences in BPI binding to these different populations. These methods are detailed in Schountz et al., 157 The Journal of Immunology 3893-3901 (1996), the teachings and content of which were incorporated by reference above.

To verify antigenic peptide binding to live APC, initial EGAD-BPI were screened for selective binding to NOD ($I\text{-}A^{g7}$) APC and assayed for simultaneous binding using monoclonal antibodies against either MHC-II or ICAM-1 by flow cytometry methods using live APC (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). In this assay, increasing concentrations of the biotinylated-BPI, -CD11a(237-247) or -GAD65(208-217) peptide were incubated overnight with spleenocytes from each inbred strain. Bound peptide was detected with amplification of avidin-FITC fluorescence by the use of a biotinylated anti-avidin reagent, followed by a second round of avidin-FITC binding. Biotinylated peptide was incubated with 10^6 viable cells at a peptide concentrations of 50 μM. Bound peptide was detected with avidin-FITC/biotinylated anti-avidin-FITC. For analysis, a forward/side scatter gate was set on live lymphocytes and 20,000 events were collected in this gate. Background fluorescence (detection reagents only) is shown in each panel of Figs. 5a-5c along with the 50 μM bio-peptide histogram (peptide-fluorescence intensity = FL1). The percentage of positive cells (M2) were determined by the CELLQuest™ program (Becton-Dickinson) and are displayed in each panel along with the median channel fluorescence (MCF) of the M2 population (all data are the direct output of the CELLQuest™ program running on an Apple G3 computer).

To demonstrate that monoclonal antibodies to MHC-II or ICAM-1 effectively block binding of the diabetes BPI (EGAD-BPI) to NOD spleenocytes, an assay identical to that used for Figs. 5a-5c was used. However, either purified 10-3.62 (anti-MHC-II) or 3E2 (anti-ICAM-1) were included in the overnight incubation of the spleen cells with the biotinylated BPI. Both antibodies were purchased from PharMingen and used at 5 μg/ml final concentration. A control containing no added monoclonal antibody was also tested. Analyses were gated on forward/side scatter dotplots for live lymphocytes and 20,000 events were analyzed for each histogram. Results for this example are given in Figs. 6a-6c.

Results and Discussion:

As illustrated by the data provided in Figs. 5a-5c, NOD spleen cells bind the diabetes BPI (EGAD-BPI) at a higher density than spleenocytes identically purified from BALB/c, A.SW, or A.BY. Previous data has shown that B cells are the major antigenic peptide binding cells in these spleen cell preparations isolated by lymphocyte separation media (LSM) density gradient centrifugation (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). There was a significant difference in the percentage of high-density binding cells, from 30% (NOD) to 6% (A.BY). In contrast, the separate Signal-1 (GAD65 peptide) and Signal-2 (CD11a peptide) moieties did not bind preferentially to NOD APC. Supporting data is given in Figs. 5b and 5c. Fig. 5b illustrates direct binding of biotinylated LFA-1 (CD11a 237-247) peptide to the same spleenocyte preparations as those shown in Fig. 5a. Note that this Signal-2 moiety bound similarly to all strain spleenocytes. Fig. 5c illustrates direct binding of biotinylated GAD65 (208-217) peptide to the same spleenocyte preparations as those depicted in Figs. 5a and 5b. These data indicate that BPI could be engineered to fit particular MHC peptide binding motifs as discussed by Corper et al. in *A Structural Framework for Deciphering the Link Between I-A^{g7} and Autoimmune Diabetes*, 288 Science 505-511 (2000), and by Dessen et al. in *X-ray Crystal Structure of HLA-DR4 (DRA*0101, DRB*0401) Complexed With a Peptide From Human Collagen II*, 7 Immunity 473-481 (1997), the respective teachings of which are incorporated by reference herein.

Additionally, as illustrated in Figs. 6a-6c, other studies have shown that monoclonal antibodies to MHC-II and ICAM-1 block peptide binding to NOD spleen cells. These data indicate that the diabetes BPI bind to both receptors on the APC surface. Thus, Monoclonal antibody to MHC-II or ICAM-1 effectively block binding of the diabetes BPI (EGAD-BPI) to NOD spleenocytes. In effect the predicted bifunctional nature of the BPI is demonstrated by these results and suggests that the BPI will link MHC-II to ICAM-1 on the APC surface. This mechanism was further demonstrated by co-capping experiments.

EXAMPLE 3

This example utilizes co-capping experiments to demonstrate simultaneous binding of the BPI to MHC-II and ICAM-1 molecules.

Materials and Methods:

Further support for simultaneous binding of the BPI to MHC-II and ICAM-1 molecules has been observed in co-capping experiments using biotinylated mAb10-3.62 and streptavidin to cap MHC-II in the presence or absence of the BPI. To test the ability of the BPI peptide to link MHC-II and ICAM-1 molecules on the APC surface, we used a modification of a co-capping experiment originally described for monoclonal antibodies. Briefly, biotinylated monoclonal antibody to MHC-II (10-3.62) is incubated with freshly-isolated APC from NOD mice previously treated by intravenous (i.v.) injection of a given BPI variant or saline. Antibody-bound cells are then incubated with streptavidin (37 C x 15 min.) to cap the MHC-II molecules on the APC surface. The cells were transferred to ice and labeled with a fluorescent (PE) monoclonal antibody to ICAM-1 (3E2). The cells are plated and observed for evidence that a given BPI links ICAM-1 into the MHC-II cap, i.e., by standard fluorescence microscopy and image analysis. In one experiment, T-depleted spleenocytes from mice treated 16 hours previously with EGAD-BPI (i.v.) exhibited co-capping of ICAM-1 with MHC-II in the presence of bio-10-3.62/streptavidin. In the other experiment, T-depleted spleenocytes from mice treated 16 hours previously with saline only did not exhibit co-capping. The results for these experiments are given in Fig. 7.

Results:

The top panels of Fig. 7 illustrate the results from the T-depleted spleenocytes from mice treated 16 hours previously with EGAD-BPI (i.v.), wherein ICAM-1 was co-capped with MHC-II in the presence of bio-10-3.62/streptavidin. The bottom panels of Fig. 7 illustrate the results from the mice treated with saline only wherein co-capping is not exhibited. This is evidenced by having ICAM-1 remain dispersed on the B-cell membranes. As is shown, B cells isolated from NOD mice treated 16 hours previously by i.v. injection of either 40 nanomoles of EGAD-BPI or saline (PBS) alone displayed two distinct patterns of ICAM-1 expression, as measured by staining with PE-labeled 3E2 (anti-ICAM-1) on ice.

On cells from BPI-treated mice, ICAM-1 appears to have co-capped with the MHC-II molecules (note single concentrated spot of ICAM-1 red fluorescence in top panels). By contrast, the nominal dispersed density of ICAM-1 is observed on cells isolated from PBS-treated NOD mice (note entire surface red fluorescence staining in bottom panels).

Therefore, it appears evident that BPI have the capacity to bind simultaneously to MHC-II and ICAM-1 structures on the surface of live APC and therefore may provide signal alterations involving pathways necessary for T_H1/T_H2 differentiation. To directly examine the effects of BPI on T_H1/T_H2 immune deviation, T-cells from mice injected with EGAD-BPI were examined for cytokine analysis.

EXAMPLE 4

This example used an ELISPOT to determine T_H1/T_H2 frequency as altered by BPI injection.

Materials and Methods:

Groups of 3-5 NOD mice were immunized subcutaneously (s.c.) with the GAD 65 peptide in CFA (40 nanomoles/mouse) at the tail base. Different groups received either the EGAD-BPI, its single TCR epitope (Signal-1 moiety), or its CD11a peptide (Signal-2 moiety) i.v. (all 40 nanomoles/mouse). After 6-8 days, another identical 40 nanomole injection was given to each mouse, and the next day lymph nodes draining the site of the s.c. injection were made into single cell suspensions for culture. Identical primary cultures were incubated for 96 hours; then, viable T-cells were recovered by density gradient centrifugation. One million of these cells were combined in nitrocellulose-bottomed 96-well plates (Millititer-HA, Millipore, Bedford, MA), previously coated (50 μl/well) with mAb to either mouse $IFN\gamma$ (clone R4-6A2), or mouse IL-4 (clone BVD4-1D11) at a concentration of 10 μg/ml in PBS. Groups of triplicate cultures were incubated with either Concanavalin-A (2 μg/ml), or the Signal-1 peptide moiety plus 20 U/ml recombinant IL2 (R & D Systems). After 72-96 hours of culture at 37°C and 5% CO_2, plates were washed three times with PBS-0.05% Tween-20. In appropriate wells are added biotinylated anti-$IFN\gamma$ (clone XMG1.2) or biotinylated anti-IL-4 (clone BVD6-24G2) at a concentration of 1 μg/ml and incubated for 1 hour at room temperature. Positive control wells receive known T_H1 or T_H2 clones in

place of normal T-cells. MAb pairs to IL12, IL10, and IL2 are also available and will be used to test for these cytokines in the same assay. All mAb and recombinant controls are purchased from PharMingen (San Diego, CA). Finally, plates were washed three times with PBS-Tween, and then exposed to 100 μl of a 1:2000 dilution of streptavidin alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA) for 1 h and washed as before. Cytokine-producing cells were enumerated by development of the membrane with BCIP/NBT substrate kit (BioRad Labs, Richmond, CA), followed by image capture and analysis using a standard stereomicroscope connected with a digital camera and NIH image software (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). In separate experiments, CD4+ T-cell clones from NOD mice immunized with the GAD65(208-217) peptide by our previously described methods can be used in the same assay. The clones were generated using the methods described in Murray et al., 24 Eur. J. Immunol. 2337-2344 (1994), the teachings of which are hereby incorporated by reference. These clones will be maintained by biweekly restimulation with irradiated NOD lymphocytes, the GAD peptide, and recombinant IL-2. For further analysis, BPI that have been substituted at predicted TCR-contact positions will be used to determine which of these BPI variants are most effective in the inhibition of proliferation and cytokine release from individual clones as analyzed above. Predicted positions that will be scanned with all amino acids except cysteine are amino acids 208, 213, and 216. These residues point toward the TCR in the recently solved crystal structure of the GAD65 peptide bound to I-A^{g7} (Corper et al., 288 *Science* 505-511 (2000) and see Fig. 8).

Results:

Importantly, this example shows the ability of a given BPI to modulate a functional immune response. It can be seen that mice treated with the BPI produce abundant IL-4, whereas the control mice did not produce this cytokine (illustrated in Figs. 9a and 9c). Since IL-4 is the signature cytokine of type-2 immunity, this example shows that the BPI have the capacity to switch dominant type-1 immunity toward T_H2 differentiation and a type-2 response. Moreover, we have developed this *in vivo* assay system to provide a relatively quick examination of a given BPI's immunoregulatory efficacy. Once T_H1/T_H2 modulation

is confirmed as in the present example, studies can then move on to the more stringent tests of BPI efficacy using adoptive transfer experiments as described below.

As shown in Fig. 9a and 9c, IL-4 production increases by approximately 10-fold when T-cells are from the BPI treated animals stimulated in vitro with mitogen. IFN-γ production also increased, although to a lesser extent (see Figs. 9b and 9d).

EXAMPLE 5

This example tested the capacity of the BPI to inhibit lymphocytic infiltration of pancreatic islets in NOD mice. Lymphocytic infiltration is a hallmark of insulitis and the development of type-1 diabetes.

Materials and Methods:

These studies sought to confirm that the BPI containing the immunodominant GAD65 TCR epitope (EGAD-BPI) was biologically active *in vivo* and inhibited the development of pancreatic inflammation. Groups of three normal glycemic NOD males (8 weeks old) were immunized with the GAD65 (208-217) peptide in CFA as described in Example 4. The control group received PBS, and separate experimental groups received either the EGAD-BPI, the GAD peptide alone (i.e., the T-cell receptor epitope), or the CD11a peptide alone(i.e., the second signal receptor moiety) by two intravenous injections as described in Example 4. On day 10, the pancreata were removed to 10% PBS-buffered formalin, embedded in paraffin, and five-micron serial sections were examined histologically for mononuclear cell infiltration as previously described by Yoon, et al., *Control of Autoimmune Diabetes in NOD Mice by GAD Expression or Suppression in β Cells*, 284 Science 1183-1187 (1999), the entirety of which is hereby incorporated by reference.

Results:

The results for this experiment are given in Figs. 11 and 12. Fig. 11 represents the cumulative data of this analysis wherein the severity of islet infiltration is scored and plotted as the percentage of islets examined. Over 100 islets from each group in greater than 5 tissue sections were analyzed by three independent observers. As shown in Fig. 11, there

was a clear inhibitory effect of the EGAD-BPI treatment on mononuclear cell infiltration (insulitis). Over 95% of the islets from the BPI treated animals were intact and did not show infiltration (i.e., grade-0 islets). All of the other groups showed some signs of insulitis even at this early stage of the disease. Notably, the GAD peptide treated animals showed the most insulitis (grade-0 islets reduced to 62% and 37.5% of islets scored grade 2 or above. This compared with 66.7% normal islets in the CD11a peptide treated group and 71.4% normal islets in PBS treated animals. Thus, compared to the PBS control, EGAD-BPI treatment provided an 84% *inhibition of insulitis* [calc. as: % islets @grade 1-4 (PBS Rx) minus % islets @grade 1-4 (EGAD-BPI Rx) divided by % islets @grade 1-4 (PBS Rx) multiplied by 100]. Representative islets from each group of the experiment are shown below in Figs. 12a-12d, as stained with hematoxylin and eosin. Note severe lymphocytic infiltration in Figs. 12b and 12d which was observed in groups treated with the single Signal-1 or Signal-2 moieties while the islets from the EGAD-BPI treated mice were predominately intact (see Fig. 12c). Taken together, these data strongly indicate that treatment with the diabetes BPI containing these Signal-1 and Signal-2 peptide moieties significantly inhibits the infiltration of lymphocytes into the pancreatic islets in this animal model of type-1 diabetes. Therefore, we would predict that BPI operate via a mechanism involved in the normal breakdown of self tolerance to pancreatic autoantigens. These data indicate that BPI may operate through immune deviation to block the autoimmune response to pancreatic antigen. To further test this theory, we transferred T-cells primed in the presence of the EGAD-BPI into NOD.Scid mice genetically programmed for diabetes development.

EXAMPLE 6

This example tested BPI blocking of diabetes development in the well described intact immune system of immunologically reconstituted NOD.Scid mice to study diabetes progression.

Materials and Methods:

BPI proven active for modulation of T_H1/T_H2 responses against known immunodominant peptides of GAD65 for blocking T-cell initiated diabetes progression in a NOD mouse model was tested. The NOD.Scid adoptive transfer model, wherein CD25-

depleted NOD spleen cells have been observed to induce diabetes as early as 2-4 weeks post adoptive transfer was used for this purpose. NOD.Scid adoptive transfers were performed by a modification of a protocol described by Solomon et al. in *B7/CD28 Costimulation is Essential for the Homeostasis of the CD4$^+$ CD25 Immunoregulatory T-cells That Control Autoimmune Diabetes*, 12 Immunity 431-440 (2000), the content of which is incorporated herein by reference. NOD spleen cells from 8 week (non-diabetic) females were used to enrich a CD25-/CTLA4-depleted population by treatment with purified monoclonal antibody (mAb 7D4, PharMingen) followed by low-tox rabbit complement (Cedarlane) (80% depletion of CTLA4+ cells by flow analysis with PE-labeled UC10-4F10-11 mAb; not shown). These inducer cells (15 x 10^6 per mouse) were injected (i.v.) into 6 week NOD.Scid females (Jackson Labs) together with 3 x 10^6 CD4+ T-cells from mice either treated with the EGAD-BPI or treated identically except with PBS in place of the EGAD-BPI. In vitro clonal expansion was with either recombinant IL-2 (R&D systems), or ConA, as described for the ELISPOT experiments. All manipulations of animals and cells were performed in laminar flow hoods, and animals were maintained continuously behind laminar flow barriers on autoclaved food and water in microisolator cages. Some experiments will deplete specific subsets of the CD4+ cells using mAb to CD154, CD25, CD62L, CD152, etc. and magnetic particles prior to adoptive transfer. CD4+ cells from mice treated with the individual moieties of the BPI can be used as negative controls along with CD4+ cells from mice treated with saline alone. Co-transfer of CD4+ T-cells enriched from matched NOD mice treated with a given BPI demonstrates that BPI treatment leads to regulatory T-cells capable of delaying the onset of diabetes. Moreover, other diseases listed in Table 1 can be tested in this same manner (i.e., by adoptive transfer of regulatory T-cells).

Results:

Significantly, we have now seen a clear difference in the development of hyperglycemia and diabetes between the two experimental groups. As can be seen in Fig. 13, at 7 weeks post adoptive transfer, 80% of mice which received the vehicle, (PBS) developed hyperglycemia and diabetes. By contrast only 40% of mice treated with the EGAD-BPI showed hyperglycemia and progression to diabetes. Therefore, these data demonstrate the blocking of diabetes progression as mediated by the BPI treatment. Further

modifications to the BPI structure may enhance their effectiveness in this model and in the treatment of type-1 diabetes. These data indicate that T-cells capable of suppressing diabetes development were generated in the presence of the BPI and operated *in vivo* to inhibit diabetes progression within the intact system of the NOD.Scid mouse. Thus we would anticipate similar regulatory T-cells to become activated by BPI containing TCR epitopes of other disease associated antigens. For example, collagen-II peptide epitopes may initiate suppressor T-cells involved in rheumatoid arthritis. Moreover, regulatory T-cells that would expand T_H1 populations may be generated by BPI containing CTLA4 second signal moieties and these could be used in diseases such as HIV1 infection or other chronic viral diseases. Extrapolation of this example to clinical trial should be straightforward, as the NOD model is recognized as a significant representation of the human disease. (See e.g., Atkinson and Leiter, The NOD Mouse Model of Type-1 Diabetes: As Good as it Gets?, 5 *Nature Medicine,* 601-604 (1999).

EXAMPLE 7

Here, the predicted examples of BPI for other autoimmune diseases are briefly detailed. Specifically, BPI containing immunodominant TCR epitopes for collagen-induced arthritis (CIA) and myelin basic protein-induced experimental allergic encephalomyelitis (EAE) will be discussed. Also, the CD40L peptide mimic is predicted to favor T_H2 immunity, as blocking the CD40 signal would be expected to decrease IL12 production (Ruedl, et al., *The Antigen Dose Determines T Helper Subset Development by Regulation of CD40 Ligand*, 30 Eur. J. Immunol. 2056-2064 (2000)). Therefore, in these autoimmune models and in the NOD model, we will attempt to favor T_H2 immunity by linking the appropriate TCR epitopes to the CD40L peptide mimic as well as the CD11a mimic used in diabetes inhibition. Mutations in this loop peptide have been shown to affect binding and function of CD40L.

Materials and Methods:

There are several autoimmune models which employ different cross-reactive immunodominant epitopes, different mouse strains, and tissues of analysis. These types of models are highly similar to our short-term model of diabetes development that we designed

to test the EGAD-BPI. For CIA: We will induce the disease by a single peptide in CFA injection protocol as described by Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994). As in the NOD system, these mice will receive either the BPI, PBS, or the single peptide moieties. The minimal immunodominant collagen-II epitope is listed above, and modifications to the BPI will be based upon the x-ray structure of this complex (Dessen et al., 7 Immunity 473-481 (1997). For EAE, the disease is induced by the method of Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994). This method also uses a single peptide (the myelin basic protein (MBP) peptide, 85-101) injection in CFA, by methods analogous to those described in our NOD system.

Results:

We would anticipate that the BPI constructed with collagen and MBP would be effective in modulating immunity to these antigens to a T_H2-dominated pattern. Since these diseases are thought to involve predominant T_H1 immunopathology (see above references), such a switch would ostensibly delay or cure the disease in these mice (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996). As in the NOD model, such results would target the development of analogous compounds for use in the human diseases of rheumatoid arthritis and multiple sclerosis, where these types of immune responses are clinically observed. Most importantly, BPI offer the capability of blocking autoimmune T-cell responses while maintaining host immunity to infectious agents and developing cancers.

EXAMPLE 8

This example describes predicted BPI for infectious diseases and certain cancers. Specifically, a general protocol for the testing of a given BPI containing immunodominant TCR epitopes of a specific human pathogen will be described using the example of HIV-1 p24 epitope (Harcourt, et. al., *HIV-1 Variation Diminishes CD4 T Lymphocyte Recognition*, 188 J. Exp. Med., 1785-1793 (1998)) (SEQ ID No. 8).

Materials and Methods:

By contrast to the previous experimental examples, these BPI will be primarily tested by their effects on long-term T-cell clones derived from human patients. Briefly, peripheral blood mononuclear cells (PBMCs) are prepared from patient and control whole blood, and CD8+ cells removed by the negative selection protocol with magnetic particles. These cells are grown in tissue culture medium at 4 x 10^6 cells in 1 ml for 6 days in the presence of 20 μM of the p24 peptide, and blast cells isolated by density gradient centrifugation. Secondary cultures of these cells contained recombinant human IL-2 (20 U/ml) and are continued for a maximum of 10-14 days. Lines were expanded by repeating the process of re-culturing with irradiated APC from histocompatible donors together with the p24 peptide and expansion of the T-cell blasts in IL-2. Clones were prepared by limiting dilution cloning in fresh plates containing irradiated APC, peptide and IL-2 (50 U/ml) (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). BPI will be tested for their ability to inhibit the proliferation and cytokine release of these established lines of human T-cells by our detailed methods described in Schountz et al., *Unique T Cell Antagonist Properties of the Exact Self-Correlate of a Peptide Antigen Revealed by Self-Substitution of Non-Self-Positions in the Peptide Sequence,* 168 Cellular Immunology 193-200 (1996). Here, we show that peptides changed by amino acid substitutions at key TCR-contact residues alter the proliferation and cytokine release of cloned T-cells. If a given BPI significantly inhibits or alters cytokine release to p24 peptide-specific clones, this will target the BPI for more stringent testing *in vivo*. Mouse models can be used to test candidate BPI for infectious diseases. These studies will be performed as described for the GAD65 BPI with the substitution of immunodominant peptides of the infectious agents for the GAD65 TCR epitope peptide. Positive results in these *in vivo* studies would substantiate animal studies with live pathogens. The Scid-human mouse model in which human tissues are adoptively transferred to the Scid mouse could be used to examine protective immunity to HIV (Jenkins, et al., Blood (1998) 8:2672, hepatitis-C virus (HCV) (Bronowicki, J., et al. Hepatology (1998)28:211), human pappiloma virus (HPV) (Tewari, et al. Gynecol. Oncol. (2000)77:137, and respiratory syncitial virus (RSV) (Nadal, et al. Clin. Exp. Immunol.85:358) infections.

Results:

It is hypothesized that incorporation of the CD28/CTLA4 peptide or the Fas-ligand peptide as the second signal moiety will shift immunity toward the T_H1 subset and induce or enhance CTL responses. (Padrid, P., et al. Am. J. Respir. Cell. Mol. Biol. (1998)18:453). CTLA4 is a negative regulator of T-cell activation and signaling through CD28 has been linked to T_H2 differentiation (Rulifson, I., et al. J. Immunol. (1997)158:658). Given the evidence that the LFA-1 signal is inhibitory to T_H2 differentiation and that CD28 signaling favors T_H2 differentiation, we will proceed with these studies using this selective differentiation paradigm to further modify BPI design. Similarly, the evidence suggests that CD95 (also called Fas) interaction with the CD95-ligand (FasL) favors T_H1 differentiation (Chattergoon, et al. Nature Biotech. (2000)18:974). BPI with the peptide mimic of the FasL, which is from the Y218 loop of FasL, predicted to interact with Fas at the T-cell:APC interface, will be used to favor T_H1 activation and CTL responses against chronic T_H2-dominated diseases.

EXAMPLE 9

Here we describe several possible BPI that contain the TCR epitopes of described allergen sequences synthetically linked to the predicted CD28/CTLA4 peptide mimic and/or the predicted FasL peptide mimic.

Materials and Methods:

These studies will again use a combination of analyses using human cloned T-cell lines and animal models of allergy to these well-known allergic agents. Allergies affect a very large population of humans, with estimates of many allergies and asthma reaching 10% of otherwise healthy individuals. We will use our previously described 7-10 day protocol to investigate BPI modulation of the immune response to the allergen peptides described in the table. As previously described, this protocol involves the injection of the allergen peptide in CFA on day 1, followed by i.v. injection of the BPI on day 1 and on day 6, 7, 8 or 9. On day 7, 8, 9 or 10, the draining lymph nodes are cultured and compared for T_H1 versus T_H2 cytokines by ELISPOT (see Example 4 for detailed methods). Once candidate BPI for a given allergen have been identified by this relatively short-term experiment, we

will test these BPI for the alteration of CD4 T-cell response using cloned lines from atopic patients. Briefly, CD4 T-cell clones will be established from patient peripheral blood lymphocytes as described under Example 8. We will test the capacity of a given BPI to inhibit or alter cytokine release by these cells using the ELISPOT as described previously. Finally, we will move the examination of a given BPI toward clinical testing using identically described procedures with these specific allergens in human MHC (HLA) transgenic mice (Svetlana, P. et al. J. Immunol. (1998)161:2032). These mice offer the advantage of having the human MHC molecules and thus, BPI binding will be very similar in this model as in the human allergic condition. If a given BPI blocks T_H2 activation in these mice against the native allergen, then adoptive transfer studies with human Scid mice (similar to those used in Example 8) can be used as a precursor to human clinical trials.

Results:

Blocking the CTLA4/CD28 pathway in preference for LFA-1 signaling will favor T_H1 differentiation against these allergens in the presence of the BPI. Also, BPI with the FasL moiety should favor type-1 immunity. It has been a long-held practice to "desensitize" atopic patients with allergens given by injection and desensitization is thought to operate via a shift toward T_H1 responses to the specific allergens (Holt, et al., Nature (1999) 402:6760 suppl:B12-17). Therefore, as in the case of the chronic T_H2-linked infectious diseases, our working hypothesis is to use the T_H1-promoting capacity of LFA-1 signaling together with the possibility to inhibit T_H2 signaling via the CTLA4/CD28 molecules to effect this result. We will also examine the effects of allergen epitopes linked to the Fas peptide mimic, as dual expression of Fas and antigen within APC has been shown to favor T_H1 immunity and CTL induction (Chattergoon, M., et. al. Nat. Biotech. (2000)18:974). It may be possible to induce cell death of APC presenting allergen epitopes, thereby limiting the activation of T_H2-type regulatory cells in allergy and asthma, including RSV-precipitated childhood and geriatric bronchiolitis.

We claim:

1. A peptide comprising a first portion and a second portion, said first portion including a sequence having at least about 10% sequence homology with a peptide derived from a TCR epitope, said second portion including a sequence having at least about 10% sequence homology with a peptide derived from a Signal-2 moiety.

2. The peptide of claim 1, further comprising a linking portion.

3. The peptide of claim 2, said linking portion comprising at least one amino acid residue.

4. The peptide of claim 3, said amino acid being a flexible, non-substrate amino acid.

5. The peptide of claim 2, said linking portion comprising a sequence of amino acid residues, said sequence comprising a non-substrate amino acid alternating with a hydrophilic amino acid.

6. The peptide of claim 2, said linking portion having the general formula $(A,B)_X$, wherein A and B are amino acid residues, and said A amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine, and said B amino acid residue is glycine, and wherein X ranges from 1 to 100.

7. The peptide of claim 6, said A amino acid residue being aminocaproic acid.

8. The peptide of claim 1, said first portion including a sequence having at least about 10% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

9. The peptide of claim 8, said peptide having at least 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

10. The peptide of claim 9, said peptide having at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

11. The peptide of claim 1, said first portion comprising a peptidomimetic of a peptide selected from the group consisting of SEQ ID Nos. 1-25.

12. The peptide of claim 1, said first portion comprising a derivative of a peptide selected from the group consisting of SEQ ID Nos. 1-25.

13. The peptide of claim 1, said TCR epitope being correlated with a known disease state.

14. The peptide of claim 1, said first portion capable of binding with a major histocompatability complex on an antigen presenting cell.

15. The peptide of claim 14, said peptide:MHC complex capable of engaging a T-cell receptor on a T-cell.

16. The peptide of claim 1, said second portion including a sequence having at least about 10% sequence homology with a sequence selected from a group consisting of SEQ ID Nos.30-41.

17. The peptide of claim 16, said peptide having at least about 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

18. The peptide of claim 17, said peptide having at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

19. The peptide of claim 1, said second portion comprising a peptidomimetic of a peptide selected from the group consisting of SEQ ID Nos. 30-41.

20. The peptide of claim 1, said second portion comprising a derivative of a peptide selected from the group consisting of SEQ ID Nos. 30-41.

21. The peptide of claim 1, said second portion capable of binding with a Signal-2 ligand on an antigen presenting cell.

22. The peptide of claim 21, said peptide capable of initiating a second signal in a T-cell.

23. The peptide of claim 1, said peptide capable of modifying an immune response from a type-1 dominated response to a type-2 dominated response.

24. The peptide of claim 1, said peptide capable of modifying an immune response from a type-2 dominated response to a type-1 dominated response.

25. The peptide of claim 1, said second portion being associated with a particular type of immune response.

26. The peptide of claim 2, said linking portion being positioned intermediate said first portion and said second portion.

27. The peptide of claim 1, said peptide being synthesizable as one continuous sequence.

28. A method of modifying an immune response comprising the steps of: (a) contacting an antigen presenting cell with a peptide, said peptide comprising a first portion binding to a major histocompatability complex on said antigen presenting cell to form a peptide:MHC complex, and a second portion binding to a second signal moiety on said antigen presenting cell; (b) causing engagement of the T-cell receptor in a T-cell in response to the formation of said peptide:MHC complex; (c) blocking or altering a second signal in a T-cell in response to the binding of said second portion to said second signal moiety; and (d) said first and second signals generating an immune response which deviates from the immune response generally associated with said peptide:MHC complex.

29. The method of claim 28, said immune response deviating from a type-1 immune response to a type-2 immune response.

30. The method of claim 28, said immune response deviating from a type-2 immune response to a type-1 immune response.

31. The method of claim 28, said first portion having at least 10% sequence homology with a peptide derived from a Signal-1 moiety.

32. The method of claim 28, said second portion having at least 10% sequence homology with a peptide derived from a Signal-2 receptor moiety.

33. A peptide having the general formula AB wherein each of said A and B represent a chain of amino acid residues, and wherein said A chain comprises at least about 5 amino acid residues and has at least about 10% sequence homology with a Signal-1 moiety, and said B chain comprises at least about 4 amino acid residues and has at least about 10% sequence homology with a peptide derived from a Signal-2 receptor moiety.

34. The peptide of claim 34, further comprising an X chain of amino acid residues, said X chain comprising at least about 1 amino acid residue.

35. The peptide of claim 34, said X chain being positioned intermediate said A chain and said B chain.

36. The peptide of claim 33, said peptide being synthesizable as one continuous sequence.

37. The peptide of claim 34, said X chain comprising a flexible, non-substrate linker having the general formula $(Y,Z)_t$, wherein Y and Z are amino acid residues, and said Y amino acid residue is individually and respectively selected from the group consisting of non-substrate amino acids, and said Z amino acid residue is individually and respectively selected from the group consisting of hydrophilic amino acids, and wherein T ranges from 1 to 100.

38. The peptide of claim 37, said non-substrate amino acids being selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine.

39. The peptide of claim 38, said non-substrate amino acid residue being aminocaproic acid.

40. The peptide of claim 37, said hydrophilic amino acid being glycine.

41. The peptide of claim 33, said A chain including a sequence having at least about 10% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

42. The peptide of claim 41, said A chain having at least 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

43. The peptide of claim 42, said A chain having at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

44. The peptide of claim 33, said A chain comprising a peptidomimetic of a peptide selected from the group consisting of SEQ ID Nos. 1-25.

45. The peptide of claim 33, said A chain comprising a derivative of a peptide selected from the group consisting of SEQ ID Nos. 1-25.

46. The peptide of claim 33, said Signal-1 moiety being associated with a known health condition.

47. The peptide of claim 33, said peptide capable of binding with a major histocompatability complex on an antigen presenting cell to form a peptide:MHC complex.

48. The peptide of claim 47, said peptide:MHC complex capable of engaging a T-cell.

49. The peptide of claim 33, said B chain including a sequence having at least about 10% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

50. The peptide of claim 49, said B chain having at least about 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

51. The peptide of claim 50, said B chain having at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

52. The peptide of claim 33, said B chain comprising a peptidomimetic of a peptide selected from the group consisting of SEQ ID Nos. 30-41.

53. The peptide of claim 33, said B chain comprising a derivative of a peptide selected from the group consisting of SEQ ID Nos. 30-41.

54. The peptide of claim 33, said peptide capable of binding with a Signal-2 ligand on an antigen presenting cell.

55. The peptide of claim 54, said peptide capable of blocking or altering a second signal to a T-cell upon binding with said Signal-2 moiety.

56. The peptide of claim 33, said peptide capable of modifying an immune response from a type-1 dominated response to a type-2 dominated response.

57. The peptide of claim 33, said peptide capable of modifying an immune response from a type-2 dominated response to a type-1 dominated response.

58. The peptide of claim 33, said peptide capable of shifting a cell-mediated immune response to a humoral immune response.

59. The peptide of claim 33, said peptide capable of shifting a humoral immune response to a cell-mediated response.

60. A method of preparing a peptide for modulating immune response, comprising the steps of:

selecting a first peptide sequence, said first peptide sequence having at least about 10% sequence homology with a sequence derived from a Signal-1 moiety;

selecting a second peptide sequence, said second peptide sequence having at least about 10% sequence homology with a sequence derived from a Signal-2 receptor moiety; and

synthesizing said peptide as a continuous peptide chain comprising said first peptide sequence and said second peptide sequence.

61. The method of claim 60, further comprising the step of selecting a third peptide sequence, said third peptide comprising at least one amino acid residue.

62. The method of claim 60, said first peptide sequence being associated with a known health condition.

63. The method of claim 60, said first peptide sequence being capable of binding with a major histocompatibility complex on an antigen presenting cell to form a peptide:MHC complex.

64. The method of claim 60, said second peptide sequence being capable of binding with a Signal-2 ligand on an antigen presenting cell.

65. The method of claim 60, further comprising the step of contacting said peptide with a major histocompatibility complex on an antigen presenting cell.

66. The method of claim 60, further comprising the step of contacting said peptide with a Signal-2 ligand on an antigen presenting cell.

67. A peptide comprising:
a first peptide sequence capable of initiating a first signal in a T-cell; and
a second peptide sequence capable of initiating a second signal in a T-cell.

68. The peptide of claim 67, further comprising a linking peptide sequence, said linking peptide sequence comprising at least one amino acid residue.

69. The peptide of claim 67, said first peptide sequence being derived from a Signal-1 moiety.

70. The peptide of claim 67, said second peptide sequence being derived from a Signal-2 receptor moiety.

71. The peptide of claim 67, said first peptide sequence having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 1-25.

72. The peptide of claim 67, said second peptide sequence having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 30-41.

73. The peptide of claim 68, said linking peptide sequence having the general formula of $(Y,Z)_t$, wherein Y and Z are amino acid residues, and wherein T ranges from 1 to 100.

74. The peptide of claim 73, said Y amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine.

75. The peptide of claim 73, said Z amino acid residue being individually and respectively selected from the group consisting of hydrophilic amino acid residues.

76. The peptide of claim 75, wherein said hydrophilic amino acid residue is glycine.

77. The peptide of claim 73, said Y amino acid residue being aminocaproic acid.

78. A method of constructing a BPI comprising the steps of: (a) selecting a known health condition; (b) identifying a TCR epitope specific for said health condition; and (c) incorporating said TCR epitope into a BPI.

79. The method of claim 78, further comprising the step of selecting a Signal-2 receptor moiety and incorporating said Signal-2 receptor moiety into said BPI.

80. The method of claim 79, further comprising the step of conjugating said TCR epitope with said Signal-2 receptor moiety.

81. The method of claim 80, said conjugating step including positioning a linker intermediate said TCR epitope and said Signal-2 receptor moiety.

82. The method of claim 81, said linker comprising at least one amino acid residue.

83. The method of claim 81, said linker having the general formula of $(Y,Z)_t$, wherein Y and Z are amino acid residues, and wherein T ranges from 1 to 100.

84. The method of claim 83, said Y amino acid residue being individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine.

85. The method of claim 83, said Z amino acid residue being individually and respectively selected from the group consisting of hydrophilic amino acid residues.

86. The method of claim 85, wherein said hydrophilic amino acid residue is glycine.

87. The method of claim 78, said TCR epitope being capable of binding with a major histocompatibility complex on an antigen presenting cell to form a peptide:MHC complex.

88. The method of claim 79, said Signal-2 receptor moiety being capable of binding with a Signal-2 ligand on an antigen presenting cell.

89. The method of claim 78, said TCR epitope having at least about 10% sequence homology with a sequence selected from SEQ ID Nos. 1-25.

90. The method of claim 79, said Signal-2 receptor moiety having at least about 10% sequence homology with a sequence selected from SEQ ID Nos. 30-41.

91. In combination:

a first peptide sequence capable of binding with a major histocompatibility complex on an antigen presenting cell; and

a second peptide sequence capable of binding with a Signal-2 ligand on an antigen presenting cell.

92. The combination of claim 91, further including a linker positioned intermediate said first peptide sequence and said second peptide sequence.

93. The combination of claim 91, said first peptide sequence being derived from a Signal-1 moiety.

94. The combination of claim 91, said second peptide sequence being derived from a Signal-2 receptor moiety.

95. The combination of claim 91, said first peptide sequence having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 1-25.

96. The combination of claim 91, said second peptide sequence having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 30-41.

97. The combination of claim 92, said linker comprising at least one amino acid residue.

98. The combination of claim 92, said linker having the general formula of $(Y,Z)_t$, wherein Y and Z are amino acid residues, and wherein T ranges from 1 to 100.

99. The combination of claim 98, wherein said Y amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine.

100. The combination of claim 99, said Y amino acid residue being aminocaproic acid.

101. The combination of claim 98, said Z amino acid residue being individually and respectively selected from the group consisting of hydrophilic amino acid residues.

102. The combination of claim 101, wherein said hydrophilic amino acid residue is glycine.

103. A peptide having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 42-46.

104. The peptide of claim 103, said peptide having at least about 50% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 42-46.

105. The peptide of claim 104, said peptide having at least about 95% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 42-46.

106. A derivative of a peptide, said peptide being selected from the group consisting of SEQ ID Nos. 42-46.

107. A peptidomimetic of a peptide, said peptide being selected from the group consisting of SEQ ID Nos. 42-46.

108. A peptide mimic of a peptide, said peptide being selected from the group consisting of SEQ ID Nos. 42-46.

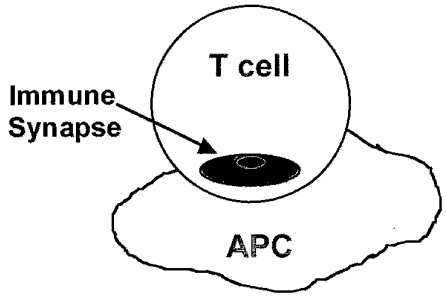

Fig. 1

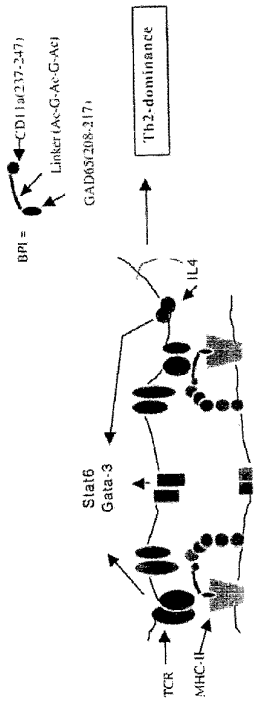
Th2-dominance
BPI =
Linker (Ac-G-Ac-G-Ac)
IL4
Stat6
Gata-3
TCR
MHC-II

Fig. 2

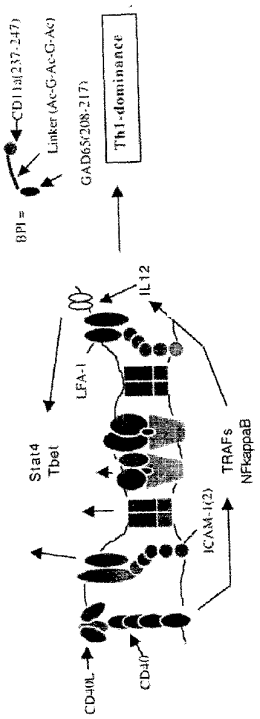
BPI =
CD11a(237-247)
Linker (Ac-G-Ac-G-Ac)
GAD65(208-217)
Th1-dominance
IL12
LFA-1
Stat4
Tbet
TRAFs
NFkappaB
ICAM-1(2)
CD40L
CD40

Fig. 3

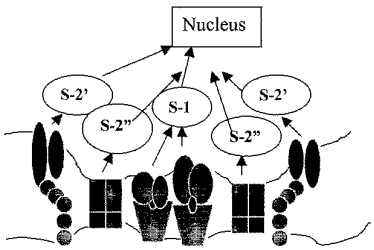

Fig. 4

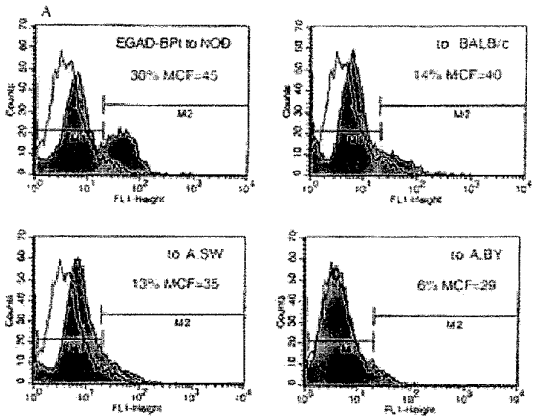
A
EGAD-BPI to NOD
30% MCF=45
M2
to BALB/c
14% MCF=40
M2
to A.SW
13% MCF=35
M2
to A.BY
6% MCF=29
M2
Counts
FL1-Height

Fig. 5a

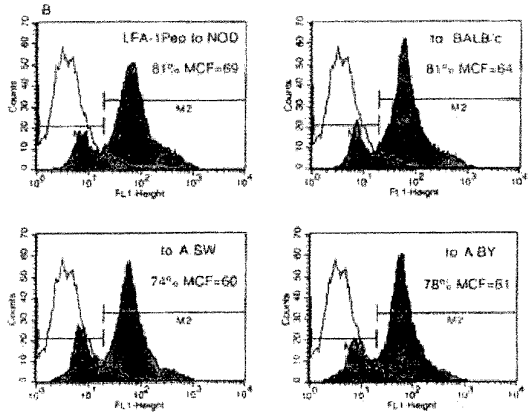
B
LFA-1Pep to NOD
81% MCF=69
M2
to BALB/c
81% MCF=64
M2
to A.SW
74% MCF=60
M2
to A.BY
78% MCF=61
M2
Counts
FL1-Height

Fig. 5b

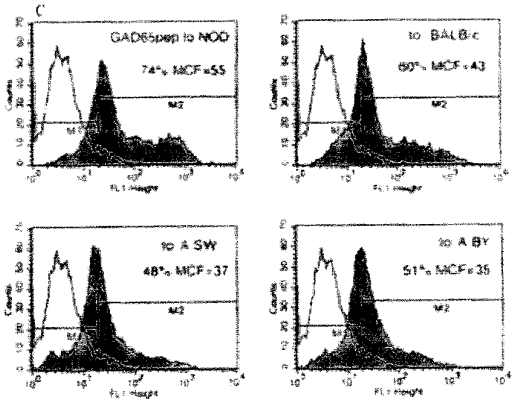
C
GAD65pep to NOD
74% MCF=55
M2
to BALB/c
60% MCF=43
M2
to A SW
48% MCF=37
M2
to A BY
51% MCF=35
M2

Fig. 5c

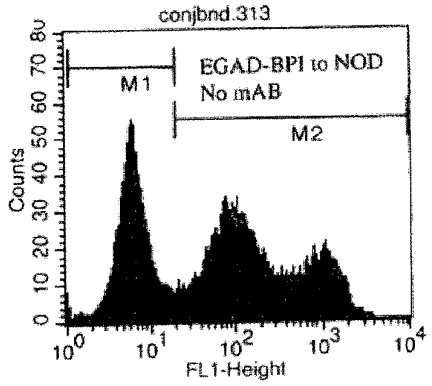
conjbnd.313
EGAD-BPI to NOD
No mAB
M1
M2
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-Height

Fig. 6a

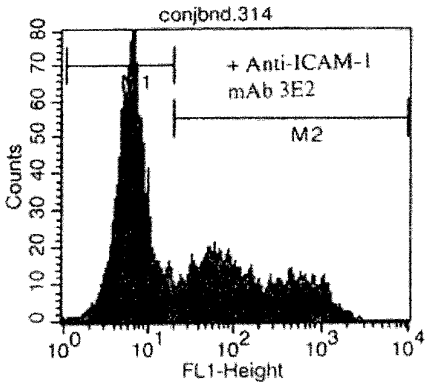
conjbnd.314
+ Anti-ICAM-1
mAb 3E2
M1
M2
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-Height

Fig. 6b

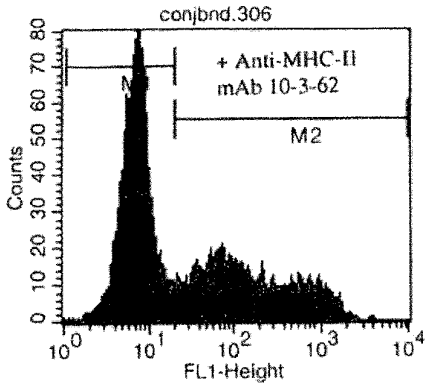
conjbnd.306
+ Anti-MHC-II
mAb 10-3-62
M2
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-Height

Fig. 6c

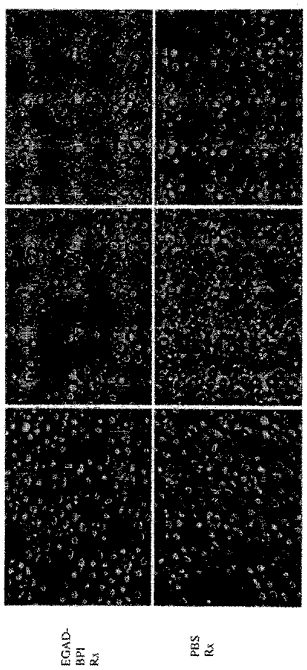

Fig. 7

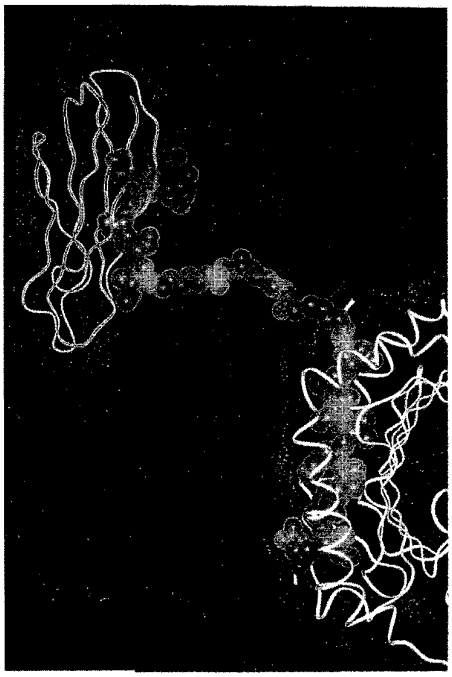

Fig. 8

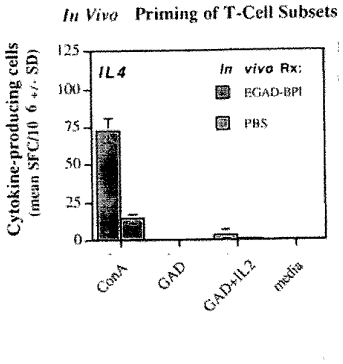
In Vivo Priming of T-Cell Subsets
Cytokine-producing cells (mean SFC/10^6 +/- SD)
IL4
In vivo Rx:
EGAD-BPI
PBS
125
100
75
50
25
0
ConA
GAD
GAD+IL2
media

Fig. 9a

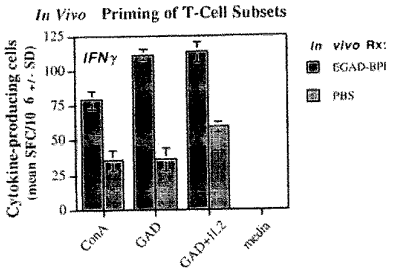
In Vivo Priming of T-Cell Subsets
IFNγ
Cytokine-producing cells (mean SFC/10^6 +/- SD)
125
100
75
50
25
0
ConA
GAD
GAD+IL-2
media
In vivo Rx:
EGAD-BPI
PBS

Fig. 9b

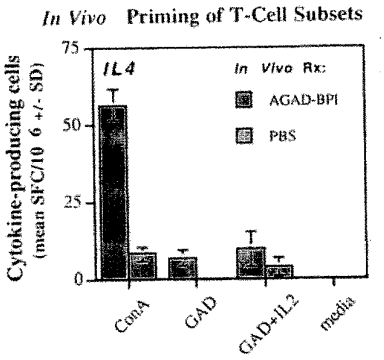
In Vivo Priming of T-Cell Subsets
Cytokine-producing cells (mean SFC/10^6 +/- SD)
75
50
25
0
IL4
In Vivo Rx:
AGAD-BPI
PBS
ConA
GAD
GAD+IL2
media

Fig. 9c

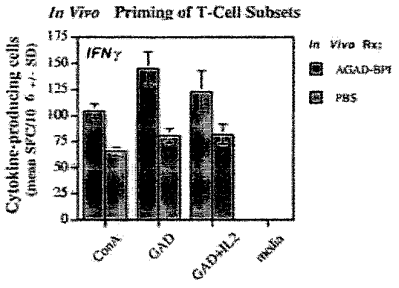
In Vivo Priming of T-Cell Subsets
IFNγ
Cytokine-producing cells (mean SFC/10^6 +/- SD)
175
150
125
100
75
50
25
0
ConA
GAD
GAD-HL2
media
In Vivo Rx:
AGAD-BPI
PBS

Fig. 9d

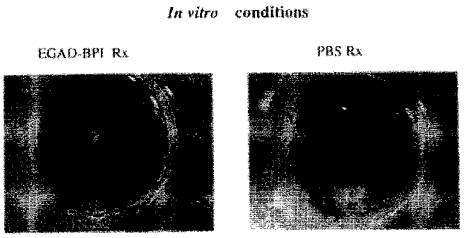

Fig. 10

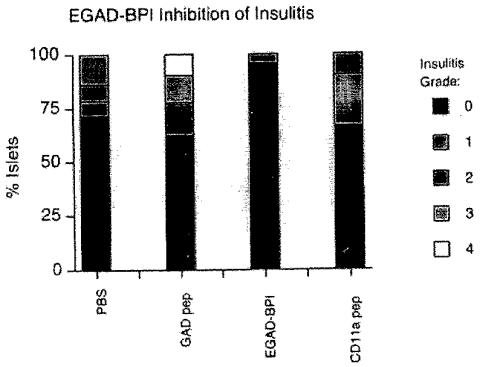
EGAD-BPI Inhibition of Insulitis
% Islets
100
75
50
25
0
PBS
GAD pep
EGAD-BPI
CD11a pep
Insulitis Grade:
0
1
2
3
4

Fig. 11

PBS

Fig. 12a

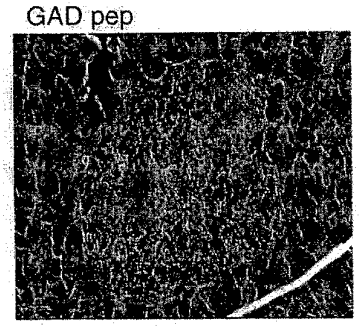

Fig. 12b

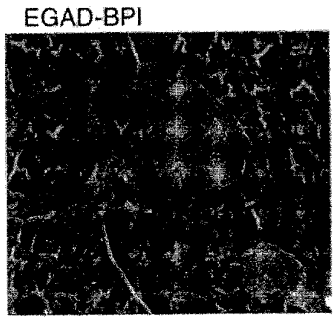

Fig. 12c

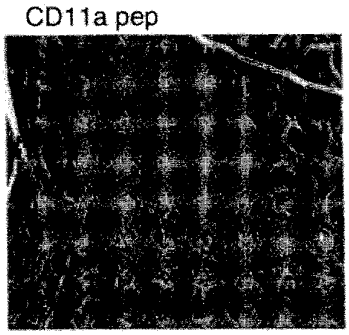

Fig. 12d

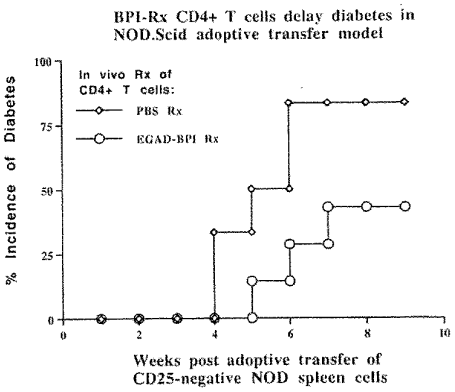
BPI-Rx CD4+ T cells delay diabetes in NOD.Scid adoptive transfer model
In vivo Rx of CD4+ T cells:
PBS Rx
EGAD-BPI Rx
% Incidence of Diabetes
100
75
50
25
0
0
2
4
6
8
10
Weeks post adoptive transfer of CD25-negative NOD spleen cells

Fig. 13

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<110> Murray, Joseph S
Siahaan, Teruna J
Hu, Yongbo

<120> Signal-1/Signal-2 Bifunctional Peptide Inhibitors

<130> 30805 Murray

<140> US 09/739,466
<141> 2000-12-18

<160> 46

<170> PatentIn version 3.1

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<212> PRT
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<400> 2
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1 5 10

<210> 3

<211> 13

<212> PRT

<213> Homo sapiens

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1 5 10

<210> 4

<211> 12

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<213> Yersinia enterocolitica

<400> 4

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<211> 14

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1 5 10

<210> 7

<211> 20

<212> PRT

<213> Human immunodeficiency virus type 1

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1 5 10 15

Met Trp Gln Glu
20

<210> 8

<211> 20

<212> PRT

<213> Human immunodeficiency virus type 1

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1 5 10 15

Pro Arg Thr Leu
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<213> Simian immunodeficiency virus

<400> 9

Ser Thr Pro Glu Ser Ala Asn Leu
1 5

<210> 10

<211> 15

<212> PRT

<213> respiratory syncytial virus

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Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly Lys Lys Thr
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<210> 11

<211> 9

<212> PRT

<213> Human papillomavirus

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Val Tyr Arg Asp Gly Asn Pro Tyr Ala
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<210> 12

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1 5 10

<210> 14

<211> 15

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<213> Human papillomavirus

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1 5 10 15

<210> 15

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<213> Homo sapiens

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Ala Glu Leu Tyr His Phe Leu Leu Lys Tyr Arg Ala Arg
1 5 10

<210> 16

<211> 14

<212> PRT

<213> Homo sapiens

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1 5 10

<210> 17

<211> 16

<212> PRT

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1 5 10 15

<210> 18

<211> 17

<212> PRT

<213> Felis sp.

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Lys Ala Leu Pro Val Val Leu Glu Asn Ala Arg Ile Leu Lys Asn Cys
1 5 10 15

Val

<210> 19

<211> 20

<212> PRT

<213> Ambrosia artemisiifolia

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Leu Val Pro Cys Ala Trp Ala Gly Asn Val Cys Gly Glu Lys Arg Ala
1 5 10 15

Tyr Cys Cys Ser
20

<210> 20

<211> 20

<212> PRT

<213> Ambrosia trifida

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Pro Ile Gly Lys Tyr Cys Val Cys Tyr Asp Ser Lys Ala Ile Cys Asn
1 5 10 15

Lys Asn Cys Thr
20

<210> 21

<211> 15

<212> PRT

<213> Cryptomeria japonica

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Lys Ser Met Lys Val Thr Val Ala Phe Asn Gln Phe Gly Pro Asn
1 5 10 15

<210> 22

<211> 19

<212> PRT

<213> Cryptomeria japonica

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Ile Asp Ile Phe Ala Ser Lys Asn Phe His Leu Gln Lys Asn Thr Ile
1 5 10 15

Gly Thr Gly

<210> 23

<211> 17

<212> PRT

<213> Apis mellifera

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Tyr Phe Val Gly Lys Met Tyr Phe Asn Leu Ile Asp Thr Lys Cys Tyr
1 5 10 15

Lys

<210> 24

<211> 20

<212> PRT

<213> Hevea brasiliensis

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Ala Ser Glu Gln Glu Thr Ala Asp Ala Thr Pro Glu Lys Glu Glu Pro
1 5 10 15

Thr Ala Ala Pro
20

<210> 25

<211> 17

<212> PRT

<213> Dermatophagoides pteronyssinus

<400> 25

Phe Gly Ile Ser Asn Tyr Cys Gln Ile Tyr Pro Pro Asn Ala Asn Lys
1 5 10 15

Ile

<210> 26

<211> 5

<212> PRT

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<220>

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<400> 26

Xaa Gly Xaa Gly Xaa
1 5

<210> 27

<211> 5

<212> PRT

<213> Artificial

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1 5

<210> 28

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<213> Artificial

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<220>
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<400> 28
Xaa Gly Xaa Gly Xaa
1 5

<210> 29
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<400> 29
Xaa Gly Xaa Gly Xaa
1 5

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Ile Thr Asp Gly Glu Ala Thr Asp Ser Gly
1 5 10

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<400> 31
Thr Asp Gly Glu Ala Thr Asp Ser Gly Asn
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<210> 32
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<212> PRT
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Ala Ser Pro Gly Lys Ala Thr Glu Val Arg
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<210> 33

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<212> PRT

<213> Mus musculus

<400> 33

Ser Pro Ser His Asn Thr Asp Glu Val Arg
1 5 10

<210> 34

<211> 12

<212> PRT

<213> Homo sapiens

<400> 34

Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu
1 5 10

<210> 35

<211> 12

<212> PRT

<213> Mus musculus

<400> 35

Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Phe Val
1 5 10

<210> 36

<211> 10

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<213> Mus musculus

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Ile Thr Asp Gly Glu Ala Thr Asp Ser Gly
1 5 10

<210> 37

<211> 13

<212> PRT

<213> Homo sapiens

<400> 37

Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu
1 5 10

<210> 38

<211> 13

<212> PRT

<213> Mus musculus

<400> 38

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1 5 10

<210> 39

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<212> PRT

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1 5 10 15

<210> 40

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<213> Mus musculus

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<223> The artificial sequence includes the GAD65 peptide sequence from homo sapiens and the CD11a peptide sequence from homo sapiens lin

ked by a series of alternating aminocaproic acid and glycine resi
dues

<220>

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<222> (15)..(15)

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<400> 42

Glu Ile Ala Pro Val Phe Val Leu Leu Glu Xaa Gly Xaa Gly Xaa Ile
1 5 10 15

Thr Asp Gly Glu Ala Thr Asp Ser Gly
20 25

<210> 43

<211> 29

<212> PRT

<213> Artificial

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<222> (9)..(9)

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<220>

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<220>

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<222> (13)..(13)

<223> X is aminocaproic acid.

<220>

<221> misc_feature

<223> The artificial sequence includes the Tat peptide from simian immu nodeficiency virus, a linker consisting of aminocaproic, aminodod ecanoic, and glycine residues and the CTLA4 (93-104) peptide sequ ence from mus musculus

<400> 43

Ser Thr Pro Glu Ser Ala Asn Leu Xaa Gly Xaa Gly Xaa Tyr Met Arg
1 5 10 15

Asn Ser Lys Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly
20 25

<210> 44

<211> 37

<212> PRT

<213> Artificial

<220>

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<220>

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<220>

<221> misc_feature

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<220>

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<223> The artificial sequence includes the p24 peptide from HIV-1, a series of alternating aminocaproic acid and glycine residues, and the FAS-ligand from homo sapiens

<400> 44

Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser
1 5 10 15

Pro Arg Thr Leu Xaa Gly Xaa Gly Xaa Lys Val Glu Leu Met Tyr Pro
20 25 30

Pro Pro Tyr Phe Val
35

<210> 45

<211> 30

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<213> Artificial

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<220>

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<222> (16)..(16)

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<223> The artificial sequence includes a collagen II (1168-1180) peptide sequence, a sequence including aminocaproic acid, aminohexanoic acid and glycine and CD40L (93-104) from homo sapiens

<400> 45

Gln Tyr Met Arg Ala Asp Gln Ala Ala Gly Gly Leu Arg Xaa Gly Xaa
1 5 10 15

Gly Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu
20 25 30

<210> 46

<211> 31

<212> PRT

<213> Artificial

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<221> misc_feature

<222> (11)..(11)

<223> X is aminocaproic acid

<220>

<221> misc_feature

<222> (13)..(13)

<223> X is aminocaproic acid

<220>

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<222> (15)..(15)

<223> X is aminocaproic acid

<220>

<221> misc_feature

<223> The artifiicial sequence includes the HPV E7 peptide sequence fro
m human papilloma virus, a series of alternating aminocaproic aci
d and glycine residues, and the CD95 (143-155) peptide sequence o
f the FAS-ligand from homo sapiens

<400> 46

Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr Xaa Gly Xaa Gly Xaa Tyr
1 5 10 15

Met Arg Asn Ser Lys Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly
20 25 30

【国際公開パンフレット（コレクトバージョン）】

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(71) Applicant (*for all designated States except US*): **UNIVERSITY OF KANSAS** [US/US]; Strong Hall, Room 207, 2385 Irving Hill Drive, Lawrence, KS 66044 (US).

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(54) Title: SIGNAL-1/SIGNAL-2 BIFUNCTIONAL PEPTIDE INHIBITORS

(57) Abstract: A novel peptide sequence having the general formula AB wherein each of A and B represent a chain of amino acid residues and wherein said A chain is capable of binding with a mojor histocompatibility comples on an antigen presenting cell, and wherein said B chain is capable of binding wiht a Signal-2 receptor on an antigen presenting cell. Preferred forms of the peptide sequence further include and Y chain positioned intermediate the A chain and the B chain. Moreover, preferred forms include and A chain which has at least about 10% sequence homology with a Signal-1 moiety, or is a peptidomimetic of a Signal-1 moiety, said B chain has at least 10% sequence homology with a Signal-2 receptor moiety, said B chain has at least 10% sequence homology with a Signal-2 receptor moiety, or is a peptidomimetic of a Signal-2 receptpr moiety, and wherein the X chain has at least one amino acid residue, or is a peptidomimetic of that amino acid residue. Advantageously, the movel peptide sequence is capable of shifting a type-1 immune response to a type-2 immune response or from a type-2 immune response to a type-1 immune response.

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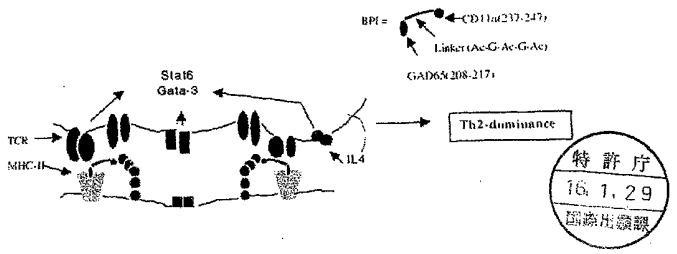

(57) Abstract: A novel peptide sequence having the general formula AB wherein each of A and B represent a chain of amino acid residues and wherein said A chain is capable of binding with a major histocompatibility complex on an antigen presenting cell, and wherein said B chain is capable of binding with a Signal-2 receptor on an antigen presenting cell. Preferred forms of the peptide sequence further include and Y chain positioned intermediate the A chain and the B chain. Moreover, preferred forms include and A chain which has at least about 10% sequence homology with a Signal-1 moiety, or is a peptidomimetic of a Signal-1 moiety, said B chain has at least 10% sequence homology with a Signal-2 receptor moiety, said B chain has at least 10% sequence homology with a Signal-2 receptor moiety, or is a peptidomimetic of a Signal-2 receptpr moiety, and wherein the X chain has at least one amino acid residue, or is a peptidomimetic of that amino acid residue. Advantageously, the novel peptide sequence is capable of shifting a type-1 immune response to a type-2 immune response or from a type-2 immune response to a type-1 immune response.

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SIGNAL-1/SIGNAL-2 BIFUNCTIONAL PEPTIDE INHIBITORS

SEQUENCE LISTING

A printed Sequence Listing accompanies this application, and has also been submitted with identical contents in the form of a computer-readable ASCII file on a floppy diskette and a CDROM.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention concerns immune responses initiated by the recognition of a peptide:MHC complex on the surface of antigen presenting cells by T-cells. The present invention also concerns immune responses initiated by the binding of a Signal-2 moiety to its complement protein on the surface of an antigen presenting cell. More particularly, the present invention concerns the immune responses initiated by the recognition of the peptide:MHC by the T-cell and by the binding of a Signal-2 moiety to its complement protein. Still more particularly, the present invention concerns the modification of the typical immune response generated by a particular individual in response to this binding. Most particularly, the present invention concerns the conjugation of peptides derived from the peptide portion of the peptide:MHC complex to the preferred Signal-2 moiety in order to modify or shift a given immune response from type-1 to type-2 or from type-2 to type-1. This may include specific phenotypes of regulatory T-cells including suppressor T-cells.

Description of the Prior Art

Autoimmune diseases are characterized by the activation of T-cells against self-antigens. These T-cells then destroy cells presenting these antigens. For example, insulin-dependent diabetes mellitus (IDDM, also called Type-I diabetes) is characterized by the activation of T-cells against the insulin-producing cells of the pancreas and their subsequent destruction by these T-cells. The diseases and conditions associated with autoimmune responses are strongly associated with specific subtypes (alleles) of cell surface proteins

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called major histocompatability complex (MHC) class II molecules. MHC molecules bind fragments (peptides) of proteins from infectious agents, allergens, and self proteins, and this MHC:peptide complex is the structure that T-cells recognize with their receptor (called the T-cell receptor, or TCR). The MHC:peptide complex is displayed on the surfaces of other cells of the immune system (i.e., B cells, dendritic cells and macrophages) which are called antigen presenting cells (APC). In order for an immune response to ensue, the major regulatory cell of the immune system, the undifferentiated T-cell, must be presented with small breakdown products (peptides) of the foreign invader. This presentation occurs on the surface of the APC. The T-cell must then interact with the APC, and this interaction stimulates the T-cell to divide and differentiate to produce molecules that attack, either directly or indirectly, cells displaying the same or highly similar MHC:peptide complex. It is well known that the genes that encode the MHC molecules are extremely variable within the species, and the different MHC alleles prefer to bind some peptides over others. Along with other genetic and environmental factors, the existence of different MHC alleles helps to explain why some members of a species develop conditions such as autoimmune diseases, allergies, asthma, and even certain infectious diseases, while others remain seemingly unaffected, or immune, to the same substances. Other differences arise because cell surface proteins distinct from the peptide:MHC complex must also bind to specific receptors on the T-cell. These other protein:protein pairs at the interface of the T-cell and APC membranes provide a costimulatory signal, known as Signal-2 which, along with the signal generated by the TCR recognition of the MHC:peptide complex (known as Signal-1), initiates an immune response.

A defining stage of the immune response is the differentiation of $CD4^+$ T-cells into either type-1 helper T-cells (T_H1 cells) or type-2 helper T-cells (T_H2 cells) as a result of the two signals. These two subtypes of T_H cells and the regulatory network of cells that they selectively activate are well-known correlates of human health conditions and disease states. Differentiation into T_H1 cells results in predominantly cell-mediated immunity while differentiation into T_H2 cells results in predominantly humoral immunity. Each of these immunity types helps to protect the body against different types of invasion. Type-1 immunity protects the body against intracellular pathogens such as bacteria, but is also implicated in organ-specific autoimmune diseases. Type-2 immunity is important for

protection against extracellular parasites, but is associated with allergic reactions as well. Development of T_H1 cells is driven by a cytokine called interleukin-12, which is produced by immune cells known as macrophages and dendritic cells. Interleukin-12 induces or stimulates the naive T-cell ($CD4^+$ T-cells) to produce interferon-γ (IFN-γ) and interleukin-2 (IL-2). These two cytokines (IL-2 and IFN-γ) are involved in classic cell-mediated functions such as clonal expansion of cytotoxic T-lymphocytes (CTLs), macrophage activation, and class switching to IgG isotypes that mediate complement lysis of sensitized cells. Commitment to a T_H1 immune response is enhanced by the presence of IFN-γ which up-regulates expression of the interleukin-12 (IL-12) receptor while inhibiting the development of T_H2 cells. T_H2 immunity results from the production of interleukin-4 (IL-4) by the naive T-cell. IL-4 induces T_H2 development and the subsequent production of interleukins-4 (IL-4), -5 (IL-5), -10 (IL-10), and -13 (IL-13). IL-4 also operates to down-regulate expression of the IL-12 receptor on developing cells, thereby inhibiting T_H1 development and helping undifferentiated T-cells to commit to T_H2 cell development. Additionally, IL-4 and IL-5 are known to activate B cells and switch to neutralizing antibody (IgG1 in the mouse) and IgE, the initiator of immediate hypersensitivity.

In order for either of these immune pathways to be activated, a two-signal mechanism is required to fully activate the T-cell. Signal-1 (S-1) occurs when the T-cell antigen receptor (TCR) recognizes the peptide:MHC-II complex on the surface of an antigen presenting cell (APC). This first signal passes through the T-cell receptor and initiates a cascade of tyrosine phosphorylation/dephosphorylation events mediated by kinases and phosphatases and leads to the activation of Ca^{++} flux, nuclear factor of activated T cells (NF-AT) and NFκB transcription factors. These factors enter the nucleus of the T-cell and bind to promoters of genes responsible for effector functions. Signal-2 (S-2) arises from the binding of Signal-2 receptors to their ligands on the surface of an APC. Signal-2 receptors include CD28 and its ligand B7 as well as LFA-1 and its ligand ICAM-1. When a Signal-2 receptor and its ligand form a complex at the interface between the T-cell and APC receptor membranes, a series of signaling events occur. These events include serine/threonine phosphorylation/dephosphory-lation and activation of guanine nucleotide exchange factors that activate adapter proteins with GTPase activity. These signaling events activate a separate set of transcription factors. The signal delivered through the CD28:B7 complex is

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distinct from that delivered from the ICAM-1:LFA-1 complex, particularly with respect to the differentiation of $CD4^+$ T-cells into T_H1 versus T_H2 effector populations. When the predominant binding occurs between LFA-1 and ICAM-1, the $CD4^+$ T-cell differentiation favors T_H1 cells which are abundant producers of IL-2 and IFNγ, the preeminent initiators of inflammatory immune responses including delayed-type hypersensitivity (DTH), immunity to intracellular pathogens, and several autoimmune diseases. When the predominant binding occurs between CD28 and B7, the $CD4^+$ T-cells differentiate into T_H2 cells. In contrast to T_H1 cells, T_H2 cells do not produce abundant IL-2 or IFNγ cytokines, but instead release the mediators of immediate-type hypersensitivity such as allergy and asthma, i.e., IL-4, IL-5, IL-10, and IL-13. Thus, the ability to manipulate the relative contribution of the complex providing the second signal has a profound effect on the type of immune response that is elicited against a given self-tissue antigen.

The associations between the TCR and APC occur at a specialized junction or interface between the TCR and the APC called the immunological synapse. An immune synapse is depicted schematically in Fig. 1. This immune synapse can be defined as the organized structure of activation molecules that assemble at the interface between the T-cell and the APC. Like a synapse in the nervous system, the immune synapse is a close association between cellular membranes. In order for an immune response to ensue, the major regulatory cell of the immune system, the undifferentiated T-cell must be presented with small breakdown products (peptides) of the foreign invader. In an unactivated T-cell, TCR and adhesion molecules are dispersed randomly on the T-cell membrane. The formation of the immunological synapse is an active and dynamic mechanism that allows T-cells to distinguish potential antigenic ligands. The immunological synapse consists of a central cluster of T-cell receptors surrounded by a ring of adhesion molecules. The stable formation of the immune synapse requires adhesion molecules such as LFA-1 and the peptide-recognition receptor (TCR) to form a doughnut-like structure with the TCR on the inside and LFA-1 on the outside. During activation, the TCR and LFA-1 molecules pass by each other within the T-cell lipid bilayer during the formation of the doughnut-like structure (this process is called translocation). If these molecules do not translocate within the immune synapse then the T-cell signal is not fully received and a different program of gene activity may ensue within the T-cell. This can drastically effect the immune response,

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especially if the T helper cell deviates from a gene program that would lead to IFNγ release (T_H1 cells and type-1 immunity) to a program that ultimately activates IL-4 production (i.e., T_H2 cells and type-2 immunity).

In more detail, to activate the pathway leading to T_H1 dominance, the TCR recognizes the peptide:MHC-II complex and sends Signal-1 to the T-cell. Additionally, LFA-1 binds to ICAM-1, and these molecules, along with the peptide:MHC-II complex, translocate to form the end-stage immune synapse. This leads to the effective expression of the CD40 ligand (CD154) by the uncommited T_H cell. CD40 interaction (expressed on the antigen presenting cell) with its ligand generates NFκB up-regulation of the inflammatory cytokine, IL-12. IL-12 then binds to its receptor on the undifferentiated T_H cell and initiates the T_H1 program, including the up-regulation of the transcription regulators, Stat4 and Tbet. This leads to T_H1 dominance against the autoantigen (e.g., glutamic acid decarboxylase, GAD65), which was initiated by the GAD65 peptide component of the TCR:peptide:MHC-II complex. For the pathway leading to T_H2 dominance, the TCR can recognize the same peptide:MHC-II complex, thereby sending Signal-1. However, in this case, a weaker strength of Signal-1 and/or altered or blocked binding between Signal-2 moieties leads to an altered form of the end-stage immune synapse. Likely, this lower strength of Signal-1 or distinct participation of the LFA-1 second signal leads to this different result, i.e., dominant T_H2 differentiation. For example, the altered immune synapse can dictate that the CD40 ligand is not expressed and IL-12 is therefore not released by the APC. This pathway is schematically represented in Fig. 2. Here, IL-4 appears to accumulate, thereby leading to the up-regulation of Stat6 and GATA-3 within the T-cell and hence commitment to a T_H2 pattern of differentiation.

A major goal of modern applied immunology is to be able to switch from T_H1-dominant immunity (e.g., as seen in autoimmune diseases and transplant rejection) to T_H2 responses against these same tissue antigens. In other cases, it would be extremely valuable to replace weak T_H2 immunity with T_H1 dominance leading to strong T-cell proliferation and the effective generation of cytotoxic T-cells (CTL). These cases may include chronic viral illnesses, like hepatitis-C and AIDS; and could include certain cancers like melanoma. Accordingly, what is needed in the art is modifiers of these immune responses so that type-2 immunity can be replaced with type-1 immunity or type-1 immunity can be replaced with

type-2 immunity, as desired in order to combat different human disease states or health conditions.

SUMMARY OF THE INVENTION

The present invention solves the problems found in the prior art and provides a distinct advance in the state of the art. Briefly, the present invention embraces a peptide which includes a portion of a Signal-1 moiety at one end and a portion of a Signal-2 moiety at the other end. These two ends can be directly connected to each other or connected via a flexible, non-substrate linker. This conjugation of the peptide portions directly and via a linker into a continuous peptide chain produces a new class of immunotherapeutic peptides termed bifunctional peptide inhibitors (BPI). These BPI are based upon the two signal mechanism of T-cell activation and link Signal-1 and Signal-2 moieties in order to alter T-cell activation. In other words, the present invention provides a method of modulating T-cells and subsequent immunity in a very specified manner such that only specific disease-associated populations of these cells are targeted by the products of the present invention. Thus, the present invention leaves necessary components of the intact immune system to operate in their nominal protective manner.

In more detail, the present invention describes constructing a peptide sequence having a TCR epitope of interest (a Signal-1 moiety) at one end and a peptide derived from the protein:protein interaction (the Signal-2 moiety) which generates Signal-2. These two peptide sequences can be connected via a flexible linker which couples the Signal-1 moiety to the Signal-2 moiety or can be directly linked together. In some cases, the linkage between the two peptides sequences may include flanking residues from each portion. The combination of the Signal-1 moiety coupled with the Signal-2 moiety constitutes a BPI. Accordingly, once a TCR epitope of interest is identified and the desired immune response (type-1 or type-2) determined, a BPI according to the present invention, can be generated.

As noted above, an important stage of the immune response is the differentiation of $CD4^+$ T-cells into either type-1 helper T-cells (T_H1 cells) or type-2 helper T-cells (T_H2 cells). Differentiation into T_H1 cells results in predominantly cell-mediated immunity while differentiation into T_H2 cells results in predominantly humoral immunity. Each of these immunity types help to protect the body against different types of invasion. T_H1 cells protect the body against intracellular pathogens such as bacteria, and are also implicated in organ-

specific autoimmune diseases. T_H2 cells are important for protection against extracellular parasites as well as allergic reactions. Development of T_H1 cells is driven by a cytokine called interleukin-12, which is produced by immune cells known as macrophages and dendritic cells. Interleukin-12 induces or stimulates the naive T-cell to produce interferon-γ (IFN-γ) and interleukin-2 (IL-2). These two cytokines (IL-2 and IFN-γ) are involved in classic cell-mediated functions such as clonal expansion of cytotoxic T-lymphocytes (CTLs), macrophage activation, and class switching to IgG isotypes that mediate complement lysis of sensitized cells. Commitment to a T_H1 immune response is enhanced by the presence of IFN-γ which up-regulates expression of the interleukin-12 (IL-12) receptor while inhibiting the development of T_H2 cells. This pathway is shown schematically in Fig. 3.

T_H2 immunity results from the production of interleukin-4 (IL-4) by the naive T-cell. IL-4 induces T_H2 development and the subsequent production of interleukins 4 (IL-4), 5 (IL-5) and 13 (IL-13), through activation of the transcription regulator Stat6. IL-4 also operates to down-regulate expression of the IL-12 receptor on developing cells, thereby inhibiting T_H1 development and helping undifferentiated T-cells to commit to T_H2 cell development. Additionally, IL-4 and IL-5 are known to activate B cells and switch to neutralizing antibody (IgG1 in the mouse) and IgE, the initiator of immediate hypersensitivity. Again, a schematic representation of this process is depicted in Fig. 2.

As noted above, a two-signal mechanism is required to fully activate the T_H cell. Signal-1 occurs when the T-cell antigen receptor (TCR) recognizes or engages the peptide:MHC-II complex on the surface of an antigen presenting cell (APC). This first signal is transmitted through the T-cell receptor and initiates a cascade of tyrosine phosphorylation/dephosphorylation events mediated by kinases and phosphatases and leads to the activation of Ca^{++} flux, NF-AT and NFκB transcription factors. These factors enter the nucleus of the T-cell and bind to promoters of genes responsible for effector functions. Signal-2 arises from the binding of a Signal-2 receptor on the T-cell to its protein ligand on the APC. Signal-2 receptors include CD28 and its ligand B7 as well as LFA-1 and its ligand ICAM-1. When a Signal-2 receptor and its ligand form a complex at the interface between the T-cell and APC membranes, a series of signaling events occurs including serine/threonine phosphorylation/dephosphorylation along with actuation of guanine nucleotide exchange factors that activate adapter proteins with GTPase activity. These

signaling events activate a separate set of transcription factors. The signal delivered through the CD28:B7 complex is distinct from that delivered from the ICAM-1:LFA-1 complex, particularly with respect to the differentiation of $CD4^+$ T-cells into T_H1 versus T_H2 effector populations. A schematic representation of this signaling is provided herein as Fig. 4. When the predominant binding occurs between LFA-1 and ICAM-1, the $CD4^+$ T-cells differentiate into T_H1 cells. The $CD4^+$ T-cells of the T_H1 differentiation state are abundant producers of IL-2 and IFNγ, two cytokines that are the preeminent initiators of inflammatory immune responses, such as delayed-type hypersensitivity (DTH), immunity to intracellular pathogens, and several autoimmune diseases. When the predominant binding occurs between CD28 and B7 (i.e., decreased LFA-1:ICAM-1 signaling), the $CD4^+$ T-cells differentiate into T_H2 cells. In contrast to T_H1 cells, T_H2 cells do not produce IL-2 and IFNγ cytokines, but instead release the mediators of immediate-type hypersensitivity such as allergy and asthma, i.e., IL-4, IL-5, IL-10, and IL-13. Thus, the ability to manipulate the relative contribution of the complex providing Signal-2 has a profound effect on the type of immune response that is elicited against a given self-tissue antigen.

The associations between the TCR and APC occur at a specialized junction called the immunological synapse (shown in Fig. 1). In order for the immune response to proceed, the undifferentiated T_H cell, must be presented with peptides of the foreign invader on the surface of the APC. In an unactivated T-cell, TCR and adhesion molecules are dispersed randomly on the T-cell membrane. The formation of the immunological synapse is an active and dynamic mechanism that allows T-cells to distinguish potential antigenic ligands. The immunological synapse consists of a central cluster of T-cell receptors surrounded by a ring of adhesion molecules. This arrangement is depicted schematically in Fig. 1. In this figure, the TCR:peptide:MHC-II complex is in the center of the dark circle which represents the protein:protein pair constituting the Signal-2 receptor and the Signal-2 ligand. The stable formation of the immune synapse requires adhesion molecules such as LFA-1 and the peptide-recognition receptor (TCR) to form a doughnut-like structure with the TCR on the inside and LFA-1 on the outside. During activation, the TCR and LFA-1 molecules actually translocate past one another within the T-cell lipid bilayer. If these molecules do not translocate within the immune synapse then the T-cell signal is not fully received and a different program of gene activity may occur within the T-cell. This can drastically effect

the immune response, especially if this causes the T helper cell (T_H) to deviate from a gene program leading to a T_H1 immune response to a program that activates a T_H2 immune response. As shown in Fig. 2, an interpretation of the BPI mechanism suggests that BPI bind to both the MHC-II and second signal ligands. This effectively tethers the MHC-II:peptide and ICAM-1 moecules thereby preventing the translocation step of immune synapse formation.

In one aspect of the present invention, known TCR epitopes are used as the first peptide portion of the BPI. In this manner, minimal peptide sequences that are potent immunogens are utilized. These minimal peptide sequences (e.g. antigenic peptides) effectively engage the TCR involved in immune responses of interest (i.e. autoimmune diseases, infectious diseases, allergies, cancers, etc). There are already many known TCR epitopes of interest (Signal-1 moieties) and their sequences have been defined in the literature. A partial list of some representative Signal-1 moieties is provided in Table 1. This list is by no means exhaustive as there are potentially thousands of Signal-1 moieties.

In another aspect of the present invention, TCR epitopes of interest are identified so that the first portion of the BPI can be synthesized. In this aspect, these dominant TCR epitopes have been so determined by previous art and the sequences are available in the literature. The peptide to which a given T-cell response is focused upon, (e.g., the response against the diabetes-associated antigen GAD65) is identified by the fact that most effector T-cells respond to this portion of the antigen and not other portions. In mouse model systems, animals are immunized with the whole protein antigen. Next, T-cells are removed after the antigen has primed the immune system. These T-cells are placed separately in cultures with short overlapping peptides of the antigen. Most of the response will be to a single peptide and this is the dominant TCR epitope. In humans, T-cells are first cloned from patients. These cloned T-cells are placed separately in cultures with overlapping peptides (again, representing individual portions of the antigen involved, e.g., HIV-1, p24 (SEQ ID No. 8)). Again, the peptide to which most T-cell clones respond is the dominant TCR epitope. The foregoing is described by Schountz et al., *MHC Genotype Controls the Capacity of Ligand Density to Switch T Helper (Th)-1/Th-2 Priming In Vivo*, 157 The Journal of Immunology 3893-3901 (1996), the teachings and content of which are hereby incorporated by reference herein.

In another aspect of the present invention, peptides derived from Signal-2 receptors are used to alter interactions between the nominal receptors on T-cells and their complementary ligands on the APC surface. Table 3 includes a representative list of some known Signal-2 receptor moieties. Of course, those of ordinary skill in the art will be able to identify other Signal-2 moieties not listed therein, as this list is representative and not all-inclusive.

Another aspect of the present invention is the linking of the TCR epitope (i.e. the Signal-1 moiety) to a Signal-2 receptor peptide mimic (i.e., the Signal-2 moiety) in order to modify the resultant immune response. This linkage can be between the Signal-1 moiety and the Signal-2 moiety directly, or through flanking residues. Alternatively, this linking can be done via a linker which is positioned between the Signal-1 moiety and the Signal-2 moiety. The linker could be any amino acid including naturally occurring or chemically synthesized amino acids. Preferably, non-substrate amino acids will be used due to their resistance to protease attack. Still more preferably, the linker will comprise a non-substrate amino acid alternating with a small or hydrophilic amino acid. Even more preferably, the linker is synthesizable as one continuous sequence along with the Signal-1 and Signal-2 moieties, which flank the linker at each respective end. Still more preferably, the linker has the general formula $(A,B)_X$, wherein A and B are amino acid residues, and the A amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine, and the B amino acid residue is a small or hydrophilic amino acid. In this formula, X can range from 1 to 100. A particularly representative B residue is glycine. In this embodiment, a linker could potentially have aminocaproic acid (Ac), aminohexanoic acid (Ahx), aminododecanoic acid (Ado), and β-alanine (βA) alternating with glycine residues (G) (e.g., Ac-G-Ahx-G-Ado-G-βA). The choice of the residues used to construct the linker can be based upon the desired length of the linker as well as steric hindrance considerations. One preferred linker comprises alternating Ac and G residues. This linker can be lengthened or shortened by the inclusion of the other amino acid residue choices (Ahx, Ado, βA). Some representative linkers are included in Table 2 as SEQ ID Nos. 26-29.

Approximately 10^9 different TCR account for protective immunity to the universe of infectious agents and contain the repertoire of TCR that may turn against self in

autoimmune diseases. Moreover, the TCR are also specific for potential tumor antigens and the myriad of allergenic substances in the environment. By changing the TCR epitope of a given BPI we direct the immunomodulating capacity of the BPI to a select group of TCR. In other words, the selection of a TCR epitope to incorporate into the BPI targets T-cells that are involved in a particular human disease in a highly specific fashion. For example, incorporating the GAD65 epitope into a BPI targets autoaggressive T-cells involved in the induction of type-1 diabetes. This targeting to specific TCR allows that T-cells necessary for immunity to infectious agents or cancers will not be significantly compromised. Thus, BPI offer the possibility to specifically modulate T-cell immunity to one antigen while leaving intact the T-cell repertoire necessary for protective immunity to infectious agents and developing cancers.

As noted above, the Signal-1 moieties of the present invention are preferably derived from TCR epitopes and a list of representative known epitopes is provided in Table 1 wherein these known epitopes are presented as SEQ ID Nos. 1-25. When a derivative of a TCR epitope is used to construct the BPI, preferably, the TCR epitope selected will be correlated with a known health condition or disease state. When using one of the representative peptides shown in Table 1 to construct the BPI, it is preferred that the peptides include a sequence having at least about 10% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. More preferably, the peptide will have at least 30% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. More preferably, the peptide will have at least 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. Even more preferably, the peptide will have at least 70% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. Most preferably, the peptide will have at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25. Of course, it is also well known in the art to use peptidomimetics to construct moieties having similar functions as the peptides derived from the TCR epitopes. In this respect, the teachings of Falcioni et al. in *Peptidomimetic Compounds That Inhibit Antigen Presentation by Autoimmune Disease-Associated Class II Major Histocompatability Molecules*, 17 Nature Biotechnology, 562-567 (1999), are incorporated by reference herein. Accordingly, all or part of this Signal-1 moiety portion of the BPI can include such

peptidomimetics. Preferably, the peptidomimetic will be a mimetic of a peptide selected from the group consisting of SEQ ID Nos. 1-25. Alternatively, the Signal-1 moiety will be a derivative of a TCR epitope or a peptide selected from the group consisting of SEQ ID Nos. 1-25. At any rate, it is desired that this first portion of the BPI (or the portion responsible for initiating the first signal) be capable of binding with a major histocompatability complex (MHC) on an antigen presenting cell (APC). Furthermore, it is preferred that this resulting peptide:MHC complex be capable of engaging important TCR and initiating some form of the signal to the T-cell.

As noted above, the peptides used on the side of the linker opposite the Signal-1 moiety are preferably derived from Signal-2 receptors. This second portion of the BPI is connected to the first portion either directly or via the linker. In preferred forms, the second portion includes a sequence having at least about 10% sequence homology with a sequence selected from a group consisting of SEQ ID Nos. 30-41. More preferably, the second portion peptide has at least about 30% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41. Still more preferably, the second portion peptide has at least about 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41. Even more preferably, the second portion peptide has at least about 70% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41. Most preferably, the second portion peptide includes a sequence having at least about 95% sequence homology with a sequence selected from the group of SEQ ID Nos. 30-41. As with the first portion, peptidomimetics can be used in place of all or some of the amino acid residues of the second portion. In preferred forms the peptidomimetic of the second portion will be a mimic of a peptide selected from the group consisting of SEQ ID Nos. 30-41. Alternatively, the second portion of the BPI will comprise a derivative of a peptide selected from the group consisting of SEQ ID Nos. 30-41. Similar to the first portion, it is preferred that the second portion be capable of binding with a complementary ligand (e.g. the Signal-2 ligand) on an antigen presenting cell. For example, when a peptide derived from LFA-1 is used as this Signal-2 moiety of the BPI, it should bind to ICAM-1 on the surface of the APC. Additionally, it is preferred that this binding with the Signal-2 ligand on the APC inhibits or alters the binding of the moiety's parent receptor (on the T-cell) to this same APC ligand.

As explained above, the immune response involves a two signal mechanism and the purpose of the present invention is to modify a given immune response, e.g., from type-1 immunity to type-2 immunity or from type-2 immunity to type-1 immunity. This modification or shifting of immune response phenotype is brought about by BPI according to the present invention. It is preferred in some cases for the BPI to modify an immune response from a T_H1 dominated or cytolytic immune response to a T_H2 dominated response; and, in other cases, it is preferred for the BPI to modify an immune response from a T_H2 dominated response to a T_H1 or cytolytic dominated response. In some cases, BPI may operate via the activation of very specific T-cell phenotypes, e.g., peptide-specific suppressor T-cells. In contrast to the nominal situation where an antigen stimulates the system toward a T_H1 response (depicted in Fig. 2), the response generated when a BPI similar to the GAD 65-CD11a BPI is introduced into the immune synapse is quite different and operates to shift the response from type-1 to type-2. This situation is depicted schematically in Fig. 2. In this manner, a BPI comprising a Signal-1 moiety, a flexible, non-substrate linker, and a Signal-2 moiety is formed and introduced into the immune synapse. The TCR recognizes the peptide:MHC complex on the APC and initiates the first signal. However, the second portion of the BPI (the Signal-2 moiety) blocks the typical Signal-2 interaction occurring between LFA-1/ICAM-1, (or for other BPI:CTLA-4/B7, or CD40L/CD40, or FasL:Fas) and the translocation of the TCR into the central cluster. Depending on whether the LFA-1/ICAM-1 or CTLA-4/B7 interaction is targeted by the specific BPI construction, perhaps by tethering the MHC-II:peptide complex to the second signal ligand, the signal will be altered in a different direction of differentiation. For example, when the Signal-2 peptide portion of the BPI is derived from LFA-1, this would favor a decrease in CD40-ligand expression and hence, a lack of IL-12 release. By contrast, IL-4 released during the initial T-cell activation will accumulate to higher levels surrounding the synapse. This accumulation of IL-4 leads to Stat6 and GATA-3 up-regulation in the naive T-cell and ultimately to commitment to a type-2 pattern. Alternatively, when the Signal-2 moiety peptide portion of the BPI is derived from CTLA-4, the normal binding of CTLA-4 and CD-28 to B7 ligands is affected and thus more CD40 ligand is expressed (i.e., a greater role for high affinity LFA-1:ICAM-1 is dictated by blocking the B7 receptors); hence, the release of IL-12 increases. Interleukin-12 induces or stimulates the naive T-cell to produce more IFN-γ

and IL-2, thus providing a positive feedback toward type-1 immunity. These two cytokines (IL-2 and IFN-γ) are involved in classic cell-mediated functions such as clonal expansion of cytotoxic T-lymphocytes (CTLs), macrophage activation, and class switching to IgG isotypes that mediate complement lysis of sensitized cells. Such responses are hallmarks of protective immunity against human viral diseases. It will also operated to link TCR epitopes to the receptor for Fas. Since Fas:FasL interaction governs apoptosis, it will be possible to increase the frequency of specific TCR-bearing cells by blocking the apoptotic event. This will be important for BPI design against HIV, HPV, HCV, and cancers.

Thus, an important aspect of the present invention is that tethering a specific TCR epitope to a Signal-2 receptor peptide mimic leads to alteration of T-cell differentiation involving T-cells bearing only these receptors and/or T-cell populations indirectly linked to these peptide specific subsets. The ability to block or alter T-cell responses to a given immunodominant peptide antigen would offer extremely precise treatments for immunopathological conditions. A major drawback to current immunotherapies is that broad specificities of T-cells are affected leaving the host more susceptible to infections and cancers. The BPI of the present invention should block and/or alter only the desired T-cell population and subsequent responses that depend on these initial T-cells. Also, BPI will target a specific TCR-bearing population for activation toward a desired effector function.

In another aspect of this invention, the relative strength of signal generated by the T-cell-APC interaction has an affect on whether the ultimate immune response is a type-1 or a type-2 response. In this regard, the teachings of Murray in *How the MHC Selects T_H1/T_H2 Immunity*, 19 Immunology Today 157-163 (1998) are hereby incorporated by reference.

In another aspect of the present invention, an immune response is modified by contacting an APC with a peptide capable of binding to an MHC and to a Signal-2 ligand on the APC and causing an altered signal to be transmitted to the T-cell. Thus, the immune response is deviated from the immune response generally associated with the immunogenic peptide and its corresponding antigen (i.e., infectious agent, self protein, or allergen).

In another aspect of the present invention, a peptide having the general formula AXB is provided. The A, X, and B represent a chain of amino acid residues wherein the A chain has at least about five residues and at least about 10% sequence homology with a TCR

epitope, the B chain has at least four residues and at least about 10% sequence homology with a peptide derived from a Signal-2 moiety, and the X chain is a linker. The linker could be any amino acid including naturally occurring or chemically synthesized amino acids. Additionally, the X chain has at least one residue. It is possible to link A to B directly without X as well, although a linker of some size is preferred in order to span the distance between the MHC-II and second signal ligands on the APC surface. As noted above, preferably, non-substrate amino acids will be used due to their resistance to protease attack. Still more preferably, the linker will comprise a non-substrate amino acid alternating with a small or hydrophilic amino acid. Even more preferably, the linker is synthesizable as one continuous sequence along with the Signal-1 and Signal-2 moieties, which flank the linker at each respective end. Still more preferably, the linker has the general formula $(A,B)_x$, wherein A and B are amino acid residues, and the A amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine, and the B amino acid residue is a small or hydrophilic amino acid. In this formula, X can range from 1 to 100. A particularly representative B residue is glycine. In this embodiment, a linker could potentially have aminocaproic acid (Ac), aminohexanoic acid (Ahx), aminododecanoic acid (Ado), and β-alanine (βA) alternating with glycine residues (G) (e.g., Ac-G-Ahx-G-Ado-G-βA). The choice of the residues used to construct the linker can be based upon the desired length of the linker as well as steric hindrance considerations, hydrophobicity, charge, etc. One preferred linker comprises alternating Ac and G residues. This linker can be lengthened or shortened by the inclusion of the other amino acid residue choices (Ahx, Ado, βA). Some representative linkers are included in Table 2 as SEQ ID Nos. 26-29. Additionally, the X chain is positioned between the A chain and the B chain and the entire peptide can be synthesized as one continuous sequence. Some preferred sequences will have an A chain having at least about 10% sequence homology with any one of SEQ ID Nos. 1-25, an X chain having at least about 2% sequence homology with any one of SEQ ID Nos. 26-29, and a B chain having at least about 10% sequence homology with any one of SEQ ID Nos. 30-41. Preferably, the peptide is capable of shifting a type-1 response to a type-2 response, or vice versa. Of course, peptidomimetics may be synthesized to mimic any part of the BPI, including the linker. Preferably, the A chain binds to the MHC on an APC to form a

peptide:MHC complex. This complex is capable of engaging the TCR on critical T-cell populations. Still more preferably, the B chain is capable of binding to a Signal-2 ligand on the APC at the same time as the formation of the peptide:MHC complex. This combined binding to the APC should be capable of altering the signal delivered to the T-cell. The combination of the first signal and the second signal are capable of fully activating a T-cell and by selecting the peptide used for the A chain and the peptide used for the B chain, the immune response can be deviated from its normal progression. In the case of a normally activated type-1 response leading to the up-regulation of T_H1 cells, the response can be altered to give a type-2 response leading to the up-regulation of T_H2 cells. In the case of a normally activated type-2 response leading to the up-regulation of T_H2 cells, the response can be altered to give a type-1 response leading to the up-regulation of T_H1 cells. Again, the A chain can be chosen based on the health condition normally associated with the sequence (for example, see Table 4).

In another aspect of the present invention, a method is provided for preparing a peptide for modulating immune responses. This method comprises the steps of selecting a first peptide sequence which has at least about 10% sequence homology with a sequence derived from a TCR epitope, selecting a second peptide sequence which has at least about 10% sequence homology with a sequence derived from a Signal-2 receptor moiety, selecting a third peptide sequence which is a flexible, non-substrate linker, and synthesizing the peptides as a continuous peptide chain. Preferably, the linker is flanked on one end with the peptide derived from the TCR epitope and flanked on the other end with the peptide derived from the Signal-2 moiety. Preferably, the first peptide sequence should be associated with a known health condition and be capable of binding with an MHC on an APC. Similarly, it is preferred that the second peptide sequence be capable of binding with a Signal-2 ligand moiety on the APC. The method can further comprise the step of contacting the nominal peptide immunogen with the TCR, thereby binding the first peptide sequence to the MHC and the second peptide sequence to the Signal-2 ligand, thereby generating APC bearing potent first and altered/blocked second signal ligands which activate a desired immune response.

Inherent in the BPI design is the antigen-specific moiety that a given T-cell population is activated to respond against (i.e., the TCR epitope), ultimately leading to the

cascade of immune reactions that generate protective or in some cases pathologic immune responses. These epitopes when bound to major histocompatibility complex (MHC) molecules on the antigen presenting cell (APC) surface provide the Signal-1 of the two-signal mechanism of T-cell activation. Thus, an important consideration is the affinity of a given peptide for MHC molecules. This affinity was directly tested by the binding of biotinylated peptide to the cells that present the peptide:MHC complex to the T-cells in mouse and man. T-cell clones to possible peptide epitopes were generated and tested for binding to the immunodominant TCR of a response and specifically stimulate T-cell functions in vitro by the ELISPOT assay. Because modifications in the peptide residues that actually contact the TCR are part of the BPI development, it is also preferable that known crystallographic structures of the epitope bound to MHC molecules are available. This allows for precise three-dimensional predictions of how a particular amino acid substitution or mimetic will affect the actual structure encountered by the developing T-cells. However, in the absence of known crystal structures, it is possible to predict the shape of a hypothetical peptide:MHC structure based upon the available coordinates of other peptide:MHC structures. This predictability is partially due to established peptide binding motifs, that allow for prediction of which residues of the epitope fit most readily into the particular binding pockets of a given MHC allele. These predictions have been accomplished using two different alleles ($I\text{-}A^s$ and $I\text{-}A^b$) binding a collagen peptide wherein each polymorphic position of the I-A molecules were substituted with the known amino acids at these positions. The overall structure was based upon the published coordinates of a reference allele $I\text{-}A^k$ binding a different peptide. Thus, in cases where the 3D structure of a particular disease-associated epitope is not known, a structure for predicting where the TCR contacts the peptide by a similar allelic-substitution and modeling approach can still be generated. It is important to identify (or at least predict) these TCR contact positions. It is well-known that certain alterations to TCR-contact positions can change the functional differentiation of T-cells into the T_H1 or T_H2 types that can determine the course of immunity (see Murray, et al., Major Histocompatibility Complex (MHC) Class II Molecules Direct TCR-Specificity for Opposite Ends of the Same Immunogenic Peptide in T_H1 or T_H2 responses (unpublished manuscript, 2000); and Murray; 19 *Immunology Today* 157-163 (1998), the teachings of which are hereby incorporated by reference.

Specifically, it was verified that the peptide binds to MHC molecules on live APC. Briefly, spleen cell density-gradient fractions (from mice), or PBL, or APC lines (from humans) were incubated in round bottom 96-well plates with increasing concentrations of individual biotinylated peptides at 37°C, 5% CO_2 for 16 hours. Following binding of the BPI to the APC, Avidin-FITC was incubated with the cells on ice for 30 minutes, followed by biotinylated anti-Avidin for 1 hour, then again with Avidin-FITC. For BPI titrations, increasing concentrations (0.1-100 μM) of the biotinylated derivatives in sterile 0.5% BSA-PBS were incubated with the APC for 16 hours as above. As shown in Fig. 5, the GAD 65 BPI binds preferentially to NOD APC as predicted. The LFA-1 moiety or the GAD 65 moiety did not display this increased binding to the diabetes strain's APC. Three-color analyses used Cy-Chrome or PE-conjugated antibodies to known surface markers of APC that were commercially available. Avidin-FITC and biotinylated anti-Avidin detection of the bound peptide was as previously described in Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996). Slight modifications of this method were used to test peptide inhibition (i.e., peptide affinity) and co-capping to the MHC and ICAM molecules directly on the live APC surface. First, monoclonal antibodies (mAb) against MHC or ICAM molecules blocked BPI binding, therefore showing that the BPI binds to these molecules (Fig. 6). Unlabeled peptide was used in a competition assay to derive the relative affinity of the BPI for the APC and the ability of the BPI to crosslink the MHC and ICAM molecules on the APC was examined by cocapping ICAM with an anti-MHC mAb. Here, cells were incubated with the BPI. Next, an anti-MHC mAb was added. After 30 minutes at 37°C, the cells were transferred to ice and stained with PE-labeled anti-ICAM (Fig. 7).

Next, T-cell clones were generated for determination of TCR epitopes for later use in BPI. These experiments utilized CD4+ or CD8+ T-cell clones from humans or mice immunized against predicted TCR epitopes using previously described methods (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). These clones were maintained by biweekly restimulation with irradiated histocompatible lymphocytes, the peptide, and recombinant IL2. To determine if a given TCR-epitope is effective for the activation to cytokine synthesis, an ELISPOT assay was used. Of course, other cytokine

assays could also be used. For analysis, BPI that have been substituted at predicted TCR-contact positions will be used to determine which of these BPI variants are most effective in the inhibition of proliferation and cytokine release from individual clones as analyzed above. Predicted positions will be scanned with different amino acids or mimetics to alter the interaction with the TCR in the structures generated by molecular modeling.

Next, Molecular Dynamics/Energy Minimizations (MDEM) will be used to study the peptide conformations. MD simulations will be used in conjunction with crystallographic data to predict the solution conformations of the BPI. MDEM simulations can predict flexibility and conformational changes during binding of BPI to MHC-II and ICAM-1. Amino acid substitutions can also be introduced into the Signal-1 and/or Signal-2 moieties in order to favor simultaneous binding to both receptors, as first predicted by these model studies. The I-A^{g7}:BPI:D1 structure (see Fig. 8) is an example of using the crystal structure coordinates of a disease-associated peptide:MHC structure (I-A^{g7}:GAD65 peptide). For this figure, docking studies were performed on a Silicon Graphic Octane workstation using InSight II (MSI/Biosym). The LFA-1-ICAM-1 structure was taken from known diffraction coordinates and the I-A^{g7}:GAD65 peptide was from known coordinates. Of course, analogous methods can be used to model the BPI listed in Tables 1, 2, 3 and 4 for binding to the various MHC molecules of mouse and man.

The second stage in the BPI process is selection of peptide mimics of established second signal receptor molecules involved in the functional differentiation of T-cells. To determine these structures, the crystallographic structures and available models of the second signal receptors bound to their physiological ligands will be used to predict the regions of the receptors that make contact with the ligand. This approach was used to design the EGAD-BPI which can be depicted as

(EIAPVFVLLE)-(AcGAcGAc)-(ITDGEATDSG)
GAD65(208-217) linker LFA-1(237-247)

These interactions can also be determined empirically by making site-directed changes in the second signal molecules and determining whether such mutant molecules still bind to the ligand in question. However, many of these studies have been performed with

known second signal receptors, including LFA-1, CD28/CTLA4, CD40L, and CD95L. Molecular modeling studies will be used as above to model the interactions of these second signal moieties with their ligands separately and when the moiety is part of the BPI structure.

Once candidate BPI moieties that bind effectively to the MHC and second signal ligands and that alter T-cell functions in vitro were identified, these moieties were synthesized at opposite ends of relatively short synthetic peptides, connecting them by a non-proteolytic substrate linker of variable length. As described by the prototypical EGAD-BPI, the Signal-1 moiety (i.e., the disease-associated TCR epitope) was linked by this flexible linker to the Signal-2 receptor mimic peptide (i.e., a second signal receptor thought to be involved in T_H1/T_H2 differentiation. These synthetic peptides were generated by conventional methods of peptide synthesis. In some cases, BPI are tested for binding to isolated MHC and second signal ligands, and NMR, molecular modeling and crystallography are used to determine their exact 3D structures. Finally, it was determined whether a given BPI was biologically active *in vivo*. Mice were treated with the BPI and immune cells isolated and tested for cytokine production by ELISPOT (see Figs. 9 & 10). Of course other cytokine assays will be well known to those of ordinary skill in the art and can be used in place of ELISPOT. *In vivo* models similar to the NOD.Scid model (i.e., human Scid, transgenic knockout strains, etc.) were used, with modifications necessary for each disease being examined, as a more stringent test of BPI efficacy. For example, in the case of the HIV-1 p24 TCR-epitope, human-Scid mice are used for the adoptive transfer experiments. In this experiment, T-cells from patients will first be cloned by conventional methods and stimulated with the BPI *in vitro*. Next, these cells will be transferred into the human-Scid mice and analyzed as with the EGAD-BPI in the NOD.Scid adoptive transfer experiment. Results from these experiments are given in Figs. 11-13.

To synthesize the BPI, Fmoc chemistry on chlorotrityl resins was used. Protected amino acids were double coupled at 8-fold excess for 1 hour. Resins were DMF and MeOH washed and cleaved in Reagent R: TFA, EDTA, Thioanisole, Anisole. The TFA mixture containing the peptide in solution was precipitated in ether and washed extensively. Preparative HPLC of peptides was accomplished by a gradient of 0-80% acetonitrile in 0.1% TFA. Lyophilization of the various fractions and verification by MALDI-TOF using a Voyager mass spec (PerSeptive, Foster City, CA) yielded the synthetic peptide as a TFA salt.

Modeling, crystallography and binding studies, as described above, were used to generate the predicted BPI complex structure.

In another example which tested the diabetes BPI *in vivo*, 15 million disease-linked lymphocytes (i.e., patient T-cells, or T-cell populations linked to the disease process) were injected with or without T-cells that received the BPI compound (in vivo or in vitro) and were expanded for 24 hours in recombinant IL-2. Some experiments will deplete specific subsets of the T-cells using mAb to CD154, CD25, CD62L, CD152, etc. and magnetic particles prior to adoptive transfer. T-cells (from mice or humans) treated with the individual moieties of the BPI will be used as negative controls along with CD4+ cells from mice treated with saline alone (Fig. 9). For blocking spontaneous diabetes, five groups of ten female NOD mice (12 weeks of age) were used and monitored for nondiabetic blood glucose levels with a standard glucometer (AccuChek-complete, Roche Diagnostic). Each mouse was labeled and individually monitored for blood glucose levels weekly for the course of the experiment. The five groups received either (a) intravenous (i.v.) injection of the BPI (100 μg in 100 μl endotoxin-free saline/injection) at 8 weeks of age, (b) same dose GAD65 (208-217) epitope alone, (c) same dose CD11a (237-247) peptide alone, or (d) saline alone. For other systems, similar treatment groups involving the different Signal-1 and Signal-2 peptides and BPI will be used. Mice will be tested by challenging with the appropriate infectious agent or antigen depending upon the particular BPI in question.

To evaluate the disease process by immunohistology (*see*, e.g., Fig. 12A-D), spleen, pancreas, or other target organs, e.g., the CNS for the MBP peptide BPI, or lung for the RSV peptide BPI, were removed from euthanized mice of each group and prepared for histology by fixing in neutral buffered formalin and embedding in paraffin, or snap frozen in O.C.T. medium. For scoring inflammation, minimums of five sections from each mouse were used to assess the blocking affect of a given BPI. For characterization of standard T-cell markers on cellular infiltrates, biotinylated mAb to various cell surface antigens will be incubated individually with the Cryostat sections (2 hours), followed by avidin-alkaline phosphatase (Vector laboratories). Alternatively, cell subsets will be phenotyped by standard flow cytometry methods as described in Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996). Finally, the students t-test or ANOVA will be used to

estimate the statistical significance of differences observed between groups and individual mice.

A few representative assembled BPI consisting of a Signal-1 moiety and a Signal-2 receptor moiety joined together via a linker are provided in Table 4 as SEQ ID Nos. 42-46. These representative BPI are operable for shifting specific immune responses from a type-1 to a type-2 response and vice-versa. Advantageously, other immune responses to other antigenic peptides will be preferably unaffected.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1 is a schematic representation of an immune synapse between a T cell and an APC illustrating the doughnut structure of the TCR:peptide:MHC location;

Fig. 2 is a schematic representation of how a representative BPI blocks differentiation leading to a T_H1 dominated immune response and shifts immunity to T_H2 dominated immune response;

Fig. 3 is a schematic representation of nominal activation of a type-1 immune response through the interactions between cell surface proteins within the immune synapse;

Fig. 4 is a simplified schematic representation of the two signal mechanism of T-cell activation;

Fig. 5a is a graph representing the results of a flow cytometry analysis comparing the binding of a representative BPI to different mouse strains;

Fig. 5b is a graph representing the results of a flow cytometry analysis comparing the binding of a representative BPI portion to different mouse strains;

Fig. 5c is a graph representing the results of a flow cytometry analysis comparing the binding of a representative BPI portion to different mouse strains;

Fig. 6a is a graph illustrating the results of a flow cytometry analysis of a representative BPI binding to the APC of a mouse strain, without antibodies to MHC-II or ICAM-1;

Fig. 6b is a graph illustrating the results of a flow cytometry analysis of a representative BPI binding to the APC of a mouse strain, when antibodies to ICAM-1 are present;

Fig. 6c is a graph illustrating the results of a flow cytometry analysis of a representative BPI binding to the APC of a mouse strain, when antibodies to MHC-II are present;

Fig. 7 is a color photograph representing the results of a fluorescent microscopy analysis of a representative BPI simultaneously binding to MHC-II and ICAM-1 structures on the NOD APC by co-capping with antibodies to MHC-II, the top panels are from mice APC treated with a representative BPI and the bottom panels are treated with just the saline vehicle;

Fig. 8 is a color photograph of the molecular model of a representative BPI binding to the NOD mouse's MHC-II ($I\text{-}A^{g7}$) and the D1 domain of ICAM-1, MHC-II is shown in pink, ICAM-1 is in light blue, the BPI is shown by atom with the carbon in green, oxygen in red, and nitrogen in blue;

Fig. 9a is a graph representing the ELISPOT analysis of IL-4 cytokine release by T-cells taken from NOD mice treated with the EGAD-BPI or the saline control;

Fig. 9b is a graph representing the ELISPOT analysis of IFN-γ cytokine release by T-cells taken from NOD mice treated with the EGAD-BPI or the saline control;

Fig. 9c is a graph representing the ELISPOT analysis of IL-4 cytokine release by T-cells taken from NOD mice treated with the AGAD-BPI or the saline control;

Fig. 9d is a graph representing the ELISPOT analysis of IFN-γ cytokine release by T-cells taken from NOD mice treated with the AGAD-BPI or the saline control;

Fig. 10 are representative photographs of the raw data of the ELISPOT analysis used for the graphs in Figs. 9a-9d;

Fig. 11 is a graph of the severity of islet infiltration as an indicator of the inhibition of insulitis by the EGAD-BPI, separate portions of the EGAD-BPI, and saline;

Fig. 12a is a representative color photograph of the histological analysis of pancreata infiltration by mononuclear cells in NOD mice treated with the saline control;

Fig. 12b is a representative color photograph of the histological analysis of pancreata infiltration by mononuclear cells in NOD mice treated with the GAD peptide;

Fig. 12c is a representative color photograph of the histological analysis of pancreata infiltration by mononuclear cells in NOD mice treated with the EGAD-BPI;

Fig. 12d is a representative color photograph of the histological analysis of pancreata infiltration by mononuclear cells in NOD mice treated with the CD11a peptide; and

Fig. 13 is a graph illustrating the results of a 10 week monitoring of blood glucose levels in NOD.Scid mice which received CD25-negative diabetes-inducer cells together with T-cells from NOD mice injected with either the EGAD-BPI or saline.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

As used herein, the following definitions will apply: "Sequence Identity" as it is known in the art refers to a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, namely a reference sequence and a given sequence to be compared with the reference sequence. Sequence identity is determined by comparing the given sequence to the reference sequence after the sequences have been optimally aligned to produce the highest degree of sequence similarity, as determined by the match between strings of such sequences. Upon such alignment, sequence identity is ascertained on a position-by-position basis, e.g., the sequences are "identical" at a particular position if at that position, the nucleotides or amino acid residues are identical. The total number of such position identities is then divided by the total number of nucleotides or residues in the reference sequence to give % sequence identity. Sequence identity can be readily calculated by known methods, including but not limited to, those described in Computational Molecular Biology, Lesk, A. N., ed., Oxford University Press, New York (1988), Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York (1993); Computer Analysis of Sequence Data, Part I, Griffin, A.M., and Griffin, H. G., eds., Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology, von Heinge, G., Academic Press (1987); Sequence Analysis Primer, Gribskov, M. et al., eds., M. Stockton Press, New York (1991); and Carillo, H., et al. Applied Math., 48:1073 (1988), the teachings of which are incorporated herein by reference. Preferred methods to determine the sequence identity are designed to give the largest match between the sequences tested. Methods to determine sequence identity are codified in publicly available computer programs which determine sequence identity between given sequences. Examples of such

programs include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research, 12(1):387 (1984)), BLASTP, BLASTN and FASTA (Altschul, S. F. et al., J. Molec. Biol., 215:403-410 (1990). The BLASTX program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al., NCVI NLM NIH Bethesda, MD 20894, Altschul, S. F. et al., J. Molec. Biol., 215:403-410 (1990), the teachings of which are incorporated herein by reference). These programs optimally align sequences using default gap weights in order to produce the highest level of sequence identity between the given and reference sequences. As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "sequence identity" to a reference nucleotide sequence, it is intended that the nucleotide sequence of the given polynucleotide is identical to the reference sequence except that the given polynucleotide sequence may include up to 5 point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, in a polynucleotide having a nucleotide sequence having at least 95% identity relative to the reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. Analogously, by a polypeptide having a given amino acid sequence having at least, for example, 95% sequence identity to a reference amino acid sequence, it is intended that the given amino acid sequence of the polypeptide is identical to the reference sequence except that the given polypeptide sequence may include up to 5 amino acid alterations per each 100 amino acids of the reference amino acid sequence. In other words, to obtain a given polypeptide sequence having at least 95% sequence identity with a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total number of amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or the carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal

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positions, interspersed either individually among residues in the reference sequence or in the one or more contiguous groups within the reference sequence. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. However, conservative substitutions are not included as a match when determining sequence identity.

Similarly, "sequence homology", as used herein, also refers to a method of determining the relatedness of two sequences. To determine sequence homology, two or more sequences are optimally aligned as described above, and gaps are introduced if necessary. However, in contrast to "sequence identity", conservative amino acid substitutions are counted as a match when determining sequence homology. In other words, to obtain a polypeptide or polynucleotide having 95% sequence homology with a reference sequence, 95% of the amino acid residues or nucleotides in the reference sequence must match or comprise a conservative substitution with another amino acid or nucleotide, or a number of amino acids or nucleotides up to 5% of the total amino acid residues or nucleotides, not including conservative substitutions, in the reference sequence may be inserted into the reference sequence.

A "conservative substitution" refers to the substitution of an amino acid residue or nucleotide with another amino acid residue or nucleotide having similar characteristics or properties including size, charge, hydrophobicity, etc., such that the overall functionality does not change significantly.

"Isolated" means altered "by the hand of man" from its natural state., i.e., if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Finally, all references and teachings cited herein which have not been expressly incorporated by reference are hereby incorporated by reference.

Sequences including or having a sequence which has at least about 10% sequence identity with any one of SEQ ID Nos. 1-46 and which exhibit similar binding properties to APC or linking properties between two peptide sequences are within the scope of the present invention. Preferably, such sequences will have at least about 30% sequence identity with any one of SEQ ID Nos. 1-46, still more preferably at least about 50% sequence identity,

even more preferably, at least about 70% sequence identity, and most preferably at least about 95% sequence identity. Alternatively, sequences including or having a sequence which has at least about 10% sequence homology with any one of SEQ ID Nos. 1-46 and which exhibit similar binding properties to APC or linking properties between the two adjacent peptide sequences are embraced in the present invention. More preferably, such sequences will have at least about 30% sequence homology with any one of SEQ ID Nos. 1-46, still more preferably at least about 50% sequence homology, even more preferably at least about 70% sequence homology, and most preferably at least about 95% sequence homology. Additionally, sequences which differ from any one of SEQ ID Nos. 1-46 due to a mutation event, a series of mutation events, or chemical derivatization but which still exhibit desired properties are also embraced in the present invention. Such mutation events or derivatizations include but are not limited to point mutations, deletions, insertions, rearrangements, peptidomimetics, and other chemical modifications.

A "linker" is defined as any amino acid including naturally occurring or chemically synthesized amino acids. Preferably, a "linker" is a flexible, non-substrate sequence of amino acid residues resistant to proteolytic degradation which can be used to conjugate and/or couple a Signal-1 moiety to a Signal-2 moiety.

A "Signal-1 moiety" is defined as a peptide epitope, i.e., the peptide portion of an antigen and/or mimetics of these antigenic peptides to which important TCRs bind.

A "Signal-2 moiety" or a "Signal-2 receptor moiety" is defined as a peptide portion of a second signal receptor known to bind to and/or affect binding of the receptor to its complimentary ligand on the APC. This can include peptide mimics and mimetics of the receptor/ligand structure of interest.

A "Signal-2 ligand" is the complementary protein of the Signal-2 receptor moiety on the APC to which the receptor portion and/or the Signal-2 receptor moiety has significant affinity and binds.

As used herein "derivative" with respect to peptides refers to changes produced by amino acid addition, deletion, replacement, substitution, and/or modification; mutants produced by recombinant and/or DNA shuffling; and salts, solvates, and other chemically synthesized/modified forms of the peptide that retain in part the activity of the isolated native peptide.

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BPI were generated using automated peptide synthesis by a robotic multiple peptide synthesizer employing Fmoc amino acid chemistry by standard methods. Wang resin (*p*-benzyloxybenzyl alcohol polystyrene) was used as the solid support. Peptides were characterized by reversed-phase HPLC and electrospray mass-spectrometry. This synthesis, referred to as Merrifield peptide synthesis, utilizes traditional organic chemical reactions carried out on a solid material so that the peptide chain is lengthened while attached to the support structure. The peptides will be cleaved from the resin using TFA, and purified by reverse-phase HPLC and analyzed by mass spectroscopy. Alternatively, these reactions can be carried out in solution when larger amounts of the peptides are desired. Of course, the peptides of the invention may be synthesized or prepared by a number of techniques which are well known in the art. See, for example, Creighton, 1983, Proteins: Structures and Molecular Principles, W. H. Freeman and Co., New York, which is incorporated herein by reference in its entirety. Short peptides, for example, can be synthesized on a solid support or in solution. Longer peptides may be made using recombinant DNA techniques. Here, the nucleotide sequences encoding the peptides of the invention may be synthesized, and/or cloned, and expressed according to techniques well known to those of ordinary skill in the art. See, for example, Sambrook, et al., 1989, Molecular Cloning, A is Laboratory Manual, Vols. 1-3, Cold Spring Harbor Press, New York.

Alternatively, the peptides of the invention may be synthesized such that one or more of the bonds which link the amino acid residues of the peptides are non-peptide bonds. These alternative non-peptide bonds may be formed by utilizing reactions well known to those in the art, and may include, but are not limited to amino, ester, hydrazide, semicarbazide, and azo bonds, to name but a few. In yet another embodiment of the invention, peptides comprising the sequences described above may be synthesized with additional chemical groups present at their amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptides is enhanced. For example, hydrophobic groups such as carbobenzoxyl, dansyl, or t-butyloxycarbonyl groups, may be added to the peptides' amino termini. Likewise, an acetyl group or a 9-fluorenylmethoxy-carbonyl group may be placed at the peptides' amino termini. Additionally, the hydrophobic group, t-butyloxycarbonyl, or an amido group may be added to the peptides' carboxy termini.

Purchasing preformed peptides provides another alternative source of peptides having 25 amino acids or less as these are easily purchased from commercial peptide synthesis laboratories. In later synthesis schemes, peptide mimetic compounds may be synthesized in place of the peptide moieties and linked by the same chemistry. The design of peptidomimetics is an established technique and known correlates of key amino acids of the peptide can be synthesized by previously published methods. Furthermore, as it is well known in the art, peptidomimetics may be developed which have the same modulation properties as the preferred peptides detailed herein. As these peptidomimetics require no more than routine skill in the art to produce, such peptidomimetics are embraced within the present application. Notably, the side chains of these peptidomimetics will be very similar in structure to the side chains of the preferred peptides herein, however, their peptide backbone may be very different or even entirely dissimilar. If resistance to degradation *in vivo* or greater conformational stability were desired, the peptides of the present invention could be cyclized by any well known method. One such method adds Penicillamine (Pen) and cysteine (Cys) residues to the N- and C-termini to form cyclic peptides via a disulfide bond between the Pen and Cys residues. The formation of this cyclic peptide restricts the peptide conformation to produce a conformational stability, thereby providing better selectivity for cell surface receptors than its linear counterpart.

The portion of the BPI which spans between the Signal-1 moiety and the Signal-2 moiety is referred to as a linker. As noted above, the linker is not essential in forming a BPI. However, when a linker is used, the linker can be any naturally occurring or chemically synthesized amino acid. Preferably, the linker is a non-substrate amino acid residue chain which helps to prevent protease attack. A particularly preferred linker is a repeating chain of the non-natural amino acid, aminocaproic acid (Ac), and the amino acid glycine (G) (e.g. Ac-G-Ac-G-Ac). If a shorter length was needed for the linker, beta-alanine residues (βAla) could be substituted for one or more of the Ac residues. If a longer chain was needed for the linker, amino-dodecanoic acid residues (Adod) could be substituted for one or more of the Ac residues. As is well known in the art, peptide mimetics of these linker amino acids may also be synthesized and inserted into the BPI structure.

The following examples set forth preferred embodiments of the present invention. It is to be understood, however, that these examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

EXAMPLE 1

This example describes the methods used to generate the BPI.

Materials and Methods:

Synthesis of peptides was via Fmoc on chlorotrity resins. Protected amino acids were double coupled at 8-fold excess for one hour. Resins were dimethylforamide (DMF) and methanol (MeOH) washed and cleaved in Reagent R: trifluorolacetic acid (TFA), ethylene diamine tetraacetic acid (EDTA), Thioanisole, Anisole. The TFA mixture containing the peptide in solution is precipitated in ether and washed extensively. Preparative HPLC of peptides was accomplished by a gradient of 0-80% acetonitrile in 0.1% TFA. Lyophilization of the various fractions and verification by mass spectroscopy yielded the synthetic peptides as a TFA salt. Modeling, crystallography and binding studies are as described above.

Results:

The peptides produced in this example are provided in Table 1 and are also listed as SEQ ID Nos. 1- 46. These peptides include the Signal-1 moiety, the Signal-2 moiety and the non-substrate linker between the two moieties. To produce the BPI, any Signal-1 moiety could be linked with any Signal-2 moiety via any linker using the peptide synthesis described above. In other words, the BPI are generated as one continuous peptide chain comprising a Signal-1 peptide sequence followed by a linker sequence followed by a Signal-2 peptide sequence. Additionally, some representative BPI were generated for later use in the experiments. These BPI are included herein in Table 4. However, it is important to note that these BPI are representative (as are each of the BPI portions listed in Tables 1-4) and not all inclusive.

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TABLE 1. Signal-1 Peptides

SEQ ID No.	Sequence	Name, Source	Organism	Health Condition
1	EIAPVFVLLE	GAD65 (208-217)	Homo sapiens	type-1 diabetes
2	EIAPVFVLIE	GAD67 (217-226)	Mus musculus	type-1 diabetes
3	QYMRADQAAGGLR	Collagen II (1168-1180)	Homo sapiens	rheumatoid arthritis
4	RVVINKDTTHI	Yersinia HSP (322-333)	Yersinia enterocolitica	reactive arthritis
5	ENPVVHFFKNIVTPR	Myelin BP (84-98)	Homo sapiens	multiple sclerosis
6	GYKVLVLNPSVAAT	HCV, NS3 (1248-61)	Hepatitis C virus	hepatitis
7	GSDTITLPCRIKQFINMWQE	HIV, gp120 (410-429)	HIV-1	AIDS
8	PIVQNLQGQMVHQAISPRTL	HIV, p24 (133-152)	HIV-1	AIDS
9	STPESANL	SIV, Tat (28-35)	Simian immunodeficiency virus	simian AIDS
10	AICKRIPNKKPGKKT	RSV, G (183-197)	Respiratory syncytial virus	asthma
11	VYRDGNPYA	HPV 16, E6 (60-68)	Human papillomavirus (HPV)	cervical cancer
12	DRAHYNI	HPV 16, E7 (48-54)	HPV	cervical cancer
13	YMLDLQPETT	HPV 16, E7(11-20)	HPV	cervical cancer
14	ASDLRTIQQLLMGTV	HPV 33, E7 (73-87)	HPV	cervical cancer
15	AELYHFLLKYRAR	MAGE (3114-3126)	Homo sapiens	melanoma

16	LLKYRAREPVTKAE	MAGE (3120-3133	Homo sapiens	melanoma
17	EQVAQYKALPVVLENA	Fel d 1 (22-37)	Felis domesticus	cat allergy
18	KALPVVLENARILKNCV	Fel d 1(28-44)	Felis domesticus	cat allergy
19	LVPCAWAGNVCGEKRAYCCS	Amb a 5 (1-20)	Ambrosia artemisiifolia	ragweed allergy
20	PIGKYCVCYDSKAICNKNCT	Amb t 5 (21-40)	Ambrosia trifida	ragweed allergy
21	KSMKVTVAFNQFGPN	Cry j 1 (211-225)	Cryptomeria japonica	cedar allergy
22	IDIFASKNFHLQKNTIGTG	Cry j 2 (182-200)	Cryptomeria japonica	cedar allergy
23	YFVGKMYFNLIDTKCYK	Phospholypase 2 (81-97)	Apis mellifera	bee allergy
24	ASEQETADATPEKEEFTAAF	Hev b 5 (37-56)	Hevia brasiliensis	latex
25	FGISNYCQIYPPNANKI	Der p 1 (111-127)	Dermatophagoides pteronyssinus	dust mites

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TABLE 2. Linkers

SEQ ID No.	Sequence
26	Ac-G-Ac-G-Ac
27	Ac-G-βAla-G-Ac
28	Ac-G-Adod-G-Ac
29	Ahx-G-Ahx-G-Ahx

TABLE 3. Signal-2 Peptides

SEQ ID No.	Sequence	Source	Organism	Shift in Immunity
30	ITDGEATDSG	CD11a (237-247)	Homo sapiens	type-1→type-2
31	TDGEATDSGN	CD11a (238-248)	Homo sapiens	type-1→type-2
32	ASPGKATEVR	CTLA4 (24-33)	Homo sapiens	type-2→type-1
33	SPSHNTDEVR	CTLA4 (24-33)	Mus musculus	type-2→type-1
34	KVELMYPPPYYL	CTLA4 (93-104)	Homo sapiens	type-2→type-1
35	KVELMYPPPYFV	CTLA4 (93-104)	Mus musculus	type-2→type-1
36	ITDGEATDSG	CD11a (237-247)	Mus musculus	type-1→type-2
37	KGYYTMSNNLVTL	CD154 (CD40L) (93-104)	Homo sapiens	type-1→type-2
38	KGYYTMSNNLVTL	CD154 (CD40L) (93-104)	Mus musculus	type-1→type-2
39	YMRNSKYRAGGAYGP G	Fas-ligand (CD95L) (143-155)	Homo sapiens	type-2→type-1
40	YMRNSKYRAGGAYGP G	Fas-ligand (CD95L) (143-155)	Mus musculus	type-2→type-1
41	TDGEATDSGN	CD11a (238-248)	Mus musculus	type-1→type-2

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Table 4. Some Representative BPI

TCR Epitope	Linker	Signal-2 Receptor Moiety	Complete BPI Sequence	SEQ ID No.	Effect
(GAD65) Diabetes: EIAPVFVLLE	AcGAcGAc	(CDIIa) ITDGEATDSG	EIAPVFVLLEAcGAcGAcITDGEATDSG	42	type-1 – type-2
(tat) AIDS: STPESANL	AcGAdodGAc	(Fas-ligand) YMRNSKYRAGGAYGPG	STPESANLAcGAdodGAc YMRNSKYRAGGAYGPG	43	↓ CTL
(p24) PIVQNLQGQMVHQAISPRTL	AcGAcGAc	(CTLA4) KVELMYPPPYFV	PIVQNLQGQMVHQAISPRTLAcGAc GAcKVELMYPPPYFV	44	↑ type-1
(collagen-II) Rheumatoid Arthritis: QYMRADQAAGGLR	AcGAhxGAc	(CD40L) KGYYTMSNNLVTL	QYMRADQAAGGLRAcGAhxGAcKG YYTMSNNLVTL	45	type-1 – type-2
(HPV-E7) Cervical Cancer: YMLDLQPETT	AcGAcGAc	(Fas-ligand) YMRNSKYRAGGAYGPG	YMLDLQPETTAcGAcGAcYMRNSK YRAGGAYGPG	46	↑ CTL

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Discussion:

Once the linker, the Signal-1 moiety, and the Signal-2 moiety are chosen, the entire BPI can be synthesized using the above-described methods. There are thousands of potential Signal-1 moieties which could be used in connection with the present invention. Each of these moieties may be associated with a distinct immunological response or disease state. Once the epitope structure and sequence are determined, an appropriate linker could be selected and the other portion of the BPI, i.e., the second signal moiety, can be chosen. Once all of these sequences have been determined, BPI can be designed using the peptide sequences themselves, peptidomimetics, or combinations of the two. Construction of appropriate peptidomimetics is detailed by Falcioni, et al, 17 *Nature Biotechnology*, 562-567 (1999), the content and teachings of which are hereby incorporated by reference herein.

Fig. 8 illustrates the structure of the GAD65 (208-217), TCR epitope linked to the CD11a (237-247) second signal moiety produced by the present methods. It is shown bound to the groove of I-A^{g7} and the D1 domain of ICAM-1. For modeling the I-A^{g7}:GAD65 peptide structure, docking studies were performed on a Silicon Graphic Ocatane work station using InSight II software (MSI/Biosym). The LFA-1 peptide:ICAM-1 domain structure is based on the docking model of Edwards, C.P. et al. *J. Biol. Chem.* 273:28937 (1998), the teachings and disclosure of which is incorporated by reference herein. The alpha carbon ribbon of I-A^{g7} is shown in pink; D1 of ICAM-1 is in light-blue; the BPI is shown by atom, carbon in green, oxygen in red, and nitrogen in blue. This structure can be denominated as GAD65 (208-217) - [Ac-G-Ac-G-Ac] - CD11a (237-247). Advantageously, the length of the linker may be modified as needed or as indicated by any experimental data obtained in order to span between the Signal-1 and Signal-2 moieties at an optimum length.

These structures are illustrated by the preliminary mechanism depicted schematically (Figs. 2 and 3), and the structural model (Fig. 8). The linker used has the sequence –[Ac-G-Ac-G-Ac]-. To lengthen the linker, one or more aminocaproic acid (Ac) resides can be substituted with aminododecanoic acid. To shorten the linker, beta-2 alanine can be used as a substitute for aminocaproic acid.

Of course, it is possible that one of ordinary skill in the art could produce any number of peptidomimetics or derivatives which would have similar activity to the BPI, and such modifications are encompassed by the present invention as described in more detail above.

EXAMPLE 2

This example uses biotinylated BPI to test for competitive inhibition of BPI binding by unlabeled peptides or monoclonal antibodies to MHC-II and ICAM-1 on live APC, and to verify antigenic peptide binding to live APC. Additionally, it was shown that monoclonal antibodies to MHC-II or ICAM-1 effectively block binding of the diabetes BPI (GAD65 (208-217) - [Ac-G-Ac-G-Ac] - CD11a (237-247)) (hereinafter referred to as EGAD-BPI) to NOD spleenocytes.

Materials and Methods:

To obtain biotinylated BPI, the synthesized EGAD BPI was biotinylated with NHS-Biotin as described in Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994). Spleen cell density-gradient fractions from normal (unimmunized) NOD, BALB/c and other MHC congenic strains were incubated in round bottom 96-well plates with increasing concentrations of individual biotinylated peptides at 37°C, 5% CO_2 for 16 hours. Following binding of the BPI to the APC, Avidin-FITC was incubated with the cells on ice for 30 minutes, followed by biotinylated anti-Avidin for 1 hour, then again with Avidin-FITC. For BPI titrations, increasing concentrations (0.1-100 μM) of the biotinylated derivatives in sterile 0.5% BSA-PBS were incubated with the APC for 16 hours as above. Three-color analyses used Cy-Chrome or PE-conjugated anti-B220 (mAb RA3-6B2 (CD45R B-cell marker), anti-MHC class II (KH74 or 10-3.62 mAb), or anti-ICAM-1 (3E2 mAb); (all purchased from PharMingen, San Diego, CA). Bound peptide was detected with avidin-FITC/biotinylated anti-avidin/avidin-FITC on live cells gated by forward/side scatter analysis. Controls contained all detecting reagents in absence of the biotinylated peptide; 20,000 events were analyzed for each histogram with a FACScan (Becton-Dickinson) flow cytometer.

To test for competitive inhibition of BPI binding with unlabeled peptides or monoclonal antibodies to MHC-II and ICAM-1 on live APC, freshly isolated fractions of spleen cells were incubated with the previously biotinylated BPI. However, for this portion of the experiment, the experimental wells contained various unlabeled peptides (e.g., antigenic peptides or LFA-1 peptides), and/or monoclonal antibody (e.g., anti-MHC-II or anti-ICAM-1 mAb) inhibitors. Negative selection methods with monoclonal antibodies

conjugated to magnetic particles were used to enrich the spleen cell fractions for B cells, macrophages, or dendritic cells as well as to examine differences in BPI binding to these different populations. These methods are detailed in Schountz et al., 157 The Journal of Immunology 3893-3901 (1996), the teachings and content of which were incorporated by reference above.

To verify antigenic peptide binding to live APC, initial EGAD-BPI were screened for selective binding to NOD (I-A^{g7}) APC and assayed for simultaneous binding using monoclonal antibodies against either MHC-II or ICAM-1 by flow cytometry methods using live APC (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). In this assay, increasing concentrations of the biotinylated-BPI, -CD11a(237-247) or -GAD65(208-217) peptide were incubated overnight with spleenocytes from each inbred strain. Bound peptide was detected with amplification of avidin-FITC fluorescence by the use of a biotinylated anti-avidin reagent, followed by a second round of avidin-FITC binding. Biotinylated peptide was incubated with 10^6 viable cells at a peptide concentrations of 50 μM. Bound peptide was detected with avidin-FITC/biotinylated anti-avidin-FITC. For analysis, a forward/side scatter gate was set on live lymphocytes and 20,000 events were collected in this gate. Background fluorescence (detection reagents only) is shown in each panel of Figs. 5a-5c along with the 50 μM bio-peptide histogram (peptide-fluorescence intensity = FL1). The percentage of positive cells (M2) were determined by the CELLQuest™ program (Becton-Dickinson) and are displayed in each panel along with the median channel fluorescence (MCF) of the M2 population (all data are the direct output of the CELLQuest™ program running on an Apple G3 computer).

To demonstrate that monoclonal antibodies to MHC-II or ICAM-1 effectively block binding of the diabetes BPI (EGAD-BPI) to NOD spleenocytes, an assay identical to that used for Figs. 5a-5c was used. However, either purified 10-3.62 (anti-MHC-II) or 3E2 (anti-ICAM-1) were included in the overnight incubation of the spleen cells with the biotinylated BPI. Both antibodies were purchased from PharMingen and used at 5 μg/ml final concentration. A control containing no added monoclonal antibody was also tested. Analyses were gated on forward/side scatter dotplots for live lymphocytes and 20,000 events were analyzed for each histogram. Results for this example are given in Figs. 6a-6c.

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Results and Discussion:

As illustrated by the data provided in Figs. 5a-5c, NOD spleen cells bind the diabetes BPI (EGAD-BPI) at a higher density than spleenocytes identically purified from BALB/c, A.SW, or A.BY. Previous data has shown that B cells are the major antigenic peptide binding cells in these spleen cell preparations isolated by lymphocyte separation media (LSM) density gradient centrifugation (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). There was a significant difference in the percentage of high-density binding cells, from 30% (NOD) to 6% (A.BY). In contrast, the separate Signal-1 (GAD65 peptide) and Signal-2 (CD11a peptide) moieties did not bind preferentially to NOD APC. Supporting data is given in Figs. 5b and 5c. Fig. 5b illustrates direct binding of biotinylated LFA-1 (CD11a 237-247) peptide to the same spleenocyte preparations as those shown in Fig. 5a. Note that this Signal-2 moiety bound similarly to all strain spleenocytes. Fig. 5c illustrates direct binding of biotinylated GAD65 (208-217) peptide to the same spleenocyte preparations as those depicted in Figs. 5a and 5b. These data indicate that BPI could be engineered to fit particular MHC peptide binding motifs as discussed by Corper et al. in *A Structural Framework for Deciphering the Link Between I-A^{g7} and Autoimmune Diabetes*, 288 Science 505-511 (2000), and by Dessen et al. in *X-ray Crystal Structure of HLA-DR4 (DRA*0101, DRB*0401) Complexed With a Peptide From Human Collagen II*, 7 Immunity 473-481 (1997), the respective teachings of which are incorporated by reference herein.

Additionally, as illustrated in Figs. 6a-6c, other studies have shown that monoclonal antibodies to MHC-II and ICAM-1 block peptide binding to NOD spleen cells. These data indicate that the diabetes BPI bind to both receptors on the APC surface. Thus, Monoclonal antibody to MHC-II or ICAM-1 effectively block binding of the diabetes BPI (EGAD-BPI) to NOD spleenocytes. In effect the predicted bifunctional nature of the BPI is demonstrated by these results and suggests that the BPI will link MHC-II to ICAM-1 on the APC surface. This mechanism was further demonstrated by co-capping experiments.

EXAMPLE 3

This example utilizes co-capping experiments to demonstrate simultaneous binding of the BPI to MHC-II and ICAM-1 molecules.

Materials and Methods:

Further support for simultaneous binding of the BPI to MHC-II and ICAM-1 molecules has been observed in co-capping experiments using biotinylated mAb10-3.62 and streptavidin to cap MHC-II in the presence or absence of the BPI. To test the ability of the BPI peptide to link MHC-II and ICAM-1 molecules on the APC surface, we used a modification of a co-capping experiment originally described for monoclonal antibodies. Briefly, biotinylated monoclonal antibody to MHC-II (10-3.62) is incubated with freshly-isolated APC from NOD mice previously treated by intravenous (i.v.) injection of a given BPI variant or saline. Antibody-bound cells are then incubated with streptavidin (37 C x 15 min.) to cap the MHC-II molecules on the APC surface. The cells were transferred to ice and labeled with a fluorescent (PE) monoclonal antibody to ICAM-1 (3E2). The cells are plated and observed for evidence that a given BPI links ICAM-1 into the MHC-II cap, i.e., by standard fluorescence microscopy and image analysis. In one experiment, T-depleted spleenocytes from mice treated 16 hours previously with EGAD-BPI (i.v.) exhibited co-capping of ICAM-1 with MHC-II in the presence of bio-10-3.62/streptavidin. In the other experiment, T-depleted spleenocytes from mice treated 16 hours previously with saline only did not exhibit co-capping. The results for these experiments are given in Fig. 7.

Results:

The top panels of Fig. 7 illustrate the results from the T-depleted spleenocytes from mice treated 16 hours previously with EGAD-BPI (i.v.), wherein ICAM-1 was co-capped with MHC-II in the presence of bio-10-3.62/streptavidin. The bottom panels of Fig. 7 illustrate the results from the mice treated with saline only wherein co-capping is not exhibited. This is evidenced by having ICAM-1 remain dispersed on the B-cell membranes. As is shown, B cells isolated from NOD mice treated 16 hours previously by i.v. injection of either 40 nanomoles of EGAD-BPI or saline (PBS) alone displayed two distinct patterns of ICAM-1 expression, as measured by staining with PE-labeled 3E2 (anti-ICAM-1) on ice.

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On cells from BPI-treated mice, ICAM-1 appears to have co-capped with the MHC-II molecules (note single concentrated spot of ICAM-1 red fluorescence in top panels). By contrast, the nominal dispersed density of ICAM-1 is observed on cells isolated from PBS-treated NOD mice (note entire surface red fluorescence staining in bottom panels).

Therefore, it appears evident that BPI have the capacity to bind simultaneously to MHC-II and ICAM-1 structures on the surface of live APC and therefore may provide signal alterations involving pathways necessary for T_H1/T_H2 differentiation. To directly examine the effects of BPI on T_H1/T_H2 immune deviation, T-cells from mice injected with EGAD-BPI were examined for cytokine analysis.

EXAMPLE 4

This example used an ELISPOT to determine T_H1/T_H2 frequency as altered by BPI injection.

Materials and Methods:

Groups of 3-5 NOD mice were immunized subcutaneously (s.c.) with the GAD 65 peptide in CFA (40 nanomoles/mouse) at the tail base. Different groups received either the EGAD-BPI, its single TCR epitope (Signal-1 moiety), or its CD11a peptide (Signal-2 moiety) i.v. (all 40 nanomoles/mouse). After 6-8 days, another identical 40 nanomole injection was given to each mouse, and the next day lymph nodes draining the site of the s.c. injection were made into single cell suspensions for culture. Identical primary cultures were incubated for 96 hours; then, viable T-cells were recovered by density gradient centrifugation. One million of these cells were combined in nitrocellulose-bottomed 96-well plates (Millititer-HA, Millipore, Bedford, MA), previously coated (50 µl/well) with mAb to either mouse IFN_γ (clone R4-6A2), or mouse IL-4 (clone BVD4-1D11) at a concentration of 10 µg/ml in PBS. Groups of triplicate cultures were incubated with either Concanavalin-A (2 µg/ml), or the Signal-1 peptide moiety plus 20 U/ml recombinant IL2 (R & D Systems). After 72-96 hours of culture at 37°C and 5% CO_2, plates were washed three times with PBS-0.05% Tween-20. In appropriate wells are added biotinylated anti-IFN_γ (clone XMG1.2) or biotinylated anti-IL-4 (clone BVD6-24G2) at a concentration of 1 µg/ml and incubated for 1 hour at room temperature. Positive control wells receive known T_H1 or T_H2 clones in

place of normal T-cells. MAb pairs to IL12, IL10, and IL2 are also available and will be used to test for these cytokines in the same assay. All mAb and recombinant controls are purchased from PharMingen (San Diego, CA). Finally, plates were washed three times with PBS-Tween, and then exposed to 100 µl of a 1:2000 dilution of streptavidin alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA) for 1 h and washed as before. Cytokine-producing cells were enumerated by development of the membrane with BCIP/NBT substrate kit (BioRad Labs, Richmond, CA), followed by image capture and analysis using a standard stereomicroscope connected with a digital camera and NIH image software (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). In separate experiments, CD4+ T-cell clones from NOD mice immunized with the GAD65(208-217) peptide by our previously described methods can be used in the same assay. The clones were generated using the methods described in Murray et al., 24 Eur. J. Immunol. 2337-2344 (1994), the teachings of which are hereby incorporated by reference. These clones will be maintained by biweekly restimulation with irradiated NOD lymphocytes, the GAD peptide, and recombinant IL-2. For further analysis, BPI that have been substituted at predicted TCR-contact positions will be used to determine which of these BPI variants are most effective in the inhibition of proliferation and cytokine release from individual clones as analyzed above. Predicted positions that will be scanned with all amino acids except cysteine are amino acids 208, 213, and 216. These residues point toward the TCR in the recently solved crystal structure of the GAD65 peptide bound to I-A^{g7} (Corper et al., 288 *Science* 505-511 (2000) and see Fig. 8).

Results:

Importantly, this example shows the ability of a given BPI to modulate a functional immune response. It can be seen that mice treated with the BPI produce abundant IL-4, whereas the control mice did not produce this cytokine (illustrated in Figs. 9a and 9c). Since IL-4 is the signature cytokine of type-2 immunity, this example shows that the BPI have the capacity to switch dominant type-1 immunity toward T_H2 differentiation and a type-2 response. Moreover, we have developed this *in vivo* assay system to provide a relatively quick examination of a given BPI's immunoregulatory efficacy. Once T_H1/T_H2 modulation

is confirmed as in the present example, studies can then move on to the more stringent tests of BPI efficacy using adoptive transfer experiments as described below.

As shown in Fig. 9a and 9c, IL-4 production increases by approximately 10-fold when T-cells are from the BPI treated animals stimulated in vitro with mitogen. IFN-γ production also increased, although to a lesser extent (see Figs. 9b and 9d).

EXAMPLE 5

This example tested the capacity of the BPI to inhibit lymphocytic infiltration of pancreatic islets in NOD mice. Lymphocytic infiltration is a hallmark of insulitis and the development of type-1 diabetes.

Materials and Methods:

These studies sought to confirm that the BPI containing the immunodominant GAD65 TCR epitope (EGAD-BPI) was biologically active *in vivo* and inhibited the development of pancreatic inflammation. Groups of three normal glycemic NOD males (8 weeks old) were immunized with the GAD65 (208-217) peptide in CFA as described in Example 4. The control group received PBS, and separate experimental groups received either the EGAD-BPI, the GAD peptide alone (i.e., the T-cell receptor epitope), or the CD11a peptide alone(i.e., the second signal receptor moiety) by two intravenous injections as described in Example 4. On day 10, the pancreata were removed to 10% PBS-buffered formalin, embedded in paraffin, and five-micron serial sections were examined histologically for mononuclear cell infiltration as previously described by Yoon, et al., *Control of Autoimmune Diabetes in NOD Mice by GAD Expression or Suppression in β Cells*, 284 Science 1183-1187 (1999), the entirety of which is hereby incorporated by reference.

Results:

The results for this experiment are given in Figs. 11 and 12. Fig. 11 represents the cumulative data of this analysis wherein the severity of islet infiltration is scored and plotted as the percentage of islets examined. Over 100 islets from each group in greater than 5 tissue sections were analyzed by three independent observers. As shown in Fig. 11, there

was a clear inhibitory effect of the EGAD-BPI treatment on mononuclear cell infiltration (insulitis). Over 95% of the islets from the BPI treated animals were intact and did not show infiltration (i.e., grade-0 islets). All of the other groups showed some signs of insulitis even at this early stage of the disease. Notably, the GAD peptide treated animals showed the most insulitis (grade-0 islets reduced to 62% and 37.5% of islets scored grade 2 or above. This compared with 66.7% normal islets in the CD11a peptide treated group and 71.4% normal islets in PBS treated animals. Thus, compared to the PBS control, EGAD-BPI treatment provided an 84% *inhibition of insulitis* [calc. as: % islets @grade 1-4 (PBS Rx) minus % islets @grade 1-4 (EGAD-BPI Rx) divided by % islets @grade 1-4 (PBS Rx) multiplied by 100]. Representative islets from each group of the experiment are shown below in Figs. 12a-12d, as stained with hematoxylin and eosin. Note severe lymphocytic infiltration in Figs. 12b and 12d which was observed in groups treated with the single Signal-1 or Signal-2 moieties while the islets from the EGAD-BPI treated mice were predominately intact (see Fig. 12c). Taken together, these data strongly indicate that treatment with the diabetes BPI containing these Signal-1 and Signal-2 peptide moieties significantly inhibits the infiltration of lymphocytes into the pancreatic islets in this animal model of type-1 diabetes. Therefore, we would predict that BPI operate via a mechanism involved in the normal breakdown of self tolerance to pancreatic autoantigens. These data indicate that BPI may operate through immune deviation to block the autoimmune response to pancreatic antigen. To further test this theory, we transferred T-cells primed in the presence of the EGAD-BPI into NOD.Scid mice genetically programmed for diabetes development.

EXAMPLE 6

This example tested BPI blocking of diabetes development in the well described intact immune system of immunologically reconstituted NOD.Scid mice to study diabetes progression.

Materials and Methods:

BPI proven active for modulation of T_H1/T_H2 responses against known immunodominant peptides of GAD65 for blocking T-cell initiated diabetes progression in a NOD mouse model was tested. The NOD.Scid adoptive transfer model, wherein CD25-

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depleted NOD spleen cells have been observed to induce diabetes as early as 2-4 weeks post adoptive transfer was used for this purpose. NOD.Scid adoptive transfers were performed by a modification of a protocol described by Solomon et al. in *B7/CD28 Costimulation is Essential for the Homeostasis of the CD4$^+$ CD25 Immunoregulatory T-cells That Control Autoimmune Diabetes*, 12 Immunity 431-440 (2000), the content of which is incorporated herein by reference. NOD spleen cells from 8 week (non-diabetic) females were used to enrich a CD25-/CTLA4-depleted population by treatment with purified monoclonal antibody (mAb 7D4, PharMingen) followed by low-tox rabbit complement (Cedarlane) (80% depletion of CTLA4+ cells by flow analysis with PE-labeled UC10-4F10-11 mAb; not shown). These inducer cells (15 x 10^6 per mouse) were injected (i.v.) into 6 week NOD.Scid females (Jackson Labs) together with 3 x 10^6 CD4+ T-cells from mice either treated with the EGAD-BPI or treated identically except with PBS in place of the EGAD-BPI. In vitro clonal expansion was with either recombinant IL-2 (R&D systems), or ConA, as described for the ELISPOT experiments. All manipulations of animals and cells were performed in laminar flow hoods, and animals were maintained continuously behind laminar flow barriers on autoclaved food and water in microisolator cages. Some experiments will deplete specific subsets of the CD4+ cells using mAb to CD154, CD25, CD62L, CD152, etc. and magnetic particles prior to adoptive transfer. CD4+ cells from mice treated with the individual moieties of the BPI can be used as negative controls along with CD4+ cells from mice treated with saline alone. Co-transfer of CD4+ T-cells enriched from matched NOD mice treated with a given BPI demonstrates that BPI treatment leads to regulatory T-cells capable of delaying the onset of diabetes. Moreover, other diseases listed in Table I can be tested in this same manner (i.e., by adoptive transfer of regulatory T-cells).

Results:

Significantly, we have now seen a clear difference in the development of hyperglycemia and diabetes between the two experimental groups. As can be seen in Fig. 13, at 7 weeks post adoptive transfer, 80% of mice which received the vehicle, (PBS) developed hyperglycemia and diabetes. By contrast only 40% of mice treated with the EGAD-BPI showed hyperglycemia and progression to diabetes. Therefore, these data demonstrate the blocking of diabetes progression as mediated by the BPI treatment. Further

modifications to the BPI structure may enhance their effectiveness in this model and in the treatment of type-1 diabetes. These data indicate that T-cells capable of suppressing diabetes development were generated in the presence of the BPI and operated *in vivo* to inhibit diabetes progression within the intact system of the NOD.Scid mouse. Thus we would anticipate similar regulatory T-cells to become activated by BPI containing TCR epitopes of other disease associated antigens. For example, collagen-II peptide epitopes may initiate suppressor T-cells involved in rheumatoid arthritis. Moreover, regulatory T-cells that would expand T_H1 populations may be generated by BPI containing CTLA4 second signal moieties and these could be used in diseases such as HIV1 infection or other chronic viral diseases. Extrapolation of this example to clinical trial should be straightforward, as the NOD model is recognized as a significant representation of the human disease. (See e.g., Atkinson and Leiter, The NOD Mouse Model of Type-1 Diabetes: As Good as it Gets?, 5 *Nature Medicine,* 601-604 (1999).

EXAMPLE 7

Here, the predicted examples of BPI for other autoimmune diseases are briefly detailed. Specifically, BPI containing immunodominant TCR epitopes for collagen-induced arthritis (CIA) and myelin basic protein-induced experimental allergic encephalomyelitis (EAE) will be discussed. Also, the CD40L peptide mimic is predicted to favor T_H2 immunity, as blocking the CD40 signal would be expected to decrease IL12 production (Ruedl, et al., *The Antigen Dose Determines T Helper Subset Development by Regulation of CD40 Ligand,* 30 Eur. J. Immunol. 2056-2064 (2000)). Therefore, in these autoimmune models and in the NOD model, we will attempt to favor T_H2 immunity by linking the appropriate TCR epitopes to the CD40L peptide mimic as well as the CD11a mimic used in diabetes inhibition. Mutations in this loop peptide have been shown to affect binding and function of CD40L.

Materials and Methods:

There are several autoimmune models which employ different cross-reactive immunodominant epitopes, different mouse strains, and tissues of analysis. These types of models are highly similar to our short-term model of diabetes development that we designed

to test the EGAD-BPI. For CIA: We will induce the disease by a single peptide in CFA injection protocol as described by Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994). As in the NOD system, these mice will receive either the BPI, PBS, or the single peptide moieties. The minimal immunodominant collagen-II epitope is listed above, and modifications to the BPI will be based upon the x-ray structure of this complex (Dessen et al., 7 Immunity 473-481 (1997). For EAE, the disease is induced by the method of Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994). This method also uses a single peptide (the myelin basic protein (MBP) peptide, 85-101) injection in CFA, by methods analogous to those described in our NOD system.

Results:

We would anticipate that the BPI constructed with collagen and MBP would be effective in modulating immunity to these antigens to a T_H2-dominated pattern. Since these diseases are thought to involve predominant T_H1 immunopathology (see above references), such a switch would ostensibly delay or cure the disease in these mice (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996). As in the NOD model, such results would target the development of analogous compounds for use in the human diseases of rheumatoid arthritis and multiple sclerosis, where these types of immune responses are clinically observed. Most importantly, BPI offer the capability of blocking autoimmune T-cell responses while maintaining host immunity to infectious agents and developing cancers.

EXAMPLE 8

This example describes predicted BPI for infectious diseases and certain cancers. Specifically, a general protocol for the testing of a given BPI containing immunodominant TCR epitopes of a specific human pathogen will be described using the example of HIV-1 p24 epitope (Harcourt, et. al., *HIV-1 Variation Diminishes CD4 T Lymphocyte Recognition*, 188 J. Exp. Med., 1785-1793 (1998)) (SEQ ID No. 8).

Materials and Methods:

By contrast to the previous experimental examples, these BPI will be primarily tested by their effects on long-term T-cell clones derived from human patients. Briefly, peripheral blood mononuclear cells (PBMCs) are prepared from patient and control whole blood, and CD8+ cells removed by the negative selection protocol with magnetic particles. These cells are grown in tissue culture medium at 4×10^6 cells in 1 ml for 6 days in the presence of 20 μM of the p24 peptide, and blast cells isolated by density gradient centrifugation. Secondary cultures of these cells contained recombinant human IL-2 (20 U/ml) and are continued for a maximum of 10-14 days. Lines were expanded by repeating the process of re-culturing with irradiated APC from histocompatible donors together with the p24 peptide and expansion of the T-cell blasts in IL-2. Clones were prepared by limiting dilution cloning in fresh plates containing irradiated APC, peptide and IL-2 (50 U/ml) (Murray et al., 24 *Eur. J. Immunol.* 2337-2344 (1994); Murray; 19 *Immunology Today* 157-163; and Schountz et al., 157 *The Journal of Immunology* 3893-3901 (1996)). BPI will be tested for their ability to inhibit the proliferation and cytokine release of these established lines of human T-cells by our detailed methods described in Schountz et al., *Unique T Cell Antagonist Properties of the Exact Self-Correlate of a Peptide Antigen Revealed by Self-Substitution of Non-Self-Positions in the Peptide Sequence,* 168 Cellular Immunology 193-200 (1996). Here, we show that peptides changed by amino acid substitutions at key TCR-contact residues alter the proliferation and cytokine release of cloned T-cells. If a given BPI significantly inhibits or alters cytokine release to p24 peptide-specific clones, this will target the BPI for more stringent testing *in vivo*. Mouse models can be used to test candidate BPI for infectious diseases. These studies will be performed as described for the GAD65 BPI with the substitution of immunodominant peptides of the infectious agents for the GAD65 TCR epitope peptide. Positive results in these *in vivo* studies would substantiate animal studies with live pathogens. The Scid-human mouse model in which human tissues are adoptively transferred to the Scid mouse could be used to examine protective immunity to HIV (Jenkins, et al., Blood (1998) 8:2672, hepatitis-C virus (HCV) (Bronowicki, J., et al. Hepatology (1998)28:211), human pappiloma virus (HPV) (Tewari, et al. Gynecol. Oncol. (2000)77:137, and respiratory syncitial virus (RSV) (Nadal, et al. Clin. Exp. Immunol.85:358) infections.

Results:

It is hypothesized that incorporation of the CD28/CTLA4 peptide or the Fas-ligand peptide as the second signal moiety will shift immunity toward the T_H1 subset and induce or enhance CTL responses. (Padrid, P., et al. Am. J. Respir. Cell. Mol. Biol. (1998)18:453). CTLA4 is a negative regulator of T-cell activation and signaling through CD28 has been linked to T_H2 differentiation (Rulifson, I., et al. J. Immunol. (1997)158:658). Given the evidence that the LFA-1 signal is inhibitory to T_H2 differentiation and that CD28 signaling favors T_H2 differentiation, we will proceed with these studies using this selective differentiation paradigm to further modify BPI design. Similarly, the evidence suggests that CD95 (also called Fas) interaction with the CD95-ligand (FasL) favors T_H1 differentiation (Chattergoon, et al. Nature Biotech. (2000)18:974). BPI with the peptide mimic of the FasL, which is from the Y218 loop of FasL, predicted to interact with Fas at the T-cell:APC interface, will be used to favor T_H1 activation and CTL responses against chronic T_H2-dominated diseases.

EXAMPLE 9

Here we describe several possible BPI that contain the TCR epitopes of described allergen sequences synthetically linked to the predicted CD28/CTLA4 peptide mimic and/or the predicted FasL peptide mimic.

Materials and Methods:

These studies will again use a combination of analyses using human cloned T-cell lines and animal models of allergy to these well-known allergic agents. Allergies affect a very large population of humans, with estimates of many allergies and asthma reaching 10% of otherwise healthy individuals. We will use our previously described 7-10 day protocol to investigate BPI modulation of the immune response to the allergen peptides described in the table. As previously described, this protocol involves the injection of the allergen peptide in CFA on day 1, followed by i.v. injection of the BPI on day 1 and on day 6, 7, 8 or 9. On day 7, 8, 9 or 10, the draining lymph nodes are cultured and compared for T_H1 versus T_H2 cytokines by ELISPOT (see Example 4 for detailed methods). Once candidate BPI for a given allergen have been identified by this relatively short-term experiment, we

will test these BPI for the alteration of CD4 T-cell response using cloned lines from atopic patients. Briefly, CD4 T-cell clones will be established from patient peripheral blood lymphocytes as described under Example 8. We will test the capacity of a given BPI to inhibit or alter cytokine release by these cells using the ELISPOT as described previously. Finally, we will move the examination of a given BPI toward clinical testing using identically described procedures with these specific allergens in human MHC (HLA) transgenic mice (Svetlana, P. et al. J. Immunol. (1998)161:2032). These mice offer the advantage of having the human MHC molecules and thus, BPI binding will be very similar in this model as in the human allergic condition. If a given BPI blocks T_H2 activation in these mice against the native allergen, then adoptive transfer studies with human Scid mice (similar to those used in Example 8) can be used as a precursor to human clinical trials.

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Results:

Blocking the CTLA4/CD28 pathway in preference for LFA-1 signaling will favor T_H1 differentiation against these allergens in the presence of the BPI. Also, BPI with the FasL moiety should favor type-1 immunity. It has been a long-held practice to "desensitize" atopic patients with allergens given by injection and desensitization is thought to operate via a shift toward T_H1 responses to the specific allergens (Holt, et al., Nature (1999) 402:6760 suppl:B12-17). Therefore, as in the case of the chronic T_H2-linked infectious diseases, our working hypothesis is to use the T_H1-promoting capacity of LFA-1 signaling together with the possibility to inhibit T_H2 signaling via the CTLA4/CD28 molecules to effect this result. We will also examine the effects of allergen epitopes linked to the Fas peptide mimic, as dual expression of Fas and antigen within APC has been shown to favor T_H1 immunity and CTL induction (Chattergoon, M., et. al. Nat. Biotech. (2000)18:974) . It may be possible to induce cell death of APC presenting allergen epitopes, thereby limiting the activation of T_H2-type regulatory cells in allergy and asthma, including RSV-precipitated childhood and geriatric bronchiolitis.

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We claim:

1. A peptide comprising a first portion and a second portion, said first portion including a sequence having at least about 10% sequence homology with a peptide derived from a TCR epitope, said second portion including a sequence having at least about 10% sequence homology with a peptide derived from a Signal-2 moiety.

2. The peptide of claim 1, further comprising a linking portion.

3. The peptide of claim 2, said linking portion comprising at least one amino acid residue.

4. The peptide of claim 3, said amino acid being a flexible, non-substrate amino acid.

5. The peptide of claim 2, said linking portion comprising a sequence of amino acid residues, said sequence comprising a non-substrate amino acid alternating with a hydrophilic amino acid.

6. The peptide of claim 2, said linking portion having the general formula $(A,B)_X$, wherein A and B are amino acid residues, and said A amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine, and said B amino acid residue is glycine, and wherein X ranges from 1 to 100.

7. The peptide of claim 6, said A amino acid residue being aminocaproic acid.

8. The peptide of claim 1, said first portion including a sequence having at least about 10% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

9. The peptide of claim 8, said peptide having at least 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

10. The peptide of claim 9, said peptide having at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

11. The peptide of claim 1, said first portion comprising a peptidomimetic of a peptide selected from the group consisting of SEQ ID Nos. 1-25.

12. The peptide of claim 1, said first portion comprising a derivative of a peptide selected from the group consisting of SEQ ID Nos. 1-25.

13. The peptide of claim 1, said TCR epitope being correlated with a known disease state.

14. The peptide of claim 1, said first portion capable of binding with a major histocompatability complex on an antigen presenting cell.

15. The peptide of claim 14, said peptide:MHC complex capable of engaging a T-cell receptor on a T-cell.

16. The peptide of claim 1, said second portion including a sequence having at least about 10% sequence homology with a sequence selected from a group consisting of SEQ ID Nos.30-41.

17. The peptide of claim 16, said peptide having at least about 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

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18. The peptide of claim 17, said peptide having at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

19. The peptide of claim 1, said second portion comprising a peptidomimetic of a peptide selected from the group consisting of SEQ ID Nos. 30-41.

20. The peptide of claim 1, said second portion comprising a derivative of a peptide selected from the group consisting of SEQ ID Nos. 30-41.

21. The peptide of claim 1, said second portion capable of binding with a Signal-2 ligand on an antigen presenting cell.

22. The peptide of claim 21, said peptide capable of initiating a second signal in a T-cell.

23. The peptide of claim 1, said peptide capable of modifying an immune response from a type-1 dominated response to a type-2 dominated response.

24. The peptide of claim 1, said peptide capable of modifying an immune response from a type-2 dominated response to a type-1 dominated response.

25. The peptide of claim 1, said second portion being associated with a particular type of immune response.

26. The peptide of claim 2, said linking portion being positioned intermediate said first portion and said second portion.

27. The peptide of claim 1, said peptide being synthesizable as one continuous sequence.

28. A method of modifying an immune response comprising the steps of: (a) contacting an antigen presenting cell with a peptide, said peptide comprising a first portion binding to a major histocompatability complex on said antigen presenting cell to form a peptide:MHC complex, and a second portion binding to a second signal moiety on said antigen presenting cell; (b) causing engagement of the T-cell receptor in a T-cell in response to the formation of said peptide:MHC complex; (c) blocking or altering a second signal in a T-cell in response to the binding of said second portion to said second signal moiety; and (d) said first and second signals generating an immune response which deviates from the immune response generally associated with said peptide:MHC complex.

29. The method of claim 28, said immune response deviating from a type-1 immune response to a type-2 immune response.

30. The method of claim 28, said immune response deviating from a type-2 immune response to a type-1 immune response.

31. The method of claim 28, said first portion having at least 10% sequence homology with a peptide derived from a Signal-1 moiety.

32. The method of claim 28, said second portion having at least 10% sequence homology with a peptide derived from a Signal-2 receptor moiety.

33. A peptide having the general formula AB wherein each of said A and B represent a chain of amino acid residues, and wherein said A chain comprises at least about 5 amino acid residues and has at least about 10% sequence homology with a Signal-1 moiety, and said B chain comprises at least about 4 amino acid residues and has at least about 10% sequence homology with a peptide derived from a Signal-2 receptor moiety.

34. The peptide of claim 34, further comprising an X chain of amino acid residues, said X chain comprising at least about 1 amino acid residue.

35. The peptide of claim 34, said X chain being positioned intermediate said A chain and said B chain.

36. The peptide of claim 33, said peptide being synthesizable as one continuous sequence.

37. The peptide of claim 34, said X chain comprising a flexible, non-substrate linker having the general formula $(Y,Z)_t$, wherein Y and Z are amino acid residues, and said Y amino acid residue is individually and respectively selected from the group consisting of non-substrate amino acids, and said Z amino acid residue is individually and respectively selected from the group consisting of hydrophilic amino acids, and wherein T ranges from 1 to 100.

38. The peptide of claim 37, said non-substrate amino acids being selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine.

39. The peptide of claim 38, said non-substrate amino acid residue being aminocaproic acid.

40. The peptide of claim 37, said hydrophilic amino acid being glycine.

41. The peptide of claim 33, said A chain including a sequence having at least about 10% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

42. The peptide of claim 41, said A chain having at least 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

43. The peptide of claim 42, said A chain having at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 1-25.

44. The peptide of claim 33, said A chain comprising a peptidomimetic of a peptide selected from the group consisting of SEQ ID Nos. 1-25.

45. The peptide of claim 33, said A chain comprising a derivative of a peptide selected from the group consisting of SEQ ID Nos. 1-25.

46. The peptide of claim 33, said Signal-1 moiety being associated with a known health condition.

47. The peptide of claim 33, said peptide capable of binding with a major histocompatability complex on an antigen presenting cell to form a peptide:MHC complex.

48. The peptide of claim 47, said peptide:MHC complex capable of engaging a T-cell.

49. The peptide of claim 33, said B chain including a sequence having at least about 10% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

50. The peptide of claim 49, said B chain having at least about 50% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

51. The peptide of claim 50, said B chain having at least about 95% sequence homology with a sequence selected from the group consisting of SEQ ID Nos. 30-41.

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52. The peptide of claim 33, said B chain comprising a peptidomimetic of a peptide selected from the group consisting of SEQ ID Nos. 30-41.

53. The peptide of claim 33, said B chain comprising a derivative of a peptide selected from the group consisting of SEQ ID Nos. 30-41.

54. The peptide of claim 33, said peptide capable of binding with a Signal-2 ligand on an antigen presenting cell.

55. The peptide of claim 54, said peptide capable of blocking or altering a second signal to a T-cell upon binding with said Signal-2 moiety.

56. The peptide of claim 33, said peptide capable of modifying an immune response from a type-1 dominated response to a type-2 dominated response.

57. The peptide of claim 33, said peptide capable of modifying an immune response from a type-2 dominated response to a type-1 dominated response.

58. The peptide of claim 33, said peptide capable of shifting a cell-mediated immune response to a humoral immune response.

59. The peptide of claim 33, said peptide capable of shifting a humoral immune response to a cell-mediated response.

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60. A method of preparing a peptide for modulating immune response, comprising the steps of:

selecting a first peptide sequence, said first peptide sequence having at least about 10% sequence homology with a sequence derived from a Signal-1 moiety;

selecting a second peptide sequence, said second peptide sequence having at least about 10% sequence homology with a sequence derived from a Signal-2 receptor moiety; and

synthesizing said peptide as a continuous peptide chain comprising said first peptide sequence and said second peptide sequence.

61. The method of claim 60, further comprising the step of selecting a third peptide sequence, said third peptide comprising at least one amino acid residue.

62. The method of claim 60, said first peptide sequence being associated with a known health condition.

63. The method of claim 60, said first peptide sequence being capable of binding with a major histocompatibility complex on an antigen presenting cell to form a peptide:MHC complex.

64. The method of claim 60, said second peptide sequence being capable of binding with a Signal-2 ligand on an antigen presenting cell.

65. The method of claim 60, further comprising the step of contacting said peptide with a major histocompatibility complex on an antigen presenting cell.

66. The method of claim 60, further comprising the step of contacting said peptide with a Signal-2 ligand on an antigen presenting cell.

62

67. A peptide comprising:

a first peptide sequence capable of initiating a first signal in a T-cell; and

a second peptide sequence capable of initiating a second signal in a T-cell.

68. The peptide of claim 67, further comprising a linking peptide sequence, said linking peptide sequence comprising at least one amino acid residue.

69. The peptide of claim 67, said first peptide sequence being derived from a Signal-1 moiety.

70. The peptide of claim 67, said second peptide sequence being derived from a Signal-2 receptor moiety.

71. The peptide of claim 67, said first peptide sequence having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 1-25.

72. The peptide of claim 67, said second peptide sequence having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 30-41.

73. The peptide of claim 68, said linking peptide sequence having the general formula of $(Y,Z)_n$ wherein Y and Z are amino acid residues, and wherein T ranges from 1 to 100.

74. The peptide of claim 73, said Y amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine.

75. The peptide of claim 73, said Z amino acid residue being individually and respectively selected from the group consisting of hydrophilic amino acid residues.

63

76. The peptide of claim 75, wherein said hydrophilic amino acid residue is glycine.

77. The peptide of claim 73, said Y amino acid residue being aminocaproic acid.

78. A method of constructing a BPI comprising the steps of: (a) selecting a known health condition; (b) identifying a TCR epitope specific for said health condition; and (c) incorporating said TCR epitope into a BPI.

79. The method of claim 78, further comprising the step of selecting a Signal-2 receptor moiety and incorporating said Signal-2 receptor moiety into said BPI.

80. The method of claim 79, further comprising the step of conjugating said TCR epitope with said Signal-2 receptor moiety.

81. The method of claim 80, said conjugating step including positioning a linker intermediate said TCR epitope and said Signal-2 receptor moiety.

82. The method of claim 81, said linker comprising at least one amino acid residue.

83. The method of claim 81, said linker having the general formula of $(Y,Z)_n$ wherein Y and Z are amino acid residues, and wherein T ranges from 1 to 100.

84. The method of claim 83, said Y amino acid residue being individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine.

64

85. The method of claim 83, said Z amino acid residue being individually and respectively selected from the group consisting of hydrophilic amino acid residues.

86. The method of claim 85, wherein said hydrophilic amino acid residue is glycine.

87. The method of claim 78, said TCR epitope being capable of binding with a major histocompatibility complex on an antigen presenting cell to form a peptide:MHC complex.

88. The method of claim 79, said Signal-2 receptor moiety being capable of binding with a Signal-2 ligand on an antigen presenting cell.

89. The method of claim 78, said TCR epitope having at least about 10% sequence homology with a sequence selected from SEQ ID Nos. 1-25.

90. The method of claim 79, said Signal-2 receptor moiety having at least about 10% sequence homology with a sequence selected from SEQ ID Nos. 30-41.

91. In combination:

a first peptide sequence capable of binding with a major histocompatibility complex on an antigen presenting cell; and

a second peptide sequence capable of binding with a Signal-2 ligand on an antigen presenting cell.

92. The combination of claim 91, further including a linker positioned intermediate said first peptide sequence and said second peptide sequence.

93. The combination of claim 91, said first peptide sequence being derived from a Signal-1 moiety.

94. The combination of claim 91, said second peptide sequence being derived from a Signal-2 receptor moiety.

95. The combination of claim 91, said first peptide sequence having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 1-25.

96. The combination of claim 91, said second peptide sequence having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 30-41.

97. The combination of claim 92, said linker comprising at least one amino acid residue.

98. The combination of claim 92, said linker having the general formula of $(Y,Z)_t$, wherein Y and Z are amino acid residues, and wherein T ranges from 1 to 100.

99. The combination of claim 98, wherein said Y amino acid residue is individually and respectively selected from the group consisting of aminocaproic acid, aminohexanoic acid, aminododecanoic acid, and β-alanine.

100. The combination of claim 99, said Y amino acid residue being aminocaproic acid.

101. The combination of claim 98, said Z amino acid residue being individually and respectively selected from the group consisting of hydrophilic amino acid residues.

102. The combination of claim 101, wherein said hydrophilic amino acid residue is glycine.

103. A peptide having at least about 10% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 42-46.

104. The peptide of claim 103, said peptide having at least about 50% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 42-46.

105. The peptide of claim 104, said peptide having at least about 95% sequence homology with a peptide selected from the group consisting of SEQ ID Nos. 42-46.

106. A derivative of a peptide, said peptide being selected from the group consisting of SEQ ID Nos. 42-46.

107. A peptidomimetic of a peptide, said peptide being selected from the group consisting of SEQ ID Nos. 42-46.

108. A peptide mimic of a peptide, said peptide being selected from the group consisting of SEQ ID Nos. 42-46.

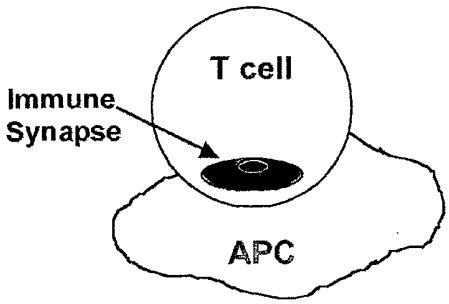


Fig. 1

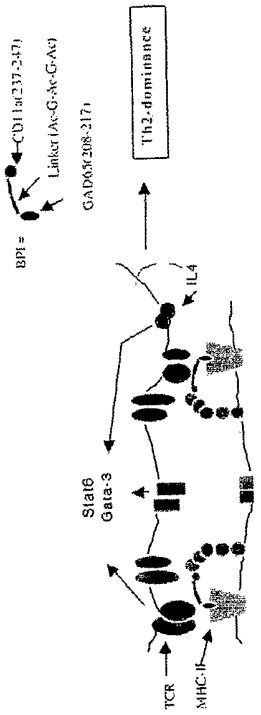
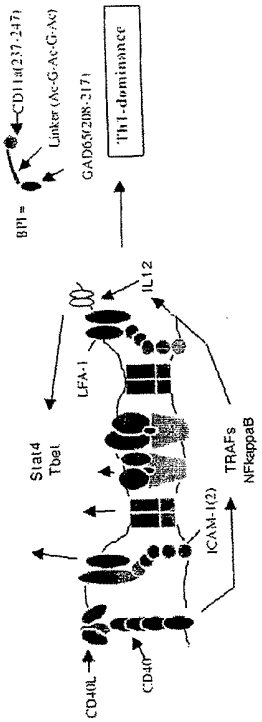


Fig. 2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GAD65(208-217)
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IL12
LFA-1
Stat4
Tbet
TRAFs
NFKappaB
ICAM-1(2)
CD40L
CD40

Fig. 3

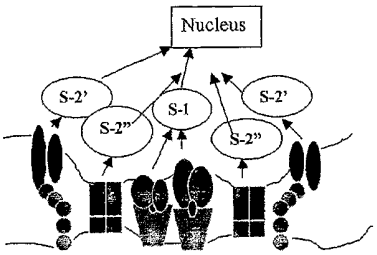

Fig. 4

71

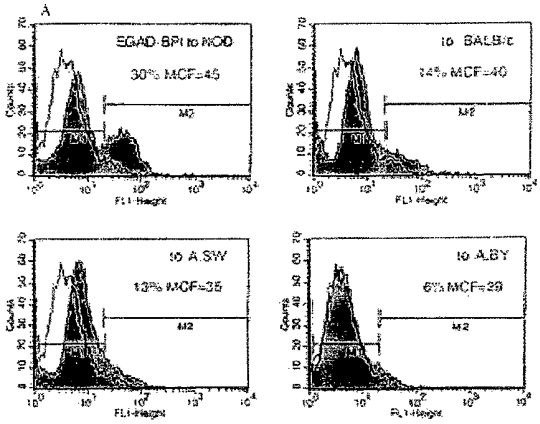
A
EGAD-SPI to NOD
30% MCF=45
M2
to BALB/c
14% MCF=40
M2
to A.SW
13% MCF=35
M2
to A.BY
6% MCF=29
M2

Fig. 5a

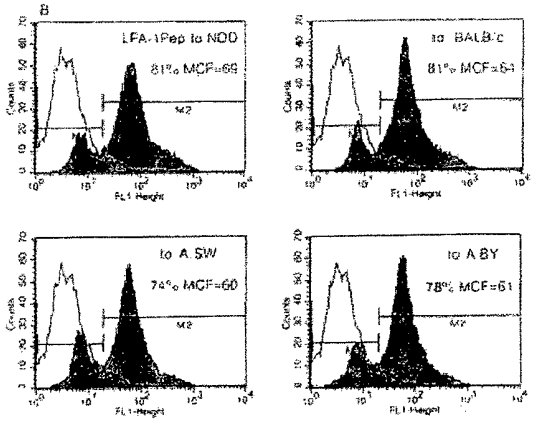
B
LFA-1Pep to NOD
81% MCF=69
M2
to BALB/c
81% MCF=64
M2
to A.SW
74% MCF=60
M2
to A.BY
78% MCF=61
M2
Counts
FL1-Height

Fig. 5b

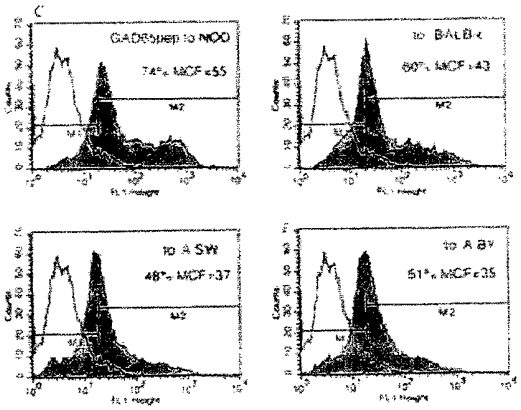
C
GAD65sp1 to NOD
74% MCF=55
M2
to BALB-c
60% MCF=43
M2
to A.SW
48% MCF=37
M2
to A.BY
51% MCF=35
M2

Fig. 5c

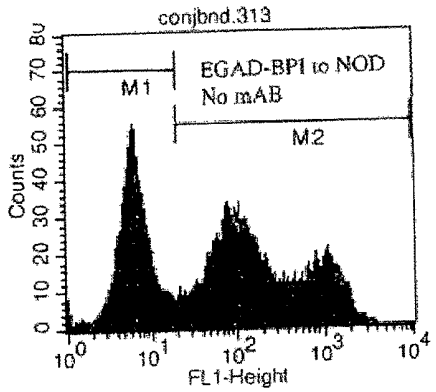
conjbnd.313
EGAD-BP1 to NOD
No mAB
M1
M2
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-Height

Fig. 6a

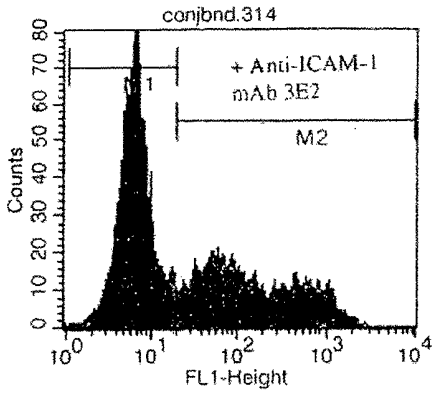
conjbnd.314
+ Anti-ICAM-1
mAb 3E2
M1
M2
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-Height

Fig. 6b

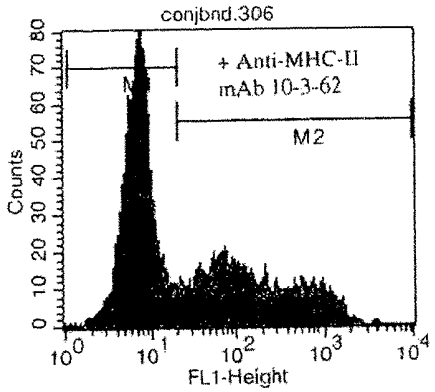
conjbnd.306
+ Anti-MHC-II
mAb 10-3-62
M2
Counts
0 10 20 30 40 50 60 70 80
10^0 10^1 10^2 10^3 10^4
FL1-Height

Fig. 6c

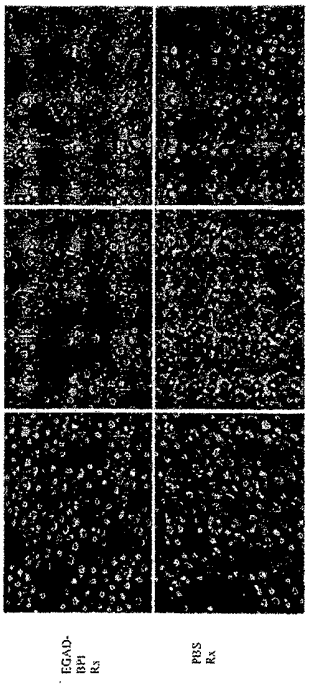

Fig. 7

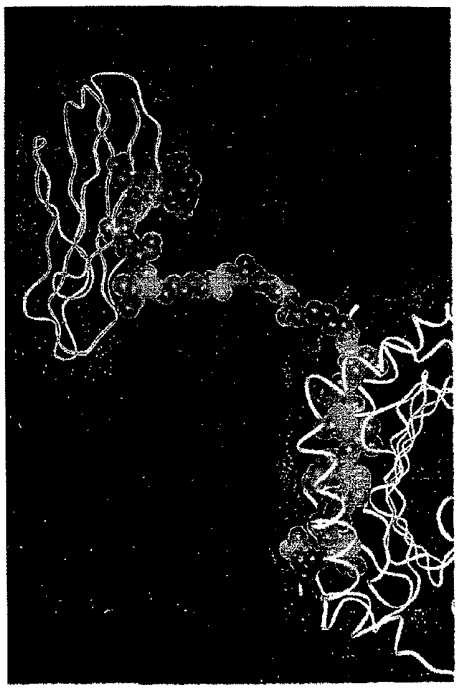

Fig. 8

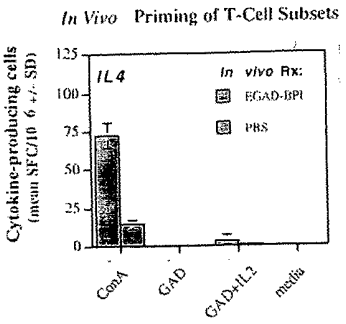
In Vivo Priming of T-Cell Subsets
Cytokine-producing cells (mean SFC/10^6 +/- SD)
125
100
75
50
25
0
IL4
In vivo Rx:
EGAD-BPI
PBS
ConA
GAD
GAD+IL2
media

Fig. 9a

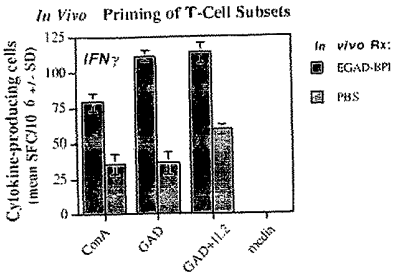
In Vivo Priming of T-Cell Subsets
IFNγ
Cytokine-producing cells (mean SFC/10^6 +/- SD)
125
100
75
50
25
0
ConA
GAD
GAD+IL-2
media
In vivo Rx:
EGAD-IPI
PBS

Fig. 9b

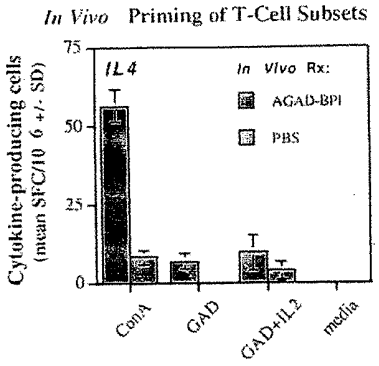
In Vivo Priming of T-Cell Subsets
Cytokine-producing cells (mean SFC/10^6 +/- SD)
75
50
25
0
IL4
In Vivo Rx:
AGAD-BPI
PBS
ConA
GAD
GAD+IL2
media

Fig. 9c

82

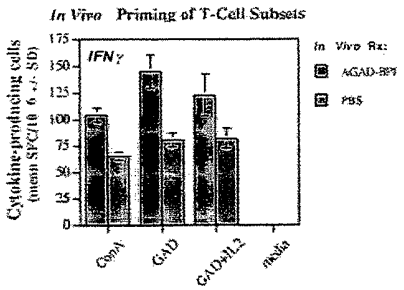
In Vivo Priming of T-Cell Subsets
IFNγ
Cytokine-producing cells (mean SFC/10^6 +/- SD)
175
150
125
100
75
50
25
0
In Vivo Rx:
AGAD-IFA
PBS
ConA
GAD
GAD+IL-2
media

Fig. 9d

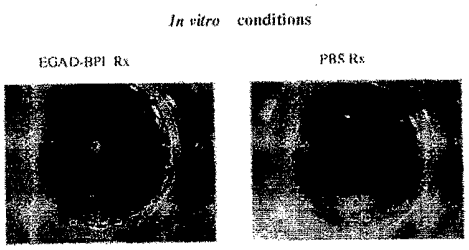

Fig. 10

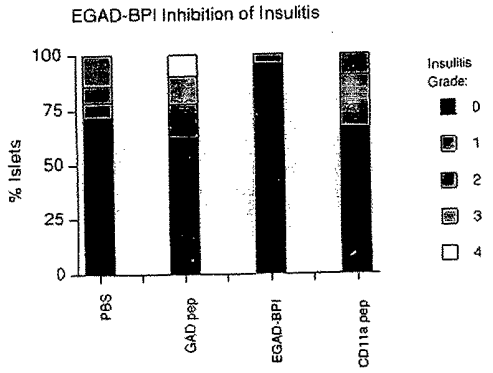
EGAD-BPI Inhibition of Insulitis
% Islets
100
75
50
25
0
PBS
GAD pep
EGAD-BPI
CD11a pep
Insulitis Grade:
0
1
2
3
4

Fig. 11

PBS

Fig. 12a

86

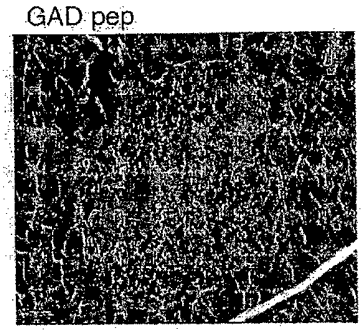

Fig. 12b

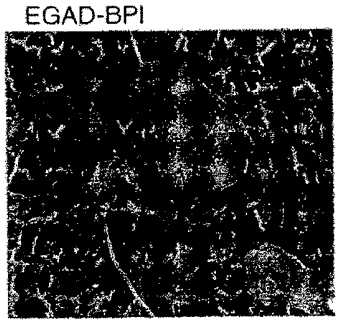

Fig. 12c

CD11a pep

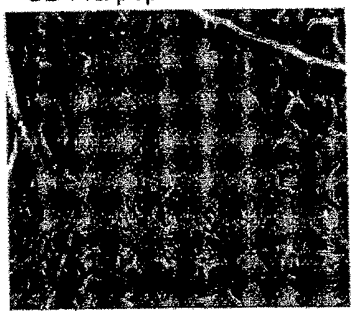

Fig. 12d

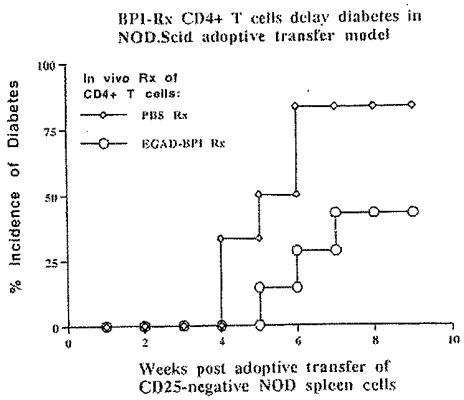
BPI-Rx CD4+ T cells delay diabetes in NOD.Scid adoptive transfer model
% Incidence of Diabetes
In vivo Rx of CD4+ T cells:
PBS Rx
EGAD-BPI Rx
100
75
50
25
0
0
2
4
6
8
10
Weeks post adoptive transfer of CD25-negative NOD spleen cells

Fig. 13

30805 Murray.ST25.txt
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Siahaan, Teruna J
Hu, Yongbo

<120> Signal-1/Signal-2 Bifunctional Peptide Inhibitors

<130> 30805 Murray

<140> US 09/739,466
<141> 2000-12-18

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1 5

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<223> The second X is aminododecanoic acid.

<220>
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<222> (5)..(5)
<223> The third X is aminocaproic acid.

<400> 28
Xaa Gly Xaa Gly Xaa
1 5

<210> 29
<211> 5
<212> PRT
<213> Artificial

<220>
<221> misc_feature
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<223> X is aminohexanoic acid

<220>

<221> misc_feature

<222> (3)..(3)

<223> X is aminohexanoic acid

<220>

<221> misc_feature

<222> (5)..(5)

<223> X is aminohexanoic acid

<400> 29

Xaa Gly Xaa Gly Xaa
1 5

<210> 30

<211> 10

<212> PRT

<213> Homo sapiens

<400> 30

Ile Thr Asp Gly Glu Ala Thr Asp Ser Gly
1 5 10

<210> 31

<211> 10

<212> PRT

<213> Homo sapiens

<400> 31

Thr Asp Gly Glu Ala Thr Asp Ser Gly Asn
1 5 10

<210> 32

<211> 10

<212> PRT

<213> Homo sapiens

<400> 32

Ala Ser Pro Gly Lys Ala Thr Glu Val Arg
1 5 10

<210> 33

<211> 10

<212> PRT

<213> Mus musculus

<400> 33

Ser Pro Ser His Asn Thr Asp Glu Val Arg
1 5 10

<210> 34

<211> 12

<212> PRT

<213> Homo sapiens

<400> 34

Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu
1 5 10

<210> 35

<211> 12

<212> PRT

<213> Mus musculus

<400> 35

Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Phe Val
1 5 10

<210> 36

<211> 10

<212> PRT

<213> Mus musculus

<400> 36

Ile Thr Asp Gly Glu Ala Thr Asp Ser Gly
1 5 10

<210> 37

<211> 13

<212> PRT

<213> Homo sapiens

<400> 37

Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu
1 5 10

<210> 38

<211> 13

<212> PRT

<213> Mus musculus

<400> 38

Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu
1 5 10

<210> 39

<211> 16

<212> PRT

<213> Homo sapiens

<400> 39

Tyr Met Arg Asn Ser Lys Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly
1 5 10 15

<210> 40

<211> 16

<212> PRT

<213> Mus musculus

<400> 40

Tyr Met Arg Asn Ser Lys Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly
1 5 10 15

<210> 41

<211> 10

<212> PRT

<213> Mus musculus

<400> 41

Thr Asp Gly Glu Ala Thr Asp Ser Gly Asn
1 5 10

<210> 42

<211> 25

<212> PRT

<213> Artificial

<220>

<221> misc_feature

<222> (11)..(11)

<223> X is aminocaproic acid

<220>

<221> misc_feature

<222> (13)..(13)

<223> X is aminocaproic acid

<220>

<221> MISC_FEATURE

<223> The artificial sequence includes the GAD65 peptide sequence from homo sapiens and the CD11a peptide sequence from homo sapiens lin

ked by a series of alternating aminocaproic acid and glycine resi
dues

<220>

<221> misc_feature

<222> (15)..(15)

<223> X is aminocaproic acid

<400> 42

Glu Ile Ala Pro Val Phe Val Leu Leu Glu Xaa Gly Xaa Gly Xaa Ile
1 5 10 15

Thr Asp Gly Glu Ala Thr Asp Ser Gly
20 25

<210> 43

<211> 29

<212> PRT

<213> Artificial

<220>

<221> misc_feature

<222> (9)..(9)

<223> The first X is aminocaproic acid.

<220>

<221> misc_feature

<222> (11)..(11)

<223> X is aminododecanoic acid.

<220>

<221> misc_feature

<222> (13)..(13)

<223> X is aminocaproic acid.

105

<220>

<221> misc_feature

<223> The artificial sequence includes the Tat peptide from simian immunodeficiency virus, a linker consisting of aminocaproic, aminododecanoic, and glycine residues and the CTLA4 (93-104) peptide sequence from mus musculus

<400> 43

Ser Thr Pro Glu Ser Ala Asn Leu Xaa Gly Xaa Gly Xaa Tyr Met Arg
1 5 10 15

Asn Ser Lys Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly
20 25

<210> 44

<211> 37

<212> PRT

<213> Artificial

<220>

<221> misc_feature

<222> (21)..(21)

<223> X is aminocaproic acid

<220>

<221> misc_feature

<222> (23)..(23)

<223> X is aminocaproic acid.

<220>

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<222> (25)..(25)

<223> X is aminocaproic acid.

<220>

<221> misc_feature

<223> The artificial sequence includes the p24 peptide from HIV-1, a series of alternating aminocaproic acid and glycine residues, and the FAS-ligand from homo sapiens

<400> 44

Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser
1 5 10 15

Pro Arg Thr Leu Xaa Gly Xaa Gly Xaa Lys Val Glu Leu Met Tyr Pro
20 25 30

Pro Pro Tyr Phe Val
35

<210> 45

<211> 30

<212> PRT

<213> Artificial

<220>

<221> misc_feature

<222> (14)..(14)

<223> X is aminocaproic acid.

<220>

<221> misc_feature

<222> (16)..(16)

<223> X is aminoaminohexanoic acid.

<220>

<221> misc_feature

<223> The artificial sequence includes a collagen II (1168-1180) peptide sequence, a sequence including aminocaproic acid, aminohexanoic acid and glycine and CD40L (93-104) from homo sapiens

<400> 45

Gln Tyr Met Arg Ala Asp Gln Ala Ala Gly Gly Leu Arg Xaa Gly Xaa
1 5 10 15

Gly Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu
20 25 30

<210> 46

<211> 31

<212> PRT

<213> Artificial

<220>

<221> misc_feature

<222> (11)..(11)

<223> X is aminocaproic acid

<220>

<221> misc_feature

<222> (13)..(13)

<223> X is aminocaproic acid

<220>

<221> misc_feature

<222> (15)..(15)

<223> X is aminocaproic acid

<220>

<221> misc_feature

<223> The artifiicial sequence includes the HPV E7 peptide sequence fro m human papilloma virus, a series of alternating aminocaproic aci d and glycine residues, and the CD95 (143-155) peptide sequence o f the FAS-ligand from homo sapiens

<400> 46

30805 Murray.ST25.txt

Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr Xaa Gly Xaa Gly Xaa Tyr
1 5 10 15

Met Arg Asn Ser Lys Tyr Arg Ala Gly Gly Ala Tyr Gly Pro Gly
20 25 30

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/48632

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 39/00
US CL : 424/192.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/192.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN/CAS: Medline, Caplus, Embase, Biosis, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOYLE et al. Enhanced responses to a DNA vaccine encoding a fusion antigen that is directed to sites of immune induction. Nature. March, 1998, Vol. 392, pages 408-411.	1-7, 13-15, 21-40, 46-48, 54-88, 91-94 and 97-102
Y	HUANG et al. Enhanced antitumor immunity by fusion of CTLA-4 to a self tumor antigen.Blood. 01 December 2000, Vol. 96, No. 12, pages 3663-3670.	1-7, 13-15, 21-40, 46-48, 54-88, 91-94 and 97-102
Y	US 5,540,926 A (ARUFFO et al.) 30 July 1996 (30.07.1996)	1-7, 13-15, 21-40, 46-48, 54-88, 91-94, 97-102
Y	WO 98/044129 (THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH) 08 October 1998 (08.10.1998)	1-7, 13-15, 21-40, 46-48, 54-88, 91-94 and 97-102

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 September 2002 (06.09.2002)	02 OCT 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Amy DeCloux Telephone No. 703 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/48632

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 8-12,16-20,41-45 and 49-53
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
These claims contain SEQ ID NOs: but no sequence CRF has been entered.

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

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(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZM,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZW

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