(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 November 2005 (17.11.2005)

PCT

(10) International Publication Number
WO 2005/108432 A2

(51) International Patent Classification[7]: C07K 16/28

(21) International Application Number:
PCT/US2005/011209

(22) International Filing Date: 30 March 2005 (30.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/557,746 30 March 2004 (30.03.2004) US

(71) Applicant *(for all designated States except US)*: **INDIANA UNIVERSITY RESEARCH & TECHNOLOGY CORPORATION** [US/US]; 351 West 10th Street, Indianapolis, IN 46202 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **SRINIVASAN, Mythily** [IN/US]; 354 Wakefield Trace, Greenwood, IN 46142 (US).

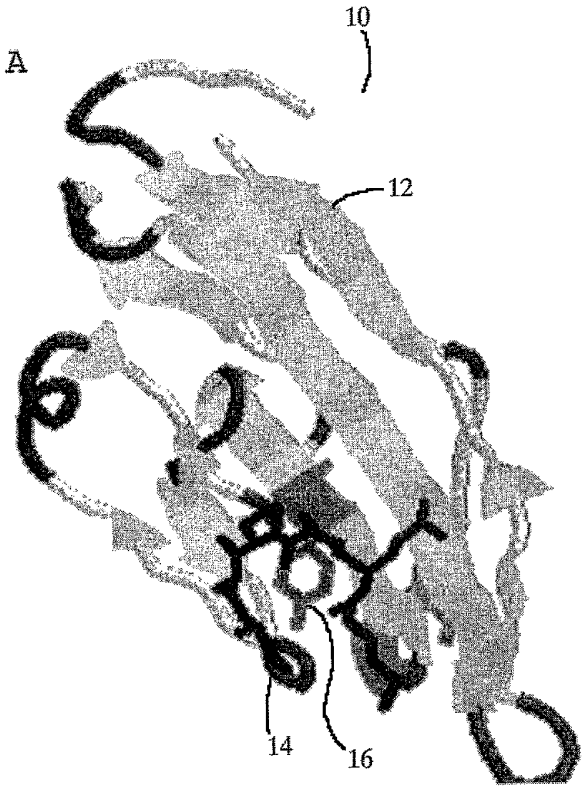
(74) Agents: **EMANUELE, John, J.** et al.; Woodard, Emhardt, Moriarty, Mcnett & Henry LLP, Bank One Center/Tower, Suite 3700, 111 Monument Circle, Indianapolis, IN 46204 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, 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, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: CD80 (B7-1) BINDING PEPTIDES AND USES THEREOF



(57) Abstract: Molecules that bind the CD80 (B7-1) ligand presented by Antigen Presenting Cells and thereby selectively inhibit costimulation of T cells. Various forms and preparations of these molecules may bind to the CD80 ligand under either or both in *vivo* or in *vitro* conditions. These molecules may be referred to as pseudo-receptors include polypeptides include polypeptides that incorporate the amino-acid sequence MQPPGC, and a retro-inverso peptide mimic that includes the D-amino acid sequence CGPPQM. These molecules have a propensity to adopt a polyproline type II helical conformation under physiological conditions. Uses for these molecules include studying the immune system, screening for other compounds that affect the immune system, or at least components of the immune system, and the treatment of autoimmune diseases such as multiple sclerosis, colitis (inflammatory bowel disease -IBD), rheumatoid arthritis, Crohn's disease, Sjorgren's syndrome, diabetes mellitus, and solid tissue transplant rejection.

WO 2005/108432 A2

GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

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.

CD80 (B7-1) BINDING PEPTIDES AND USES THEREOF

PRIORITY CLAIM

This Application claims the benefit of U.S. Provisional Patent Application No. 60/557,746 filed on March 30, 2004, which is incorporated herein in its entirety. The United States government has certain rights to this invention pursuant to Grant No. RO3 AR051411-02, obtained from the National Institute of Health-National Institute of Arthritis Musculoskeletal and Skin Diseases.

BACKGROUND

Persistent activation of auto reactive T cells plays a central role in the pathogenesis of many autoimmune diseases. Martin, R., H. F. McFarland, D. E. McFarlin, (1992), "Immunological aspects of demyelinating diseases," *Annu. Rev. Inimunol.* 10:153. Bar-Or, A., E. M. Oliveira, D. E. Anderson, D. A. Hafler, (1999), "Molecular pathogenesis of multiple sclerosis," *J. Neuroimmunol.* 100:252. Complete activation of T cells requires an antigen specific primary signal and a costimulatory signal. Lenschow, D. J., T. L. Walunas, J. A. Bluestone, (1996), "CD28/B7 system of T cell costimulation," *Annu. Rev. Inimunol.* 14:233. CD80 (B7-1) is a membrane-bound protein expressed primarily on antigen presenting cells (APCs) that binds the costimulatory receptors CD28 and CD152 on T cells. Binding of CD80 to CD28 or CD152 affects T-cell behavior and helps to regulate the immune response. Boise, L. H., A. J. Minn, P. J. Noel, C. H. June, M. A. Accavitti, T. Lindsten, C. B. Thompson, (1995), "CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-xL," *Immunity* 3:87. Karandikar, N. J., C. L. Vanderlugt, T. L. Walunas, S. D. Miller, J.A. Bluestone. 1996. CTLA-4: a negative regulator of autoimmune disease. *J. Exp. Med.* 184:783. Boussiotis, V. A., G. J. Freeman, J. G. Gribben, L. M. Nadler, (1996), "The role of B7-1/B7-2:CD28/CLTA-4 pathways in the prevention of anergy, induction of productive immunity and down-regulation of the immune response," *Immunol. Rev.* 153:5. Chang, T. T., C. Jabs, R. A. Sobel, V. K. Kuchroo, A. H. Sharpe,

(1999), "Studies in B7-deficient mice reveal a critical role for B7 costimulation in both induction and effector phases of experimental autoimmune encephalomyelitis," *J. Exp. Med.* 190:733. Truneh, A., M. Reddy, P. Ryan, S. D. Lyn, C. Eichman, D. Couez, M. R. Hurle, R. P. 40 Sekaly, D. Olive, R. Sweet, (1996), "Differential recognition by CD28 of its cognate counter receptors CD80 (B7.1) and B70 (B7.2): analysis by site directed mutagenesis," *Mol. Irnmunol.* 33:321.

Two structurally and functionally well characterized T- cell surface costimulatory molecules are CD 28 and the cytotoxic T lymphocyte associated antigen (CTLA4)/CD152, both of which bind the same ligands, CD80 (B7-1) and CD 86 (B7-2), on antigen presenting cells (ATC). Signaling via the CD 28 pathway mediates T cell activation, ligation of CD152 down-regulates T-cell proliferation and function. Lenschow, D. *et al.* (1996) *Annu. Rev. Immunol.* 14, 233-258. Given, their importance in immune regulation CD80/CD86-CD28/CD152 costimulatory molecules are potential therapeutic targets for modulating T cell responses.

Blockade of the B7-1:CD28/CD152 costimulatory pathway by CD152-fusion protein or anti-B7-1 monoclonal antibody suppresses symptoms of some diseases in some models of pathologies. Hohlfeld, R, (1997), "Biotechnological agents for the immunotherapy of multiple sclerosis: principles, problems and perspectives," *Brain* 120:865. Perrin, P. J., D. Scott, C. H. June, M. K. Racke, (1995), "B7-mediated costimulation can either provoke or prevent clinical manifestations of experimental allergic encephalomyelitis," *Immunol. Res.* 14:189. Perrin, P. J., C. H. June, J. H. Maldonado, R. B. Ratts, M. K. Racke, (1999), "Webb, L. M., Walmsley, M. J., and Feldmann, M. (1996) *Eur J Immunol* 26, 2320-2328". Potential problems associated with the use of mAb and fusion proteins to regulate diseases involving T cell regulation include their unstability, high molecular weight, poor bioavailability, and tendency to elicit an unintended immune response (Quan, C., N. J. Skelton, K. Clark, D. Y. Jackson, M. E. Renz, H. H. Chiu, S. M. Keating, M. H. Beresini, S. Fong, D. R. Artis, (1998), "Transfer of a protein binding epitope to a minimal designed peptide," *Biopolymers* 47:265. Goodman, M., S. Ro, T. Yamazaki, J. R. Spencer, A. Toy, Z. Huang, Y. He, T.

Reisine, (1992), "Topochemical design of bioactive peptides and peptidomimetics," *Bioorg. Khim.* 18:1375). There is a need then for additional molecules that can interact with key portions of the immune system. One aspect of the present invention addresses this need.

SUMMARY

One embodiment is a molecule that interacts with the antigen presenting cell (APC) ligand CD80 (B7-1), in which a molecule is, for example, a polypeptide that includes the L-amino acid sequence MQPPGX (SEQ ID 1), where X is at least one amino acid selected from the group of amino acids consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V. The polypeptide MQPPGC (SEQ ID 2) at least substantially adopts a polyproline type 11 helical conformation under physiological conditions and interacts with the receptor binding regions of molecules such as CD80 and other member of the B-1 family. The molecules can contain between about 20 to about 6 amino acid residues. In one embodiment the molecule is end capped. In one embodiment the molecule is water soluble. In one embodiment of the molecule includes the sequence MQPPGC.

Another embodiment is a molecule that interacts with the antigen presenting cell (APC) ligand CD80 (B7-1), in which a molecule includes for example, a retro-inverso peptide mimic that includes the D-amino acid sequence XGPPQM (SEQ ID 3), where X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V. The polypeptide XGPPQM (SEQ ID 3) at least substantially adopts a polyproline type 11 helical conformation under physiological conditions and interacts with the receptor binding regions of molecules such as CD80 and other member of the B-1 family. The molecules can contain between about 20 to about 6 amino acid residues. In one embodiment the molecule is end capped. In one embodiment the molecule is water soluble. In one embodiment of the molecule includes the sequence CGPPQM.

Still another embodiment is a method of modulating the immune response in animals, including down regulating and in some instances completely blocking or almost completely blocking T-cell costimulation by antigen presenting cell (APC) bearing, for example, the ligand CD80 (B7-1). This method comprises the steps of providing a molecule that interacts with CD80 and in some embodiments with other or additional molecules in the B-1 family to varying degrees. In one embodiment these molecule include the sequence MQPPGX (SEQ ID 1), where X

is at least one amino acid selected from the group of amino acids consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V. These molecules may adopt a polyproline II helical structure that interacts with molecules such as CD80. Other steps in the method include contacting at least a portion of the immune system with these modulating molecules. These molecules depending upon there composition may be end blocked, and/or water soluble and/or include between about 20 to about 6 amino acids. In still another embodiment the molecule used to modulate the immune system includes the retro-inverso peptide mimic that includes the D-amino acid sequence XGPPQM (SEQ ID 3), where X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V. The polypeptide XGPPQM (SEQ ID 3) at least substantially adopts a polyproline type 11 helical conformation under physiological conditions and interacts with the receptor binding regions of molecules such as CD80 and other member of the B-1 family.

Still another embodiment is a method treating a patient comprising the steps of providing at least one molecule that interacts with CD80 or similar molecules and administering at least one therapeutically effective dose of the molecule to a patient. These molecules may include sequences such as the L-amino acid sequence MQPPGX (SEQ ID 1), where X is at least one amino acid selected from the group of amino acids consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V. These molecules have a tendency to at least substantially adopts a polyproline type 11 helical conformation under physiological conditions and interacts with the receptor binding regions of molecules such as CD80 and other member of the B-1 family.

In still another embodiment is a method treating a patient comprising the steps of providing at least one molecule that interacts with CD80 or similar molecules and administering at least one therapeutically effective dose of the molecule to a patient. These molecules include the D-amino acid sequence XGPPQM (SEQ ID 3), where X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V. The polypeptide XGPPQM (SEQ ID 3) has a tendency to adopt a polyproline type 11

helical conformation under physiological conditions and interacts with the receptor binding regions of molecules such as CD80 and other member of the B-1 family.

Diseases that can be treated using these methods include, for example, but are not necessarily limited to autoimmune diseases and inflammatory diseases that at least implicate portions of the immune system. Specific diseases include, but are not limited to, multiple sclerosis, colitis (inflammatory bowel disease IBD), Crohn's Disease, rheumatoid arthritis, diabetes mellitus, Sjorgren's syndrome and solid tissue transplant rejection.

Yet another embodiment provides methods for either the in *vitro* or in *vivo* study of the immune system. These methods include methods for modeling at least some components of the immune system. The steps comprise providing a molecule that interacts with CD80 or similar components of the immune system and contacting the molecules with at least a portion of the immune system. Molecules that may be used to interact with the immune system include molecules that substantially adopt a polyproline type II helical conformation under physiological local conditions. The include molecules that include the L-amino acid sequence L-amino acid sequence MQPPGX (SEQ ID 1), where X is at least one amino acid selected from the group of amino acids consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V.

In still another embodiment the molecules used in modeling or otherwise studying features of the immune system include the D-amino acid sequence XGPPQM (SEQ ID 3), where X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V. The polypeptide XGPPQM (SEQ ID 3).

Still another embodiment is a method of screening for drug candidates that interact with components of the immune system such as CD80 and similar molecules. These methods may include the steps of providing molecules that interact with the immune system and the contacting prospective drug candidates and assaying for change in the behavior of the assay system. In one embodiment the molecules that may be screen for drug candidates include the L-amino acid

sequence MQPPGX (SEQ ID 1), where X is at least one amino acid selected from the group of amino acids consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V.

In still another embodiment the molecules are used in screening for additional molecules that interact with the immune system include the contacting prospective drug candidates with molecules that include the D-amino acid sequence XGPPQM (SEQ ID 3), where X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V. The polypeptide XGPPQM (SEQ ID 3).

BRIEF DESCRIPTION OF THE FIGURES AND TABLES

FIG. 1. Panels A, B, C and D superimposition of the CD80-CAP1 (SEQ ID 2) (1) with the polyproline motif of the free murine CD152 (SEQ ID 6) (A) (2), free human CD152 (SEQ ID 13) (B) (4), human 152 (6) complexed with CD80 Chain A (SEQ ID 8) (C) (6), and (D) CD 86 chain C (SEQ ID 9) (D) (8). The proteins are presented as a trace of the sequences. The RMSD for each fit is as indicated in the figure.

FIG 2 A. Computer generated image of the docked complex of CD80-CAP1 (SEQ ID 2) with the human CD80 (SEQ ID 8) (10). A schematic diagram of the molecular model of CD80 ECD (12) is complexed with CD80-CAP1 (14). Tyrosine 28 (16) is close to CD80-CAP1 (14).

FIG 2 B. Computer generated image (18) of all atoms within 5 Å of CD80-CAP1 (SEQ ID 2) (14). This image indicates that CD80-CAP1 is in contact with the CD80 receptor amino acid residues critical for receptor binding. For further discussion please see Srinivasan (2005) JBC, vol. 280 pp. 10152.

FIG. 3. The circular dichroism (CD) spectrum (20) of CD80-CAP1 (SEQ ID 2) (22) and CD80-CAP3 (SEQ ID 17) (24) peptide (l00μM in water) at 5°C in a q mM sodium citrate, 1 mM sodium borate, 1 mM sodium phosphate buffer and 15 mM NaCl with the pH adjusted at 7.0 and CD spectrum of CD80-CAP1 the presence of 6 M $CaCl_2$ (26).

FIG. 4. Graphical summary of binding data collected using an Enzyme Linked Immunoabsorbent Assay (ELISA). Panel A shows the percent CD80 binding CD28 in the presence of either control peptide or CD80-CAP1; data were collected using 3 concentrations 125, 250, or 500 μM of either CD80-CAP1 or control. Panel B shows the percent CD80 binding CD162 in the presence of either control peptide or CD80-CAP1; data were collected using 3 concentrations 125, 250, or 500 μM of either CD80-CAP1 or control. Panel C shows the percent CD86

binding CD28 in the presence of either control peptide or CD80-CAP1; data were collected using 3 concentrations 125, 250, or 500 μM of either CD80-CAP1 or control. Panel D shows the percent CD86 binding CD28 in the presence of either control peptide or CD80-CAP1; data were collected using 3 concentrations 125, 250, or 500 μM of either CD80-CAP1 or control.

FIG. 5. Shows change in absorbance over time for CD28-CD80 (panel A) and CD152-CD80 (panel B) reactions were measured over the time period of 1 to 300 seconds (s) with a mix time of 0.30 s. Readings were made at various times with a 5 s interval between data points. Concentrations of CD80-CAP1 used in the assays were as follows: 0, 25, 50 and 500 μM. The maximal velocity (mean optical density min^{-1}) of CD28-CD80 (panel C) and CD152-CD80 (panel D) was measured at the following concentrations of CD80-CAP1 0, 25, 50, 100, and 200 μM.

FIG. 6. The CD80-CAP1 inhibits T cell proliferation. Panel A shows the counts per minute of [^{3}H]-thymadine produced by mouse lymph node cells (LNC) sensitized to collagen II in the presence of one of the following CD80-CAP1, CD80-CAP3 or control peptide; these molecules were added at the following concentrations 0, 125, 250, or 500 μM. Panel B shows the counts per minute of [^{3}H]-thymadine mouse lymph node cells (LNC) sensitized to collagen V in the presence of one of the following CD80-CAP1, CD80-CAP3 or control peptide; these molecules were added at the following concentrations 0, 125, 250, or 500 μM.

FIG. 7. Treatment with CD80-CAP1 reduces the symptoms of collagen induced arthritis (CIA): DBA/1 Lac J mice were immunized with 100μg of bcII in CFA. Groups of mice were administered intravenously 500μg of CD80-CAP1, control peptide or carrier on the day of immunization. Severity of arthritis was evaluated by assigning a score of 0 to 4 based on the degree of inflammation for each limb, with 4 indicating severe arthritis and 0 indicating no arthritis and a maximum score of 16 per mouse. The numbers of mice in each treatment group

(N) are indicated. Data are presented in panel A as mean severity of arthritic mice (total clinical score per group divided by the number of arthritic mice in the group) and in panel B as average severity per arthritic mouse per group (* = $p<0.05$).

FIG. 8. Treatment of lymph node cells (LNC) with CD80-CAP1 SEQ ID 2 inhibits T cells. Synovial cells from CIA induced mice were treated with 500μg of CD80-CAP, control peptide or carrier. Cells from treated mice were isolated 30 days post-CII immunization and were cultured with 20μg/ml bCII for a total of 72 h including a 16 h pulse with [^{3}H]-thymidine. Base line proliferation of LNC cultures in the absence of bCII was 2846 cpm. Data represents mean stimulation index +/- SE (* = $p<0.05$).

FIG. 9. Results of enzyme-linked immunosorbent assay (ELISA) showing evidence that CIA mice responded to treatment with CD80-CAP1. Tissue culture supernatants from either the stimulated CD4 T cells or the sera from the CIA mice were assayed by ELISA for murine IL-12 (Panel A), INF-γ (Panel B) and IL-6 (Panel C). Assays were performed using commercially available paired antibodies in accordance with the manufacturer's instructions (eBioscience, San Diego, CA).

FIG. 10. CD80-CAP1 can be used to treat an animal model of colitis (Inflammatory Bowel Disease - IBD). Colitis was induced in population of mice by injecting T cells purified from normal mouse spleens into SCID mice. One group of mice was treated with 500 μg of CD80-CAP1 on the same day that the spleen cells were injected into the mice. Panel A shows percent gain in weight in individual mouse followed for the 10 to 27 day post transfer period. Panel B shows average percent gain in weight was followed for the 14 to 27 day post transfer period.

TABLE 1. A summary of (A) the phi (ϕ) and psi (ψ) angles of the residues constituting the conserved polyproline motif in the solution structures of murine CD152 (SEQ ID 6) (PDB 1DQT), human CD152 (SEQ ID 13) in solution (PDB IAH1), in complex with CD80 (SEQ ID 8) (PDB 118 1, chain C) and CD86 (SEQ ID 9) (PDB 1185, chain C) and in the molecular model of murine CD28 (SEQ ID

5). These data were determined using the SWISS Model. (B). Angles of phi (ϕ) and psi (ψ) for B7-CAP are also shown.

TABLE 2. Summary of estimated physical parameters such as molecular weight, accessible surface area and surface volume of the hydrophobic hexapeptide "MYPPPY" (SEQ ID 12) and CD80-CAP1 (SEQ ID 2).

TABLE 3. A summary of the root RMSD between the ligand binding polyproline residues of the free murine CD152 (SEQ ID 6), free human CD152 (SEQ ID 13), CD80/CD86 ligand bound human CD152 (SEQ ID 7), murine CD28 (SEQ ID 5) model and the B7-CAP (SEQ ID 2). These data were determined using the modeling program Chemera.

DETAILED DESCRIPTION

For the purposes of promoting an understanding of the principles of the invention, reference will now be made to the embodiments described herein and specific language will be used to describe the same. It will, nevertheless, be understood that no limitation of the scope of the invention is thereby intended. Any alterations and further modifications in the described devices, systems, and treatment methods, and any further applications of the principles of the invention as described herein, are contemplated as would normally occur to one skilled in the art to which the invention relates. While aspects of the invention may be discussed in terms of specific or general theories or principles, the invention is in no way bound by these theories or principles. Such discussion is purely illustrative and in no way limiting.

The following one letter or three letter abbreviations are used to refer to either Dextrorotary (D) or Levorotary (L) amino acids these abbreviations are as follows: Alanine: Ala, A; Arginine: Arg, R; Asparagine: Asn, N; Aspartic Acid: Asp, D; Asparagine or aspartic acid: Asx, B; Cysteine: Cys, C; Glutamine: Gln, Q; Glutamic acid: Glu, E; Glutamine or glutamic acid: Glx, Z; Glycine: Gly, G; Histidine: His, H; Isoleucine: Ile, I; Leucine: Leu, L; Lysine: Lys, K; Methionine: Met, M; Phenylalanine: Phe, F; Proline: Pro, P; Serine: Ser, S; Threonine: Thr, T; Tryptophan: Trp, W; Tyrosine: Tyr, Y; and Valine: Val, V.

Amino acids may be joined together by peptide bonds or by other bonds so long as the structure of the polypeptide, peptide or peptide mimic formed is such that it interacts with the APC CD80 (B7-1) ligand and effects the behavior of T-cells that interact with the CD80 ligand.

Creating a retro-inverso peptide mimic may involve using D in place of L amino acids and reversing of amide bonds within the peptide backbone. One effect of this is to create an analog such that the reversed amide bonds (NHCO) in the modified peptide retains both the planarity and conformational restriction of peptide bonds (CONH). In this manner, the spatial orientation of the side chains remains closely related to that of the original peptide, Goodman, M., S. Ro, T. Yamazaki, J. R. Spencer, A. Toy, Z. Huang, Y. He, T. Reisine, (1992),

"Topochemical design of bioactive peptides and peptidomimetics," *Bioorg. Khim.* 18:1375.

The terms "subject" and "patient" are used interchangeably and are intended to include organisms in which an immune response can be elicited, for example, mammals. Examples of subjects or patients include humans, monkeys, dogs, cats, mice, rats, and transgenic species thereof. An individual need not be ill in order to be classified as either a subject or patient; for example, a healthy subject of a test involving the molecules disclosed herein may also be considered to be a patient.

All sequences submitted on Compact disk or on a diskette are incorporated herein by reference. Some sequences are identified and or referred to by use of their Protein Data Bank (PDB) numbers these sequences are incorporated herein by reference from their source for example PDB or Genebank. Some sequences are included in incorporated by reference references accordingly these sequences are themselves incorporated by reference.

The B-7 family of molecules includes for example B7-1, B7-2 and the like. CD80 is a member of the B-7 family and is identical to B7-1. Accordingly, CD80, B7-1, CD80 (B7-1) used in the context of same animal or synthetic source all of these labels refer to the same molecule. Similarly, the names or labels B-7-1-CAP, CD80-CAP, and CD80 (B7-1)-CAP, used in the same context, refer to the same molecule.

As used herein a competitive antagonist of a receptor ligand may function as a pseudo receptor by effectively competing with the receptor for binding of the ligand, thereby interfering with the receptor ligand binding. In some instances CAP molecules such as CD80-CAP1 are also referred to as pseudoreceptors.

Classical methodologies for the identification and development of useful therapeutic agents include, but are not limited to rational structure based drug design, high throughput screening and virtual screening. Which approach is the best is generally not known at the onset of the process of identifying a new drug or new drug candidates. Which approach is best to try is a function of many variable including how much is known about the biology and structure of the drug target and class of drug candidates.

When the drug target is a protein based receptor that is thought to interact with proteins, peptides, polypeptides, and/or protein mimics one useful approach is to construct a combinatorial library of peptides or peptide mimics. Library compounds are then screened to determine which molecules interact with the target molecule. A number of high throughput screens including some that use phage display technology have been developed to help identify drug candidates. If a suitable assay is available, or can be developed, it is also possible to carryout high throughput screening of the library to identify molecules that are likely to be good candidates for drug development. While this approach for drug identification is oftentimes effective there are certain drawbacks to this approach which tend to limit its utility.

Sometimes the combinatorial approach and the large number of peptides or peptide mimics involved in random peptide screening by phage display compromises the process of identification and development of candidate molecules. Additionally, sometimes a structure based rational candidate design approach offers an attractive alternative to more stochastic approaches of candidate identification. This is especially true when at least some aspect of the target's structure is known or can be estimated with sufficient certainty to serve as the basis for a screen. Screens that involve using molecular models to identify candidates based on docking or fitting programs may be referred to as virtual screens. The process of using virtual screens to identify candidate molecules is referred to as virtual screening.

If the protein-protein interaction between the receptor-ligand is mediated by a subset of critical residues found in the native receptor-ligand pair then it may be possible to develop or identify a relatively small molecule that can effect the receptor-ligand interaction. If enough information is known about the physical characteristics of a receptor-ligand interface, one structure based approach for developing or identifying molecules that interfere with the interaction involves constructing pseudo-receptors or mini-receptors. These molecules may be modeled on what is learned by solving the structure of the interface or by at least modeling the interface. Mini-receptors mimic the physiological receptors in their binding of ligand. One promising target for this approach to candidate design and

identification is the interaction between certain types of T-cells and certain types of Antigen Presenting Cells (APC).

The stimulation and proliferation of T-cells is thought to involve two binding events. One event includes an interaction between the receptor CD3 on the T-cell surface and an antigenic peptide presented to the receptor by either a class I or class II MCH protein localized to the surface of an Antigen Presenting Cell (APC). Antigenic stimulation alone produces anegy and T-cell death by apoptosis. In order to insure T-cell survival and/or proliferation, a second T cell APC interaction must occur. The second interaction is oftentimes referred to as a costimulatory signal and involves an interaction between receptor-ligand pairs expressed on the surface of APCs and T cells. T cell receptors involved in the costimulatory response include the receptors CD28 and CD152.

The T cell costimulatory receptors CD28 and CD152 are members of the immunoglobulin superfamily (IgSF). These receptors include an IgV like extracellular domain. Huang, Z., S. Li, R. Korngold, (1997), "Iinmunoglobulin superfamily proteins: structure, mechanisms, and drug discovery," *Biopolymers* 43:367. Significantly, these receptors share a highly conserved "MYPPPY" (SEQ ID 12) motif in the CDR-3 like loop region that forms the ligand-binding core (Bajorath, J., W. J. Metzler, P. S. Linsley, (1997), "Molecular modeling of CD28 and three-dimensional analysis of residue conservation in the CD28/CD152 family," *J. Mci. Graph. Model.* 15:135); WO 02/042915 Kaumaya; Pravin, T., P.

Such short segments of proline rich regions are often found in situations requiring the rapid recruitment or interchange of several proteins, such as during the initiation of transcription, signaling cascades and cytoskeletal rearrangements (Kay, B. K., M. P. Williamson, M. Sudol, (2000), "The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains," *FASEB J.* 14:23 1, Creamer, T. P., and Campbell, M. N. (2002) *Adv Protein Chem* 62, 263-282. These types of proline rich regions often play a critical role in positioning proteins so as to increase the probability that the two proteins will interact with one another. Typically, these types of proline rich regions are seen in exposed portions of globular proteins which have fast on and

off rates for binding to other proteins. For example, these proline rich regions can be found in some portions of IgSF superfamily members that are involved in receptor-ligand interactions (Terasawa, H., Kohda, D., Hatanaka, H., Tsuchiya, S., Ogura, K., Nagata, K., Ishii, S., Mandiyan, V., Ullrich, A., Schlessinger, J., and et al. (1994) *Nat Struct Biol* 1, 891-897). Structurally, polyproline stretches within the loop like structures are known to have a tendency to adopt a polyproline type II (PPII) helical conformation (Creamer, T. P., and Campbell, M. N. (2002) *Adv Protein Chem* 62, 263-282). Oftentimes PPII helices are super-secondary structural elements that serve as flexible links between other secondary structures such as alpha helices and beta sheets. It is worth noting that the MYPPPY (SEQ ID 12) motif of CD152 adopts a polyproline type II (PPII) helical conformation upon binding the ligand 17. Stamper, C. C., Zhang, Y., Tobin, J. F., Erbe, D. V., Ikemizu, S., Davis, S. J., Stahl, M. L., Seehra, J., Somers, W. S., Mosyak, L.: Crystal Structure of the B7-1/Ctla-4 Complex that Inhibits Human Immune Responses Nature 410 pp. 608 (2001). Steric interactions between adjacent residues in the sequence mediate PP II helix formation. Previously, certain synthetic polyproline peptides that adopt PP II helical conformation have been shown to bind conserved protein subdomains (Stanfield, R. L., and Wilson, I. A. (1995) *Curr Opin Struct Biol* 5, 103-113). Weak binding interactions can be advantageous to receptor-ligand interactions as they increase the rate of structural modulation allowing the binding pair to readily sample a number of possible binding motifs. Weak binding interactions may also permit relatively small changes in the sequence of the proline-rich sequence or its binding domain to dramatically effect the receptor and ligand dissociation constant (K_d) (Kay, B. K., M. P. Williamson, M. Sudol, (2000), "The importance of being proline: the interaction of proline-rich motifs in signaling proteins with their cognate domains," *FASEB J.* 14:23 1).

One aspect of the invention is a peptide, polypeptide, or peptide mimic designed with reference to amino acid residues present at the interface of CD80(B7-1)/CD152:CD28 complex. Residues in the binding region may have a propensity to adopt a PP II helical conformation. Accordingly, if a peptide or peptide mimic has the same or similar propensity to adopt a PP II helical conformation it may be able to interfere with the binding or the receptor and

ligand. For example, a pseudo-receptor with PP II helical or PP II helical like structure may be able to disrupt the binding of T-cells to APCs. Such molecules have the potential to help regulate aspects of the immune response related to T-cell interaction with antigen presenting cells (APCs) (Hohlfeld, R, (1997), "Biotechnological agents for the immunotherapy of multiple sclerosis: principles, problems and perspectives," *Brain* 120:865).

One embodiment, provides a peptide, of about 20 amino acid residues, including an amino acid sequence corresponding to MQPPGC (SEQ ID 2), or a retro-inverso peptide mimic thereof (a corresponding D form of the L amino acid including the D-amino acid sequence CGPPQM (SEQ ID 4).

Another embodiment provides a peptide of about 10 amino acid residues, including the amino acid sequence corresponding to MQPPGC (SEQ ID 2), or a retro-inverso peptide mimic thereof.

Still another embodiment includes a peptide that includes about ten amino acid residues, such that the peptide may adopt a PP II helical conformation and interact with CD80 (B7-1). One such peptide which interacts with the CD80 receptor includes a polypeptide with the amino acid sequence MQPPGC (SEQ ID 2), or a retro-inverso peptide mimic thereof.

Yet another embodiment includes a CD80 (B7-l) binding molecule, which includes the amino acid sequence of MQPPGC (SEQ ID 2), or a retro-inverso peptide mimic thereof. In one embodiment the ligand is a protein or short poly-peptide having, for example, about 20 amino acids. In one embodiment the molecule is in a water-soluble form.

In so far as using molecules of the present invention in screening for drug candidates or studying the immune system or components of the immune system either in *vivo* or in *vitro* an effective amount of the compounds can be defined as the amount of the compound necessary to affect T-cell costimulation. Means for measuring this include, but are not limited to, measuring the effect of the compounds on T cell proliferation and T cell apoptosis.

One aspect provides pharmaceutical compositions that include at least one of the proteins, polypeptides, or peptide mimics or retro-inverso peptides or other CD80 (B7-1) pseudo-receptors mentioned in the disclosure. These molecules may be combined with a pharmaceutically acceptable carrier. In this regard, the peptide

or peptide-containing molecule of the invention can be incorporated into compositions suitable for administration to human or animal patients. The peptide in such compositions is in a biologically compatible form suitable for pharmaceutical administration in *vivo*.

The definition of a therapeutically active amount of the therapeutic compositions according to the present invention includes, but is not limited to, an amount effective to partially or completely relieve the symptoms associated with a specific disease or disorder. The actual amount of a compound of the invention necessary to elicit a desired therapeutic response in a particular patient may vary according to factors such as the disease state, age, sex, and weight of the individual patient. Dosage regimes may be adjusted to optimize therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The amount of the therapeutic required to treat a given condition will depend upon the nature and severity of the condition being treated, the type of defect or disease being treated and what if any additional conditions or treatments a given patient is, or has been, subject to. Ultimately, the size of the therapeutically effective dosages will likely be determined during clinical trials. Initially, human patients will be administered doses in accord with those derived from animal studies. In some situations it may be possible to deliver a single effective amount of the therapeutic composition. In still other situations it may only be possible is achieve a satisfactory result administrating multiple dosages of the composition to a given patient.

The actual amounts of the compositions and/or the optimal dosing regimes required to best clinical effect is likely to be at least partially dependent upon the specific disease or condition being treated. For example, in rheumatoid arthritis, the therapeutic composition may be administered when patients exhibit clinical symptoms of the disease. In the case of insulin-induced diabetes mellitus, the therapeutic composition may be administered when patients have clinical symptoms, or when a genetic mutation in a patient is indicative of diabetes mellitus. In cases of allograft rejection it may be advantageous to begin dosing before the manifestation of complication due to the introduction of grafted tissues or cells.

Diseases which may be treatable using the molecules and compositions of the present invention include, but are not limited to autoimmune diseases, such as multiple sclerosis, experimental autoimmune encephalomyelitis, rheumatoid arthritis, and insulin-dependent diabetes mellitus, inflammatory bowel disease and allograft transplant rejection.

The therapeutic compound may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the therapeutic compound may be coated with a material which, for example, protects the compound from or at least slows the degradation or inactivation of the compound. Degraditive processes include, for example, the action of proteolytic enzymes or other naturally occurring agents or conditions which may tend to degrade the therapeutic compound.

In order to increase the effectiveness and/or half-life of the therapeutic compound, or at least slows its inactivation, under in *vivo* conditions it may be necessary to administer the compounds coated with, or co-administer with, additional compounds. For example, a therapeutic peptide compound made in accordance with at least one embodiment may be administered to an individual in an appropriate carrier, diluent or adjuvant, co-administered with enzyme inhibitors or in an appropriate carrier such as liposomes.

Pharmaceutically acceptable diluents for the practice of at least some aspects and embodiments include saline and aqueous buffer solutions.

The term 'adjuvant' is used in its broadest sense and includes any immune stimulating compound, such as interferon. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether, and n-hexadecyl polyethylene ether.

Some embodiments include enzyme inhibitors, for example, pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP), trasylol, and other inhibitors of enzyme activities that may reduce the half-life and/or effectiveness of the therapeutic compounds.

The term 'liposomes' used herein includes water-in-oil-in-water emulsions, aqueous solutions-in-hydrohopic compounds-in aqueous solutions as well as a vesicles comprised at least in part of a phospholipids bilayer.

Therapeutic compositions in accordance with various embodiments may be administered parenterally, or intraperitoneally or both parenterally and intraperitoneally.

Compounds of various embodiments may also be prepared by dispersing the compounds in glycerol, liquid polyethylene glycols, oils, other hydrophobic compounds and mixtures thereof.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where the active ingredient is sufficiently water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition should be aseptic and fluid enough to promote syringability exists. The composition must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi.

Suitable carriers for the active ingredient include, but are not limited to, to solvents or dispersion media containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity of the composition can be maintained, by for example, the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. Aseptic condition can be achieved and/or promoted by the addition of antibacterial and antifungal agents including, but limited to, parabens, chiorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearaté and gelatin.

Sterile injectable solutions can be prepared by incorporating the peptide in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by, for example, filter sterilization. Generally, dispersions are prepared by incorporating the active ingredients into a sterile vehicle that includes a basic dispersion medium and additional or required other ingredients from those enumerated above. In the case

of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (e.g., peptide) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

When the peptide is suitably protected, as described above, the peptide may be orally administered, for example, with an inert diluent or an assimilable edible carrier. As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the peptide and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Polypeptide, peptides, and peptide mimics of the invention can be prepared in any suitable manner including, for example, by synthetic methods such as solid-phase chemical methods, by recombinant production in a host cell that expresses a nucleic acid encoding the amino acid sequence of the peptide, or a combination thereof. For recombinant production, a suitable nucleic acid (e.g. DNA) molecule can be obtained or prepared having a nucleotide sequence encoding the amino acid sequence of the desired peptide. Such nucleotide sequences can be readily identified with reference to the known, degenerative genetic code for humans and other animals, and/or by reference to known coding regions possibly in combination. The nucleotide sequence can be incorporated into a suitable vector,

such as a viral vector, along with a promoter (e.g. a constitutive promoter) operably linked to the sequence and effective to drive the expression of the encoded peptide in a host cell, such as a bacterial (e.g. E. coli) or animal (e.g. human or non-human animal) host cell. The vector can then be used to transfect the host cell, and the host cell cultured under conditions effective to express the peptide.

The synthetically or recombinantly produced peptides can be purified for use if desired, e.g. purified to substantial homogeneity or otherwise suitable for pharmaceutical and/or research purposes, using chromatographic and/or other standard techniques thereof. Peptides, peptide mimics, and retro-inverso mimics of the invention may also have free ends, or may be end-blocked as well known in the art. As well, peptides of the invention may be incorporated as a part of larger polypeptides or proteins, or other molecules, having utility in binding B7-1 (CD80) or otherwise.

Peptides of the invention and compositions containing them may be used, for example, to modulate T cell activation in vivo by blocking T cell costimulation, and to suppress chronic inflammatory processes in autoimmune disease, graft versus host disease, and/or transplant rejection. Peptides of the invention may also be used, for example, in vitro in experimental studies relating to T cell activation. Peptides of the invention, owing to their binding properties, may also be used for purposes identified in numerous patent publications and patents in fields relating to the inhibition of T cell stimulation, including for example WO 02/42415 A2 dated May 30, 2002, and U.S. Patent Nos. 6,641,809 and 6,444,792.

EXAMPLES

Example 1

Comparative Modeling

Three dimensional modeling of the mouse CD28 (SEQ ID 5) and CD152 (SEQ ID 6) incorporating the CD80 pseudo-receptor sequence was carried out with the Geno3D program. The program was run at the Pole Bio-informatique Lyonnais server (http://geno3D-pbil.ibcp.fr). The unbound mouse CD152 (SEQ ID 6) (PDB 1DQT, chain A) and human CD152 (PDB 1AH1) and ligand bound human CD152 (SEQ ID 7) (PDB 118 L chain C) were selected for use as

templates. The structural overlap for the secondary structure comparison for the CD28 and CD152 queries and the three templates were between about 51 and 67 percent. The Geno3D program produces comparative protein structure by satisfying spatial restraints (distances and dihedrals). The Geno3D prediction system uses 'topology mapping' to predict a 3D structure from a supplied amino acid sequence.

Essentially what the Geno 3D program does is blast the sequence in a database of protein sequences composed of proteins whose 3D structures are known. It maps a sequence onto close relatives and then does an energy minimization of the tentative structure using the amino acid side chains in the searched sequence. This approach first extracts homology derived spatial constraints on many atom-atom distances and dihedral angles from the template structures. An alignment is used to derive equivalent residues between the target and the template. The homology derived and the stereochemical constraints are then used to generate protein models that best satisfy the criteria. CD28 residues (1-119 from the start of translation) were modeled using the human CD152 (PDB) as the templates. While Geno 3D does not providing exact 3D structures of proteins, it can provide useful insight into what the structure might be.

Similarity between various receptors was estimated by superimposing the molecular model of murine CD28 (SEQ ID 5) with the free mouse CDI52 (SEQ ID 6) (PDB 1DQT) and human ligand bound CD152 (SEQ ID 7) (PDB 118L, Chain B). The Root Mean Square Deviation (RMSD) of the structures with the molecular model of CD28 after sequence alignment between the model and the protein is 3.58 Å.

The similarity of the molecular model of MQPPGC (SEQ ID 2) CD80-CAP1, free CD152 (SEQ ID 6) (PDB 1DQT) and the wild type mouse CD28 (SEQ ID 5) model was estimated. The RMSD of the CD80-CAP1 (SEQ ID 2) after sequence alignment with each structure was determined. The RMSD value with free human CD152 (Protein Data Bank code 1AH1) is 0.03Å and with human CD152 complexed with CD80 (Protein Data Bank code 118L, chain A) is 0.31Å.

Example 2

Design and Synthesis of the CD80-CAP

As a preliminary note, although some of the inventive molecules are referred to as CD80-competitive antagonist peptides (CD80-CAP), it will be understood that while its mechanism of action is believed to be competitive antagonism, the invention is not necessarily limited as such. The CD-80 CAP molecules are differently named as the B7-1 CAP molecules these molecules are sometimes referred to herein as pseudo-receptors.

The interface between both CD152/CD80 and CD152/CD86 is large, burying a total of 1255 and 1290 Å of the solvent-accessible surfaces of CD80 and CD86, respectively. The proline-rich region of the CD152 CDR-3-like loop packs against the hydrophobic patch of residues that form a shallow cavity on the front face of CD80 and CD86. Similar proline-rich regions commonly occur in globular domains involved in transient protein-protein interactions. Typically, proline-rich regions preferentially adopt a PP_{II} helical conformation.

Significantly, in the complex with CD80, the Pro^{101} of CD152 is in PP_{II} helical conformation with the dihedral angles of Φ and ψ measuring -75 and 164, respectively (Table 1). A competitive antagonist for this receptor ligand interaction should not only be small as to occupy the shallow binding site of CD80 (surface area of 655 Å), but it should also mimic the PP_{II} helical conformation of the physiological receptor. Amino acids exhibit varied frequencies of occurrence at the protein interface and distinct pairing preferences at sites of protein-protein interactions. In addition, residues vary in the propensity to form PP_{II} helix. A polypeptide backbone possesses both α helix and PP_{II} helix propensity. The extent to which the PP_{II} helix is adopted is determined by the degree of backbone solvation and modulated by side chain interactions. Studies of the synthetic peptide made up of MYPPPY sequence do not exhibit PP_{II} helical conformation and had no inhibitory potential in cellular assays. This maybe attributed to the lower potential of aromatic amino acids such as tyrosine to propagate PP_{II} helix. Some of the estimated physical properties of MYPPPY (SEQ ID 12) are included in Table 2. Integrating the residue preferences and propensities, novel CD80-CAP hexapeptides were designed in the hope that they would possess significant PP_{II} helical content in the context of the CD80 binding interface.

Substituting the CD80-CAP1 residues for the hydrophobic motif in the mouse CD152, comparative modeling of the modified CD152 was performed using the mouse CD152 (Protein Data Bank code 1DQT (SEQ ID 6) as template. This gives a structural representation of the CD80-CAP with reference to the adjacent residues of CD152. Each CD80-CAP was superimposed with the ligand binding motif of free murine CD152 (Protein Data Bank code 1DQT) (SEQ ID 6), free human CD152 (Protein Data Bank code 1AH1) (SEQ ID 13), and CD80 (Protein Data Bank code 118L) (12)/CD86 (Protein Data Bank code 1185) (SEQ ID 9), bound CD 152 (SEQ ID 7). Superimposition of the CD80-CAP1 (MQPPGC) with the free mouse and human CD152 and CD80- and CD86-bound CD152 yielded r.m.s. deviation values of 0.03, 0.79, 0.31, and 4.40 Å, respectively (Fig. 1). These values suggest that the CD80-CAP1 is a close mimic of the ligand binding regions of the mouse CD152 and the CD80-bound human CD152 structures. Similar superimposition of CD80-CAP3 (MAVPAT) over free mouse CD152 and free human CD152 yielded an r.m.s. deviation value of 1.47 and 1.19 Å, respectively. All CD80-CAPs that were within 5-Å r.m.s. deviation when superimposed over free murine or human CD152 and <1-Å r.m.s. deviation when superimposed over CD80-bound CD152 were selected for *in silico* docking. Some estimated physical properties of CD80-CAP1 (SEQ ID 2) and the consensus sequence MYPPPY (SEQ ID 12) are presented in Table 2. For a more thorough discussion and colorized images equivalent to the data presented in figure 1 see Srinivasan, M. *et al.* (2005) "CD80 Binding Polyproline Helical Peptide Inhibits T cell Activation," *JBC* Vol. 280, No. 11 pp 10249-10155, incorporated herein by reference in its entirety.

Example 3

Docking of mouse CD80: CD28

The extracellular domain of mouse CD80 (SEQ ID10) was modeled by Geno3D using the solution structure of human CD80 (B7-1) (SEQ ID11), human B7-2 (SEQ ID 14) (PDB 1NCN) and the docked human CD80 (B7-1) (SEQ ID 8) as the templates. Superimposition of the residues in the CDR1 and CDR3 like regions thought to be involved in receptor binding yielded an RMSD of 1.87A. A

RMSD value of this magnitude suggests high similarity between the searched sequence and the proteins structures that were used to model it.

Similarly, the extracellular domain of mouse CD28 (SEQ ID 5) was modeled by Geno3D using the solution structure of mouse and human CTLA-4 (SEQ ID 6 and SEQ ID 13) (PDB: 1 DQT and PDB 1AH1) as the templates. Interestingly, although the MYPPPY (SEQ ID 6 and 12) motif was localized to the FG loop in both, the proline in the YPP sequence had the phi and psi angles of a PPII type II helix in CD28 and not in CD152. The PPP sequence in CD28 is in a cis-trans-trans orientation favoring PP II helical conformation, while in CD152 it is in a cis-trans-cis orientation.

Docking of the mouse CD80 and a six-residue peptide having the amino acid sequence MQPPGC (SEQ ID 2) CD80-CAP1 was performed using the known BIGGER software, which utilizes the soft docking algorithm. Superimposition of the mouse CD80 molecular model with the unbound CD80 (Protein Data Bank code 1DR9) (22) and bound human CD80 (from the complex Protein Data Bank code 118L) (12) yielded an r.m.s. deviation of 6.13 and 1.87 Å, respectively (data not shown). The difference in the r.m.s. deviation values may be attributed to the structural differences between the monomer (Protein Data Bank code 1DR9) and homodimer (Protein Data Bank code 118L) of CD80.

The program "BIGGER" was used to generate and evaluate plausible binding modes between the predicted mouse CD80 ECD and the CD80-CAP (24). The coordinates of each CD80-CAP were systematically rotated (in discrete steps of 15) and translated against the surface of CD80. The top 100 docked structures of each complex generated were screened by superimposition with the human CD80-CD152 complex (Protein Data Bank code 118L). Potential CD80-CAP-CD80-docked structures with r.m.s. deviation of <5 Å were then subjected to energy minimization to optimize the binding conformation of the amino acids by Grammos. Figure 2 shows the energy-minimized CD80-CAP1 occupying the binding cleft of CD80. The conserved CD80 residues critical for binding (Tyr^{28}, Val^{89}, and Leu^{93}) are within 5 Å of the CD80-CAP1, suggesting near native docking (12). Incorporation of glutamine increased both the PP_{II} helical propensity and the potential for interaction with the interface residues (Val^{79} and Gln^{81}) at the binding pocket of CD80. The docked interface also suggests covalent interactions

between methionine and cysteine of the CD80-CAP1 with the conserved Tyr^{28} and Gln^{33}, respectively, in the binding pocket of CD80. For further discussion see Srinivasan Et al, (2005) JBC, vol. 280 pp. 10149. This analysis revealed that the energy minimized CD80-CAP1 occupies the same binding cleft formed by the residues shown to be critical for receptor binding in the receptor-ligand complex.

Example 4

Structure of the CD80-CAP1

A molecular model of the mouse CD28 was generated by homology modeling by a web based server (Geno3D) that uses distant geometry, simulated annealing and energy minimization algorithms to build the protein 3D model. The mouse CD152 (PDB 1DQT) and the ligand bound human CD152 (PDB: 118L Chain A and B) were specified as the templates. Superimposition of the model with the chain A of 1DQT yielded an RMSD of 1.10Å and with chain B of 1DQT yielded an RMSD of 1.04Å validating the accuracy of the model. The phi and psi angles of the CD28 (SEQ ID 5) model and the bound and unbound CD152 molecules are given in Table 1. Consistent with previous reports the prolines are in tran-cis-trans orientation in CD152 and in cis-cis-trans orientation in CD28.

Substituting the CD80-CAP-1 residues for the hydrophobic motif in the mouse CD28 (SEQ ID 5), comparative modeling of the modified CD28 was performed using both the mouse CD152 (SEQ ID 6) and the CD28 model as templates by Swiss-Model server. This technique gives a structural representation of the CD80-CAP with reference to the adjacent residues of CD28. The dihedral angles of the CD80-CAP residues are given in Table 2. Referring now to Table 3, superimposition of CD80-CAP1 with the hydrophobic motif of free murine CD152 (SEQ ID 6) (PDB 1DQT), free human CD152 (PDB 1AH1) (SEQ ID 12) and ligand bound CD152 (SEQ ID 7) (PDB 118L) yields a root mean square deviation of 0.11Å, 0.84Å and 0.34Å respectively. These values suggest a close structural mimic of CD80-CAP1 with the ligand bound human CD152 and the mouse CD28 model.

Example 5

Circular Dichroism (CD) spectrum of CD80-CAP1

One way to unambiguously reveal the PP_{II} structure in solution is to use spectroscopies based on optical activity such as CD. CD spectra were recorded at 5 °C with CD80-CAPs dissolved in citrate buffer with pH adjusted at 7.0. Referring now to Figure 3 the CD spectrum of CD80-CAP1 presented a strong negative band (θ = -48,000) at 207 nm and a weak positive band (θ = 17,000) at 223 nm. These are characteristic features of a PP_{II} helix. The CD spectrum of CD80-CAP3 presented a much reduced negative band (θ = -19,000) at 209 nm and a weak positive band (θ = 17,000) at 225 nm suggestive of lower PP_{II} helical content as compared with CD80-CAP1. The molar ellipticity minimum of both CD80-CAP1 and CD80-CAP3 at 207 and 209 nm, respectively decreased drastically in the presence of 6 M $CaCl_2$ presumably due to the disruption of the PP_{II} helix. Interestingly, some additional CD80-CAPs with predicted structural similarity to the binding motif of CD152 in the docking studies lacked definitive secondary structure and exhibited random coil conformation (data not shown).

Example 6

The CD80-CAP1 competes with the physiological receptors for binding CD80

The ability of the CD80-CAP1 (SEQ ID 2) to compete with physiological receptors (CD28/CD152) for binding the ligands (CD80/CD86) was evaluated by enzyme-linked immunosorbent assay. Referring now to figure 4 panels A, B, C and D, the ability of the CD80-CAP1 to compete with physiological receptors (CD28/CD152) for binding the ligand (CD80/CD86) was assayed by ELISA. The synthetic CD80-CAP1 significantly inhibited the binding of both CD28-Fc and CD152-Fc to CD80. There was no inhibition of the receptor binding to CD86 ligand. Interestingly, the prolines in the MYPPPY motif do not adopt a PPII helical conformation upon binding CD86, suggesting that the PPII helical conformation may be selective for binding CD80. Previously, certain synthetic peptides derived from the ligand binding regions of the receptor have been shown to block receptor-ligand interactions. In addition, specific inhibition of CD80 interactions has been observed with the use of certain small molecule inhibitors.

Previously, it has been shown that relative to its affinity for binding CD152, CD80 binds CD28 with slower kinetics and lower affinity. Referring now to figure 5 panels A and B a plot of change in absorbance with time showed that the maximum absorbance was reached at a later time point in the binding of CD80 to CD28 (220 s) than to CD152 (79 s). This is consistent with previous observations of slower association rate for the former interaction than the latter. Significantly, CD80-CAP1 at 25 μM drastically reduced the rate of association of binding of CD80 to both CD28 and CD 152. As illustrated in figure 4 panel C, the maximum velocity (mean optical density (MOD)/min) of CD80 binding to CD28 derived from overlapping segments of data points in the reaction was significantly reduced from 501.3 MOD/min to 258.3 MOD/min in the presence of CD80-CAP1 (25 μM). Similar reduction in the maximum velocity was observed for the interaction between CD152 and CD80, from 575.9 MOD/min to 350.1 MOD/min in the presence of CD80-CAP1 (25 μM) (Fig. 5 panel D). CD80-CAP3 (50 μM) also competed effectively, decreasing with the maximum velocity of CD80 binding to CD28 and CD152 to 435.5 and 406 MOD/min, respectively (data not shown). The optical density experiments showed significant reduction in the association rate of select CD80-CAP and to CD28 or CD152 consistently in multiple experiments. Taken together, these data suggest that both CD80-CAP1 and CD80-CAP3 can selectively block CD80-CD28/CD152 interactions. Previously, small molecule inhibitors thought to bind at the "MYPPPY" binding site on CD80 exhibited relatively weak inhibition of CD152-CD80 interactions as compared with CD28-CD152 interaction.

The ability of the synthetic CD80-CAP1 to compete with physiological receptors (CD28/CD152) for binding the ligand (CD80/CD86) was assayed by ELISA. As illustrated by the data summarized in FIG. 7, synthetic B7-CAP significantly inhibited the binding of both CD28-Fc (SEQ ID 15) and CD152-Fc (SEQ ID 16) to CD80. There was no inhibition of the receptor binding to CD86 ligand. Interestingly, the prolines in the MYPPPY (SEQ ID 9) motif do not adopt a PPII helical conformation upon binding CD86 this suggests that the PPII helical conformation may be selective for binding CD80. Previously, certain synthetic peptides derived from the ligand binding regions of the receptor have been shown

to block receptor-ligand interactions. In addition, specific inhibition of CD80 interactions has been observed with the use of certain small molecule inhibitors.

Example 7

The CD80-CAP1 inhibits T cell response

The ability of the CD80-CAP1 to block the CD80-CD28/CD152 interactions on lymph node cells were assessed by T cell proliferation assays. LNC from bCII-sensitized mice were restimulated *in vitro* in the presence of varying concentrations of CD80-CAP. Referring now to figure 6, panel A, a significant decrease in the lymph node cell proliferative responses to collagen II was observed in cells treated with CD80-CAP1. A dose response was observed with maximum inhibition (55%) at 500 μM CD80-CAP1 (mean Δ cpm = 21,985) as compared with cells stimulated with bCII only (mean Δ cpm = 39,947) with the unstimulated cultures measuring 5648 cpm (Fig. 5). Interestingly, CD80-CAP3 (SEQ ID 15) was not inhibitory at all concentrations tested, as was the control peptide. Although CD80-CAP3 adopted PP_{II} helical conformation, none of the top 100 predicted docked structures exhibited significant proximity to the critical residues (Tyr^{28}, Val^{79}) at the binding pocket of CD80. This may explain the lack of inhibitory potential of CD80-CAP3 (SEQ ID 15) despite its ability to compete with CD80-Fc for binding CD28 and CD 152.

To further investigate the inhibitory potential of the synthetic CD80-CAP1, in *vitro* T cell proliferation assays were performed using collagen V. Collagen V was used in part because it is present in lung tissue and is thought elicit an immune response with a patient undergoes a lung transplant. Referring now to figure 6, panel B, the proliferative responses of collagen V sensitized rat LNC were measured as a function of varying concentrations of CD80-CAP1. As illustrated by the data summarized in panel B, there is a significant decrease in response to collagen V when the assay included a 500μM concentration of CD80-CAP1. These data suggest that CD80-CAP1 blocks the CD80:CD28/CD152 costimulatory signals required for sustained T cell proliferation.

Example 8

Treatment with CD80-CAP1 prevents Collagen Induced Arthritis (CIA)

The biological potential of CD80-CAP1 to block the development of inflammatory CIA during antigen priming *in vivo* was tested. Groups of DBA/1 Lac J mice were immunized with 100μg of bCII in CFA. Groups of mice were administered intravenously 500μg of CD80-CAP1 or control peptide or vehicle on the day of immunization. Severity of arthritis was evaluated by assigning a score of 0 to 4 based on the degree of inflammation for each limb, with 4 indicating severe arthritis and 0 indicating no arthritis and a maximum score of 16 per mouse. Referring now to figure 7, the numbers of mice in each treatment group are indicated. Data are presented in panel A as mean severity of arthritic mice (total clinical score per group divided by the number of arthritic mice in the group) and in panel B as average severity per arthritic mouse per group (* = $p<0.05$).

Example 9

The CD80-CAP inhibits T cells recovered lymph node cells of mice with CIA.

Synovial cells from CIA induced and treated with 500μg of CD80-CAP or control peptide or vehicle peptide treated mice were isolated 30 days post-CII immunization were cultured with 20μg/ml bCII for a total of 72 h of culture including a 16 h pulse with ^{3}H thymidine. Referring now to figure 8, base line proliferation of LNC cultures in the absence of bCII was 2846 cpm. Data represents mean stimulation index +/- SE (* = $p<0.05$).

Example 10

CD80-CAP1 down regulates the production of molecules linked to the inflammatory response

A wide array of cytokines and chemokines has been reported to be involved in inflammation associated with arthritis (Tellander, A. C., Pettersson, U., Runstrom, A., Andersson, M., and Michaelsson, E. (2001) *J Autoimmun* 17, 39-50). Cell culture supernatants form simulated CD4 T cells or sera recovered from CIA mice were assayed by ELISA for murine IL 12, interferon INF-γ and IL-6.

Assays were run using paired antibodies obtained from eBiiosciences, San Diego, CA following the suppliers suggested protocols.

As illustrated by the data summarized in figure 9, panel A, treatment with CD80-binding pseudo-receptor significantly reduced serum levels of IL-12 (panel A), INF-γ (panel B) and IL-6 (panel C). Amongst the mice with collagen induced arthritis, lymph node cells from CD80-binding pseudo-receptor treated mice secreted significantly lower amounts of pro-inflammatory cytokine IL-12 as compare to vehicle treated mice following restimulation in vitro with type II collagen figure 9, panel B.

Example 11

Treatment with CD80-CAP1 reduces symptoms of colitis in animal models of the disease

Inflammatory Bowel Disease (IBD) is a chronic relapsing and remitting inflammatory condition widespread in the Untied States and Europe. Considerable evidence suggests that a dysregulated immune response by activated CD4+ T cells and increased pro-inflammatory cytokines are involved in the pathogenesis of IBD. T-cell activation involves a multitude of signaling molecules, including the CD28 and cytotoxic T-lymphocyte antigen (CTLA) receptors on the T-cell surface, and their ligands, the B7 molecules. When a B7 molecule binds CD28, it acts as a positive signal for T-cell activation, but at the CTLA receptor it delivers negative feedback to the immune system. Blockade of the CD28:ligand interactions ameliorate autoimmune diseases in multiple animal models including IBD

The effect of the CD80-CAP1 on the mouse model for IBD was measured. Naive $CD4^+CD45RB^{lo}$and $CD4^+CD45RB^{hi}$ T cells were purified from the spleens of normal C57/Bl mice and injected intraperitoneally (5×10^5 cells/mouse) into SCID mice. A group of mice administered 500ug of select CD80-CAP1 on the day of T cell transfer. The mice were monitored for disease development as reflected in body weight. Weight loss is a common system of IBS and accordingly body weight measurements were used to asses the progress of the disease. Referring now to figure 10, change in body weight is expressed as percentage of the original weight (A) for individual mice and (B) as average for individual groups, data represented as mean +/_ standard deviation.

All publications, patents, and patent applications cited in this specification whether specifically incorporated by reference or are not are incorporated herein by reference as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference and set forth in its entirety herein.

Unless specifically identified to the contrary, all terms used herein are used to include their normal and customary terminology. Further, while various embodiments of experimental tests and medical treatment embodiments having specific features and steps are described and illustrated herein, it is to be understood that any selected embodiment can include one or more of the specific features and/or steps described for another embodiment where possible.

Further, any theory of operation, proof, or finding stated herein is meant to further enhance understanding of the present invention and is not intended to make the scope of the present invention dependent upon such theory, proof, or finding.

While the invention has been illustrated and described in detail in the figures, tables and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiments have been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected. And while the invention was illustrated using specific examples, models, structures, molecules, theoretical arguments, protein sequences, accounts and illustrations these examples, arguments, illustrations sequences, molecules, accounts and the accompanying discussion should by no means be interpreted as limiting the invention. The Abstract of the Disclosure is included for the convenience of the persons searching for the document; the Abstract is not a summary of the invention it should not be used to interpret or to limit either the claims or the specification.

CLAIMS

We claim:

1. A molecule that interacts with CD80 (B7-1), comprising:

the polypeptide MQPPGX (SEQ ID 1), wherein

M, Q, P, P, and G, are L-amino acids,

X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, and V; and

said polypeptide MQPPGX at least substantially adopts a polyproline type II helical conformation under physiological conditions and interacts with the receptor binding region of CD80.

2. The molecule according to claim 1, wherein X is the L-amino acid C.

3. The molecule according to claim 1, wherein the termini of said molecule are end blocked.

4. The molecule according to claim 1, wherein said molecule includes no more than about 20 amino acids.

5. The molecule according to claim 1, wherein said molecule includes no more than about 10 amino acids.

6. The molecule according to claim 1, wherein said molecule includes no more than about 6 amino acids.

7. The molecule according to claim 1, wherein said molecule is water soluble.

8. A molecule that interacts with the CD80 (B7-1), comprising:

a retro-inverso peptide mimic XGPPQM (SEQ ID 3), wherein

G, P, P, Q, M are D-amino acids;

X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V; and

said retro-inverso peptide mimic XGPPQM at least substantially adopts a polyproline type II helical conformation under physiological conditions and interacts with the receptor binding region of CD80.

9. The molecule according to claim 8, wherein X is the D-amino acid C.

10. The molecule according to claim 8, wherein the termini of said molecule are end blocked.

11. The molecule according to claim 8, wherein said molecule includes no more than about 20 amino acids.

12. The molecule according to claim 8, wherein said molecule includes no more than about 10 amino acids.

13. The molecule according to claim 8, wherein said molecule includes no more than about 6 amino acids.

14. The molecule according to claim 8, wherein said molecule is water soluble.

15. A method for modulating an immune response, comprising the steps of:

providing a molecule that interacts with CD80 (B7-1), said molecule includes the polypeptide sequence MQPPG-X (SEQ ID 1), wherein

M, Q, P, P, and G, are L-amino acids,

X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, and

said polypeptide MQPPGX at least substantially adopts a polyproline type II helical conformation under physiological

conditions and interacts with the receptor binding region of CD80; and

contacting said molecule with components of said immune system.

16. The method for modulating an immune response according to claim 15, wherein X is the L-amino acid C.

17. The method for modulating an immune response according to claim 15, wherein the termini of said molecule are end blocked.

18. The method for modulating an immune response according to claim 15, wherein said molecule includes no more than about 20 amino acids.

19. The method for modulating an immune response according to claim 15, wherein said molecule includes no more than about 10 amino acids.

20. The method for modulating an immune response according to claim 15, wherein said molecule includes no more than about 6 amino acids.

21. The method for modulating an immune response according to claim 15, wherein said molecule is water soluble.

22. A method for modulating an immune response, comprising the steps of:

providing a molecule that interacts with CD80 (B7-1), said molecule includes;

a retro-inverso peptide mimic XGPPQM (SEQ ID 3), wherein

G, P, P, Q, M are D-amino acids,

X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, and

said retro-inverso peptide mimic XGPPQM at least substantially adopts a polyproline type II helical conformation under

physiological conditions and interacts with the receptor binding region of CD80; and

contacting said molecule with components of said immune system.

23. The method of modulating an immune response according to claim 22, wherein X is the D-amino acid C.

24. The method of modulating an immune response according to claim 22, wherein the termini of said molecule are end blocked.

25. The method of modulating an immune response according to claim 22, wherein said molecule includes no more than about 20 amino acids.

26. The method of modulating an immune response according to claim 22, wherein said molecule includes no more than about 10 amino acids.

27. The method of modulating an immune response according to claim 22, wherein said molecule includes no more than about 6 amino acids.

28. The method of modulating an immune response according to claim 22, wherein said molecule is water soluble.

29. A method for treating a patient, comprising the steps of:

providing a molecule that interacts with CD-80, said molecule includes;

the polypeptide sequence MQPPG-X (SEQ ID 1), wherein

M, Q, P, P, and G, are L-amino acids,

X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, and

said polypeptide MQPPGX at least substantially adopts a polyproline type II helical conformation under physiological

conditions and interacts with the receptor binding region of CD80; and

administering at least one therapeutically effective dose of said therapeutic compound to said patient.

30. The method of treating a patient according to claim 29, wherein X is the L-amino acid C.

31. The method of treating a patient according to claim 29, wherein the terminal amino acids of said molecule are end blocked.

32. The method of treating a patient according to claim 29, wherein said molecule includes about 20 amino acids.

33. The method of treating a patient according to claim 29, wherein said molecule includes about 10 amino acids.

34. The method of treating a patient according to claim 29, wherein said molecule includes about 6 amino acids.

35. The method of treating a patient according to claim 29, wherein said molecule is water soluble.

36. The method of treating a patient according to claim 29, wherein said patient has a medical condition selected from the group consisting of: multiple sclerosis, colitis (inflammatory bowel disease IBD), Crohn's Disease, rheumatoid arthritis, diabetes mellitus, Sjorgren's syndrome and solid tissue transplant rejection.

37. The method of treating a patient according to claim 29, wherein said patient has rheumatoid arthritis.

38. The method of treating a patient according to claim 29, wherein said patient has colitis (inflammatory bowel disease IBD).

39. The method of treating a patient according to claim 29, wherein said patient is at risk for solid tissue transplant rejection.

40. A method of treating a patient, comprising the steps of:

providing a molecule that interacts with CD80 (B7-1), said molecule includes the retro-inverso peptide mimic X-GPPQM (SEQ ID 3), wherein

G, P, P, Q, M are D-amino acids,

X is at least one the amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, and

said retro-inverso peptide mimic XGPPQM at least substantially adopts a polyproline type II helical conformation under physiological conditions and interacts with the receptor binding region of CD80; and

administering at least one therapeutically effective dose of said molecule to said patient.

41. The method of treating a patient according to claim 40, wherein X is the D-amino acid C.

42. The method of treating a patient according to claim 40, wherein the terminal amino acids of said molecule are end blocked.

43. The method of treating a patient according to claim 40, wherein said molecule includes about 20 amino acids.

44. The method of treating a patient according to claim 40, wherein said molecule includes about 10 amino acids.

45. The method of treating a patient according to claim 40, wherein said molecule includes about 6 amino acids.

46. The method of treating a patient according to claim 40, wherein said molecule is water soluble.

47. The method of treating a patient according to claim 40, wherein said patient has a medical condition selected from the group consisting of: multiple sclerosis, colitis (inflammatory bowel disease IBD), Crohn's Disease, rheumatoid arthritis, diabetes mellitus, Sjorgren's syndrome and solid tissue transplant rejection.

48. The method of treating a patient according to claim 40, wherein said patient has rheumatoid arthritis.

49. The method of treating a patient according to claim 40, wherein said patient has colitis (inflammatory bowel disease IBD).

50. The method of treating a patient according to claim 40, wherein said patient is at risk for solid tissue transplant rejection.

51. A method of modeling components of the immune system comprising the steps of:

providing a molecule that interacts with CD80, said molecule includes,

the polypeptide sequence MQPPGX (SEQ ID 1), wherein

M, Q, P, P, and G, are L-amino acids,

X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, and

said polypeptide MQPPGX at least substantially adopts a polyproline type II helical conformation under physiological conditions and interacts with the receptor binding region of CD80; and

contacting said molecule with at least a portion of said immune system.

52. The method of modeling components of the immune system according to claim 51, wherein X is the L-amino acid C.

53. The method of modeling components of the immune system according to claim 51, wherein the terminal amino acids of said molecule are end blocked.

54. The method of modeling components of the immune system according to claim 51, wherein said molecule includes about 20 amino acids.

55. The method of modeling components of the immune system according to claim 51, wherein said molecule includes about 10 amino acids.

56. The method of modeling components of the immune system according to claim 51, wherein said molecule includes about 6 amino acids.

57. The method of modeling components of the immune system according to claim 51, wherein said molecule is water soluble.

58. A method of modeling components of the immune system comprising the steps of:

providing a molecule that interacts with CD80 (B7-1), said molecule includes;

a retro-inverso peptide mimic XGPPQM (SEQ ID 3), wherein

G, P, P, Q, M are D-amino acids,

X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, and

said retro-inverso peptide mimic XGPPQM at least substantially adopts a polyproline type II helical conformation under physiological conditions and interacts with the receptor binding region of CD80; and

contacting said molecule with at least a portion of the immune system.

59. The method of modeling components of the immune system according to claim 58, wherein X is the D-amino acid C.

60. The method of modeling components of the immune system according to claim 58, wherein the termini of said molecule are end blocked.

61. The method of modeling components of the immune system according to claim 58, wherein said molecule includes no more than about 20 amino acids.

62. The method of modeling components of the immune system according to claim 58, wherein said molecule includes no more than about 10 amino acids.

63. The method of modeling components of the immune system according to claim 58, wherein said molecule includes no more than about 6 amino acids.

64. The method of modeling components of the immune system according to claim 58, wherein said molecule is water soluble.

65. A method of screening for drug candidates that interact with components of the immune system, comprising the steps of:

providing a molecule that interacts with components of the immune system, said molecule includes,

a polypeptide MQPPGX (SEQ ID 1), wherein

M, Q, P, P, and G, are L-amino acids,

X is at least one amino acid selected from the group

consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, and

said polypeptide MQPPGX at least substantially adopts a polyproline type II helical conformation under physiological conditions and interacts with at least one component of the immune system;

contacting said candidates with said molecule; and

assaying for interactions between said candidates and said molecule.

66. The method of screening for drug candidates that interact with components of the immune system according to claim 65, wherein X is the L-amino acid C.

67. The method of screening for drug candidates that interact with components of the immune system according to claim 65, wherein the termini of said molecule are end blocked.

68. The method of screening for drug candidates that interact with components of the immune system according to claim 65, wherein said molecule includes no more than about 20 amino acids.

69. The method of screening for drug candidates that interact with components of the immune system according to claim 65, wherein said molecule includes no more than about 10 amino acids.

70. The method of screening for drug candidates that interact with components of the immune system according to claim 65, wherein said molecule includes no more than about 6 amino acids.

71. The method of screening for drug candidates that interact with components of the immune system according to claim 65, wherein said molecule is water soluble.

72. A method of screening for drug candidates that interact with components of the immune system, comprising the steps of:

providing a molecule that interacts with CD80 (B7-1), said molecule includes;

a retro-inverso peptide mimic XGPPQM (SEQ ID 3), wherein

G, P, P, Q, M are D-amino acids,

X is at least one amino acid selected from the group consisting of A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V, and

said retro-inverso peptide mimic XGPPQM at least substantially adopts a polyproline type II helical conformation under physiological conditions and interacts with the receptor binding region of CD80; and

contacting said molecule with said CD80 (B7-1).

73. The method of modeling components of the immune system according to claim 72, wherein X is the D-amino acid C.

74. The method of modeling components of the immune system according to claim 72, wherein the termini of said molecule are end blocked.

75. The method of modeling components of the immune system according to claim 72, wherein said molecule includes no more than about 20 amino acids.

76. The method of modeling components of the immune system according to claim 72, wherein said molecule includes no more than about 10 amino acids.

77. The method of modeling components of the immune system according to claim 72, wherein said molecule includes no more than about 6 amino acids.

78. The method of modeling components of the immune system according to claim 72, wherein said molecule is water soluble.

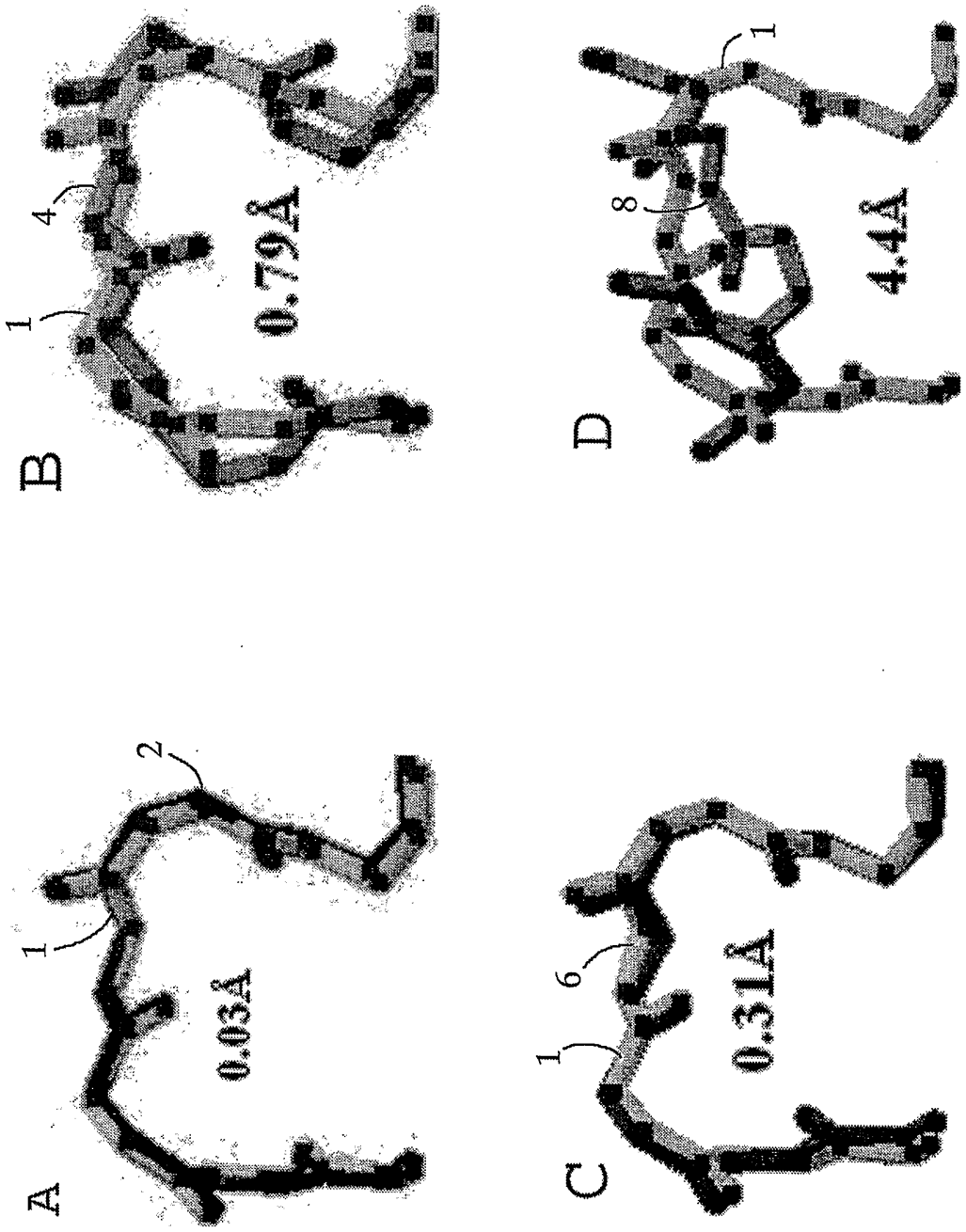


Fig. 1

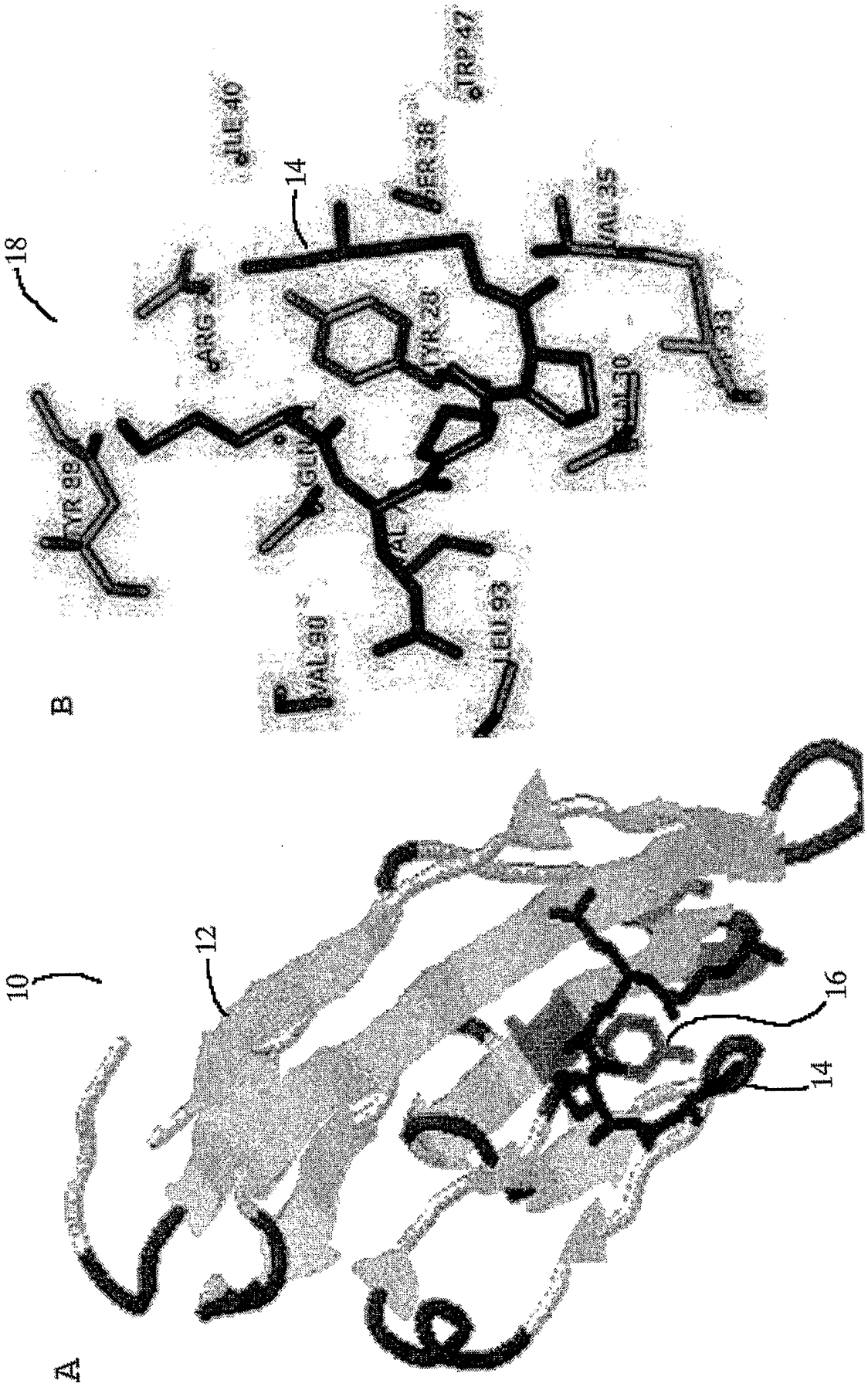

A
10
12
14
16
B
18
14
eILE 40
eTRP 47
eSER 38
eARG 2
eTYR 28
eVAL 25
eTYR 99
eGLN
eILE 30
eVAL 90
eILE 93

Fig. 2

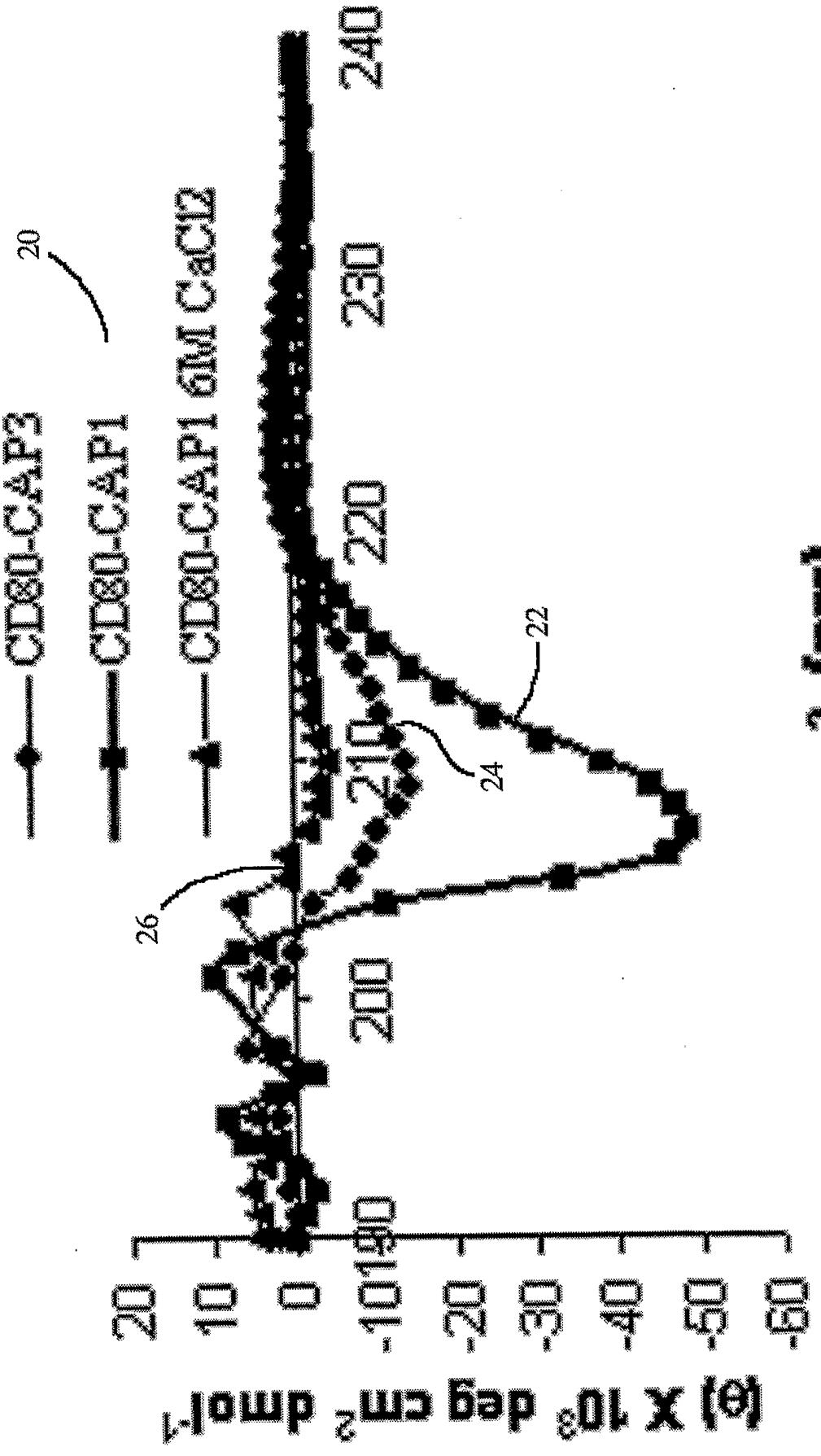

CD&D-CAP3
CD&D-CAP1
CD&D-CAP1 6M CaCl2
20
22
24
26
λ (nm)
[θ] X 10³ deg cm² dmol⁻¹
240
230
220
210
200
190
20
10
0
-10
-20
-30
-40
-50
-60

Fig. 3

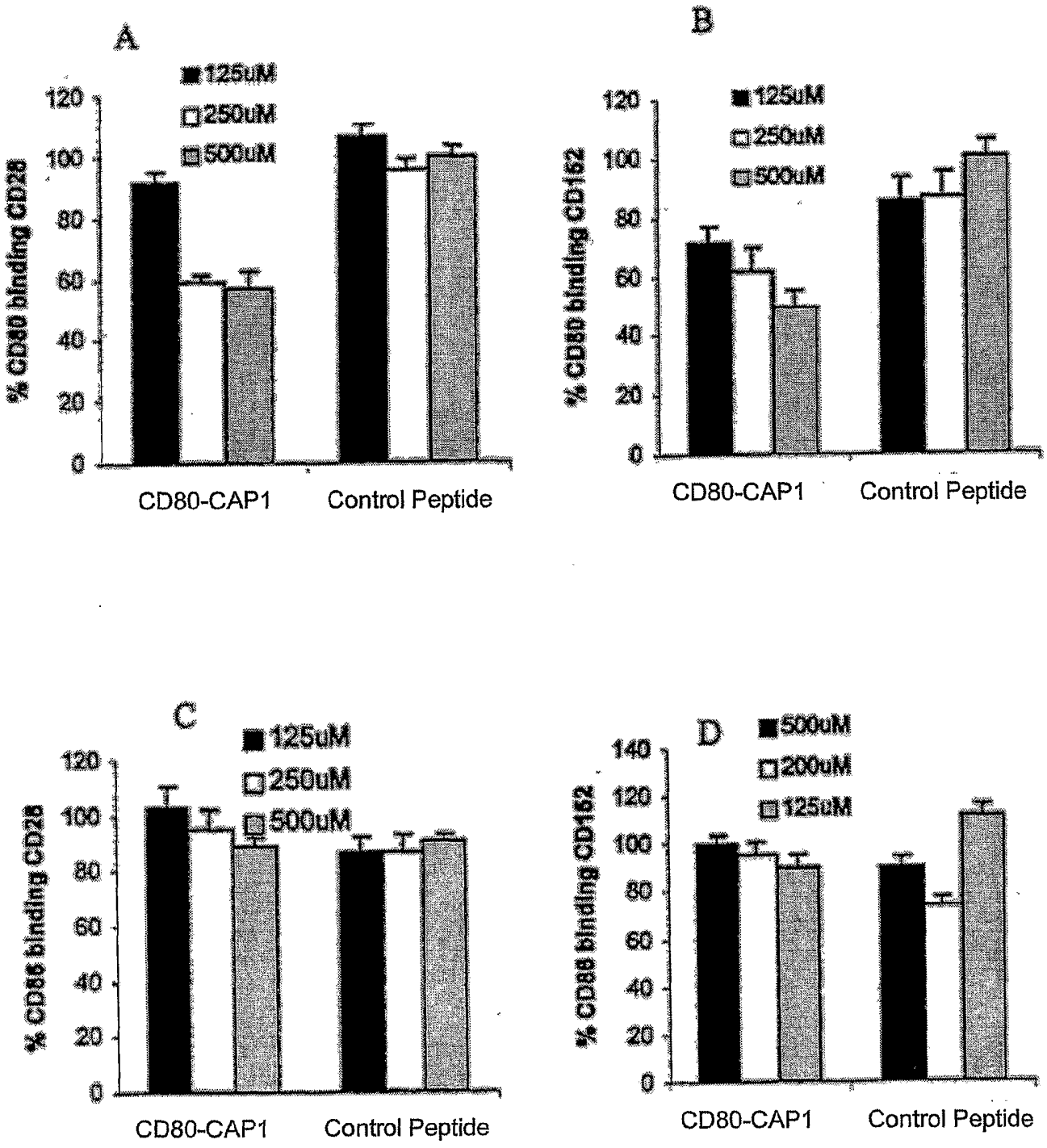
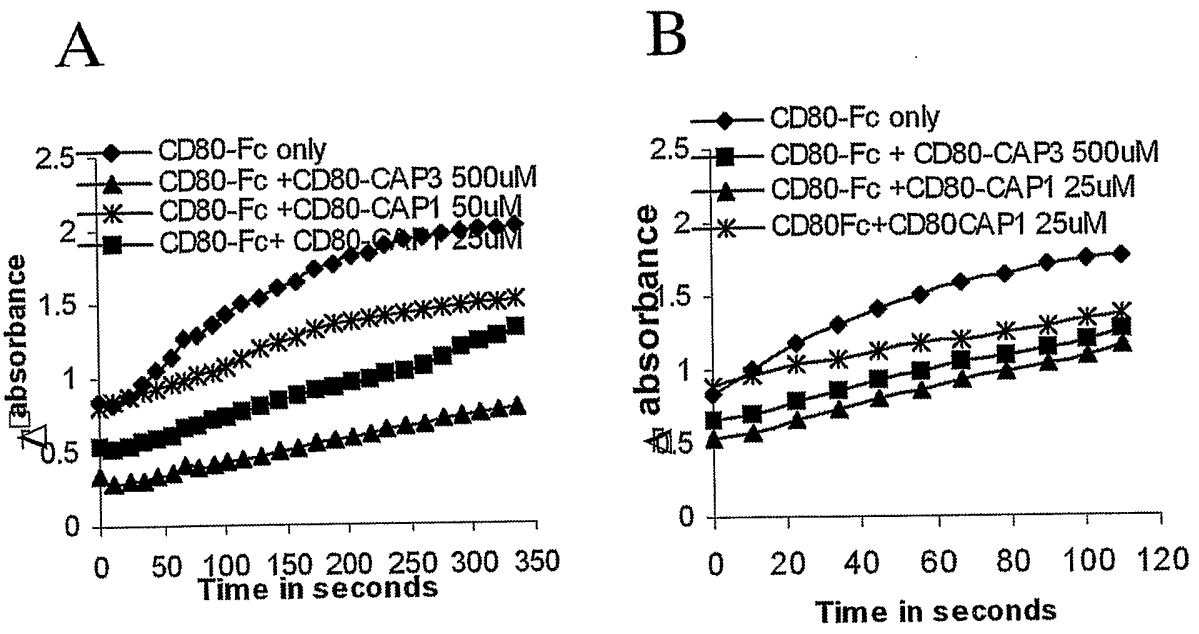

A
125uM
250uM
500uM
% CD80 binding CD28
120
100
80
60
40
20
0
CD80-CAP1
Control Peptide
B
125uM
250uM
500uM
% CD80 binding CD152
120
100
80
60
40
20
0
CD80-CAP1
Control Peptide
C
125uM
250uM
500uM
% CD86 binding CD28
120
100
80
60
40
20
0
CD80-CAP1
Control Peptide
D
500uM
200uM
125uM
% CD86 binding CD152
140
120
100
80
60
40
20
0
CD80-CAP1
Control Peptide

Fig. 4


A
CD80-Fc only
CD80-Fc +CD80-CAP3 500uM
CD80-Fc +CD80-CAP1 50uM
CD80-Fc+ CD80-CAP1 25uM
absorbance
Time in seconds
B
CD80-Fc only
CD80-Fc + CD80-CAP3 500uM
CD80-Fc +CD80-CAP1 25uM
CD80Fc+CD80CAP1 25uM
absorbance
Time in seconds

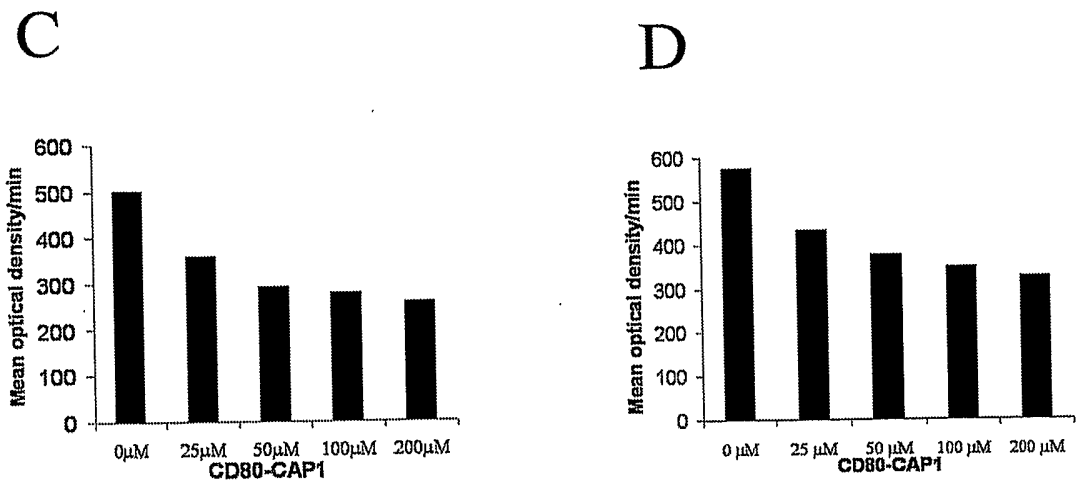
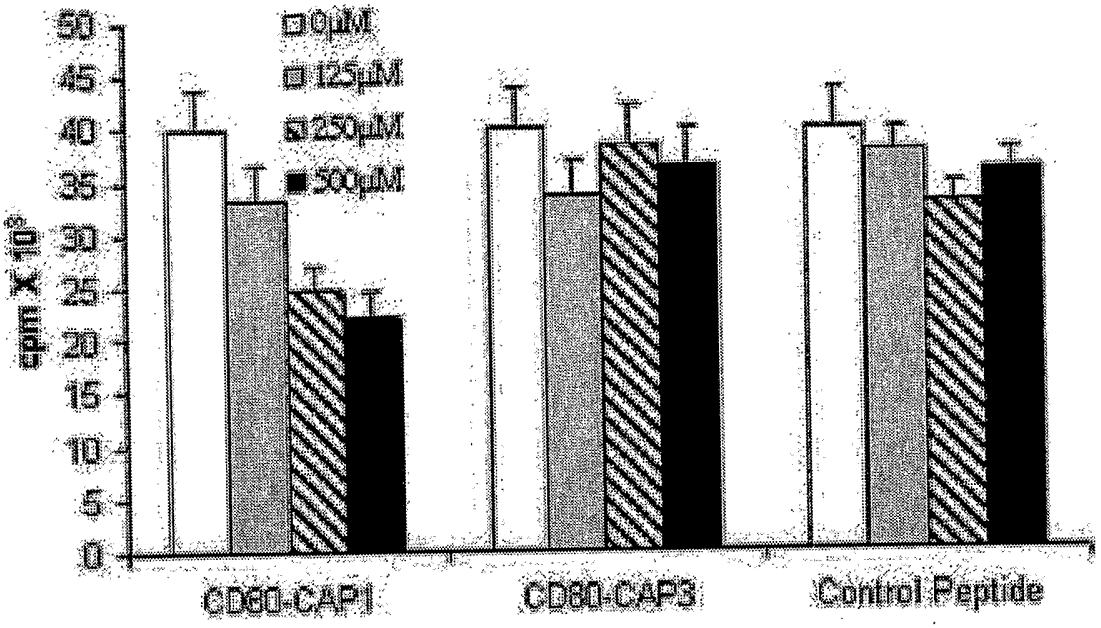

C
Mean optical density/min
0μM 25μM 50μM 100μM 200μM
CD80-CAP1
D
Mean optical density/min
0 μM 25 μM 50 μM 100 μM 200 μM
CD80-CAP1

Fig. 5

A


0μM
125μM
250μM
500μM
cpm X 10³
50
45
40
35
30
25
20
15
10
5
0
CD80-CAP1
CD80-CAP3
Control Peptide

B

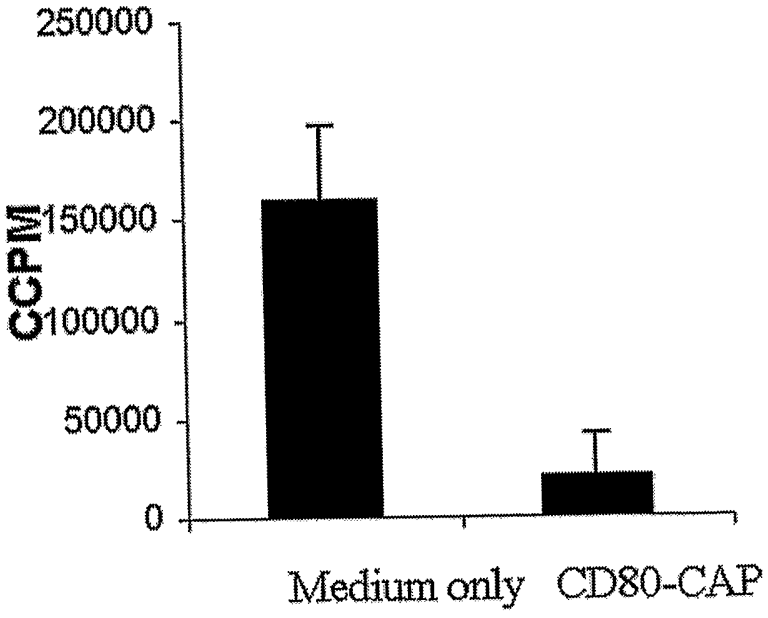
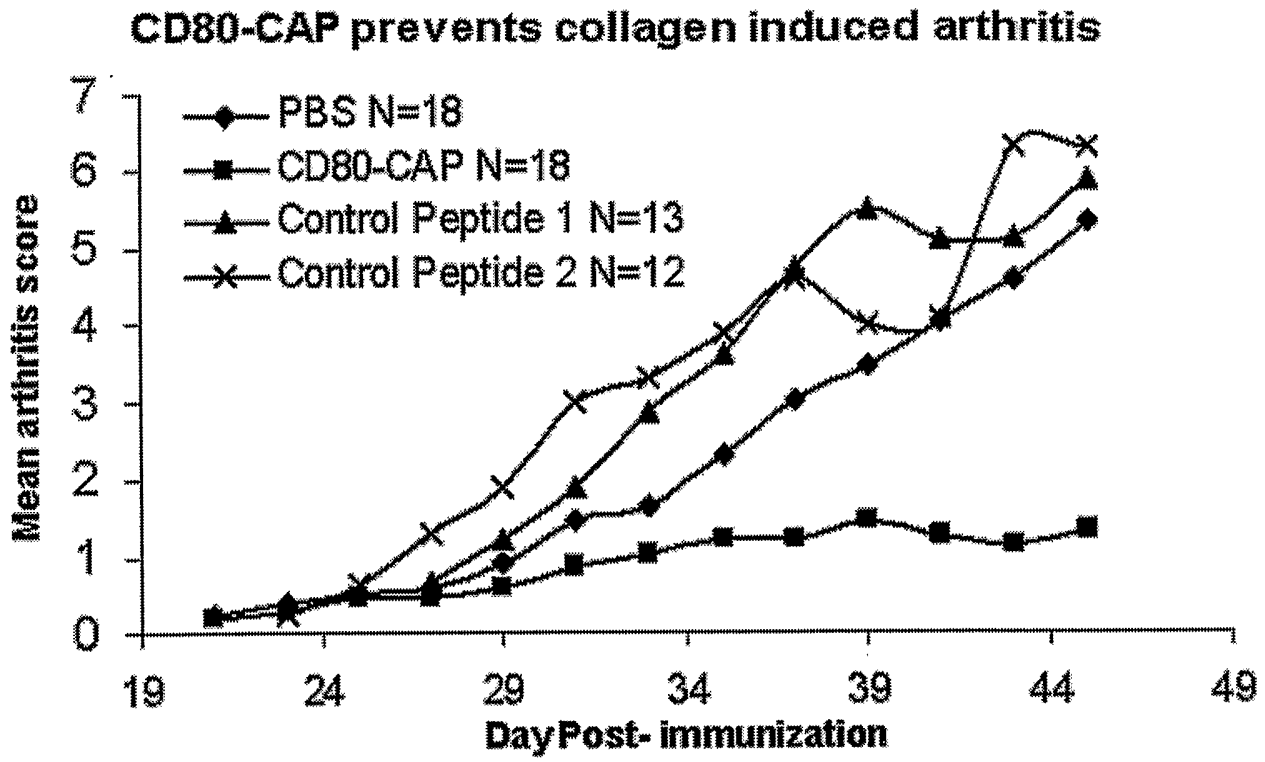

CCPM
250000
200000
150000
100000
50000
0
Medium only
CD80-CAP

Fig. 6

A


CD80-CAP prevents collagen induced arthritis
PBS N=18
CD80-CAP N=18
Control Peptide 1 N=13
Control Peptide 2 N=12
Mean arthritis score
0
1
2
3
4
5
6
7
19
24
29
34
39
44
49
Day Post- immunization

B

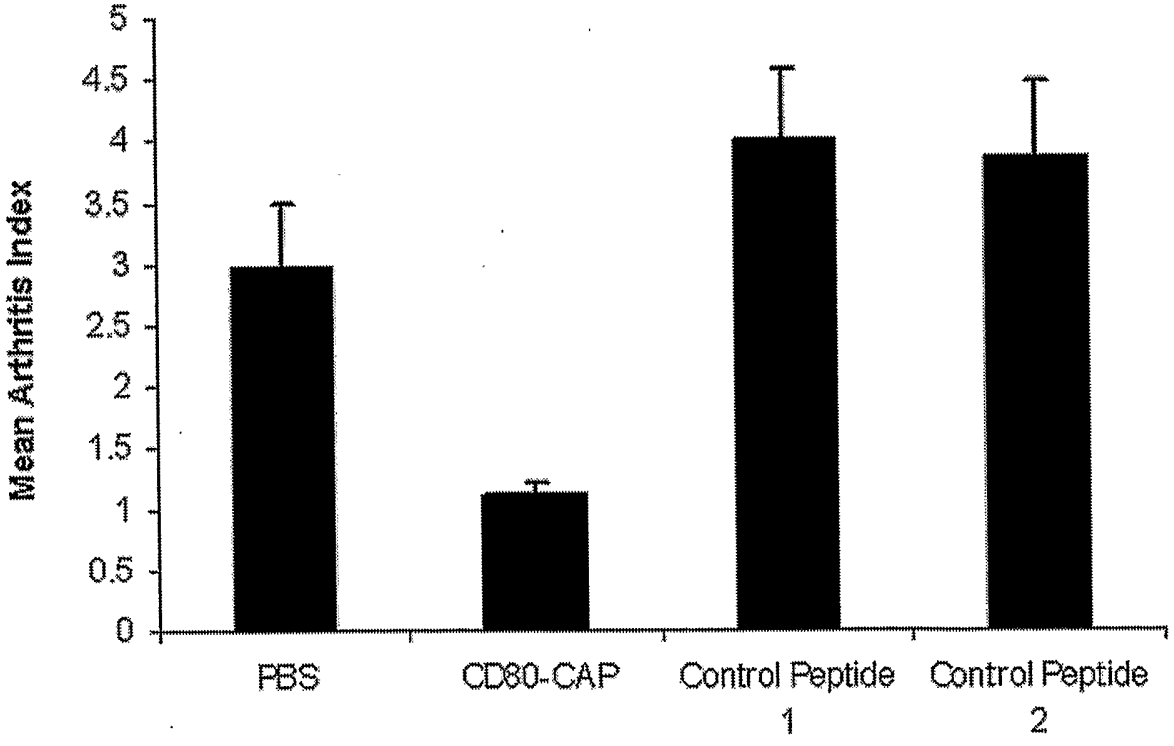

Mean Arthritis Index
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
PBS
CD80-CAP
Control Peptide 1
Control Peptide 2

Fig. 7

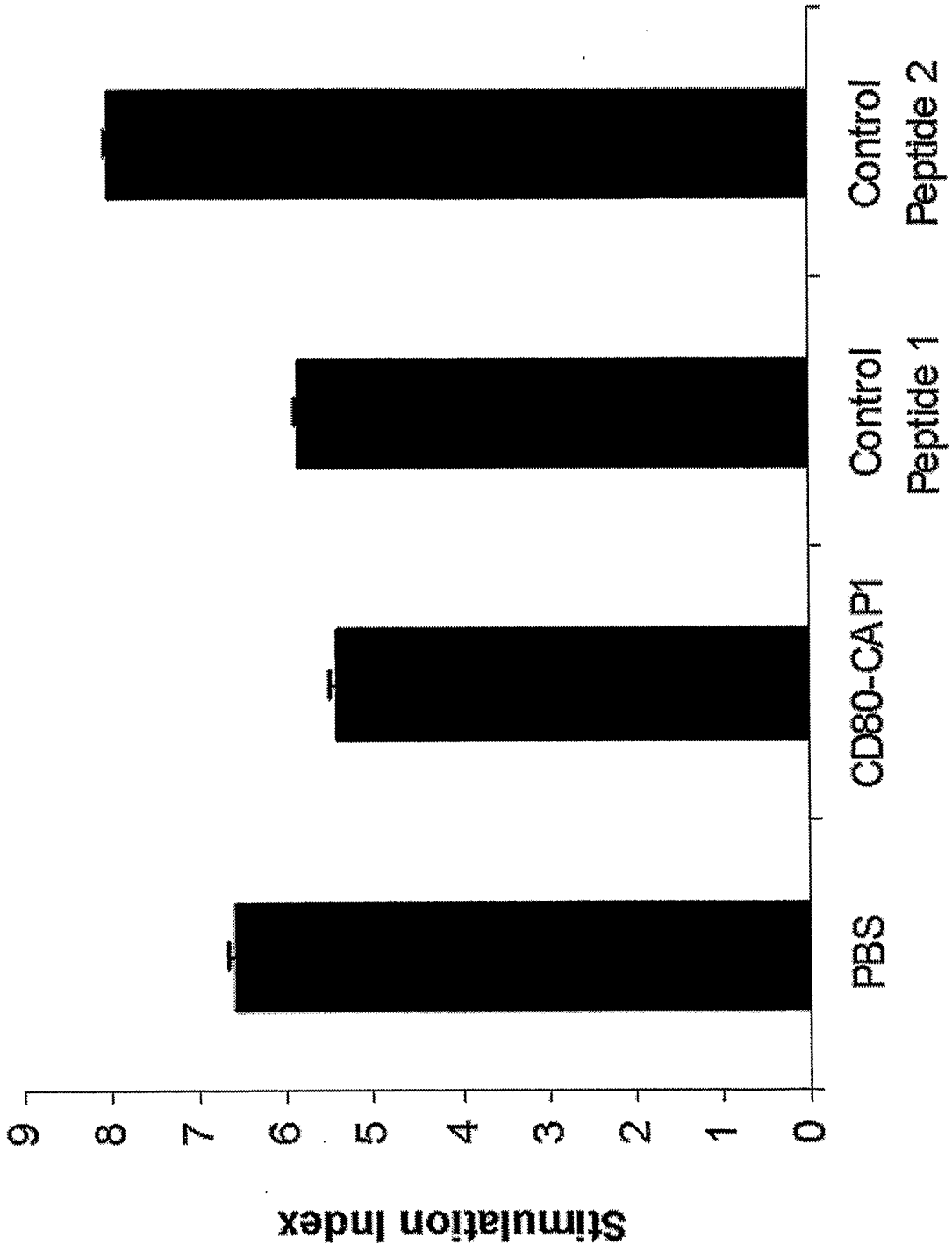

Stimulation Index
0
1
2
3
4
5
6
7
8
9
PBS
CD80-CAP1
Control Peptide 1
Control Peptide 2

Fig. 8

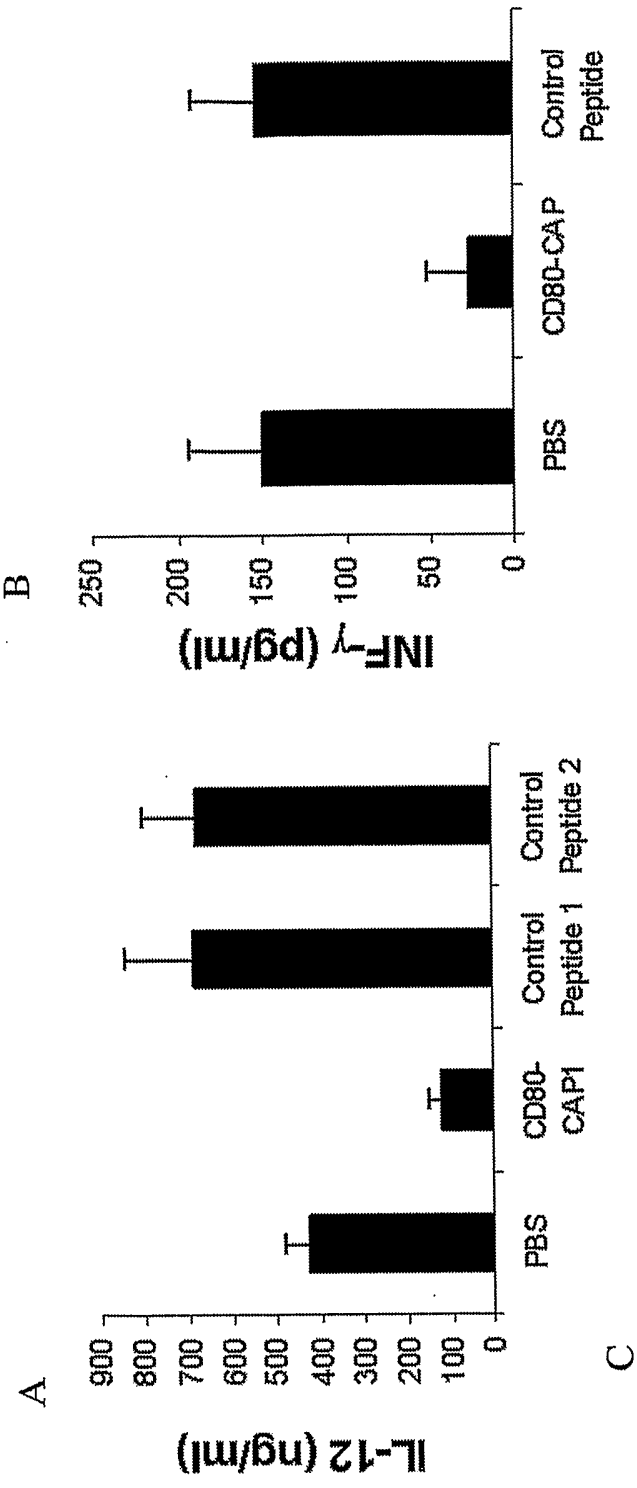
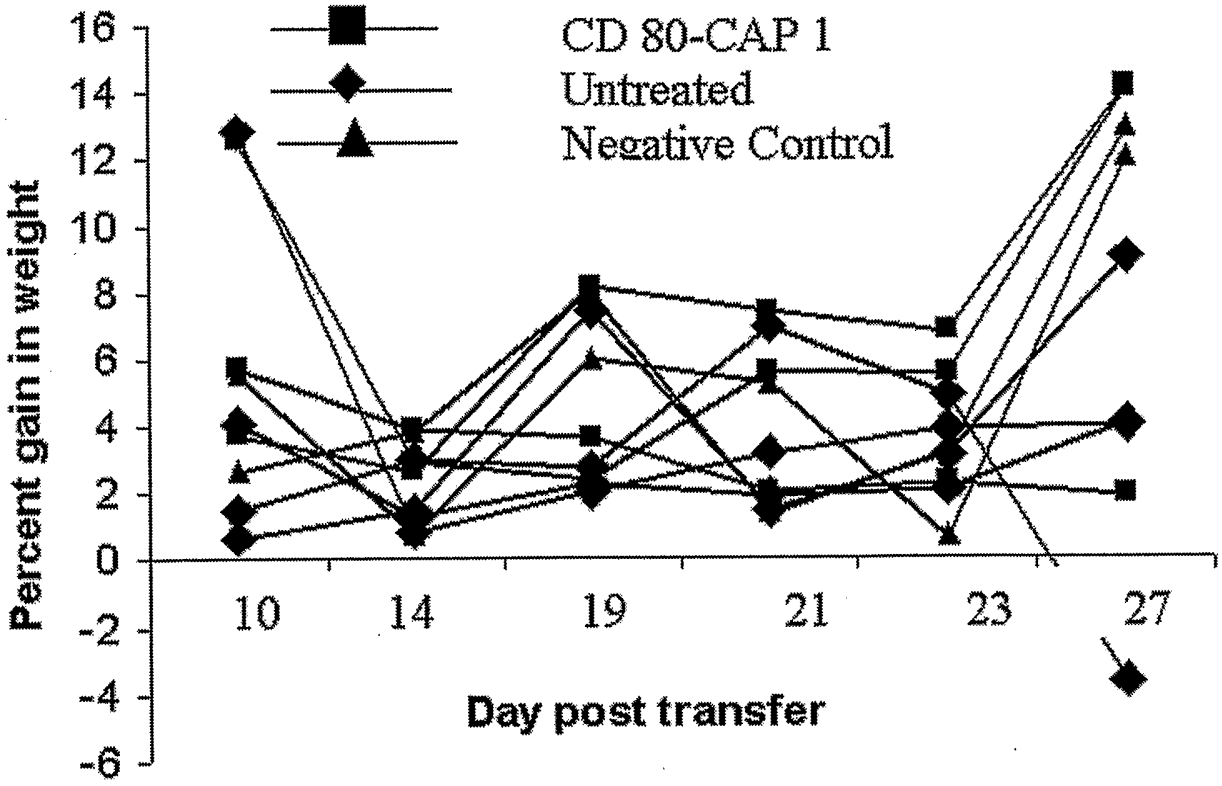

A
IL-12 (ng/ml)
900
800
700
600
500
400
300
200
100
0
PBS
CD80-CAP1
Control Peptide 1
Control Peptide 2
B
INF-γ (pg/ml)
250
200
150
100
50
0
PBS
CD80-CAP
Control Peptide
C
IL-6 (pg/ml)
450
400
350
300
250
200
150
100
50
0
PBS
CD80-CAP
Control Peptide1
Control Peptide 2

Fig. 9

A


CD 80-CAP 1
Untreated
Negative Control
Percent gain in weight
16
14
12
10
8
6
4
2
0
-2
-4
-6
10
14
19
21
23
27
Day post transfer

B

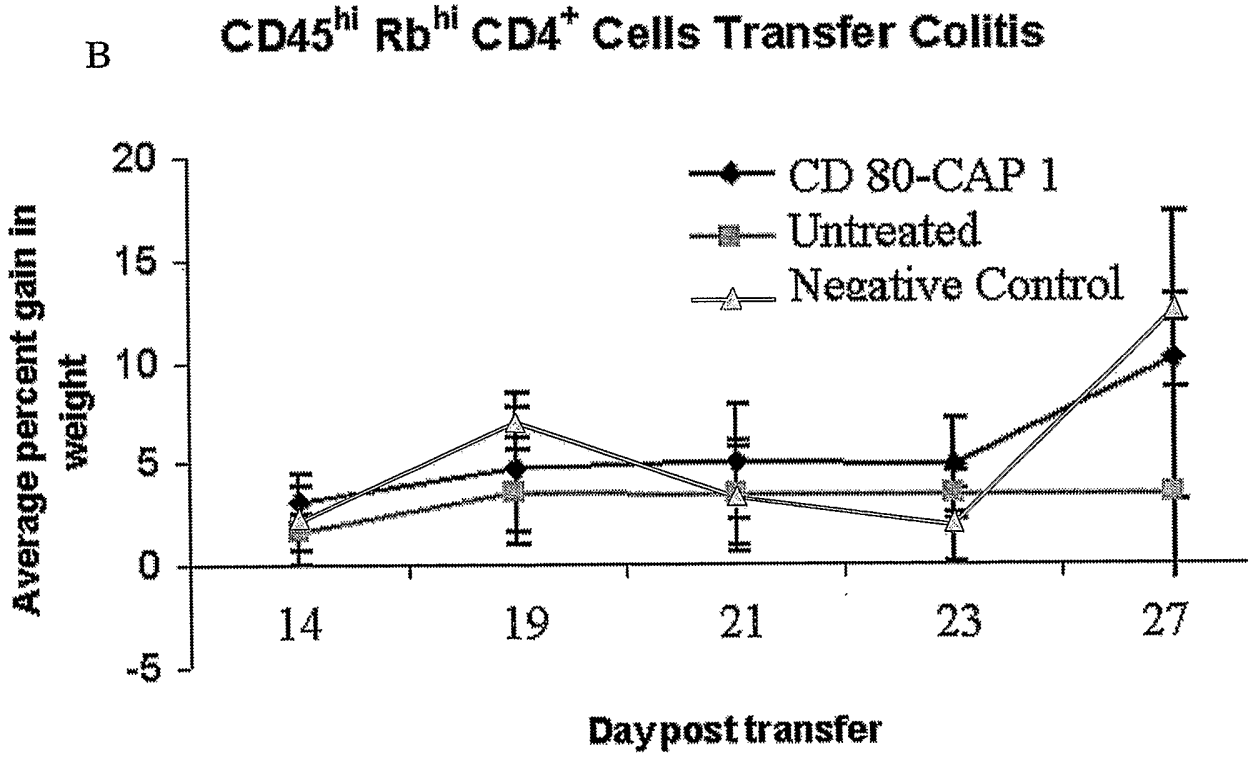

CD45hi Rbhi CD4+ Cells Transfer Colitis
CD 80-CAP 1
Untreated
Negative Control
Average percent gain in weight
20
15
10
5
0
-5
14
19
21
23
27
Day post transfer

Fig. 10

A	Mouse CD152 (Free, PDB 1DQT) (SEQ ID 6)		Human CD152 (Free PDB 1AH1) (SEQ ID 13)		Human CD80:CD152 (PDB 1I8L Chain C) (SEQ ID 7)		Human CD86:CD152 (PDB 1185 Chain C) (SEQ ID 9)		Mouse CD28 (Molecular model) (SEQ ID (5))	
	Φ	ψ	Φ	ψ	Φ	ψ	Φ	ψ	Φ	ψ
M	- 85.14	-24.872	-78.386	- 38.31	- 78.02	- 39.60	- 64.05	-158.45	- 87.98	- 80.20
Y	- 168.51	144.32	- 67.51	- 44.96	- 160.97	142.53	- 44.08	140.04	- 46.89	- 56.50
P	- 84.02	166.25	- 80.8	137.18	- 75.87	164.55	- 85.31	153.27	- 68.28	113.74
P	- 65.55	156.51	- 89.98	143.81	- 69.46	169.91	- 58.02	167.13	- 72.41	143.36
P	- 79.16	153.95	-114.41	64.68	- 69.65	160.51	- 80.63	160.50	- 89.38	170.01
Y	- 70.22	148.76	48.14	111.85	- 86.76	135.31	- 67.25	-176.49	- 62.44	90.29

B	CD80-CAP1 (SEQ ID 2)	
	Φ	ψ
Q	-166.942	146.289
P	- 79.882	165.698
P	- 55.98	152.374
G	- 86.409	156.677

Table 1

	Molecular Weight	Surface Area	Surface Volume
MYPPPY (SEQ ID 12)	748	1080 Å	888.2
CD80-CAP1 (SEQ ID 2)	613	865 Å	700.7

Table 2

	1DQT (SEQ ID 6)	1AH1 (SEQ ID 13)	1I8L (SEQ ID 8)	1185 (SEQ ID (9))	CD28 (SEQ ID (6))
1DQT	0	.84	.29	.52	.70
1AH1	.84	0	.97	.90	.54
118L	.29	.97	0	.33	.75
1185	.52	.90	.33	0	.55
CD28	.70	.54	.75	.55	0
CD80-CAP1	.11	.84	.32	.52	.69

Table 3

SEQUENCE LISTING

SEQ ID 1: CD80-CAP

MQPPGX

SEQ ID 2: CD80-CAP1

MQPPGC

SEQ ID 3: RETRO INVERSO CD80-CAP

XGPPQM

SEQ ID 4: RETRO INVERSO CD80-CAP1

CGPPQM

SEQ ID 5: MOUSE CD28

1 MTLRLLFLAL NFFSVQVTEN KILVKQSPLL VVDSNEVSLS CRYSYNLLAK EFRASLYKGV
61 NSDVEVCVGN GNFTYQPQFR SNAEFNCDGD FDNETVTFRL
WNLHVNHTDIYFCKIEFMYP
121 PPYLDNERSN GTIIHIKEKH LCHTQSSPKL FWALVVVAGV LFCYGLLVTV ALCVIWTNSR
181 RNRLLQVTTM NMTPRRPGLT RKPYQPYAPA RDFAAYRP

SEQ ID 6: MOUSE CD152

1 MACLGLRRYK AQLQLPSRTW PFVALLTLLF IPVFSEAIQV TQPSVVLASS HGVASFPCEY
61 SPSHNTDEVR VTVLRQTNDQ MTEVCATTFT EKNTVGFLDY PFCSGTFNES
RVNLTIQGLR
121 AVDTGLYLCK VELMYPPPYF VGMGNGTQIY VIDPEPCPDS DFLLWILVAV SLGLFFYSFL
181 VSAVSLSKML KKRSPLTTGV YVKMPPTEPE CEKQFQPYFI PIN

SEQ ID 7: PDB 1I8L CHAIN D

1 KAMHVAQPAV VLASSRGIAS FVCEYASPGK ATEVRVTVLR QADSQVTEVC
51 AATYMMGNEL TFLDDSICTG TSSGNQVNLT IQGLRAMDTG LYICKVELMY
101 PPPYYLGIGN GAQIYVIDPE PCPDSD

SEQ ID 8: PDB 1I8L CHAIN A

HUMAN CD80

1 VIHVTKEVKE VATLSCGHNV SVEELAQTRI YWQKEKKMVL TMMSGDMNIW
51 PEYKNRTIFD ITNNLSIVIL ALRPSDEGTY ECVVLKYEKD AFKREHLAEV
101 TLSVKADFPT PSISDFEIPT SNIRRIICST SGGFPEPHLS WLENGEELNA
151 INTTVSQDPE TELYAVSSKL DFNMTTNHSF MCLIKYGHLR VNQTFNWNTT
201 KQEHFPDN

SEQ ID 9: PDB 1I85

HUMAN CD86

1 MLKIQAYFNE TADLPCQFAN SQNQSLSELV VFWQDQENLV LNEVYLGKEK
51 FDSVHSKYMG RTSFDSDSWT LRLHNLQIKD KGLYQCIIHH KKPTGMIRIH
101 QMNSELSVLA

SEQ ID 10: MOUSE CD80

1 MACNCQLMQD TPLLKFPCPR LILLFVLLIR LSQVSSDVDE QLSKSVKDKV LLPCRYNSPH
61 EDESEDRIYW QKHDKVVLSV IAGKLKVWPE YKNRTLYDNT TYSLIILGLV LSDRGTYSCV
121 VQKKERGTYE VKHLALVKLS IKADFSTPNI TESGNPSADT KRITCFASGG FPKPRFSWLE
181 NGRELPGINT TISQDPESEL YTISSQLDFN TTRNHTIKCL IKYGDAHVSE DFTWEKPPED
241 PPDSKNTLVL FGAGFGAVIT VVVIVVIIKC FCKHRSCFRR NEASRETNNS LTFGPEEALA
301 EQTVFL

SEQ ID 11: PDB 1DR9

1 VIHVTKEVKE VATLSCGHNV SVEELAQTRI YWQKEKKMVL TMMSGDMNIW
51 PEYKNRTIFD ITNNLSIVIL ALRPSDEGTY ECVVLKYEKD AFKREHLAEV
101 TLSVKADFPT PSISDFEIPT SNIRRIICST SGGFPEPHLS WLENGEELNA
151 INTTVSQDPE TELYAVSSKL DFNMTTNHSF MCLIKYGHLR VNQTFNWNTA
201 K

SEQ ID 12 : SYNTHETIC PEPTIDE

MYPPPY

SEQ ID 13: PDB 1AH1

1 AMHVAQPAVV LASSRGIASF VCEYASPGKA TEVRVTVLRQ ADSQVTEVC
51 ATYMMGNELT FLDDSICTGT SSGNQVNLTI QGLRAMDTGL YICKVELMYP
101 PPYYLGIGNG TQIYVIDPEP CPDSDQEPK

SEQ ID 14: PDB 1 NCN

1 MLKIQAYFNE TADLPCQFAN SQNQSLSELV VFWQDQENLV LNEVYLGKEK
51 FDSVHSKYMG RTSFDSDSWT LRLHNLQIKD KGLYQCIIHH KKPTGMIRIH
101 QMNSELSVLA

SEQ ID 15: CD80-CAP3

MAVPAT