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(57) Resumo: POLIPEPTÍDEO TENDO NÃO MAIS DO QUE 100 AMINOÁCIDOS, POLIPEPTÍDEO IMUNOGÊNICO DE EPÍTOPO MÚLTIPLO, COMPOSIÇÃO DE POLIPEPTÍDEO, MÉTODO PARA PRODUZIR UM POLIPEPTÍDEO, CONSTRUÇÃO DE POLIPEPTÍDEO, MÉTODO PARA PRODUZIR A MESMA, COMPOSIÇÃO DE VACINA OU MEDICAMENTO CONTRA UM VÍRUS HIV, MÉTODO PARA PRODUZIR UMA COMPOSIÇÃO DE VACINA OU MEDICAMENTO, USO DE UM POLIPEPTÍDEO, UMA COMPOSIÇÃO DE POLIPEPTÍDEO, UMA CONSTRUÇÃO DE POLIPEPTÍDEO OU UMA COMPOSIÇÃO DE VACINA OU MEDICAMENTO. E fornecido um polipeptídeo tendo não mais do que 100 aminoácidos, polipeptídeo este que compreende uma ou mais sequências tendo pelo menos 60% de homologia com qualquer uma das SEQ ID 1 a 4, ou compreende dois ou mais epítopos tendo 7 aminoácidos ou mais, cada epítipo tendo pelo menos 60% de homologia com uma sub-sequência de qualquer uma das SEQ ID 1 a 4 que tem o mesmo comprimento como o epítipo: SEQ ID 1 GDTWAGVEAHRILQQLFT HIFRIGCQHSR; SEQ ID 2 KVGSLQYLALTAITPKKJKYPLPSVKKL TEDRWNPQKT; SEQ TD 3 EPVPLQLPPLERLTLDCEDCGTSGT Q; SEQ ID 4 YKGALDLSHTLKEKGGLEGLIYSQKRQDILDWVYIT TQGYFPD em que, o polipeptídeo é imunogênico em um vertebrado que expressa um alelo de complexo de histocompatibilidade maior (MHC), e em que o polipeptídeo não é uma proteína do vírus HIV completa.

“POLIPEPTÍDEO TENDO NÃO MAIS DO QUE 100 AMINOÁCIDOS,
POLIPEPTÍDEO IMUNOGÊNICO DE EPÍTOPO MÚLTIPLO,
COMPOSIÇÃO DE POLIPEPTÍDEO, MÉTODO PARA PRODUZIR UM
POLIPEPTÍDEO, CONSTRUÇÃO DE POLIPEPTÍDEO, MÉTODO PARA
5 PRODUZIR A MESMA, COMPOSIÇÃO DE VACINA OU
MEDICAMENTO CONTRA UM VÍRUS HIV, MÉTODO PARA
PRODUZIR UMA COMPOSIÇÃO DE VACINA OU MEDICAMENTO,
USO DE UM POLIPEPTÍDEO, UMA COMPOSIÇÃO DE POLIPEPTÍDEO,
UMA CONSTRUÇÃO DE POLIPEPTÍDEO OU UMA COMPOSIÇÃO DE
10 VACINA OU MEDICAMENTO”

A invenção diz respeito a sequências de peptídeo, composições
que compreendem as sequências de peptídeo, e em particular vacinas contra o
vírus da imunodeficiência, tais como vacinas contra o vírus da
imunodeficiência humana (HIV) e/ou outros vírus da imunodeficiência que
15 também podem ser úteis contra a síndrome da imunodeficiência adquirida
(AIDS). As vacinas compreendem as sequências e as composições. A
invenção também diz respeito aos usos das sequências e composições. A
mesma especialmente diz respeito às vacinas que são protetoras contra uma
pluralidade de cepas virais, incluindo os vírus existentes assim como os vírus
20 futuros que tenham mutado a partir dos vírus existentes (tais como as formas
mutadas de uma cepa do HIV existente).

A defesa contra doença é crítica para a sobrevivência de todos
os animais, e o mecanismo de defesa utilizado para este propósito é o sistema
imune do animal. Entender o sistema imune é portanto uma chave para o
25 desenvolvimento de novos e mais sofisticados tratamentos para seres
humanos e animais semelhantes.

O mecanismo de operação do sistema imune tem estado sob
investigação por muitos anos. O sistema é composto de vários tipos de célula
e uma variedade de moléculas, tornando-o extremamente complexo. Mesmo
30 depois de muitos anos de estudo, a extensão completa dos componentes do

sistema imune, e a sua interação um com o outro, são imperfeitamente entendidas.

A muitos anos atrás foi reconhecido que uma pessoa que se recupera de uma doença particular pode adquirir alguma proteção no futuro contra esta doença, mas não contra uma doença que esta pessoa ainda não contraiu. Este aspecto fundamental do sistema imune foi interpretado naquele tempo pela consideração de que o sistema imune adquiriu um tipo de ‘memória’ contra certos patógenos uma vez que a exposição a tais patógenos tivesse ocorrida, esta memória sendo específica para uma certa doença.

Gradualmente, tornou-se conhecido que a exposição a variantes menos nocivas de um patógeno poderia induzir proteção contra as variantes mais nocivas (por exemplo, a exposição à varíola bovina para proteger contra a varíola, ou exposição a um antraz inativado para proteger contra o antraz vivo). Assim, a idéia de vacinação contra uma doença surgiu.

É agora conhecido que o sistema imune tem pelo menos duas divisões: a imunidade inata e a imunidade adaptiva. O sistema inato é totalmente funcional antes que um patógeno entra no sistema, enquanto que o sistema adaptivo é ligado depois que o patógeno entra no sistema. Este depois desenvolve um ataque específico para o patógeno. O sistema inato compreende vários componentes, incluindo fagócitos tais como macrófagos, que (como o nome sugere) ‘come’ ou engolfa corpos estranhos tais como patógenos.

Tipicamente, mas não exclusivamente, a presente invenção está relacionada com o sistema imune adaptivo, e a menos que especificamente de outro modo indicado, o ‘sistema imune’ no presente contexto refere-se ao sistema imune adaptivo.

De modo a entender mais completamente como o sistema imune funciona, o papel de seus componentes individuais deve ser cuidadosamente considerado. Em relação ao sistema imune adaptivo, é bem

conhecido que a imunidade contra patógenos é fornecida pela ação de linfócitos, que constituem o tipo de célula mais comum no sistema imune. Existem dois tipos de linfócito: o linfócito B e o linfócito T. Estes são no geral chamados de células B e células T, respectivamente.

5 As células B têm a capacidade para se desenvolver em células plasmáticas, que fabricam anticorpos. Os anticorpos são componentes muito importantes do sistema imune do animal. Estes são produzidos em resposta a alguma porção de assinatura do patógeno invasor (um antígeno do patógeno – antígenos aqui sendo definido como qualquer substância estranha reconhecido
10 pelo sistema imune) e são usualmente específicos para este patógeno. Entretanto, se dois patógenos são muito similares, ou pelo menos contêm o mesmo antígeno, então os anticorpos produzidos contra um não obstante pode ser eficaz contra o outro (eles podem ‘reagir cruzado’). Isto explica porque a inoculação com varíola bovina pode proteger contra a varíola. É importante
15 compreender que os anticorpos ‘reconhecem’ apenas uma pequena porção da molécula antigênica do patógeno ao invés do patógeno como um todo. Estas porções são chamadas de epítomos.

 As células T não possuem ou produzem anticorpos. Ao invés, elas reconhecem fragmentos (isto é, epítomos) do antígeno estranho
20 complexados com o complexo de histocompatibilidade maior (MHC) (ou no caso de seres humanos, antígeno de leucócito humano (HLA)) por intermédio de um receptor especializado conhecido como TCR (receptor de célula T). As células T são elas próprias divisíveis em subconjuntos que podem ter uma função regulatória ou uma função efetora. As células efetoras estão
25 envolvidas com o ‘efetuar’ a remoção das substâncias estranhas. Por exemplo, as células T citotóxicas (CTL) são células efetoras que são capazes de matar células infectadas, assim como outras espécies indesejadas tais como células de tumor. As células T reguladoras, por outro lado, desempenham um papel em ajudar as células T e B efetoras a se tornarem mais eficazes. Devido a esta

função, estas células T reguladoras são freqüentemente chamadas de células T 'auxiliares'. Outras células T reguladoras, chamadas de células T 'supressora', são consideradas inibir respostas imunes, mas estas são menos bem entendidas. As células T reguladoras também podem interagir com componentes do sistema imune inato para reforçar a sua atividade.

Em um indivíduo saudável normal, os linfócitos no sistema imune permanecem em um estado de 'repouso' inativo até que uma resposta imune seja deflagrada. Quando uma resposta imune é requerida, os linfócitos se tornam ativadas, proliferam e começam a realizar as suas funções designadas. Por exemplo, qualquer célula T em repouso que demonstra na sua superfície um TCR que reconhece um epítipo do patógeno invasor complexado com uma molécula MHC é ativada, prolifera (isto sendo chamado de expansão clonal) e a progênie resultante começa a realizar ativamente as suas funções efetoras pré determinadas requeridas para combater os organismos invasores.

Quando a resposta imune é completada, (isto é, os patógenos e/ou células infectadas foram eliminados) os linfócitos reverterem mais uma vez a um estado de repouso. Este estado de repouso não é, entretanto, equivalente ao estado de repouso inativo inicial. Os linfócitos ativados, mas em repouso, podem ser rapidamente recrutados e induzidos a proliferar em resposta a uma infecção pelo mesmo patógeno, ou um intimamente relacionado, em um tempo posterior.

Esta capacidade dos linfócitos ativados em repouso, para liberar uma resposta mais rápida e mais poderosa a seguir de um segundo encontro com um patógeno invasor, eficazmente provê o sistema imune com 'memória'. A utilização da memória do sistema imune é a base para todos os medicamentos imunoproliféricos de longa duração (por exemplo, vacinas) e permanece a meta de todo desenvolvimento de medicamento imunoterapêutico de longa duração.

De modo que as células realizem as suas funções dentro do sistemas complexo de um animal, as células precisam ter ‘receptores’ nas suas superfícies. Estes receptores são capazes de ‘reconhecer’ substâncias específicas que controlam vários processos essenciais tais como a ativação, proliferação e aderência a outras células ou substratos. Por exemplo, no caso do sistema imune, os receptores nas células T e B permitem que elas não apenas reconheçam antígeno mas também interajam entre si e assim regulem suas atividades. Sem estes receptores, as células careceriam de um meio essencial de comunicação e seriam incapazes de atuar eficazmente no modo combinado que é essencial para o sistema imune de um organismo multicelular.

De modo a ser capaz de reconhecer especificamente e lidar com a ampla faixa de patógenos presentes no ambiente, o sistema imune desenvolveu dois tipos de receptor de antígeno altamente variável nos linfócitos: anticorpos em células B e receptores em célula T, ou TCRs, em células T.

Existe muitos receptores de antígeno diferentes possíveis presentes no corpo, para permitir que o sistema imune reconheça uma ampla variedade de patógenos invasores. De fato existem aproximadamente 10^{12} receptores de célula B e célula T diferentes em um indivíduo. Cada célula B individual tem apenas um tipo de receptor, e assim para lidar com um patógeno particular, uma célula B tendo o receptor ‘que melhor se ajusta’ para um antígeno deste patógeno deve ser selecionada. Este processo é chamado de ‘seleção clonal’. Em teoria, apenas um único clone pode responder (uma resposta monoclonal) ou diversos (uma resposta oligoclonal) ou muitos (uma resposta policlonal) dependendo do número de antígenos/epítomos exibidos pelo patógeno, e da especificidade das várias células B selecionadas para estes antígenos/epítomos.

Existe uma diferença maior entre os tipos de antígeno que

podem ser reconhecidos pelas células B e células T. Até agora como é conhecido, apenas os receptores na superfície dos linfócitos B (isto é, os anticorpos) são capazes de reconhecer diretamente antígenos tais como as proteínas nos vírus e bactérias ou moléculas estranhas dissolvidas no fluido corporal. Os anticorpos também podem ser produzidos em uma forma solúvel pelas células B quando elas são ativadas e desenvolvem em células plasmáticas. Os anticorpos também são chamados de imunoglobulinas (abreviado para Ig). Os receptores de célula T, por outro lado, reconhecem apenas peptídeos curtos, também conhecidos como epítopos de célula T, na superfície das células do corpo. Estes epítopos de célula T são produzidos pela degradação de proteínas maiores que são próprias (isto é, proteínas do corpo que ocorrem naturalmente) ou não próprias (isto é, derivadas de organismos estranhos que infetam o corpo). Apenas aqueles derivados de proteínas estranhas, isto é, antígenos, são normalmente capazes de induzir uma resposta imune no corpo. Uma vez produzidos, estes epítopos são ligados a um tipo especial de molécula, o MHC (complexo de histocompatibilidade maior) e o complexo resultante é depois apresentado na superfície da célula para ligar o receptor de célula T.

Deve estar claro que devido à natureza destrutiva da resposta imune, a resposta tem que atuar apenas contra patógenos estranhos, não contra as células ou proteínas do próprio corpo. Assim, o sistema imune precisa distinguir entre próprio e não próprio. Tem sido proposto que embora os clones de linfócitos que reagem contra o próprio corpo sejam produzidos, eles são deletados antes que qualquer reação possa ocorrer. Este processo é chamado de 'deleção clonal'. Também tem sido proposto que quaisquer linfócitos que reajam contra o próprio corpo podem ser retidos mas apenas em um estado 'desligado'. Este mecanismo é chamado de 'anergia clonal'. Qualquer que seja o processo considerado, permanece incerto qual é o mecanismo subjacente exato que permite que os tecidos linfóides, tais como o

timo, identifique os clones de célula T individuais que reagem contra o próprio corpo do agrupamento de linfócitos T que reagem apenas contra o próprio corpo. Os presentes inventores têm agora investigado mais completamente o mecanismo de discriminação própria/não própria, que tem
5 levado ao desenvolvimento da presente invenção. Os inventores estabeleceram agora um método de prognosticar a imunogenicidade de uma substância tal como um peptídeo, que tem permitido a identificação mais rápida de seqüências imunogênicas de peptídeo dentro das proteínas grandes.

Foi conhecido por muitos anos que o complexo de
10 histocompatibilidade maior (MHC) desempenha um papel chave no sistema imune de animais. As moléculas do MHC permitem que as células T reconheçam antígenos, como já foi debatido acima. Existem três tipos gerais de molécula MHC, classe I, classe II e classe III. As moléculas MHC de classe I e classe II são glicoproteínas que estão presentes na superfície da
15 célula, enquanto que a classe III são usualmente moléculas solúveis presentes dentro da célula. Existe um grande número de tipos diferentes de molécula MHC. Por exemplo nos seres humanos (onde o MHC é chamado de HLA ou Antígeno de Leucócito Humano) existem várias centenas de alelos diferentes dos genes que codificam as moléculas MHC, significando que na população
20 humana existem muitos tipos diferentes de HLA. Os MHCs de espécies diferentes são tipicamente chamadas de acordo com convenções diferentes, assim o MHC para camundongo é chamado de H-2, para rato RT1 e para coelho RLA. As regiões de genes diferentes que codificam moléculas MHC diferentes em um indivíduo são denominadas de modo usualmente individual,
25 tais como HLA-A, HLA-C etc. em seres humanos.

A molécula de MHC é uma molécula do sistema imune crítica, visto que é esta molécula que apresenta os epítopos dos antígenos ao sistema imune. Por exemplo, se uma célula T deve responder a um patógeno particular, o patógeno deve ter pelo menos um antígeno (tal como uma

proteína) que tenha pelo menos um epítopo (tal como uma porção de peptídeo da proteína) que possa ligar a uma molécula de MHC na superfície de uma célula e assim interagir com uma célula T que liga ao complexo de MHC-peptídeo. Assim, a resposta imune é dependente da capacidade do MHC para ligar a um epítopo. Se não existe nenhum epítopo que o MHC ligar-se-á ou se não existe nenhuma célula T que ligar-se-á ao complexo de MHC-peptídeo, então nenhuma resposta imune ocorrerá.

Com respeito às ‘auto’ proteínas, entretanto, um de vários epítopos pode ser capaz de ligar à molécula de MHC e conseqüentemente induzir potencialmente uma resposta imune. Nestas ocasiões um “sinal” específico deve ser fornecido para que os clones de linfócito auto-reativos sejam deletados ou “desligados”.

Visto que, como indicado acima, os peptídeos tanto próprios quanto estranhos (isto é, não próprios) podem se ligar às moléculas de MHC, a ligação de vários peptídeos às moléculas de MHC tem recebido exame minucioso particular no campo da imunologia. Muitas investigações têm procurado calcular ou predizer a força de ligação entre certos tipos de MHC (particularmente HLA e H-2) e as seqüências de peptídeo, para tentar esclarecer quanto as respostas imunes ou a falta delas (i.e. o “sinal” requerido para a discriminação entre próprio e estranho). Os exemplos destas incluem as seguintes:

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G. E. Meister, C. G. P. Roberts, J. A. Berzofsky, A. S. De Groot, “Two novel T cell epitope prediction algorithms based on MHC-

binding motifs; comparison of predicted and published epitopes from *Mycobacterium tuberculosis* and HIV protein sequences" *Vaccine*, 13: 581-591, (1995).

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Sturniolo T, Bono E, Ding J, Raddrizzani L, Tuereci O, Sahin U, Braxenthaler M, Gallazzi F, Protti MP, Sinigaglia F, Hammer J. 1999. "Generation of tissue-specific and promiscuous HLA ligand databases using DNA microarrays and virtual HLA class II matrices". *Nat. Biotechnol.* 17: 10 555-561.

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15 T. Tana, N. Kamikawaji, C. J. Savoie, T. Sudo, Y. Kinoshita, T. Sasazuki, "A HLA binding motif-aided peptide epitope library: A novel library design for the screening of HLA-DR4- restricted antigenic peptides recognized by CD4+ cells T." *J. Human Genet.*, 43: 14-21 (1998).

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Y. Chen *et al.* "Naturally processed peptides longer than nine

amino acid residues bind to the class I MHC molecule HLA-A2.1 com high affinity and in different conformations”, J. Immunol., 152, 2874-2881, (1994).

D. F. Hunt *et al.* “Characterização of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry”, Science, Vol. 255,
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No geral, a técnica anterior tenta prognosticar a imunogenicidade de peptídeos particulares pelo cálculo da força de ligação entre este peptídeo e o ambiente de ligação conhecido de uma molécula de MHC particular. O ambiente de ligação envolve uma ‘bolsa’ na molécula de
10 MHC que é adaptada para aceitar um peptídeo de um certo comprimento (tal como 7 a 15 aminoácidos). A estrutura da bolsa já pode ser conhecida de estudos cristalográficos de raio X anteriores. Esta força pode ser calculada matematicamente usando algoritmos apropriados para a interação atômica e molecular. Alternativamente, a técnica anterior pode tentar ‘contar’ a força de
15 ligação de um peptídeo com base em motivos existentes no peptídeo, tais como aminoácidos particulares que estão presentes em posições particulares em um peptídeo de um certo comprimento, por exemplo, uma prolina presente na posição 3 em um peptídeo de 8 aminoácidos que se liga a uma molécula de HLA particular conhecida. No geral estes métodos têm atingido
20 sucesso limitado.

Os presentes inventores acreditam que possam ter melhorado nas teorias acima a partir de um melhor entendimento de como as células T reagem contra substâncias próprias tais como as proteínas próprias são identificadas antes da sua eliminação (deleção clonal) ou silenciamento
25 (anergia clonal). Conseqüentemente, os inventores foram capazes de identificar seqüências de peptídeo imunogênicas específicas que podem fornecer proteção contra patógenos específicos, e desenvolveram vacinas para estes patógenos, usando as seqüências identificadas. No caso da presente invenção, os inventores desenvolveram peptídeos úteis em vacinas do HIV

que evocam uma resposta de célula T.

Por muitos anos a infecção pelo HIV não foi uma condição suscetível de tratamento. Quando a doença foi identificada em 1981, as terapias anti-virais existentes foram ineficazes em controlar o dano que o vírus poderia causar a uma sistema imune do paciente. O vírus causa a destruição direta e indireta das células T CD4 positivas, que são essenciais para um funcionamento completo do sistema imune. Conforme uma contagem de célula T CD4+ do paciente diminui, a doença se torna progressivamente pior. Quando a contagem de célula T CD4+ cai abaixo de certo nível, o paciente é considerado ter deteriorado até o ponto de ter AIDS completamente desenvolvida. As definições do ponto em que isto ocorre varia, mas no geral é considerado que é atingido em pessoas HIV positivas saudáveis com uma contagem de célula T CD4 + de menos do que 200 por ml de sangue. Mais recentemente, as terapias anti-virais têm melhorado, e a progressão da doença tem sido consideravelmente diminuída. As terapias combinatórias modernas (em alguns pacientes) podem retardar o início da AIDS completamente desenvolvida indefinidamente. Entretanto, estas terapias são caras, e coloca uma carga sobre o paciente requerendo que ele ou ela tome um número significativo de tabletes por dia (muitos pacientes acham difícil lembrar de tomar os remédios requeridos) e em muitos casos levando a efeitos colaterais desagradáveis (não surpreendente quando se toma um coquetel contendo muitos produtos farmacêuticos diferentes, que devem ser tomados para o resto da vida). Não apenas as terapias correntes sofrem destes problemas, mas elas não são curativas, meramente retardam o início da AIDS completamente desenvolvida. Além disso, em anos recentes houve um número aumentado de relatos indicando o aparecimento e disseminação de medicamentos contra o HIV resistente a medicamento. Conseqüentemente, existe uma necessidade real e urgente quanto a uma vacina que pudesse prevenir e/ou curar os vírus da imunodeficiência, tais como HIV, e também prevenir e/ou curar a AIDS.

Anteriormente, tentativas para se desenvolver vacinas contra a HIV têm sido feitas pela identificação de uma cepa do HIV existente e depois produzindo uma vacina específica para aquele vírus. No geral, as vacinas foram fundamentadas em uma resposta de B célula (anticorpo), o anticorpo

5 que é reativo com os antígenos de superfície da cepa do HIV específica contra a qual a mesma foi desenvolvida. Tipicamente, as proteínas de superfície que compreendem os antígenos são variáveis de uma cepa do HIV para a seguinte, visto que a mutação do vírus para produzir um novo vírus tende a ocorrer nas proteínas de superfície. A consequência disto é que as vacinas contra o HIV

10 convencionais, se elas fossem funcionais de qualquer modo, no geral protegeriam apenas contra uma cepa viral específica, e não contra uma nova cepa que resulta de uma mutação. Assim, uma nova vacina seria requerida para a proteção contra uma cepa emergente. O período de tempo entre a emergência da nova cepa viral pela mutação é muito curto. Dentro de um

15 indivíduo infectado novas cepas estão surgindo todo o tempo, como um resultado da pressão seletiva imposta por aquele sistema imune do indivíduo. Como um resultado, a população viral encontrada em um indivíduo infectado muda com o tempo (em um período de semanas, meses e anos) sem qualquer exigência para a re-infecção devido à alta taxa de mutação. Assim, o único

20 caminho provável para alvejar a infecção por vírus da imunodeficiência em um indivíduo ou para alvejar novas cepas virais potenciais que poderiam infectar ou desenvolver em um indivíduo, é alvejar uma região conservada do proteoma. Este método é inerentemente problemático, visto que a resposta imune dominante para o HIV está direcionada às seções que estão elas

25 próprias sob uma alta taxa de mutação, devido (a) à pressão imunológica e (b) às taxas de fidelidade baixa do mecanismo replicativo do vírus.

Vários estudos direcionados ao desenvolvimento de vacinas para os vírus da imunodeficiência (incluindo vacinas para o HIV) foram empreendidos no passado. Estes foram na maior parte fundamentados em

anticorpo e concentrados em vacinas com base na glicoproteína 120 e glicoproteína 160 do HIV (considerados ser os melhores candidatos para uma vacina contra o HIV). Particularmente estudos relevantes são listados nos seguintes:

5 Blood. 7 de fevereiro de 2006; “HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T-cells”. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, Lederman MM, Benito JM, Goepfert PA, Connors M, Roederer M, Koup RA.

10 Indian J Med Res. Abril de 2005; 121(4): 287-314. “Impact of genetic diversity of HIV-1 on diagnosis, antiretroviral therapy & vaccine development”. Lal RB, Chakrabarti S, Yang C.

15 J Virol. Abril de 2005; 79(8): 4580-8. “Vaccine-elicited memory cytotoxic T lymphocytes contribute to Mamu-A*01-associated control of simian/human immunodeficiency virus 89.6P replication in rhesus monkeys”. Seaman MS, Santra S, Newberg MH, Philippon V, Manson K, Xu L, Gelman RS, Panicali D, Mascola JR, Nabel GJ, Letvin NL.

20 J Infect Dis. 1 de Março de 2005; 191(5): 666-77. Epub 27 de Janeiro de 2005. “Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial”. Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, Heyward WL, Jobes DV, Popovic V, Self SG, Sinangil F, Burke D, Berman PW.

25 J Infect Dis. 1 de Março de 2005; 191(5): 654-65. Epub 27 de Janeiro de 2005. “Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection”. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF.

 J Exp Med. 21 de Junho de 2004; 199(12): 1709-18. “Cytotoxic T lymphocyte-based control of simian immunodeficiency virus replication in a preclinical AIDS vaccine trial”. Matano T, Kobayashi M,

Igarashi H, Takeda A, Nakamura H, Kano M, Sugimoto C, Mori K, Iida A, Hirata T, Hasegawa M, Yuasa T, Miyazawa M, Takahashi Y, Yasunami M, Kimura A, O'Connor DH, Watkins DI, Nagai Y.

Immunol Res. 2004; 29(1-3): 161-74. "Insights into the role of
5 host genetic and T-cell factors in resistance to HIV transmission from studies of highly HIV-exposed Thais". McNicholl JM, Promadej N.

J. Virol. Dez de 2005; 9(24): 15368-75: "Genetic and
Stochastic influences on the interaction of human immunodeficiency virus
type 1 e cytotoxic T lymphocytes in identical twins". Yang 00, Church J,
10 Kitchen CM, Kilpatrick R, Ali A, Geng Y, Killian MS, Sabado RL, Ng H,
Suen J, Bryson Y, Jamieson BD, Krogstad P.

J. Virol. Setembro de 2005. 79 (17): 11523-8. "Human
immunodeficiency virus mutations during the first month of infection are
preferentially found in known cytotoxic T-lymphocyte epitopes". Bernardin
15 F, Kong D, Peddada L, Baxter-Lowe LA, Delwart E

Entretanto, embora os epítomos conhecidos tenham sido
extensivamente estudados, nenhum foi ainda satisfatório para formar a base
de uma vacina do HIV. Além disso, as vacinas com base em qualquer epítomo
único, mesmo se ela fosse para fornecer alguma proteção, provavelmente
20 seria específica para um HLA particular, tornando a vacina ineficaz em uma
grande proporção da população humana.

Conseqüentemente, é um objetivo da presente invenção
resolver os problemas associados com a técnica anterior conhecida como
apresentados acima. É ainda um objetivo da presente invenção fornecer um
25 polipeptídeo que seja capaz de evocar uma resposta imune CTL em
vertebrados contra uma pluralidade de cepas virais da imunodeficiência e/ou
em uma pluralidade de indivíduos que expressam MHCs diferentes (HLAs). É
um outro objetivo da presente invenção fornecer uma vacina de vírus da
imunodeficiência (tal como uma vacina do HIV) usando o polipeptídeo da

invenção. Preferivelmente a vacina é capaz de proteção contra uma pluralidade de cepas virais e/ou é eficaz em uma pluralidade de indivíduos que expressam MHCs diferentes (HLAs).

Conseqüentemente, a presente invenção fornece um
 5 polipeptídeo tendo não mais do que 100 aminoácidos, polipeptídeo este que compreende uma ou mais seqüências tendo pelo menos 60 % de homologia com qualquer uma das SEQ ID 1 a 4 ou compreende dois ou mais epítomos tendo 7 aminoácidos ou mais, cada epítopo tendo pelo menos 60 % de homologia com uma sub-seqüência de qualquer uma das SEQ ID 1 a 4 que
 10 tem o mesmo comprimento como o epítopo:

SEQ ID 1 GDTWAGVEAIIRILQQLLFIEFRIGCQHSR

SEQ ID 2 KVGSLQYLALTALITPKKIKPPLPSVKKLTEDRWNPQKT

SEQ ID 3 EPVPLQLPPLERLTLDCEDCGTSGTQ

SEQ ID 4 YKGALDLSHFLKEKGGLEGLIYS
 15 QKRQDILDLWVYHTQGYFPD

em que, o polipeptídeo é imunogênico em um vertebrado que expressa um alelo de complexo de histocompatibilidade maior (MHC), e em que o polipeptídeo não é uma proteína do vírus HIV completa.

Assim, o polipeptídeo é um que pode compreender o todo de
 20 (ou pode compreender pelo menos duas partes de 7 ou mais resíduos) qualquer uma das seqüências acima, mas não pode ter mais do que 100 resíduos de aminoácido no total. O polipeptídeo também deve ser imunogênico em um vertebrado que expressa um alelo MHC (HLA em seres humanos). Um polipeptídeo imunogênico é entendido no presente contexto
 25 significar um polipeptídeo que evoca uma resposta imune em um vertebrado, tal como pela ligação a uma MHC de vertebrado e fazendo com que a mesma reaja com um linfócito de célula T citotóxico. Um método para determinar se um polipeptídeo possui imunogenicidade é apresentado no Experimento 1 abaixo. Entretanto, a presente invenção não é limitada a tais métodos, e a

pessoa habilitada pode selecionar qualquer método conhecido para determinar a imunogenicidade, como desejado.

Como mencionado acima, o polipeptídeo pode ser um que compreende dois epítomos de 7 ou mais resíduos que reagem com uma ou mais MHCs e assim evocam uma resposta CTL ampla. A resposta pode ser em um único indivíduo ou pode ser em pelo menos dois indivíduos diferentes (os indivíduos podem ser da mesma espécie ou de espécie diferente). Assim, o polipeptídeo pode compreender pelo menos dois epítomos de 7 ou mais resíduos diferentes, cada um dos quais individualmente fornece uma resposta a um paciente diferente. Um epítomo no contexto da presente invenção é uma parte de um polipeptídeo que é capaz de ligar a um MHC em um vertebrado, preferivelmente evocar uma resposta imune, tal como fazendo com que o complexo de MHC-epítomo reaja com um CTL. Um método para determinar se um polipeptídeo é um epítomo é apresentado no Experimento 1 abaixo. Entretanto, a presente invenção não é limitada a tais métodos, e a pessoa habilitada pode selecionar qualquer método conhecido para determinar se um polipeptídeo é um epítomo, como desejado.

Os presentes inventores verificaram que as seqüências acima compreendem uma pluralidade de epítomos CTL, que podem proporcionar proteção contra os vírus da imunodeficiência, especialmente o HIV, para uma ampla variedade de vertebrados em uma população, e para um amplo grupo representativo da população humana. Além disso, os inventores analisaram todas as seqüências de cepas do HIV conhecidas, e verificaram que as seqüências especificadas são acentuadamente conservadas através de todas as cepas do HIV conhecidas. Como tal, estas seqüências são muito improváveis de serem significativamente alteradas em novas cepas que resultem da mutação de cepas existentes. Conseqüentemente, os epítomos dentro destas seqüências que fornecem proteção são altamente prováveis de estarem presentes em forma inalterada em novas cepas, visto que a mutação

normalmente não ocorre nestas regiões. Conseqüentemente, estes epítomos fornecem excelente oportunidade não apenas para fornecer proteção contra as cepas do HIV existentes (tais como as cepas de referência, por exemplo, no caso do HIV-1 Clado B, a referência padrão HXB-2 - mais informação sobre as cepas de referência pode ser encontrada na base de dados <http://hiv-web.lanl.gov>), mas também proteção contra cepas até agora desconhecidas, tais como formas mutadas das cepas de referência acima.

Como debatido acima, as seqüências foram identificadas depois da análise de todas as seqüências de cepa do HIV conhecidas. As seqüências são assim seqüências de consenso desenvolvidas a partir da análise acima. Apesar de serem seqüências de consenso, as seqüências em alguns casos correspondem exatamente às seqüências naturais em algumas das cepas do HIV conhecidas. Devido à conservação extraordinária nas seqüências através de todos os vírus, as seqüências de consenso, mesmo quando diferindo das seqüências reais, apenas diferem em um pequeno número de resíduos, e assim contêm muitos epítomos menores (8-mers, 9-mers, 10-mers etc.) para os quais não existem diferenças das seqüências naturais. As seqüências de consenso acima como um todo assim contêm muitos epítomos eficazes que são os mesmos como os epítomos naturais, assim como epítomos eficazes que diferem apenas levemente dos epítomos naturais. Estará evidente para a pessoa habilitada que a invenção estende-se não apenas às seqüências de consenso e seus epítomos, mas também às seqüências reais correspondentes em quaisquer cepas do vírus da imunodeficiência (por exemplo, HIV). Assim, as seqüências com alguma homologia com as seqüências de consenso também estão dentro do escopo da invenção. Tal homologia permite a substituição, por exemplo, de até 3 aminoácidos em um epítopo de 8 mer (62,5 % de homologia) ou em um epítopo de 9-mer, 10-mer ou 11-mer. É preferido que não mais do que 10 de tais substituições sejam identificáveis em uma seqüência da invenção que corresponde às seqüências completas das SEQ ID 1 a 4 (66,6 % de homologia

para uma 30-mer). Tais substituições são preferivelmente substituições conservativas em linha com esquemas de substituição conhecidos.

5 Tendo em mente que a invenção estende-se da seqüência de consenso para as seqüências naturais correspondentes, então a invenção também fornece um polipeptídeo tendo não mais do que 100 aminoácidos, polipeptídeo este que compreende uma ou mais seqüências definidas pelos seguintes resíduos de aminoácido de uma proteína do vírus HIV ou compreende dois ou mais epítomos tendo 7 aminoácidos ou mais de uma seqüência definida pelos seguintes resíduos de aminoácido de uma proteína
10 do vírus HIV:

resíduos 51 a 80	de uma proteína VPR
resíduos 142 a 181	de uma proteína VLF
resíduos 69 a 95	de uma proteína REV
resíduos 81 a 123	de uma proteína NEF

15 em que, o polipeptídeo é imunogênico em um vertebrado que expressa um alelo de complexo de histocompatibilidade maior (MHC), e em que o polipeptídeo não é uma proteína do HIV completa.

A numeração de seqüência aludida na presente invenção é definida de acordo com princípios bem reconhecidos. Assim, a numeração
20 começa em 1 a partir do códon de início de tradução (ATG) reconhecido. Isto corresponde a uma Metionina (M), para o segmento do genoma do vírus HIV que codifica a proteína de interesse. Em outras palavras, a mesma começa em 1 em relação à Metionina mostrada como o primeiro aminoácido na seqüência de proteína de interesse como usado e definido pelas bases de dados nas quais
25 as seqüências foram apresentadas (isto é, GenBank, SwissProt, etc.).

A presente invenção será descrita em mais detalhes por via de exemplo apenas com referência às seguintes Figuras, em que:

A Figura 1 mostra a produção de IFN- γ pelas culturas de esplenócito primárias de camundongos vacinados com HIV-v e NRP

estimulados com Con A (10 $\mu\text{g/ml}$), Lisozima solúvel (5 $\mu\text{g/ml}$), polipeptídeos solúveis purificados (P1, P2, P3 e P4; 5 $\mu\text{g/ml}$) e células humanas T1 emparelhadas (T1) e JURKAT desemparelhadas (Ju) com HLA transfectadas com Lisozima, P1, P2, P3 ou P4 de acordo com o protocolo descrito no texto (a razão de esplenócito para célula transfectada é 10:1); a produção de IFN- γ é representada como o diferencial entre o nível de produção em resposta ao antígeno considerado menos a IFN- γ produzido em resposta à Lisozima solúvel ou à célula correspondente transfectada com Lisozima. Os níveis de fundo de produção mediada por Lisozima de IFN- γ foram para antígeno solúvel 25 ± 10 pg/ml, para antígeno em T1 316 ± 43 pg/ml, e para antígeno em Jurkat 19 ± 6 pg/ml;

A Figura 2 mostra a produção de IFN- γ pelas culturas de esplenócito primárias de camundongos vacinados com HIV-v e NRP estimulados com Con A (10 $\mu\text{g/ml}$), Lisozima solúvel (5 $\mu\text{g/ml}$), solução equimolar purificada de proteínas do HIV recombinantes (51,1, g/ml) e células humanas T1 emparelhadas (T1) e JURKAT desemparelhadas (Ju) com HLA transfectadas com Lisozima ou solução equimolar purificada de proteínas do HIV recombinantes de acordo com o protocolo descrito no texto (a razão de esplenócito para célula transfectada é de 10:1); a produção de IFN- γ é representada como o diferencial entre o nível de produção em resposta ao antígeno considerado menos a IFN- γ produzida em resposta à Lisozima solúvel ou à célula correspondente transfectada com Lisozima; os níveis de fundo da produção mediada por Lisozima de IFN- γ foram para o antígeno solúvel 25 ± 10 pg/ml, para o antígeno em T1 316 ± 43 pg/ml, e para o antígeno em Jurkat 19 ± 6 pg/ml.

A Figura 3 mostra a produção de IFN- γ similar como para as Figures 1 e 2 em respeito ao grupo de teste 3, confirmando a reatividade do peptídeo da SEQ ID 3;

A Figure 4 mostra a produção de IFN- γ similar como para as

Figures 1 e 2 em respeito ao grupo de teste 4, confirmando a reatividade do peptídeo da SEQ ID 4;

A Figure 5 mostra a produção de IFN- γ similar como para as Figures 1 e 2 em respeito ao grupo de teste 5, confirmando a reatividade dos antígenos derivados das proteínas VIF, REV e NEF HIV completas; e

A Figure 6 mostra a produção de IFN- γ pelas culturas de esplenócito primárias de camundongos vacinados com HIV-v e NRP-v estimulados com Ionomicina (0,78 μ g/ml) e PMA (0,05 μ g/ml), Con A (5 μ g/ml) ou células humanas CEM emparelhadas com HLA infectadas com isolados do HIV-1 do Glado D (UG21-R5) ou Glado A (LTG-29-X4) de acordo com o protocolo descrito nos Exemplos (a razão de esplenócito para célula infectada é de 10:1). A produção de IFN- γ é representada como, o número de células que produzem IFN- γ no grupo HIV-v menos o número de células que produzem IFN- γ no grupo NRP-v. O número de valores de células que produzem IFN- γ para os esplenócitos tanto de HIV-v quanto de NRP-v estimulados com Con A ou Ionomicina e PMA foram acima de 4900 e acima de 8000 respectivamente. A significância estatística foi estabelecida pela análise de Mann-Whitney não paramétrica dos valores de IFN- γ estabelecidos para os grupos HIV-v e NRP-v.

Os polipeptídeos da invenção descritos acima tipicamente compreendem um ou mais (preferivelmente dois ou mais). Estes epítomos são preferivelmente epítomos de célula T, tais como epítomos de linfócito T citotóxicos (CTL). No geral o polipeptídeo é imunogênico a uma cepa do vírus da imunodeficiência (preferivelmente HIV), e preferivelmente a uma pluralidade de tais cepas. No presente contexto, um polipeptídeo imunogênico a uma cepa do vírus da imunodeficiência é entendido significar um polipeptídeo que é parte de uma proteína viral e que evoca uma resposta imune, tal como pela exibição da reatividade de CTL quando ligado a um MHC. Um método para determinar se um polipeptídeo possui tal

imunogenicidade é apresentado no Experimento 1 abaixo. Entretanto, a presente invenção não é limitada a tais métodos, e a pessoa habilitada pode selecionar qualquer método conhecido para determinar imunogenicidade, como desejado.

5 Na presente invenção, o polipeptídeo compreende duas ou mais seqüências como descritas acima. Tipicamente, duas, três, quatro, cinco ou mais de tais seqüências podem estar presentes no polipeptídeo, se desejado. Quanto mais tais epítomos estão presentes, maior a amplitude de proteção proporcionada dentro de uma população de seres humanos e/ou
10 animais individuais com HLAs ou MHCs divergentes.

O polipeptídeo de acordo com a presente invenção também pode compreender uma ou mais seqüências adicionais que não são epítomos, se desejado. Tipicamente as seqüências adicionais são de uma ou mais proteínas virais da imunodeficiência (preferivelmente proteínas do HIV).
15 Estas seqüências podem estar situadas entre duas ou mais das seqüências (os epítomos) descritas acima, e/ou podem estar situadas em uma ou ambas as extremidades do polipeptídeo. A presença de tais seqüências adicionais não devem afetar a função do polipeptídeo, contanto que o polipeptídeo como um todo não se torne muito grande, que interfira com a apresentação dos epítomos
20 no sistema imune do vertebrado. Em formas de realização específicas da invenção, quando o polipeptídeo é homólogo à SEQ ID 1, as seqüências adicionais são preferivelmente uma ou mais de uma proteína VPR (preferivelmente de uma cepa do HIV), quando o polipeptídeo é homólogo à SEQ ID 2, as seqüências adicionais são preferivelmente uma ou mais de uma
25 proteína VIF (preferivelmente de uma cepa do HIV), quando o polipeptídeo é homólogo à SEQ ID 3, as seqüências adicionais são preferivelmente uma ou mais de uma proteína REV (preferivelmente de uma cepa do HIV), e quando o polipeptídeo é homólogo à SEQ ID 4, as seqüências adicionais são preferivelmente uma ou mais de uma proteína NEF (preferivelmente de uma

cepa do HIV).

Nas formas de realização mais preferidas, as seqüências adicionais das proteínas mencionadas acima são aquelas dentro das seguintes seqüências de consenso ou aquelas tendo pelo menos 60 % de homologia com uma seqüência dentro das seguintes seqüências de consenso:

VPR HIV Consenso — SEQ ID 5

MEQAPEDQGPQREPYNEWTLLEELKNEAVRHFPRPWLHGLGQHIY
ETYGDTWAGVEABRILQQLFIHFRIGCQHSRIGIIRQRRARNGASRS

VIF HIV Consenso — SEQ ID 6

MENRWQVMIVWQVDRI VIR1RTWKSLVKHHMYISKKAKGWFYRHH
YESTHPRISSEVHIPLGDDAKLVITTYWGLHTGERDWHLGQGVSIWR
KKRYSTQVDPDLADQLIHLYYFDCFSESAIRKAILGHIVSPRCEYQAG
HNKVGSLQYLALTALITPKKIKPPLPSVKKLTEDRWKPKTKGHRG
SHTMNGH

REV HIV Consenso — SEQ ID 7

MAGRSGDSDEELLKAVRIIKILYQSNPYPSPEGTRQARRNRRRRWRAR
QRQIRSISERILSTCLGRPAEPVPLQLPPLERLTLDCEDCGTSGTQQSQ
GTEEGVGSPQILVESPTVLESGTKE

NEF HIV Consenso — SEQ ID 8

MGGKWSKSSVVGWPAVRERMRRAPAAEGVGAVSRDLEKHGAITSS
NTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKGALDLSHFLK
EKGGLEGLIYSQKRQDILDLWVYHTQGYFPDWQNYTPGPORYPLTFG
WCFKLVPVEPEKVEEANEKENNCLLHPMSQHGMEDPEREVLVWKFD
SRLAFHHMARELHPEYYKDC

A homologia aludida acima em relação às seqüências é preferivelmente de 75 %, 85 %, 95 % ou substancialmente 100 %.

Na presente invenção, a cepa do vírus da imunodeficiência não é especialmente limitada, e os polipeptídeos podem ser imunogênicos contra, e/ou derivada de, qualquer cepa do vírus HIV conhecida. As cepas futuras que

tenham mutado de qualquer uma destas cepas existentes também podem ser aquelas contra as quais os polipeptídeos são imunogênicos ou das quais os polipeptídeos são derivados.

As seqüências que definem os polipeptídeos da presente
5 invenção são situadas dentro das proteínas VPR, VIF, REV e NEF de qualquer cepa do vírus HIV (as seqüências de consenso das quais para todas as seqüências analisadas ou alternativamente as posições das quais dentro da proteína, são descritas acima). As seguintes seqüências específicas foram analisadas pelos inventores, e preferivelmente as seqüências do HIV aludidas
10 na invenção são selecionadas destas seqüências ou mutações específicas destas seqüências. Assim, as seqüências específicas homólogas às SEQ ID 1 a 4 descritas acima são preferivelmente aquelas nas posições apropriadas dentro das seguintes proteínas. Similarmente, as seqüências da presente invenção definidas pelas posições de resíduo dentro das proteínas de qualquer cepa
15 viral, a saber os resíduos 51 a 80 da proteína VPR, resíduos 142 a 181 da proteína VIF, resíduos 69 a 95 da proteína REV, e resíduos 81 a 123 da proteína NEF. A lista está na forma |versão número (número gi)|identificação da base de dados (por exemplo, gb para GenBank)|número de acesso NCBI|informação adicional opcional (por exemplo, o número de acesso da
20 seqüência de nucleotídeo a partir da qual a seqüência de proteína é derivada). As seqüências e cepas virais correspondentes a partir das quais elas derivam podem ser todas encontradas a partir da base de dados de proteína NCBI pública, que pode ser acessada *online* nos seguintes endereços URL <http://www.ncbi.nlm.nih.gov/entrez/query/static/help/helpdoc.html#Protein>. As
25 bases de dados de proteína contêm dados de seqüência das regiões codificadoras traduzidas de seqüências de DNA no GenBank, EMBL e DDBJ assim como seqüências de proteína submetidas ao Protein Information Resource (PIR), SWISS-PROT, Protein Research Foundation (PRF), e Protein Data Bank (PDB) (seqüências de estruturas resolvidas).

Proteínas VPR

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 |58700473|gb|AAW80948.1|, |58700471|gb|AAW80947.1|, |58700469|gb|AAW80946.1|,
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 |58700461|gb|AAW80942.1|, |328639|gb|AAA45064.1|, |328424|gb|AAA44993.1|,
 |392792|gb|AAA03700.1| HIV-1 **consenso**, |82294586|sp|Q85737|Q85737_9HIV1 HIV-1
consenso, |18699252|gb|AAL78493.1|AF414006_5, |1732492|gb|AAB38839.1|,
 |1732483|gb|AAB38831.1|, |33358354|gb|AAQ16648.1|, |33358352|gb|AAQ16647.1|,
 |33358350|gb|AAQ16646.1|, |33358348|gb|AAQ16645.1|, |33358346|gb|AAQ16644.1|,
 |33358344|gb|AAQ16643.1|, |33358342|gb|AAQ16642.1|, |33358340|gb|AAQ16641.1|,
 |33358338|gb|AAQ16640.1|, |33358336|gb|AAQ16639.1|, |33358334|gb|AAQ16638.1|,
 |33358331|gb|AAQ16637.1|, |33358329|gb|AAQ16636.1|, |37725255|gb|AAR02317.1|,
 |37725235|gb|AAR02299.1|, |37725225|gb|AAR02290.1|, |37725215|gb|AAR02281.1|,
 |37725205|gb|AAR02272.1|, |37725193|gb|AAR02261.1|, |27227859|dbj|BAC45032.1|,
 |27227849|dbj|BAC45023.1|, |23954644|gb|AAN40113.1|truncada,
 |23954624|gb|AAN40103.1|truncada, |1794250|gb|AAC56429.1|, |1794248|gb|AAC56428.1|,
 |1794246|gb|AAC56427.1|, |1794244|gb|AAC56426.1|, |1794242|gb|AAC56425.1|,
 |1794240|gb|AAC56424.1|, |1794238|gb|AAC56423.1|, |1794236|gb|AAC56422.1|,
 |1794234|gb|AAC56421.1|, |1794232|gb|AAC56420.1|, |1794230|gb|AAC56419.1|,
 |1794228|gb|AAC56418.1|, |1794225|gb|AAC56417.1|, |328448|gb|AAA83398.1|,
 |328038|gb|AAA44858.1|, |82304199|sp|Q9E526|Q9E526_9HIV1,
 |82295433|sp|Q8AC97|Q8AC97_9HIV1, |82295432|sp|Q8AC89|Q8AC89_9HIV1,
 |82287838|sp|Q6WWX7|Q6WWX7_9HIV1, |82287837|sp|Q6WWX6|Q6WWX6_9HIV1,
 |82287836|sp|Q6WWX5|Q6WWX5_9HIV1, |82287835|sp|Q6WWX4|Q6WWX4_9HIV1,
 |82287834|sp|Q6WWX3|Q6WWX3_9HIV1, |82287833|sp|Q6WWX2|Q6WWX2_9HIV1,
 |82287832|sp|Q6WWX1|Q6WWX1_9HIV1, |82287831|sp|Q6WWX0|Q6WWX0_9HIV1,
 |82287830|sp|Q6WWW9|Q6WWW9_9HIV1, |82287829|sp|Q6WWW8|Q6WWW8_9HIV1,
 |82287828|sp|Q6WWW7|Q6WWW7_9HIV1, |82287827|sp|Q6WWW6|Q6WWW6_9HIV1,
 |82287826|sp|Q6WWW5|Q6WWW5_9HIV1, |82281651|sp|P90287|P90287_9HIV1,
 |82281644|sp|P90278|P90278_9HIV1, |82281643|sp|P90277|P90277_9HIV1,
 |82281642|sp|P90276|P90276_9HIV1, |82281641|sp|P90275|P90275_9HIV1,
 |82281640|sp|P90274|P90274_9HIV1, |82281638|sp|P90273|P90273_9HIV1,
 |82281637|sp|P90272|P90272_9HIV1, |82281636|sp|P90271|P90271_9HIV1,
 |82281635|sp|P90270|P90270_9HIV1, |82281634|sp|P90269|P90269_9HIV1,
 |82281633|sp|P90268|P90268_9HIV1, |82281632|sp|P90267|P90267_9HIV1,
 |82281631|sp|P90266|P90266_9HIV1, |82281285|sp|P88528|P88528_9HIV1,
 |82281284|sp|P88526|P88526_9HIV1, |82281283|sp|P88524|P88524_9HIV1,
 |82281268|sp|P88425|P88425_9HIV1, |82281267|sp|P88424|P88424_9HIV1,
 |82281266|sp|P88423|P88423_9HIV1, |82281265|sp|P88422|P88422_9HIV1,
 |82281264|sp|P88421|P88421_9HIV1, |82281263|sp|P88420|P88420_9HIV1,

82281262 sp P88419 P88419_9HIV1,	82281261 sp P88418 P88418_9HIV1,
82281260 sp P88417 P88417_9HIV1,	82281259 sp P88416 P88416_9HIV1,
82281258 sp P88415 P88415_9HIV1,	82281257 sp P88414 P88414_9HIV1,
82281256 sp P88413 P88413_9HIV1,	82281255 sp P88412 P88412_9HIV1,
82281254 sp P88411 P88411_9HIV1,	82281253 sp P88410 P88410_9HIV1,
82281252 sp P88409 P88409_9HIV1,	82281251 sp P88408 P88408_9HIV1,
82281250 sp P88407 P88407_9HIV1,	82281249 sp P88406 P88406_9HIV1,
82281248 sp P88405 P88405_9HIV1,	82281247 sp P88204 P88204_9HIV1,
82281243 sp P88156 P88156_9HIV1,	82278599 sp O11910 O11910_9HIV1,
82278598 sp O11909 O11909_9HIV1,	82278597 sp O11908 O11908_9HIV1,
82278596 sp O11907 O11907_9HIV1,	82278595 sp O11906 O11906_9HIV1,
4205077 gb AAD10948.1 ,	4205068 gb AAD10940.1 ,
4205050 gb AAD10924.1 ,	4205041 gb AAD10916.1 ,
4205023 gb AAD10900.1 ,	4205032 gb AAD10908.1 ,
4204996 gb AAD10876.1 ,	4205005 gb AAD10884.1 ,
329397 gb AAB53951.1 ,	328667 gb AAB59883.1 ,
328458 gb AAA45001.1 ,	326425 gb AAB59752.1 ,
326431 gb AAA44222.1 ,	328574 gb AAA45058.1 ,
82320734 sp Q52S57 Q52S57_9HIV1,	328414 gb AAA44984.1 ,
82320728 sp Q52S38 Q52S38_9HIV1,	328163 gb AAA44874.1 ,
82308345 sp Q9YTC4 Q9YTC4_9HIV1,	82320738 sp Q52S68 Q52S68_9HIV1,
82308241 sp Q9YP47 Q9YP47_9HIV1,	82320731 sp Q52S48 Q52S48_9HIV1,
82308239 sp Q9YP38 Q9YP38_9HIV1,	82320725 sp Q52S27 Q52S27_9HIV1,
82308236 sp Q9YP24 Q9YP24_9HIV1,	82308242 sp Q9YP51 Q9YP51_9HIV1,
82307554 sp Q9WJU7 Q9WJU7_9HIV1,	82308240 sp Q9YP45 Q9YP45_9HIV1,
82304713 sp Q9IDV1 Q9IDV1_9HIV1,	82308238 sp Q9YP30 Q9YP30_9HIV1,
82296624 sp Q8AQP7 Q8AQP7_9HIV1 truncada,	82308047 sp Q9YJ66 Q9YJ66_9HIV1,
82296614 sp Q8AQN7 Q8AQN7_9HIV1 truncada,	82307552 sp Q9WJU3 Q9WJU3_9HIV1,
82290362 sp Q77695 Q77695_9HIV1,	82299117 sp Q8USH4 Q8USH4_9HIV1,
82287920 sp Q6XDS4 Q6XDS4_9HIV1,	82291331 sp Q79789 Q79789_9HIV1,
82282600 sp Q5EFT7 Q5EFT7_9HIV1,	82287922 sp Q6XDS8 Q6XDS8_9HIV1,
82282598 sp Q5EFT5 Q5EFT5_9HIV1,	82282601 sp Q5EFT8 Q5EFT8_9HIV1,
82282596 sp Q5EFT3 Q5EFT3_9HIV1,	82282599 sp Q5EFT6 Q5EFT6_9HIV1,
82282594 sp Q5EFT1 Q5EFT1_9HIV1,	82282597 sp Q5EFT4 Q5EFT4_9HIV1,
62548196 gb AAX86748.1 ,	82282595 sp Q5EFT2 Q5EFT2_9HIV1,
62548186 gb AAX86739.1 ,	62548206 gb AAX86757.1 ,
62548166 gb AAX86721.1 ,	62548176 gb AAX86730.1 ,
8920160 emb CAB96346.1 ,	34330007 gb AAO65893.1 ,
34330001 gb AAO65888.1 ,	82291091 sp Q79149 Q79149_9HIV1

As cepas do HIV preferidas aludidas na presente invenção, por exemplo contra as quais os presentes polipeptídeos devem ser imunogênicos, são aquelas contendo estas proteínas específicas. Os números de acesso acima especificam explicitamente a identidade da cepa além da sequência de proteína específica.

A homologia percentual de uma primeira sequência de polipeptídeo a uma segunda sequência de polipeptídeo, como aludido no contexto da presente invenção, é definida como o número de resíduos de aminoácido na segunda sequência que se iguala tanto em posição (absoluta ou relativa) quanto em identidade com aquele na primeira sequência, dividido

pelo número total de resíduos de aminoácido no segundo polipeptídeo (tanto o primeiro quanto o segundo polipeptídeos deve ter o mesmo número de resíduos de aminoácido) e multiplicado por 100. Embora não uma ocorrência comum, nem todas as seqüências de proteína do HIV são idênticas no comprimento para todas as cepas conhecidas. Existem casos de proteínas que são um ou dois aminoácidos mais longos ou mais curtos do que o agrupamento geral. A pessoa habilitada pode facilmente identificar estas proteínas. Por esta razão, a definição acima estende-se à posição absoluta para as proteínas do mesmo comprimento e posição relativa para proteínas de comprimentos diferentes.. Isto, entretanto, não afeta a definição da posição reativa como definido com respeito à localização (por exemplo, do aminoácido 7 ao 37) dada acima. Na presente invenção, é preferido que a homologia de polipeptídeo para as seqüências definidas seja 75 % ou mais, 85 % ou mais, 95 % ou mais ou 100 % (ou substancialmente 100 %).

Os epítomos dentro das seqüências definidas acima não são especialmente limitadas, contanto que contenham 7 resíduos de aminoácido ou mais. Preferivelmente os epítomos são de um comprimento que seja é apropriado para os epítomos CTL em uma espécie de vertebrado particular, tal como em um ser humano, tendo um MHC específico. Tipicamente os epítomos contêm 8, 9, 10 ou 11 resíduos de aminoácido, mas podem conter mais se desejado. No geral um epítopo apropriado é um que é um epítopo de CTL em um vertebrado tal como um ser humano.

Tipicamente, o polipeptídeo compreende entre 7 e 100 aminoácidos, e preferivelmente de 8 a 50 aminoácidos. O tamanho não deve ser tão grande que epítomos úteis sofram de competição com epítomos não protetores no sistema imune (por esta razão proteínas completas não são incluídas), nem deve o tamanho ser tão pequeno que apenas uma faixa muito estreita de proteção seja oferecida. As faixas mais preferidas são de 8 a 45 aminoácidos, 15 a 45 aminoácidos e 20 a 45 aminoácidos e 15 a 35

aminoácidos. O comprimento mais preferido é de 25 a 45 resíduos de aminoácido. É particularmente preferido que o polipeptídeo consista substancialmente de uma seqüência selecionada das seqüências nas posições definidas acima na lista específica de proteínas apresentadas acima.

5 Além dos polipeptídeos descritos acima, que não devem ser maiores do que 100 resíduos de aminoácido no comprimento, a invenção também fornece polipeptídeos imunogênicos de epítipo múltiplo que compreendem dois ou mais polipeptídeos da presente invenção. Estes polipeptídeos de epítipo múltiplo não são limitados no tamanho. Assim, eles
10 se estendem não apenas aos polipeptídeos tendo de 7 a 100 resíduos de aminoácido como esboçado acima, mas também aos polipeptídeos maiores, contanto que estes polipeptídeos maiores compreendam duas ou mais unidades, cada unidade consistindo de um polipeptídeo da invenção. Assim, um polipeptídeo tendo 100 unidades de repetição de um 7-mer de acordo com
15 a presente invenção é abrangido pela presente invenção, como é um polipeptídeo tendo, digamos 52 unidades de um epítipo de 8-mer, e 23 unidades de um segundo epítipo de 10-mer. Os polipeptídeos deste tipo não sofrerão dos problemas de competição associados com polipeptídeos de comprimento similar que compreendem apenas um ou dois epítopos. Para se
20 evitar dúvidas, o polipeptídeo de epítipo múltiplo pode compreender cópias múltiplas do mesmo epítipo ou cópias únicas de uma pluralidade de epítopos diferentes ou cópias múltiplas de 2 ou mais epítopos.

 Também é fornecido pela invenção uma composição de polipeptídeo que compreende dois ou mais polipeptídeos diferentes como
25 definido acima. Assim, a composição de polipeptídeo pode compreender qualquer número de polipeptídeos da presente invenção juntos na mesma mistura ou formulação. A presença de uma pluralidade de polipeptídeos juntos é útil visto que cada um pode evocar a sua própria resposta imune, ampliando o efeito protetor da composição. É particularmente preferido que a

composição contenha todas as seqüências das SEQ ID 1 a 4 cada uma em um peptídeo separado ou diversas em um número menor de peptídeos (por exemplo, 3 combinadas em um peptídeo maior e as outras três 3 em um outro peptídeo maior, etc.).

- 5 A invenção também fornece uma construção de polipeptídeo, construção esta que compreende um polipeptídeo como definido acima e um carreador. A construção pode ser formada combinando-se dois ou mais epítopos e/ou um polipeptídeo como definido acima com o carreador. O carreador pode ser uma molécula, tal como um adjuvante e/ou um excipiente.
- 10 A combinação neste contexto significa misturar junto ou ligar junto (por exemplo, por intermédio de uma ligação covalente).

- A presente invenção fornece ainda um polipeptídeo como definido acima para o uso na medicina. Também é fornecido um medicamento ou composição de vacina contra um vírus da imunodeficiência,
- 15 tal como o HIV, que compreenda um polipeptídeo como definido acima, e um ou mais excipientes e/ou adjuvantes apropriados (se a parte carreadora da construção é por si só um excipiente ou adjuvante, então um outro excipiente ou adjuvante pode não ser necessário). O excipiente ou adjuvante não é especialmente limitado, e quaisquer excipientes ou adjuvantes habitualmente
- 20 usados em medicamentos e vacinas podem ser utilizados. A composição de vacina ou medicamento podem ser produzidos de acordo com qualquer método conhecido apropriadamente adaptado para a presente invenção, tal como misturando-se um polipeptídeo da invenção com um excipiente ou adjuvante apropriados.

- 25 Um método para produzir um polipeptídeo como definido acima também é fornecido pela invenção. O método não é especialmente limitado, e tipicamente compreende unir dois ou mais epítopos para formar o polipeptídeo. O polipeptídeo, entretanto, pode ser sintetizado pela síntese direta química (por exemplo, incorporando um aminoácido em um tempo até

que o polipeptídeo completo seja formado) ou pelos métodos recombinantes. Tais métodos gerais são bem conhecidos para a pessoa habilitada e podem ser adaptados para a presente invenção como desejado. Em alguns casos, o polipeptídeo da presente invenção pode compreender seqüências de aminoácido adicionais em um ou ambos os terminais para ajudar na síntese do polipeptídeo. Estas seqüências adicionais são preferivelmente de 1 a 5 aminoácidos no comprimento. Tipicamente 3 aminoácidos são envolvidos. Por exemplo, um pode incluir uma cisteína (C) ou uma cadeia Gly-Cys (G-C) no terminal N ou C de modo a permitir a ligação química a outros componentes de proteína.

A invenção fornece ainda o uso de um polipeptídeo ou composição como definidos acima, na fabricação de um medicamento ou vacina, eficaz no tratamento ou prevenção de um vírus da imunodeficiência, tal como o HIV. Também é fornecido um método para tratar ou prevenir um vírus da imunodeficiência tal como o HIV, método este que compreende administrar um polipeptídeo, uma composição, um medicamento ou uma vacina como definidos acima a um vertebrado. O método de administração não é especialmente limitado, e pode compreender a administração subcutânea, intramuscular, intravenosa, intradérmica, transdérmica ou intranasal ou pode ser administrado oralmente (por exemplo, na forma de uma pílula ou uma preparação líquida) ou pode estar na forma de um supositório, se desejado. A forma de tais preparações de administração não é especialmente limitada, e formas conhecidas podem ser utilizadas com modificações apropriadas que estarão evidentes para a pessoa habilitada. A dosagem não é especialmente limitada e pode variar de 1 ng a 100 g do polipeptídeo por indivíduo, dependendo do tamanho, peso e espécie do indivíduo envolvido.

A invenção pode ser aplicada a qualquer vertebrado, visto que os sistemas imunes de vertebrados operam em uma maneira relacionada.

Tipicamente, o vertebrado aludido no presente contexto é um mamífero. É especialmente preferido que o vertebrado seja um ser humano.

Os exemplos de MHCs humanos (HLAs) que podem ser utilizados com a presente invenção incluem os seguintes:

HLA-A

A*010101, A*010102, A*010103, A*0102, A*0103, A*0104N, A*0106, A*0107, A*0108, A*0109, A*0110, A*02010101, A*02010102L, A*020102, A*020103, A*020104, A*020105, A*020106, A*020107, A*020108, A*020109, A*020110, A*020111, A*0202, A*020301, A*020302, A*0204, A*0205, A*020601, A*020602, A*020603, A*0207, A*0208, A*0209, A*0210, A*0211, A*0212, A*0213, A*0214, A*0215N, A*0216, A*021701, A*021702, A*0218, A*0219, A*022001, A*022002, A*0221, A*0222, A*0224, A*0225, A*0226, A*0227, A*0228, A*0229, A*0230, A*0231, A*0232N, A*0233, A*0234, A*023501, A*023502, A*0236, A*0237, A*0238, A*0239, A*0240, A*0241, A*0242, A*0243N, A*0244, A*0245, A*0246, A*0247, A*0248, A*0249, A*0250, A*0251, A*0252, A*0253N, A*0254, A*0255, A*0256, A*0257, A*0258, A*0259, A*0260, A*0261, A*0262, A*0263, A*0264, A*0265, A*0266, A*0267, A*0268, A*0269, A*0270, A*0271, A*0272, A*0273, A*03010101, A*03010102N, A*03010103, A*030102, A*030103, A*0302, A*0303N, A*0304, A*0305, A*0306, A*0307, A*0308, A*0309, A*0310, A*0311N, A*0312, A*0313, A*0314, A*110101, A*110102, A*1102, A*1103, A*1104, A*1105, A*1106, A*1107, A*1108, A*1109, A*1110, A*1111, A*1112, A*1113, A*1114, A*1115, A*1116, A*1117, A*1118, A*1119, A*2301, A*2302, A*2303, A*2304, A*2305, A*2306, A*2307N, A*2308N, A*2309, A*2310, A*2311N, A*2312, A*24020101, A*24020102L, A*240202, A*240203, A*240204, A*240205, A*240206, A*240301, A*240302, A*2404, A*2405, A*2406, A*2407, A*2408, A*2409N, A*2410, A*2411N, A*2413, A*2414, A*2415, A*2417, A*2418, A*2419, A*2420, A*2421, A*2422, A*2423, A*2424, A*2425, A*2426, A*2427, A*2428, A*2429, A*2430, A*2431, A*2432, A*2433, A*2434, A*2435, A*2436N, A*2437, A*2438, A*2439, A*2440N, A*2441, A*2442, A*2443, A*2444, A*2445N, A*2446, A*250101, A*250102, A*2502, A*2503, A*2504, A*2601, A*2602, A*2603, A*2604, A*2605, A*2606, A*260701, A*260702, A*2608,

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HLA-B

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HLA-C

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HLA-E

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HLA-F

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HLA-G

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HLA-DRB1

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HLA-DRB2-9

DRB2*0101, DRB3*010101, DRB3*01010201, DRB3*01010202, DRB3*010103, DRB3*010104, DRB3*0102, DRB3*0103, DRB3*0104, DRB3*0105, DRB3*0106,

DRB3*0107, DRB3*0108, DRB3*0109, DRB3*0110, DRB3*0111, DRB3*0201, DRB3*020201, DRB3*020202, DRB3*020203, DRB3*020204, DRB3*0203, DRB3*0204, DRB3*0205, DRB3*0206, DRB3*0207, DRB3*0208, DRB3*0209, DRB3*0210, DRB3*0211, DRB3*0212, DRB3*0213, DRB3*0214, DRB3*0215, DRB3*0216, DRB3*0217, DRB3*0218, DRB3*0219, DRB3*030101, DRB3*030102, DRB3*0302, DRB3*0303, DRB4*01010101, DRB4*0102, DRB4*01030101, DRB4*01030102N, DRB4*010302, DRB4*010303, DRB4*010304, DRB4*0104, DRB4*0105, DRB4*0106, DRB4*0107, DRB4*0201N, DRB4*0301N, DRB5*010101, DRB5*010102, DRB5*0102, DRB5*0103, DRB5*0104, DRB5*0105, DRB5*0106, DRB5*0107, DRB5*0108N, DRB5*0109, DRB5*0110N, DRB5*0111, DRB5*0112, DRB5*0113, DRB5*0202, DRB5*0203, DRB5*0204, DRB5*0205, DRB6*0101, DRB6*0201, DRB6*0202, DRB7*010101, DRB7*010102, DRB8*0101, DRB9*0101.

HLA-DQA1

DQA1*010101, DQA1*010102, DQA1*010201, DQA1*010202, DQA1*0103, DQA1*010401, DQA1*010402, DQA1*0105, DQA1*0106, DQA1*0107, DQA1*0201, DQA1*030101, DQA1*0302, DQA1*0303, DQA1*040101, DQA1*040102, DQA1*0402, DQA1*0403N, DQA1*0404, DQA1*050101, DQA1*050102, DQA1*0502, DQA1*0503, DQA1*0504, DQA1*0505, DQA1*060101, DQA1*060102, DQA1*0602.

HLA-DQB1

DQB1*020101, DQB1*020102, DQB1*0202, DQB1*0203, DQB1*030101, DQB1*030102, DQB1*030201, DQB1*030202, DQB1*030302, DQB1*030303, DQB1*0304, DQB1*030501, DQB1*030502, DQB1*030503, DQB1*0306, DQB1*0307, DQB1*0308, DQB1*0309, DQB1*0310, DQB1*0311, DQB1*0312, DQB1*0313, DQB1*0401, DQB1*0402, DQB1*050101, DQB1*050102, DQB1*050201, DQB1*050202, DQB1*050301, DQB1*050302, DQB1*0504, DQB1*060101, DQB1*060102, DQB1*060103, DQB1*0602, DQB1*0603, DQB1*060401, DQB1*060402, DQB1*060501, DQB1*060502, DQB1*0606, DQB1*0607, DQB1*0608, DQB1*0609, DQB1*0610, DQB1*061101, DQB1*061102, DQB1*0612, DQB1*0613, DQB1*0614, DQB1*0615, DQB1*0616, DQB1*0617, DQB1*0618, DQB1*0619, DQB1*0620, DQB1*0621, DQB1*0622, DQB1*0623.

HLA-DPA1

DPA1*010301, DPA1*010302, DPA1*010303, DPA1*0104, DPA1*0105, DPA1*0106, DPA1*0107, DPA1*0108, DPA1*020101, DPA1*020102, DPA1*020103, DPA1*020104, DPA1*020105, DPA1*020106, DPA1*020201, DPA1*020202, DPA1*020203, DPA1*0203, DPA1*0301, DPA1*0302, DPA1*0303, DPA1*0401.

HLA-DPB1

DPB1*010101, DPB1*010102, DPB1*010103, DPB1*0102, DPB1*020102, DPB1*020103, DPB1*020104, DPB1*020105, DPB1*020106, DPB1*0202, DPB1*0203, DPB1*030101, DPB1*030102, DPB1*0302, DPB1*040101, DPB1*040102, DPB1*0402, DPB1*0501, DPB1*0601, DPB1*0801, DPB1*0901, DPB1*1001, DPB1*110101, DPB1*110102, DPB1*1301, DPB1*1401, DPB1*1501, DPB1*1601, DPB1*1701, DPB1*1801, DPB1*1901, DPB1*200101, DPB1*200102, DPB1*2101, DPB1*2201, DPB1*2301, DPB1*2401, DPB1*2501, DPB1*260101, DPB1*260102, DPB1*2701, DPB1*2801, DPB1*2901, DPB1*3001, DPB1*3101, DPB1*3201, DPB1*3301, DPB1*3401, DPB1*3501, DPB1*3601, DPB1*3701, DPB1*3801, DPB1*3901, DPB1*4001, DPB1*4101, DPB1*4401, DPB1*4501, DPB1*4601, DPB1*4701, DPB1*4801, DPB1*4901, DPB1*5001, DPB1*5101, DPB1*5201, DPB1*5301, DPB1*5401, DPB1*5501, DPB1*5601, DPB1*5701, DPB1*5801, DPB1*5901, DPB1*6001, DPB1*6101N, DPB1*6201, DPB1*6301, DPB1*6401N, DPB1*6501, DPB1*6601, DPB1*6701, DPB1*6801, DPB1*6901, DPB1*7001, DPB1*7101, DPB1*7201, DPB1*7301, DPB1*7401, DPB1*7501, DPB1*7601, DPB1*7701, DPB1*7801, DPB1*7901, DPB1*8001, DPB1*8101, DPB1*8201, DPB1*8301, DPB1*8401, DPB1*8501, DPB1*8601, DPB1*8701, DPB1*8801, DPB1*8901, DPB1*9001, DPB1*9101, DPB1*9201, DPB1*9301, DPB1*9401, DPB1*9501, DPB1*9601, DPB1*9701, DPB1*9801, DPB1*9901.

HLA-DMA

DMA*0101, DMA*0102, DMA*0103, DMA*0104.

HLA-DMB

DMB*0101, DMB*0102, DMB*0103, DMB*0104, DMB*0105, DMB*0106.

HLA-DOA

DOA*010101, DOA*01010201, DOA*01010202, DOA*01010203, DOA*010103,
DOA*01010401, DOA*01010402, DOA*010105.

HLA-DOB

DOB*01010101, DOB*01010102, DOB*010102, DOB*010201, DOB*010202, DOB*0103,
DOB*01040101, DOB*01040102.

A invenção não é limitada a tais moléculas de HLA, e pode ser adaptada a tais moléculas recém descobertas, se desejado, simplesmente estabelecendo-se a reatividade de substâncias tais como peptídeos com as moléculas. Isto pode ser facilmente obtido usando-se técnicas conhecidas que são padrão no campo. Os alelos HLA particularmente preferidos para o uso com a presente invenção incluem os seguintes:

HLA Classe I

<u>HLA A</u>	<u>HLA B</u>	<u>HLA Cw</u>
A*6802	B*5801	Cw*1701
A*6801	B*5701	Cw*1601
A*6601	B*5501	Cw*1502
A*3303	B*5201	Cw*1402
A*3301	B*5101	Cw*1203
A*3201	B*5001	Cw*0802
A*310102	B*4901	Cw*0801
A*3002	B*4501	Cw*0704
A*3001	B*4403	Cw*0703
A*2902	B*4402	Cw*0702
A*2608	B*4101	Cw*0701
A*2601	B*4002	Cw*0602
A*2501	B*4001	Cw*0501
A*2402	B*3901	Cw*0401
A*2301	B*3801	Cw*0304
A*1101	B*3701	Cw*0303
A*0302	B*3503	Cw*0202
A*0301	B*3501	Cw*0102
A*0205	B*2705	
A*0201	B*1801	
A*0101	B*1501	
	B*1402	
	B*1401	
	B*1302	
	B*0801	
	B*0705	
	B*0702	

HLA Classe II

<u>HLA DPB</u>	<u>HLA DQA</u>	<u>HLA DQB</u>	<u>HLA DRB</u>
DPB1*1701	DQA1*0505	DQB1*0604	DRB1*1601
DPB1*1301	DQA1*0501	DQB1*0603	DRB1*1501
DPB1*1001	DQA1*0401	DQB1*0602	DRB1*1401
DPB1*0601	DQA1*0303	DQB1*0503	DRB1*1302
DPB1*0501	DQA1*0302	DQB1*0502	DRB1*1301
DPB1*0402	DQA1*0301	DQB1*0501	DRB1*1201
DPB1*0401	DQA1*0201	DQB1*0402	DRB1*1104
DPB1*0301	DQA1*0104	DQB1*0303	DRB1*1101
DPB1*0201	DQA1*0103	DQB1*0302	DRB1*0801
DPB1*0101	DQA1*0102	DQB1*0301	DRB1*0701
	DQA1*0101	DQB1*0202	DRB1*0404
		DQB1*0201	DRB1*0401
			DRB1*0301
			DRB1*0103
			DRB1*0102
			DRB1*0101

Os alelos mais preferidos de acordo com a invenção são os seguintes:

HLA-A*0201, HLA-A*0206, HLA-A*0301, HLA-A*1101, HLA-A*2402, HLA-A*3401, HLA-B*0702, HLA-B*0801, HLA-B*1301, HLA-B*27, HLA-B*4002, HLA-B*5101, HLA-Cw*03, HLA-cW*07

HLA-DRB1*0301, HLA-DRB1*0401, HLA-DRB1*0701, HLA-DRB1*1501, HLA-DRB1*1104, HLA-DRB1*1101, HLA-DRB4*0101

HLA-DQA1*01, HLA-DQA1*02, HLA-DQA1*05

HLA-DQB1*03, HLA-DQB1*04, HLA-DQB1*05, HLA-DQB1*06

HLA-DPA1*01, HLA-DPA1*02

HLA-DPB1*02, HLA-DPB1*04

- 5 A invenção será agora descrita por via de exemplo apenas, com referência às seguintes formas de realização específicas.

EXEMPLOS

EXPERIMENTO 1 – Reatividade contra antígenos do HIV

O propósito do estudo é avaliar a reatividade dos polipeptídeos de HIV descritos acima e a sua capacidade para induzir uma resposta de

citocina tipo Th1 específica contra proteínas do HIV naturalmente processadas e apresentadas no contexto de HLA humana (HLA A*0201).

Como fundo para os experimentos, é útil entender que as respostas Th1 e Th2 são definidas pelo padrão de citocinas produzidas pelas células auxiliares T envolvidas nelas. Isto, entretanto, não significa que os linfócitos remanescentes (células T e B) envolvidos nestas respostas específicas também não produzam citocinas que ajudam a direcionar o padrão característico de resposta em que elas estão envolvidas. Deste modo, uma resposta equivalente a Th1 é caracterizada pela produção de IFN- γ e IL-2, levando à estimulação de uma resposta CTL CD8⁺ e uma resposta de anticorpo IgG2a associada (em camundongos). A resposta IFN γ pode ser produzida tanto pelas células auxiliares T CD4⁺ assim como pelas células T CD8⁺ que também formam parte desta. Neste caso o componente de IFN- γ da resposta produzida pelas células T CD8⁺ foi investigado. Isto foi porque o experimento primariamente investigou epítomos de célula T CD8⁺ e foi desejável provar que a resposta observada foi causada por aquelas células. Visto que as células T CD8⁺ reagem com epítomos apenas nas moléculas MHC de classe I, as células humanas que compartilham com o camundongo transgênico apenas uma molécula MHC de classe I (isto é, HLA-A*0201) foram usados. Uma resposta equivalente a Th2 é caracterizada pela produção de IL-4 e IL-10, levando à estimulação de uma resposta de anticorpo IgG_E, IgG1 e (em camundongos) IgG2b. Ambas as respostas são antagonísticas com IFN- γ e IL-10 infra-regulando a produção um do outro.

Todos os experimentos descritos abaixo foram realizados em duplicata.

Materiais e métodos

Peptídeos e proteínas recombinantes

Todos os polipeptídeos usados neste estudo (isto é, P1: VPR aa 51 a 80 (SEQ ID 1); P2: VIF: aa 142 a 181 (SEQ ID 2); P3: REV aa 69 a 95

(SEQ ID 3); P4: NEF: aa 81 a 123 (SEQ ID 4); e NRP: um polipeptídeo não relevante de controle foram sintetizados pela química de Fmoc e recolocados em suspensão em DMSO a 10 % em PBS.

5 As proteínas recombinantes VIF do HIV-1 (ref: EVA659), REV (fusão de rótulo His; ref: ARP663.2) e NEF (ref: EVA650) foram obtidas do Repository of HIV reagents (NIBSC, UK). As preparações de proteína foram misturadas em quantidades equimolares em PBS a 1 mg/ml e armazenadas a -80° C até o uso.

Linhagens de Célula

10 As linhagens de célula T T1 e JURKA são linhagens de linfoblastóides humanos derivados de indivíduos que carregam e que não carregam HLA-A*0201 respectivamente. A T1 foi mantida em meio IMDM (Invitrogen) enquanto a JURKAT foi mantida em meio RPMI-1640 (Sigma) contendo 10 mM de HEPES e 1 mM de piruvato de sódio. Ambos os meios
15 foram suplementados com 50 IU/50 mg/ml de penicilina/estreptomicina (Sigma) e, como meio completo, 10 % de FCS. As culturas celulares foram mantidas a 37° C em uma atmosfera umidificada de 5 % de CO₂.

Os esplenócitos primários foram mantidos em meio IMDM (Invitrogen) suplementado com 0,02 mM de β-mercaptoetanol (Sigma), 50
20 IU/50 mg/ml de penicilina/estreptomicina (Sigma) e 10 % de FCS (Sigma) a 37° C em uma atmosfera umidificada de 5 % de CO₂.

Preparação de células alvo para a análise de citocina

As culturas de célula na fase exponencial foram colhidas pela centrifugação (250 g, 5 min) e recolocadas em suspensão a uma densidade de
25 10⁶ células/ml em meio isento de soro. As alíquotas das suspensões de célula foram transfectadas com uma faixa de antígenos de polipeptídeo a uma concentração de 5 µg por 10⁶ células usando Lipofectina (Invitrogen) de acordo com as instruções do fabricante e incubadas em meio completo por 8 a 10 horas antes do tratamento com Mitomicina C (MMC).

Para o tratamento com MMC, as células foram colhidas pela centrifugação (250 g, 5 min) e recolocadas em suspensão em meio IMDM isento de soro contendo 50 µg/ml de Mitomicina C (Sigma). Depois de 45 min de incubação a 37° C, as suspensões de célula foram lavadas quatro vezes em meio de IMDM isento de soro (250 g, 5 min) e finalmente recolocadas em suspensão em meio IMDM completo.

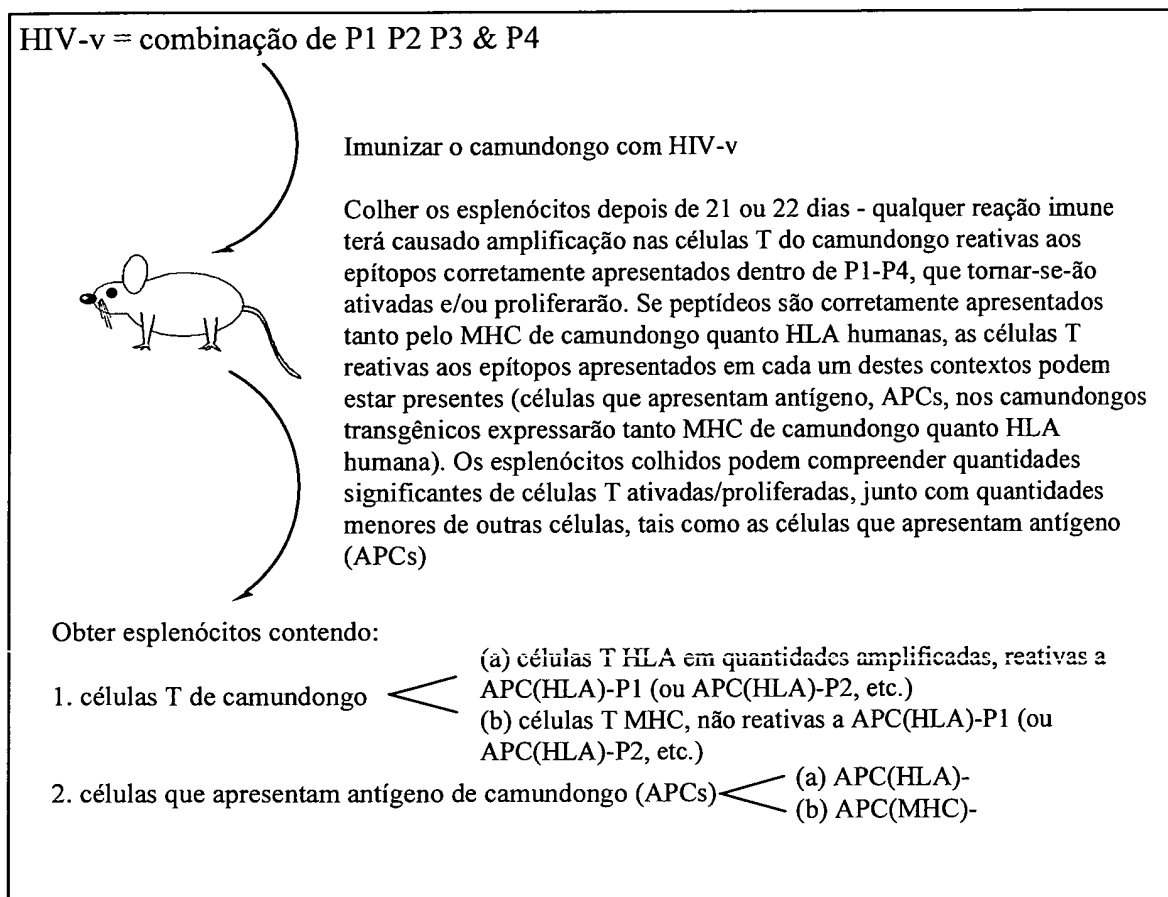
Imunizações

Camundongos C57BL/6-Tg(HLA-A2.1)1Enge/J de sete a dez semanas de idade (transgênicos em HLA-A*0201 em um fundo C57/BL6, Jackson Labs) foram imunizados intradermicamente com uma dose de 100 µl (liberada em 5 locais a 20 µl/local) por camundongo da preparação de antígeno. No grupo de teste, cada dose da preparação de antígeno conteve 40 nmoles de uma mistura equimolar de todos os quatro peptídeos (HIV-v) (10 nmoles de cada) e 0,5 nmoles de Subunidade B da Toxina da Cólera (>99 % pura, BioMol) e foi preparada em Lipofectina (Invitrogen) de acordo com as instruções do fabricante. No grupo de controle, cada dose da preparação de antígeno conteve 40 nmoles do polipeptídeo não relevante e 0,5 nmol da Subunidade B da Toxina da Cólera (>99 % pura, BioMol) e foi preparada em Lipofectina (Invitrogen) de acordo com as instruções do fabricante.

No dia 14 após a imunização, todos os animais receberam uma imunização de reforço usando as mesmas doses e via de liberação como usadas originalmente. Finalmente, nos dias 21 ou 22, todos os animais foram escolhidos e seus baços coletados.

O protocolo de imunização pode ser representado esquematicamente como segue:

Esquema 1 - inoculação



ELISA de Citocina

Os baços de camundongo que pertencem ao mesmo grupo experimental foram reunidos, suavemente prensados através de peneiras de célula e as células sangüíneas vermelhas removidas pelo tratamento com tampão de lise de célula vermelha (nove partes de $\text{NH}_4\text{cloreto}$ 0,16 M e uma parte de Tris 0,17 M, pH 7,2). As suspensões de esplenócito de cada grupo experimental foram plaqueadas em placas de 24 reservatórios a uma densidade de 4×10^6 células/reservatório contendo uma faixa de antígenos de polipeptídeo (5 $\mu\text{g/ml}$) ou, alternativamente, linhagens de célula tratadas com MMC (a razão de esplenócito para célula (S:C) de 10:1) transfectada com antígenos de polipeptídeo como descrito acima.

Depois de 4 dias de incubação a 37°C , o sobrenadante foi coletado e analisado quanto a $\text{IFN-}\gamma$ e IL-4 por um ELISA de citocina intercalado de acordo com o protocolo do fabricante (Pharmingen). Os limites de detecção inferiores para o ensaio foram 9,77 pg/ml para IL-4 e 39,06 pg/ml

para IFN- γ .

Depois de 4 dias de incubação a 37° C, o sobrenadante foi coletado e analisado quanto IFN- γ e IL-4 por um ELISA de citocina intercalado de acordo com o protocolo do fabricante (Pharmingen). Os limites de detecção inferiores para o ensaio foram 9,77 pg/ml para IL-4 e 39,06 pg/ml para IFN- γ .

Resultados

Reatividade do peptídeo 1

Cada um dos polipeptídeos descritos neste pedido de patente (incluindo P1, P2, P3 e P4 testados neste Exemplo) contém diversos epítomos de célula T CD8+ dos quais diversos são específicos para HLA-A*0201. No processamento interno do polipeptídeo pelas células que apresentam antígeno (APCs) de um receptor que carrega HLA-A*0201, estes epítomos específicos de célula T CD8+ são apresentados na superfície da APC de onde eles se originam para ativar as células T CD8+ ingênuas e induzem uma resposta imune equivalente a Th1 específica para o peptídeo que é testado, neste caso P1, P2, P3 e P4.

Para confirmar isto, as linhagens de célula humanas que carregam (T1) e que não carregam (JURKAT) HLA-A*0201 foram intracelularmente carregadas com P1 por meio de um veículo de lipídeo (Lipofectina, INVITROGEN). Os esplenócitos de animais imunizados com a preparação de polipeptídeo do HIV (HIV-v) foram descobertos produzir níveis aumentados de IFN- γ comparados com esplenócitos de animais imunizados com NRP quando co-cultivadas com células humanas que carregam HLA-A*0201 (T1) tratadas com MMC transfectadas com P1, mas não quando co-cultivadas com células humanas que não carregam HLA-A*0201 (JURKAT) tratadas do mesmo modo (ver a Figura 1, os dados para os quais são apresentados na Tabela 1 abaixo).

Tabela 1

Δ IFN- γ para Lys (pg/ml)	NRP	HIV-v
Con A	2345,3 $\pm$ 45,9	2711,1 $\pm$ 44,5
HIV Peptideo 1 (sol)	46,2 $\pm$ 4,0	46,7 $\pm$ 7,1
T1-HIV pep 1 (pro)	52,8 $\pm$ 7,3	1413,5 $\pm$ 43,4
Ju-HIV pep 5 (pro)	< 39	53,3 $\pm$ 2,7

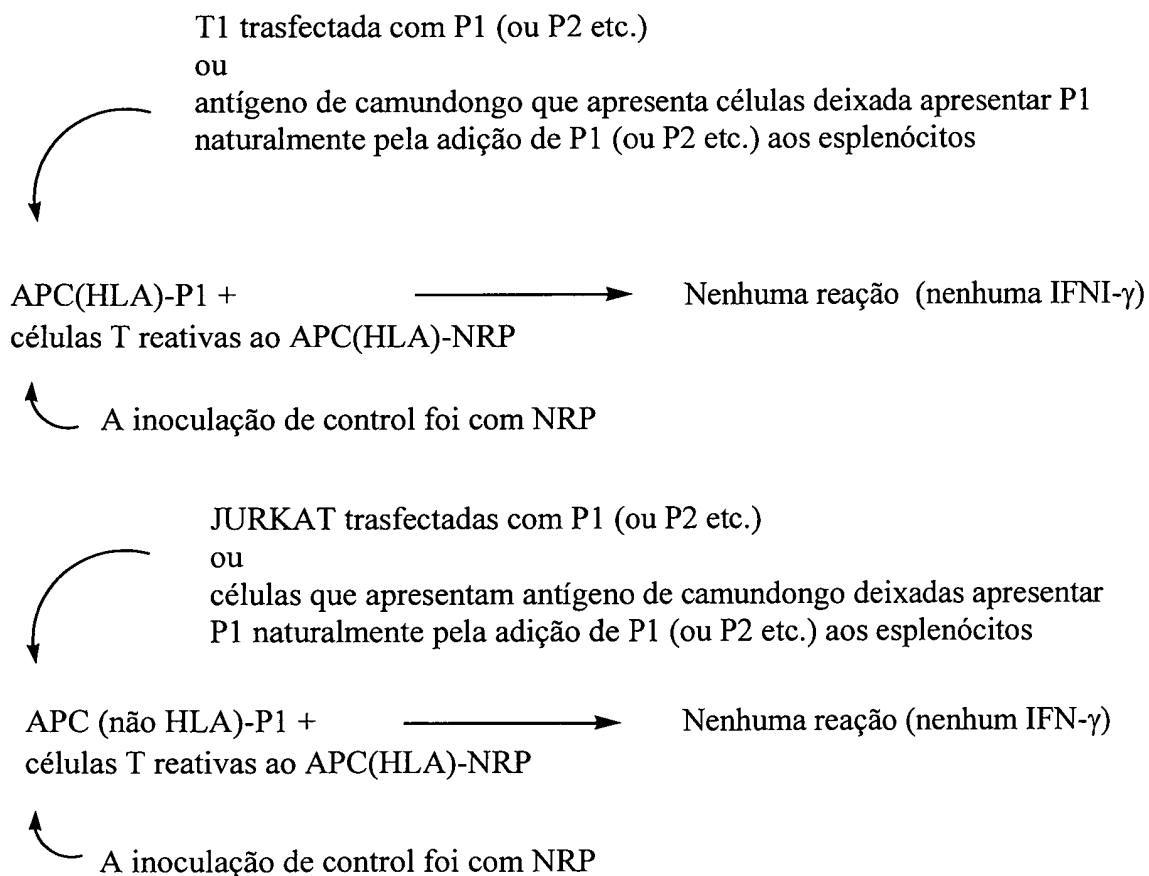
Nota:

“Lys” significa o fundo do controle negativo no qual todos os valores são calculados. “Sol” significa peptídeo solúvel apresentado à população de esplenócito primária. “Pro” significa que o peptídeo está sendo apresentado complexado com as moléculas de células HLA a seguir de processamento interno e carregamento dos epítopos resultantes nas moléculas de MHC. Os valores representam a média $\pm$ erro padrão da Δ IFN- γ para Lys (pg/ml).

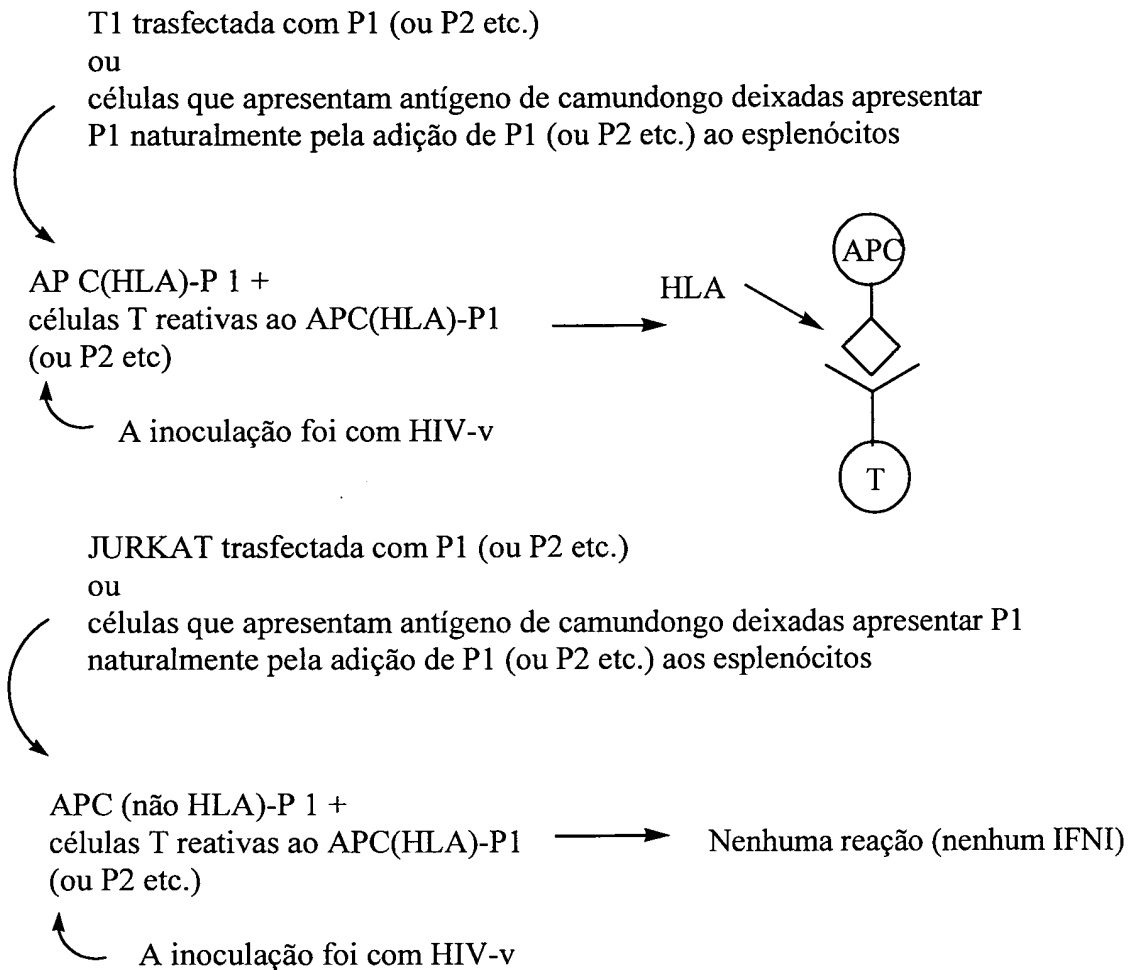
10

O experimento pode ser representado esquematicamente como segue:

Esquema 2 – teste de controle para T1 e JURKAT



Esquema 3 - resultados de teste do HIV-v para T1 e JURKAT



Visto que os camundongos transgênicos usados nestes experimentos não carregam nenhuma outra HLA humana e a capacidade destas células T CD8⁺ para reconhecer especificamente epítopos derivados de P1 no contexto de outras HLAs humanas que elas nunca encontraram é baixo, estes resultados claramente mostram que a resposta de IFN- γ observada é especificamente causada pelas células T CD8⁺ para reconhecer epítopos derivados de P1 em associação com moléculas HLA-A*0201.

Também é importante mencionar que nenhuma resposta de IL-4 pôde ser detectada contra as células transfectadas com P1 em animais imunizados com HIV-v ou NRP (dados não mostrados). Visto que a produção de IL-4 é antagonística em relação às respostas de Th1 e por este motivo para a criação de respostas de célula T CD8⁺ específicas de antígeno, a falta da produção de IL-4 em cada grupo claramente mostra que a imunização com

HIV-v induz uma resposta equivalente a Th1 específica para o componente P1 da preparação.

De maneira interessante, nenhum diferencial na produção de IFN- γ pôde ser observado no HIV-v comparado com os grupos imunizados com NRP quando P1 é simplesmente adicionado à cultura (na ausência de células T1 ou JURKAT). Esta situação, entretanto, pode ser explicada com base em que P1 contém primariamente epítomos que são reativos no contexto de HLAs humanas e não de camundongo. Os camundongos transgênicos usados nestes experimentos contêm um complemento completo de moléculas de MHC de camundongo além das moléculas HLA-A*0201. Portanto, as culturas de esplenócito primárias aqui consideradas contêm diferentes populações de célula T (CD8+ e CD4+) capazes de responder ao antígeno apresentado no contexto das moléculas de MHC tanto humanas quanto de camundongo presentes na população de APC de camundongo também encontrados nestas culturas. Apenas uma pequena fração do antígeno P1 capturado e processado por estes APCs seriam esperados estar disponíveis para os linfócitos T CD8+ específicos de P1 no contexto de HLA-A*0201 requerido. Visto que o nível de uma resposta imune *in vitro* é primariamente determinada pela disponibilidade de antígeno, os linfócitos T CD8+ específicos de P1 detectados quando células humanas foram usadas como estimuladores simplesmente seriam incapazes de responder devido à falta de antígeno suficiente apresentado a elas no contexto de HLA-A*0201 apropriado.

Reatividade de peptídeo 2

Os esplenócitos de animais imunizados com a preparação de polipeptídeo do HIV (HIV-v) foram descobertos produzir níveis aumentados de IFN- γ comparados com esplenócitos de animais imunizados com NRP quando co-cultivados com células humanas que carregam HLA-A*0201 tratadas com MMC (T1) transfectadas com P2, mas não quando co-cultivadas

com células humanas que não carregam HLA-A*0201 (JURKAT) tratadas do mesmo modo (ver a Figura 2, os dados para os quais são apresentados na Tabela 2 abaixo).

Tabela 2

A IFN- γ para Lys (pg/ml)	NRP	HIV-v
Con A	2345,3 $\pm$ 45,9	2711,1 $\pm$ 44,5
HIV Peptideo 2 (sol)	446,7 $\pm$ 10,6	2721,3 $\pm$ 22,7
T1-HIV pep 2 (pro)	737,4 $\pm$ 39,7	2027,8 $\pm$ 22,2
Ju-HIV pep 2 (pro)	62,1 $\pm$ 30,6	95,6 $\pm$ 2,3

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Nota:

“Lys” significa o fundo do controle negativo no qual todos os valores são calculados. “Sol” significa peptídeo solúvel apresentado à população de esplenócito primária. “Pro” significa que o peptídeo está sendo apresentado complexado com as moléculas de células HLA a seguir de processamento interno e carregamento dos epítopos resultantes nas moléculas de MHC. Os valores representam a média $\pm$ erro padrão da Δ IFN- γ para Lys (pg/ml).

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Como foi o caso para P1, estes resultados claramente mostram que a resposta IFN- γ observada é especificamente causada pelas células T CD8⁺ que reconhecem epítopos derivados de P2 em associação com moléculas HLA-A*0201.

15

Também é importante observar que nenhuma resposta IL-4 pôde ser detectada contra as células transfectadas com P2 em animais imunizados com HIV-v ou NRP (dados não mostrados). Visto que a produção de IL-4 é antagonística em relação às respostas Th1 e por este motivo à criação de respostas de célula T CD8⁺ específicas de antígeno, a falta de produção de IL-4 em cada grupo claramente mostra que a imunização com HIV-v induz uma resposta equivalente a Th1 específica ao componente P2 da preparação.

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Ao contrário de P1, a produção de IFN- γ aumentada pôde ser observada no HIV-v comparada com os grupos imunizados com NRP quando

25

P2 é simplesmente adicionado à cultura de esplenócito. Esta situação claramente mostra que P2 abriga não apenas epítomos de HLA-A*0201 fortes mas também epítomos (H2-D) de camundongo. É importante, entretanto, salientar que a resposta de IFN- γ medida neste caso pode não estar unicamente associada com as moléculas de MHC de Classe I. As células dendríticas mostraram (Peachman KK, Rao M, Alving CR, Palmer DR, Sun W, Rothwell SW, “Human dendritic cells and macrophages exhibit different intracellular processing pathways for soluble and liposome-encapsulated antigens.” Immunobiology. 2005; 210(5): 321-33) serem capazes de processar *in vitro* no antígeno solúvel do caminho de MHC Classe II capturado do meio. Como um resultado a resposta de IFN- γ aumentada observada neste caso pode ser originada por cada uma ou ambas das células T CD8+ e CD4+ específicas de P2.

Reatividade de peptideo 3

Similarmente ao P2, a produção de IFN- γ aumentada pôde ser observada nos grupos imunizados com HIV-v comparado com NRP quando P3 é simplesmente adicionado à cultura de esplenócito. As causas para isto foram explicadas anteriormente e por este motivo não devem ser mais aqui desenvolvidas.

Com respeito ao componente específico de HLA-A*0201 da resposta, mais uma vez como foi o caso para P1 e P2, a produção aumentada de IFN- γ é observada em animais imunizados com HIV-v comparados com aqueles que recebem NRP, mas a um grau menor do que em P1 (ver a Figura 3, os dados para os quais são apresentados na Tabela 3 abaixo). Esta observação é explicada em termos similares àqueles fornecidos para P1.

Tabela 3

A IFN- γ para Lys (pg/ml)	NRP	HIV-v
Con A	2345,3 $\pm$ 45,9	2711,1 $\pm$ 44,5
HIV Peptideo 3 (sol)	976,3 $\pm$ 10,5	2027,5 $\pm$ 8,0
T1-HIV pep 3 (pro)	172,2 $\pm$ 40,2	329,3 $\pm$ 10,5
Ju-HIV pep 3 (pro)	< 39	< 39

Nota:

“Lys” significa o fundo do controle negativo no qual todos os valores são calculados. “Sol” significa peptídeo solúvel apresentado à população de esplenócito primária. “Pro” significa que o peptídeo está sendo
 5 apresentado complexado com as moléculas de células HLA a seguir de processamento interno e carregamento dos epítomos resultantes nas moléculas de MHC. Os valores representam a média $\pm$ erro padrão da Δ IFN- γ para Lys (pg/ml).

As culturas de esplenócito primárias dos animais transgênicos
 10 aqui usados contêm populações de célula T diferentes (CD8 $^{+}$ e CD4 $^{+}$) capazes de responder ao antígeno apresentado no contexto das moléculas de MHC tanto humanas quanto de camundongo presentes na população de APC de camundongo também encontrada nestas culturas. A seguir da vacinação, os epítomos derivados de P3 tanto da Classe I quanto da Classe II poderiam ter
 15 sido apresentadas às células T CD8 $^{+}$ e CD4 $^{+}$. Visto que o número de moléculas HLA-A*0201 nas APCs de camundongo apenas representam uma pequena fração do número total de moléculas de MHC presentes (isto é, todas as moléculas H2-D mais as moléculas HLA-A*0201), apenas uma fração do antígeno P3 total liberado estaria disponível às células T CD8 $^{+}$ com uma
 20 especificidade para HLA-A*0201. Tanto *in vivo* como *in vitro*, a intensidade da resposta de célula T é primariamente dependente da disponibilidade do antígeno. Portanto, em uma situação onde haveria competição intensa entre as moléculas de MHC quanto aos epítomos P3, segue que estas moléculas que representam uma pequena fração da população seriam responsáveis apenas
 25 por uma pequena fração da resposta IFN- γ total.

Reatividade do peptídeo 4

Similarmente à P1, a produção de IFN- γ aumentada pode ser observada na HIV-v comparada com os grupos imunizados com NRP quando P4 é simplesmente adicionado à cultura de esplenócito assim como quando é

apresentado por intermédio de linhagens de células humanas transfectadas (ver a Figura 4, os dados para a qual são apresentados na Tabela 4 abaixo). Visto que as causas para estas observações já foram explicadas para o caso de P1 as mesmas não devem ser mais aqui desenvolvidas e deve-se referir a esta seção mais no início.

Tabela 4

A IFN- γ para Lys (pg/ml)	NRP	HIV-v
Con A	2345,3 $\pm$ 45,9	2711,1 $\pm$ 44,5
HIV Peptideo 4 (sol)	112,2 $\pm$ 2,0	141,6 $\pm$ 7,0
T1-HIV pep 4 (pro)	391,9 $\pm$ 25,7	1843,8 $\pm$ 15,5
Ju-HIV pep 4 (pro)	< 39	62,5 $\pm$ 5,8

Nota:

“Lys” significa o fundo do controle negativo no qual todos os valores são calculados. “Sol” significa peptídeo solúvel apresentado à população de esplenócito primária. “Pro” significa que o peptídeo está sendo apresentado complexado com as moléculas de células HLA a seguir de processamento interno e carregamento dos epítomos resultantes nas moléculas de MHC. Os valores representam a média $\pm$ erro padrão da Δ IFN- γ para Lys (pg/ml).

Reatividade para as proteínas recombinantes do HIV

Até agora os experimentos aqui descritos têm claramente mostrado que a imunização com HIV-v induz uma resposta de IFN- γ específica de célula T CD8⁺ contra cada um de seus componentes constituintes. Entretanto, é útil estabelecer se os camundongos vacinados com HIV-v são capazes de reconhecer, e conseqüentemente induzir uma resposta imune específica para, epítomos criados na captura e processamento das proteínas do HIV completas das quais alguns deles são derivados. Devido aos conceitos de segurança de usar vírus HIV vivo em células humanas foi decidido estudar a resposta de IFN- γ em camundongos vacinados com HIV-v e NRP usando o mesmo método usado para P1, P2, P3 e P4, mas neste caso usando como antígenos estimuladores uma mistura equimolar de VIF, REV e

NEF.

Mais uma vez, a vacinação com HIV-v de animais deu origem a um aumento específico na produção de IFN- γ comparada com a vacinação de NRP independentemente se a preparação de antígeno equimolar foi simplesmente adicionada à cultura ou apresentada às células T CD8⁺ ativadas por intermédio de células humanas transfectadas (ver a Figura 5, os dados para os quais são apresentados na Tabela 5 abaixo). De maneira interessante, a produção de fundo de IFN- γ em todos os casos foi maior do que anteriormente observado, provavelmente refletindo a capacidade da preparação de proteína equimolar para induzir uma resposta IFN- γ não específica nas culturas de esplenócito primárias ou mudanças nas células humanas transfectadas que as tornam mais suscetíveis para induzir a produção de IFN- γ não específica nos esplenócitos com os quais elas foram co-cultivadas. Esta observação, entretanto, não deprecia o fato evidente de que a vacinação com HIV-v leva ao reconhecimento específico de epítipo HLA-A*0201 do HIV naturalmente processado e apresentado a partir de proteínas do HIV completas.

Tabela 5

A IFN- γ para Lys (pg/ml)	NRP	HIV-v
Con A	2345,3 $\pm$ 45,9	2711,1 $\pm$ 44,5
HIV rProt mix (sol)	724,6 $\pm$ 14,3	1086,6 $\pm$ 2,5
T1-HIV rProt mix (pro)	848,0 $\pm$ 8,5	1324,5 $\pm$ 14,4
Ju-HIV rProt mix (pro)	911,6 $\pm$ 10,5	940,9 $\pm$ 17,9

Nota:

“Lys” significa o fundo do controle negativo no qual todos os valores são calculados. “Sol” significa peptídeo solúvel apresentado à população de esplenócito primária. “Pro” significa que o peptídeo está sendo apresentado complexado com as moléculas de células HLA a seguir de processamento interno e carregamento dos epítopos resultantes nas moléculas de MHC. Os valores representam a média $\pm$ erro padrão da Δ IFN- γ para Lys (pg/ml).

EXPERIMENTO 2 - A imunização pela HIV-v induz uma resposta específica de antígeno contra células humanas infectadas com isolados de campo do HIV

O propósito deste estudo é avaliar se a imunização com os polipeptídeos de epítipo de célula T conservados do HIV identificados (HIV-v) induz e resposta específica de antígeno contra células humanas infectadas com isolados de campo do HIV.

Materiais e métodos

Peptídeos, vírus e linhagem de célula

A vacina candidata (HIV-v) usada neste estudo é composta de diversos polipeptídeos (isto é, P1: VPR aa 51 a 80 (SEQ ID 1); P2: VIF: aa 142 a 181 (SEQ ID 2); P3: REV aa 69 a 95 (SEQ ID 3); P4: NEF: aa 81 a 123 (SEQ ID 4)) que foram todos sintetizados pela química de Fmoc e recolocada em suspensão em DMSO em PBS (a concentração de DMSO na preparação final foi menor do que 10 %). A Lisozima (Sigma) desnaturada pela ebulição foi usada como a preparação não relevante de controle (NRP-v).

As cepas HIV-1 infecciosas foram originadas de NIBSC e representaram isolados Ugandenses de HIV-1 Glado D (UG21-R5) e Glado A (UG-29-X4). CEM é uma linhagem de célula T linfoblastóide CD4+ susceptível à infecção pelos isolados do HIV denominados acima. Esta linhagem de célula foi mantida em meio RPMI-1640 (Sigma) suplementado com 50 IU/50 mg/ml de penicilina/estreptomicina (Sigma) e, como meio completo, 10 % de FCS. As culturas de célula foram mantidas a 37° C em uma atmosfera umidificada de 5 % de CO₂.

Os esplenócitos primários foram mantidos em meio IMDM (Invitrogen) suplementado com 0,02 mM de β-mercaptoetanol (Sigma), 50 IU/50 mg/ml de penicilina/estreptomicina (Sigma) e 10 % FCS (Sigma) a 37° C em uma atmosfera umidificada de 5 % de CO₂.

Imunizações

No dia 1, camundongos C57BL/6-Tg(HLA-A2.1)1Enge/J de sete a dez semanas de idade (transgênicos em HLA-A*0201 em um fundo C57/BL6, Jackson Labs) foram imunizados subcutaneamente na base da cauda com uma dose de 200 µl da preparação de antígeno emulsificada em IFA (Sigma). No grupo de teste (n = 4), cada dose da preparação de antígeno conteve 40 nmoles de uma mistura equimolar de todos os quatro peptídeos (10 nmoles cada) enquanto que no grupo de controle (n = 4), cada dose da preparação de antígeno conteve 40 nmoles do polipeptídeo não relevante.

No dia 15 todos os animais receberam uma imunização de reforço usando as mesmas doses e vias de liberação como usadas originalmente.

No Dia 21 todos os animais foram escolhidos e seus baços colhidos.

Tingimento de citocina intracelular

Os baços de camundongo pertencente ao mesmo grupo experimental foram reunidos, suavemente prensados através de peneiras de célula e as células sangüíneas vermelhas removidas pelo tratamento com tampão de lise de célula vermelha (nove partes de NH₄Cl 0,16 M e uma parte de Tris 0,17 M, pH 7,2). As suspensões de esplenócito de cada grupo experimental foram plaqueadas em placas de 96 reservatórios (falcon, BD discovery labware) a uma densidade de 2×10^5 células/reservatório contendo Ionomicina (0,78 µg/ml) e PMA (0,05 µg/ml), Concanavalina A a 5 µg/ml ou 2×10^4 células CEM irradiadas com UV infectadas e não infectadas com HIV (razão de esplenócito para célula (S:C) 10:1).

Depois de 3 horas de incubação a 37° C com 5 % de CO₂, Brefeldin A foi adicionado a todos os reservatórios a 10 µg/ml de concentração final. As placas foram depois incubadas por mais 3 horas placas a 37° C e depois as placas transferidas a um refrigerador a +4° C para armazenagem durante a noite. Depois da centrifugação (200 g, 5 min), o

sobrenadante foi removido dos reservatórios e as células contra tingidas com anticorpo anti-CD3-FITC por 15 a 20 minutos na temperatura ambiente no escuro. As células foram depois lavadas com PBS pela centrifugação (200 g, 5 min), fixadas por 20 min na temperatura ambiente (reagente de fixação A, Caltag) e lavadas mais uma vez. Depois de permeabilizar as células por 20 min (reagente de permeabilização B, Caltag) na temperatura ambiente, as células foram lavadas e os anticorpos anti-IFN γ -PE e anti-IL4-APC adicionados. Depois de 30 min de incubação, as células foram lavadas duas vezes e recolocadas em suspensão em 40 % (p/v) de formaldeído em PBS antes de ser analisado em um citômetro de fluxo.

Resultados

O propósito deste estudo foi avaliar a capacidade da preparação de HIV-v para induzir a imunidade específica de antígeno em camundongos transgênicos HLA*A-0201 contra células humanas infectadas com HIV compatíveis com HLA.

Como mostrado na Fig. 6, os esplenócitos de animais imunizados com HIV-v produziram níveis mais altos de IFN- γ quando co-cultivados contra células humanas infectadas com isolados do vírus HIV-1 Glado D (UG21-R5) ou Glado A (UG-29-X4) do que os esplenócitos de animais imunizados com um polipeptídeo não relevante. Nenhuma resposta de IL-4 contra as células alvo foi observada em nenhum dos grupos experimentais (dados não mostrados).

Os resultados deste estudo indicam que a vacinação com a preparação de peptídeo HIV-v induz uma resposta de célula T tipo Th1 específica de patógeno contra diferentes clados de vírus HIV circulantes.

REIVINDICAÇÕES

1. Polipeptídeo tendo não mais do que 100 aminoácidos, caracterizado pelo fato de que compreende uma ou mais seqüências tendo pelo menos 60 % de homologia com qualquer uma das SEQ ID 1 a 4 ou
5 compreende dois ou mais epítomos tendo 7 aminoácidos ou mais, cada epítopo tendo pelo menos 60 % de homologia com uma subsequência de qualquer uma das SEQ ID 1 a 4 que tem o mesmo comprimento como o epítopo:

SEQ ID 1 GDTWAGVEAIRILQQLLFHFRIGCQHSR

SEQ ID 2 KVGSLQYLALTALITPKKIKPPLPSVKKLTEDRWNK PQ KT

10 SEQ ID 3 EPVPLQLPPLERLTLDCEDCGTSGTQ

SEQ ID 4 YKGALDLSHFLKEKGGLEGLIYSQKRQDILDLWVYHT
QGYFPD

em que, o polipeptídeo é imunogênico em um vertebrado que expressa um alelo de complexo de histocompatibilidade maior (MHC), e em
15 que o polipeptídeo não é uma proteína do vírus HIV completa.

2. Polipeptídeo tendo não mais do que 100 aminoácidos, caracterizado pelo fato de que compreende uma ou mais seqüências definidas pelos seguintes resíduos de aminoácido de uma proteína do vírus HIV ou
compreende dois ou mais epítomos tendo 7 aminoácidos ou mais de uma
20 seqüência definida pelos seguintes resíduos de aminoácido de uma proteína do vírus HIV:

resíduos 51 a 80 de uma proteína VPR

resíduos 142 a 181 de uma proteína VIF

resíduos 69 a 95 de uma proteína REV

25 resíduos 81 a 123 de uma proteína NEF

em que, o polipeptídeo é imunogênico em um vertebrado que expressa um alelo de complexo de histocompatibilidade maior (MHC), e em que o polipeptídeo não é uma proteína do vírus HIV completa.

3. Polipeptídeo de acordo com as reivindicações 1 ou 2,

caracterizado pelo fato de que compreende um linfócito T citotóxico (CTL), epítipo de célula T CD8+ e/ou célula T CD4+.

4. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que compreende 2, 3, 4, 5 ou mais epítopos.

5. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que é imunogênico a um epítipo dentro de qualquer uma das seqüências das SEQ ID 1 a 4.

10 6. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que é imunogênico a uma cepa do HIV.

7. Polipeptídeo de acordo com a reivindicação 6, caracterizado pelo fato de que é imunogênico a uma pluralidade de cepas do HIV.

15 8. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que compreende ainda uma ou mais seqüências adicionais de uma proteína viral da imunodeficiência.

20 9. Polipeptídeo de acordo com a reivindicação 8, caracterizado pelo fato de que quando o polipeptídeo é pelo menos 60 % homólogo à SEQ ID 1, a proteína é uma proteína VPR, quando o polipeptídeo é pelo menos 60 % homólogo à SEQ ID 2, a proteína é uma proteína VIF, quando o polipeptídeo é pelo menos 60 % homólogo à SEQ ID 3, a proteína é uma proteína REV, e quando o polipeptídeo é pelo menos 60 % homólogo à SEQ ID 4, a proteína é uma proteína NEF.

25 10. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que a homologia é de 75 % ou mais.

11. Polipeptídeo de acordo com a reivindicação 9, caracterizado pelo fato de que a homologia é de 85 % ou mais.

12. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que a homologia é substancialmente de 100 %.

5 13. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que o epítopo compreende 8, 9, 10 ou 11 aminoácidos ou mais.

14. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que não tem mais do que 50 resíduos de aminoácido.

10 15. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que compreende de 15 a 45 resíduos de aminoácido.

15 16. Polipeptídeo imunogênico de epítopo múltiplo, caracterizado pelo fato de que compreende dois ou mais polipeptídeos como definidos em qualquer uma das reivindicações precedentes.

17. Composição de polipeptídeo, caracterizada pelo fato de que compreende dois ou mais polipeptídeos como definidos em qualquer uma das reivindicações precedentes.

20 18. Composição de polipeptídeo de acordo com a reivindicação 17, caracterizada pelo fato de que os polipeptídeos tendo as seqüências das SEQ ID 1 a 4 são todos presentes.

19. Polipeptídeo de acordo com qualquer uma das reivindicações precedentes, caracterizado pelo fato de que é para o uso na medicina.

25 20. Método para produzir um polipeptídeo como definido em qualquer uma das reivindicações precedentes, caracterizado pelo fato de que compreende combinar dois ou mais epítopos para formar o polipeptídeo.

21. Construção de polipeptídeo, caracterizada pelo fato de que compreende um polipeptídeo como definido em qualquer uma das

reivindicações precedentes e um carreador.

22. Método para produzir uma construção de polipeptídeo como definida na reivindicação 21, caracterizado pelo fato de que compreende combinar dois ou mais epítomos e/ou um polipeptídeo com o carreador.

23. Construção de polipeptídeo ou um método de acordo com as reivindicações 21 ou 22, caracterizada pelo fato de que o carreador é uma molécula.

24. Construção de polipeptídeo ou um método de acordo com as reivindicações 22 ou 23, caracterizada pelo fato de que o carreador compreende um adjuvante e/ou um excipiente.

25. Composição de vacina ou medicamento contra um vírus HIV, caracterizada pelo fato de que compreende um polipeptídeo ou composição de polipeptídeo como definido(a) em qualquer uma das reivindicações de 1 a 19, e um excipiente e/ou adjuvante apropriados.

26. Composição de vacina ou medicamento contra um vírus HIV, caracterizada pelo fato de que compreende uma construção de polipeptídeo como definida nas reivindicações 21, 23 ou 24 e opcionalmente um excipiente e/ou adjuvante apropriados.

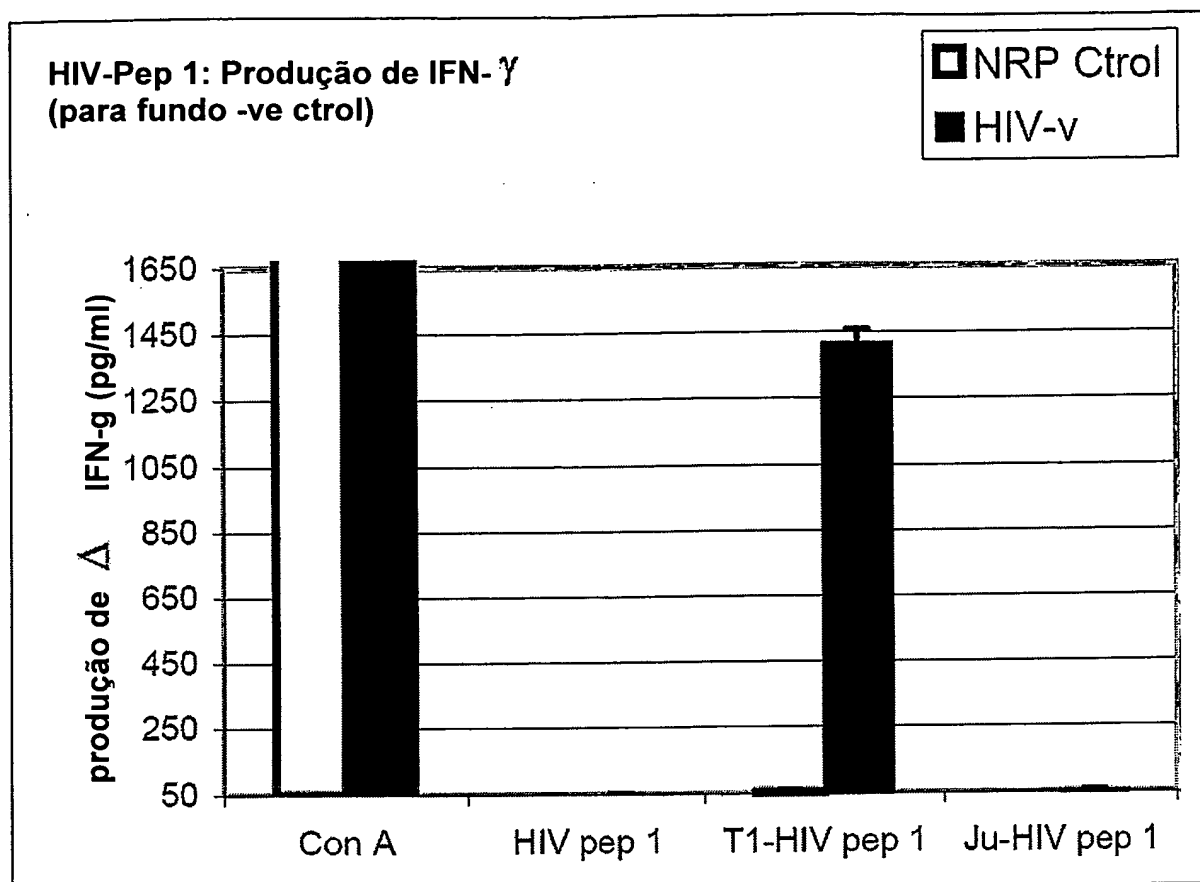
27. Método para produzir uma composição de vacina ou medicamento como definida nas reivindicações 25 ou 26, caracterizado pelo fato de que compreende misturar um polipeptídeo como definido em qualquer uma das reivindicações de 1 a 19 com um excipiente e/ou adjuvante apropriados.

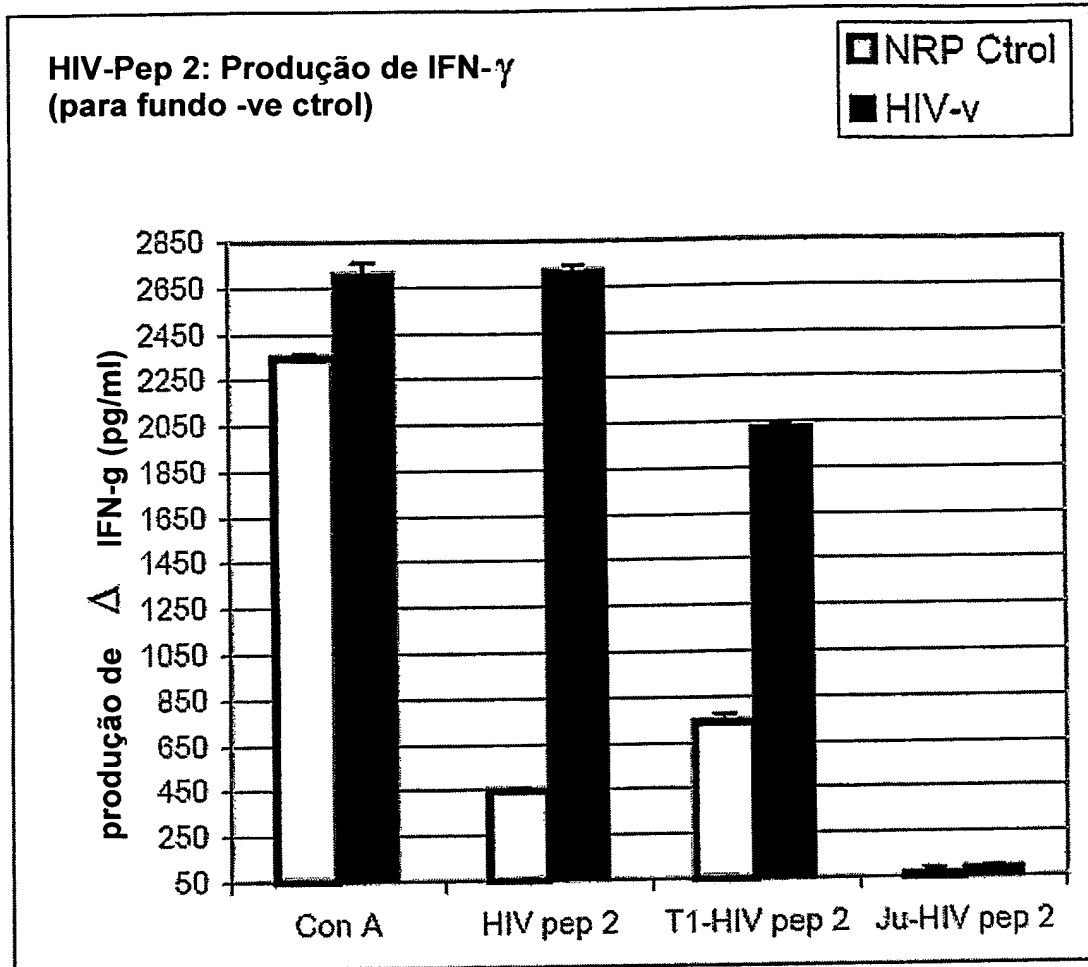
28. Uso de um polipeptídeo, uma composição de polipeptídeo, uma construção de polipeptídeo ou uma composição de vacina ou medicamento como definido(a) em qualquer uma das reivindicações de 1 a 19, 21 e 23 a 26, caracterizado pelo fato de ser na fabricação de um medicamento ou vacina, eficaz no tratamento ou prevenção de um vírus HIV.

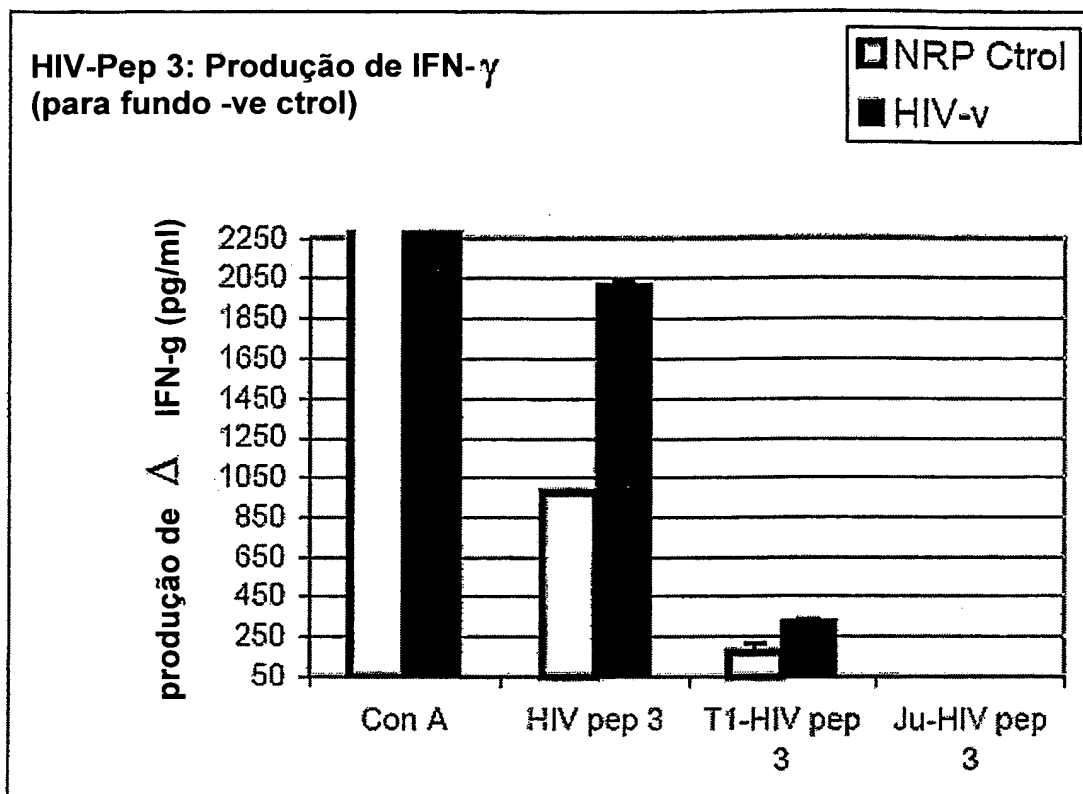
29. Método para tratar ou prevenir uma infecção por vírus HIV, caracterizado pelo fato de que compreende administrar um polipeptídeo, uma composição de polipeptídeo, uma construção de polipeptídeo, uma composição de vacina ou medicamento, como definido(a) em qualquer uma das reivindicações de 1 a 19, 21 e 23 a 26 a um vertebrado.

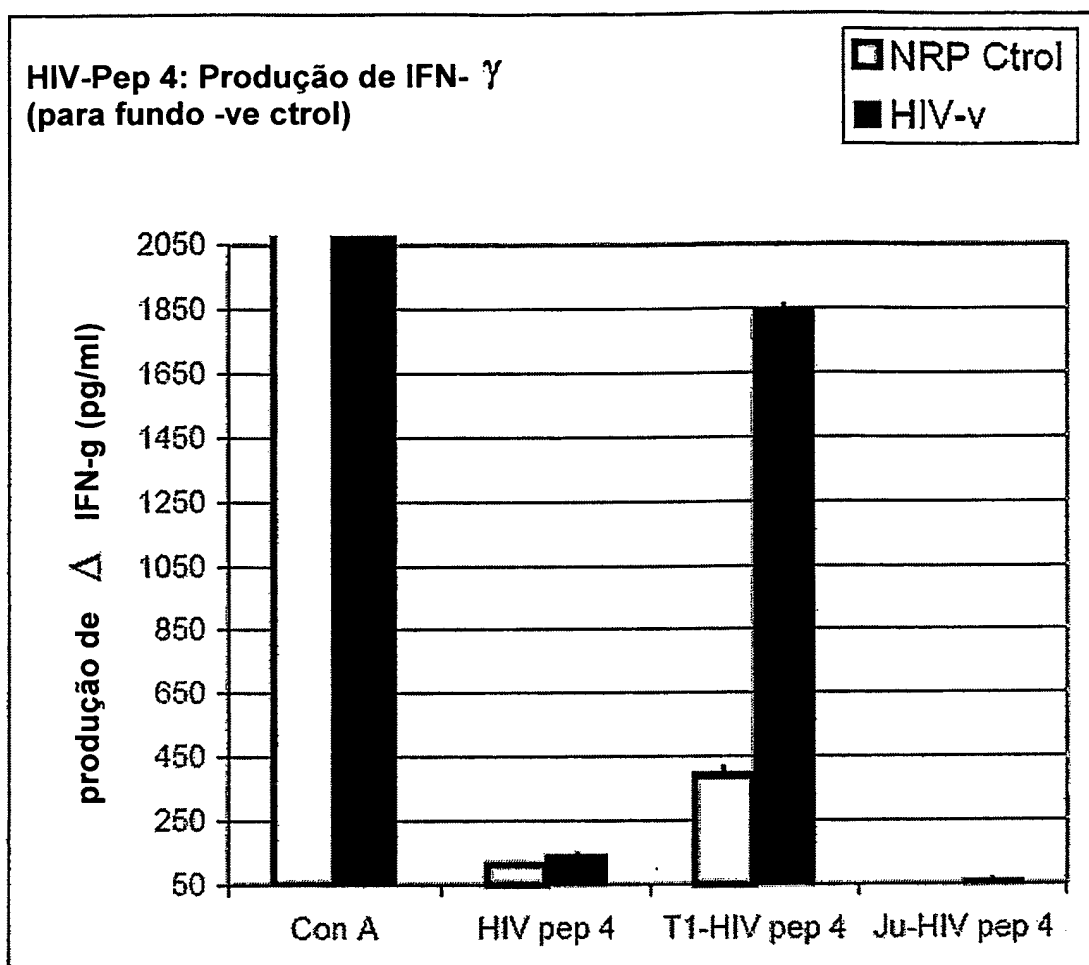
5

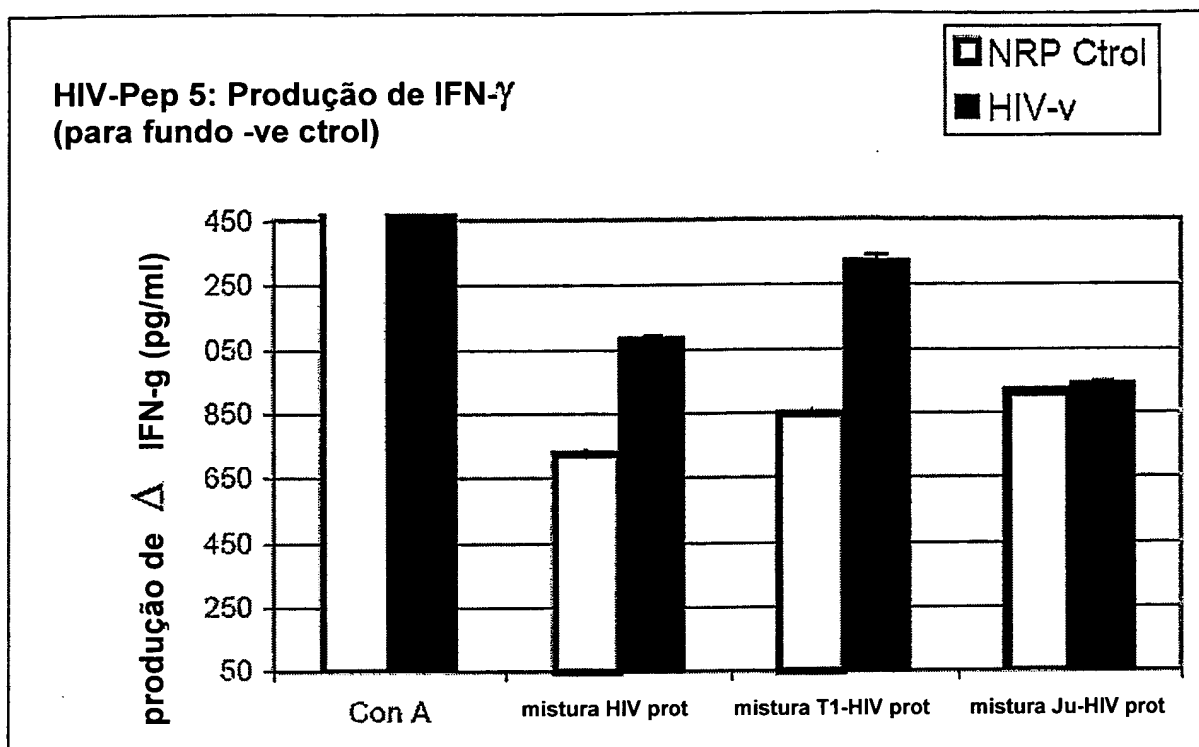
30. Método de acordo com a reivindicação 29, caracterizado pelo fato de que o vertebrado é um ser humano.

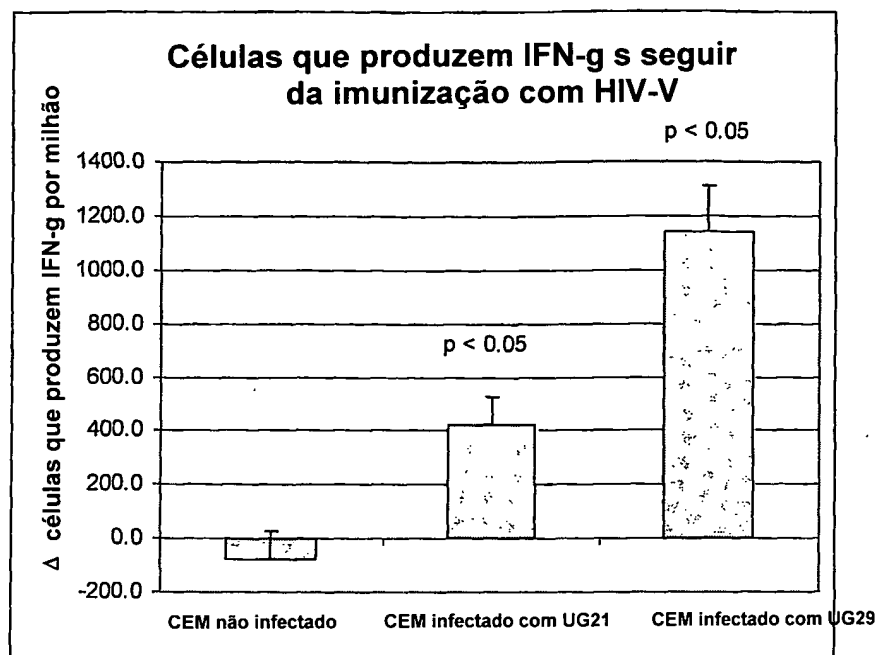
**Figura 1**

**Figura 2**

**Figura 3**

**Figura 4**

**Figura 5**

**Figura 6**

RESUMO

“POLIPEPTÍDEO TENDO NÃO MAIS DO QUE 100 AMINOÁCIDOS, POLIPEPTÍDEO IMUNOGÊNICO DE EPÍTOPO MÚLTIPLO, COMPOSIÇÃO DE POLIPEPTÍDEO, MÉTODO PARA PRODUZIR UM
5 POLIPEPTÍDEO, CONSTRUÇÃO DE POLIPEPTÍDEO, MÉTODO PARA PRODUZIR A MESMA, COMPOSIÇÃO DE VACINA OU MEDICAMENTO CONTRA UM VÍRUS HIV, MÉTODO PARA PRODUZIR UMA COMPOSIÇÃO DE VACINA OU MEDICAMENTO, USO DE UM POLIPEPTÍDEO, UMA COMPOSIÇÃO DE POLIPEPTÍDEO,
10 UMA CONSTRUÇÃO DE POLIPEPTÍDEO OU UMA COMPOSIÇÃO DE VACINA OU MEDICAMENTO”

É fornecido um polipeptídeo tendo não mais do que 100 aminoácidos, polipeptídeo este que compreende uma ou mais sequências tendo pelo menos 60% de homologia com qualquer uma das SEQ ID 1 a 4, ou
15 compreende dois ou mais epítopos tendo 7 aminoácidos ou mais, cada epítipo tendo pelo menos 60% de homologia com uma sub-sequência de qualquer uma das SEQ ID 1 a 4 que tem o mesmo comprimento como o epítipo: SEQ ID 1 GDTWAGVEAHRILQQLLFT HFRIGCQHRS; SEQ ID 2 KVGSLQYLALTALITPKKIKPPLPSVKKL TEDRWNPQKT; SEQ ID 3
20 EPVPLQLPPLERLTLDCEDCGTSGT Q; SEQ ID 4 YKGALDLSHFLKEKGGLEGLIYSQKRQDILDWVYH TQGYFPD em que, o polipeptídeo é imunogênico em um vertebrado que expressa um alelo de complexo de histocompatibilidade maior (MHC), e em que o polipeptídeo não é uma proteína do vírus HIV completa.