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(54) Title: DISTINCT HIV-1 GAG AND ENV EPITOPES OF HLA ALLELES ASSOCIATED WITH DIFFERENTIAL SUSCEPTIBILITY TO HIV-1 INFECTION

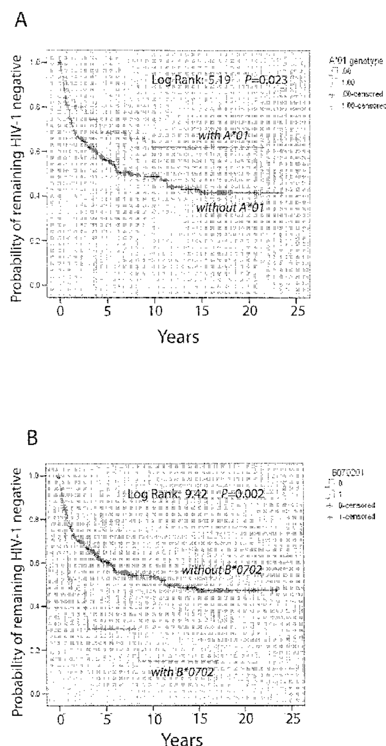

Figure 1

(57) Abstract: We investigated the peptide binding profile of two HLA class I alleles, A*0101 and B*0702, that are associated with differential outcomes of HIV-1 infection. HLA-A*0101 is significantly associated with HIV-1 resistant women (p=0.016, odds ratio: 1.7, 95% CI:1.1-2.7), while HLA-B*0702 is associated with susceptibility to HIV-1 infection.

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Distinct HIV-1 gag and env epitopes of HLA alleles associated with differential susceptibility to HIV-1 infection

PRIOR APPLICATION INFORMATION

5 The instant application claims the benefit of US Provisional Patent Application 60/977,128, filed October 3, 2007.

BACKGROUND OF THE INVENTION

Since the Human Immunodeficiency Virus (HIV) was identified over two 10 decades ago as the cause of Acquired Immunodeficiency Syndrome (AIDS), the disease has been ravaging populations worldwide. Nowhere is this more devastating than in sub-Saharan Africa, where in some countries over 35 percent are affected [1]. UNAIDS has estimated that more than 60 million people have been infected with the HIV virus. Of these, over 20 million have died, while almost 15 40 million are currently living with the disease [1]. Public health awareness and the development of a variety of anti-HIV drugs has played an important role in ameliorating the effects of this virus in developed countries, however, in developing countries, the HIV/AIDS epidemic continues to grow at an alarming rate. In an effort to control the spread of this disease, work has been ongoing for 20 more than two decades to develop an effective vaccine. The task has proved to be extremely difficult and today there is still no effective vaccine for HIV-1. Part of the difficulty is due to the fact that the HIV genome undergoes rapid mutation, the consequence of an error-prone replication enzyme. Another obstacle is that we still do not fully understand the correlates of the immune responses that can control 25 HIV.

Almost all vaccines to date have been derived out of an understanding, no matter how primitive, of what results in protective immunity. Heterogeneity in susceptibility to HIV-1 has been observed in several cohort studies. Despite repeated exposures, some individuals do not seem to become infected with HIV-1, 30 and among those that do, there is marked variation in how the virus is handled and in the time-course of progression to AIDS [2]. The understandings of why some individuals can escape HIV-1 infection and how their immune system works will

help us to unveil the parameters of protective immunity to HIV, which will contribute to develop effective vaccines and control strategies.

SUMMARY OF THE INVENTION

5 According to a first aspect of the invention, there is provided a method of preparing a reagent for inoculating an individual against an HIV infection comprising mixing at least one resistance-linked peptide having an amino acid sequence as set forth in any one of SEQ ID Nos 1-32 or natural variant thereof with a suitable adjuvant, excipient or carrier.

10 Preferably, the reagent is free of peptides having an amino acid sequence as set forth in any one of SEQ ID Nos. 33-124.

The peptide may have an amino acid sequence as set forth in any one of SEQ ID Nos. 1-12 or as set forth in any one of SEQ ID Nos. 6-12 or as set forth in SEQ ID No. 1 or as set forth in SEQ ID No. 6 or natural variants thereof.

15 According to a second aspect of the invention, there is provided a reagent comprising:

a peptide consisting of or consisting essentially of an amino acid sequence as set forth in SEQ ID Nos. 1-32 or a naturally occurring variant thereof;

at least one MHC class I molecule; and

20 a detectable label.

According to a third aspect of the invention, there is provided a reagent comprising:

a peptide consisting of or consisting essentially of an amino acid sequence selected from the group consisting of any one of SEQ ID Nos. 33-124;

25 at least one MHC class I molecule; and

a detectable label.

BRIEF DESCRIPTION OF THE DRAWINGS AND TABLES

30 Figure 1. **A**. Kaplan-Meier survival analysis showed that individuals who have A*0101 allele were significantly less likely to become infected by HIV-1.
B. Kaplan-Meier survival analysis showed that individuals who have B*0702 allele were significantly more likely to become infected by HIV-1.

Figure 2. A*0101-NSSKVSQNY Tetramer staining showed that the tetramer binds to the intended CD8+ T cell population expressing HLA class I at a frequency of <1%. Using a multicolor flow cytometry panel, along with CD3 and CD8 to gate out our populations of interest, we also costained with CD62L and CD45RA. These markers are used to phenotypically define distinct subsets of T cells, including Naive (CD62L+ CD45RA+), Effector (CD62L-/CD45RA+/-) and Central memory cells (CD62L+/CD45RA-). These various subsets have distinct functional and phenotypic profiles.

Figure 3. B*0701-APRKKGCWK and B*0702-SPRTLNAW tetramer staining showed that the tetramer binds to the intended CD8+ T cell population expressing HLA class I at a frequency of <1%. Using a multicolor flow cytometry panel, along with CD3 and CD8 to gate out our populations of interest, we also costained with CD62L and CD45RA. These markers are used to phenotypically define distinct subsets of T cells, including Naive (CD62L+ CD45RA+), Effector (CD62L-/CD45RA+/) and Central memory cells (CD62L+/CD45RA-). These various subsets have distinct functional and phenotypic profiles.

Figure 4.A schematic drawing of a MHC (HLA in humans) tetramer consisting of Avidin, 4 MHC (HLA) class I molecules and 4 peptide epitopes binding to the MHC (HLA) class I molecules.

Table 1. HLA genotypes associated with resistance or susceptibility to HIV infection.

Table 2. A*0101 and B*0702 epitopes in HIV-1 gag protein.

Table 3. A*0101 epitopes in HIV-1 env.

Table 4. B*0702 epitopes in HIV-1 env

Table 5. HIV subtype sequence variants at gag A*0101 epitope location.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

For almost 20 years, we have been closely monitoring HIV-infections in a cohort of women in Nairobi, Kenya: the Pumwani Sex-worker cohort. A subset of women within this cohort remain HIV-1 seronegative and PCR negative despite being highly exposed to the virus through active sexwork [3-6]. Previous studies from our group have shown that resistance to HIV-1 infection is associated with several HLA alleles and HIV-specific $CD8^{+}$ and $CD4^{+}$ T-cell responses, as well as mucosal neutralizing antibody to HIV-1 [7-12].

Human Leukocyte Antigens (HLAs) are a group of host proteins that are responsible for presenting foreign matter within the body, including infectious pathogens to the immune system. HLA molecules are central in regulating the immune response through the binding and presentation of peptides known as epitopes derived from self and foreign proteins to T cells. The genes coding for HLA are extremely polymorphic. This genetic diversity ensures that no pathogens can escape detection at the population level. For instance, homozygosity for class I HLA alleles, which limits the breadth of the Cytotoxic T Lymphocyte (CTL) response, has been shown to accelerate disease progression and increase susceptibility to HIV infection [13]. The importance of different HLA alleles to virus control varies because of differences in antigenic recognition. Studies have shown that among HIV-1 infected people some HLA alleles are over represented in rapid progressors, such as B*0702, whereas other genotypes such as B*57 are associated with long-term non-progression [14-16]. To thoroughly investigate the role of HLA in resistance to HIV-1 infection observed in the Pumwani Sexworker Cohort we genotyped class I and class II antigens of more than 1000 women including 132 women who remain HIV-1 negative despite prolonged exposure (with an average follow-up time of 9.6 +/- 4.3 years). We have identified several HLA class I and class II alleles that are significantly associated with resistance or susceptibility to HIV-1 infection and disease progression (Table 1). Since HLA class I and class II antigens regulate immune responses through binding and presenting antigens to CD8+ and CD4+ T cells, the associations of HLA alleles with different outcomes of HIV-1 infection are most likely due to the differences in the antigenic peptides or epitopes of HIV being presented and the resulting immune responses that are engaged following immune recognition. In other words, HLA genotypes play an important role in susceptibility and resistance to HIV-1

infection. While several group have identified HLA types that seem to protect against disease progression, we have identified several that protect against infection and also against disease progression.

The traditional methods for epitope identification include algorithmic identification, peptide elution, and cell-based binding assay techniques. The low throughput, poor reproducibility, labour intensity, and restriction to only the most commonly studied MHC types have impeded the success of these methods. The iTopia® Epitope Discovery System by Beckman Coulter is a cell free system that can be used to identify, rapidly and reproducibly, epitopes for specific HLA alleles. The identified epitopes can then be confirmed with patient samples using ELISPOT and cytokine flow cytometry (CFC) assays.

Using this system, we investigated the peptide binding profile of two HLA class I alleles, A*0101 and B*0702, that are associated with differential outcomes of HIV-1 infection. HLA-A*0101 is significantly associated with HIV-1 resistant women (p=0.016, odds ratio: 1.7, 95% CI:1.1-2.7) and significantly less likelihood of seroconversion (Figure 1A), while HLA-B*0702 is associated with susceptibility to HIV-1 infection in the Pumwani Sexworker Cohort ((p=0.035, odds ratio: 0.38, 95%CI:0.14-1.1), rapid seroconversion (Figure 1B) and high viral loads and rapid disease progression in several different populations [17].

After screening 616 overlapping peptides of HIV-1 clade A and D gag polyprotein and 1820 overlapping peptides of clade A and D env protein with this system, we discovered that HLA*A0101 recognized fewer peptides than B*0702 (Tables 2-4). Among 616 peptides overlapping HIV-1 gag a total of 12 peptides including 7 peptide variants were recognized by A*0101, while 29 peptides were recognized by B*0702. Among 1820 peptides overlapping HIV-1 env 63 peptides were recognized by B*0702, whereas A*0101 recognizes only 20 peptides and peptide variants. The peptides recognized by these two alleles associated with different outcomes of HIV-1 infection do not overlap (Tables 2-4). Among 12 peptides recognized by A*0101 in HIV gag only a single conserved peptide, NSSKVSQNY and 7 variants of this peptide, with sufficient affinity and normal off-rate, which are measures of binding strength (Table 2), This peptide (not its variants) has been previously identified as a B*3501 epitope in a Gambian study [18] although this may be a misassignment (since we could not detect any

interferon gamma positive ELISPOT response from PBLs of 7 B*3501 positive patients tested) or it is possible that both A*0101 and B*3501 recognize the same peptide. However, our study has clearly shown that A*0101, an HLA allele associated with resistance to infection by HIV-1, not only recognizes this peptide,
5 but the multiple variants of this peptides (Table 2). We continue to determine if B*35 indeed recognizes this peptide and to investigate the perhaps altered immune responses generated by the same peptide presented by two different alleles. The peptide recognition by B*3501 was previously identified in HIV-exposed but uninfected Gambian women (Rowland-Jones et al., 1995 HIV-specific
10 cytotoxic T-cells in HIV-exposed but uninfected Gambian women. Nature Medicine 1,59-64). It was considered that the CTL activity may represent protective immunity against HIV infection. If both A*0101 and B*3501 can recognize the same epitope and the epitope is associated with protection from HIV-1 infection in both populations, the A*0101 and B*3501 epitope associated immune responses
15 represent protective immune responses to HIV-1. It will provide support to include this epitope in peptide-based HIV vaccines and A*0101- NSSKVSQNY (SEQ ID No. 6), A*0101-NSSKVSQNY variant tetramers can be used to monitor immune responses of successful vaccination. As will be apparent to one of skill in the art, as used herein, 'natural variant' refers to an amino acid sequence corresponding to
20 SEQ ID No. 6, that is, at the same location within the HIV genome but having one or more amino acid changes. Examples of such natural variants may be seen in SEQ ID Nos. 7-12.

We were able to show that B*0702 recognized 29 different peptides with high to moderate affinity and low off-rate in gag (Table 2). Within the gag polyprotein the
25 epitope NSSKVSQNY (SEQ ID No. 6) and its variant recognized by A*0101 with high affinity are located in the 3' end of p17 and contains part of the signal sequences that are essential to cleave gag polyprotein into individual gag proteins (Table 2), while the B*0702 epitopes are scattered on the entire gag polyprotein (Table 2). For the env protein the peptides recognized by A*0101 mostly located
30 on the N-terminal and C1 region (5 out of 20 peptides) and in gp41 (11 out of 20) (Table 3). Whereas, B*0702 recognizes more env peptides (63 env peptides) than A*0101 (20 env peptides) with only 3 out of 63 peptides are in the N-terminal region (Tables 4-1 and 4-2). Importantly, the peptides recognized by these two

alleles associated with different outcomes of HIV-1 infection do not overlap (Tables 2-4).

Contrary to the traditional view that a broadly reactive CTL of high magnitude is necessary to provide protection against HIV-1 infection, we have shown here a susceptible allele shown to associate with HIV disease progression, and an increased likelihood of infection. B*0702, in fact, recognizes many epitopes with high affinity, low off-rate and probably results in stronger and persistent CTL responses. This indicates that these broadly reactive responses, which in some cases may be strong immune responses, are not protective, and may in fact exacerbate HIV disease. Thus, this data suggests that recognition of multiple HIV-1 *gag* and *env* peptides with high affinity and low off-rate does not lead to a better outcome than restricted recognition of a few critical epitopes, such as NSSKVSQNY (SEQ ID No. 6), it's variants (SEQ ID Nos. 7-12 and SEQ ID No. 1) and the like. Thus we are proposing that these critical epitopes, such as NSSKVSQNY and its variants elicit protective immune responses and thus are potent vaccinogens and necessary components of an HIV vaccine. The concept of a focused immune response is relatively unexplored, but fits well with recent concepts emerging from our research and of others in the field. We believe that it's the type and context of the immune response elicited by an epitope that is more important in addition to the epitope itself. The A*0101 epitopes NSSKVSQNY and its variants are relatively conserved in HIV-1 subtype M group including A, B, D, G, and H subtypes with the exception of subtypes C, F and K (Table 5). As many of the B*0702 epitopes are also conserved, it is likely that the differences between these two HLA alleles associated with different outcomes of HIV-1 infection are epitope location, number of epitopes, magnitude and persistence and durability of the resulting immune responses. This is consistent with previous observations showing that HIV-1 resistant women have a global hyporesponsiveness to IL-4 production [19], lower expression of IRF-1 [20] and lower magnitude and narrow spectrum of CD8+ and CD4+ T cell responses to p24 [21-23].

The current strategy for HIV-1 vaccine development is to generate potent cellular and humoral immune responses, both mucosally and systematically, to provide defense against infection and contain transmission [2]. Until now, the efforts to develop HIV-1 vaccines all been aimed at generating pan or broad and

potent polyfunctional immune responses to HIV-1 antigens. Our data indicates that people who are susceptible to HIV-1 infection or rapid disease progression likely make these pan immune responses through recognition of multiple epitopes, which may lead to elevated immune activation, which in turn is a key driver of HIV
5 pathogenesis. On the other hand, a focused, strong but temporally lived immune response (such as in the case of A*0101) may be sufficient to fend off the initial infection without unnecessary immune activation. Therefore, in contrast to the conventional idea that an effective vaccine should generate a pan strong and multivalent immune responses to HIV-1, we propose an effective vaccine or
10 treatment should be narrow in spectrum, directed to a few critical conserved epitopes (such as in the case of A*0101) and block or neutralize (or interfere with) the wide spectrum of immune activation due to recognition of multiple epitopes (such as in the case of B*0702).

We classified immune responses to HIV-1 into good (protective) and bad or
15 susceptible (harmful or non-protective) immune responses and propose that harmful immune responses should be neutralized and good immune responses should be promoted. This is a novel strategy for HIV-1 vaccine development and to the development of tools to monitor effectiveness of vaccine trials.

In a first aspect of the invention, there is provided a purified or isolated
20 peptide consisting of or consisting essentially of or comprising an amino acid sequence selected from the group consisting of: (N/S/H)S(S/N)(K/Q)VS(Q/R)NY (SEQ ID No. 1); GTEELRSLF (SEQ ID No. 2); TTEGCQQIM (SEQ ID No. 3); YVDRFFKTL (SEQ ID No. 4) and KTGTEELKS (SEQ ID No. 5). (N/S/H)S(S/N)(K/Q)VS(Q/R)NY (SEQ ID No. 1) is a consensus sequence of
25 NSSKVSQNY (SEQ ID No. 6); SSSKVSQNY (SEQ ID No. 7); NSSQVSQNY (SEQ ID No. 8); HSNQVSQNY (SEQ ID No. 9); HSSQVSQNY (SEQ ID No. 10); NSSQVSRNY (SEQ ID No. 11) and SSSQVSQNY (SEQ ID No. 12) (Table 2). As discussed herein, these peptides correspond to regions of the gag protein that are linked to a protective immune response. Accordingly and as discussed herein,
30 such peptides can be used as a component of an HIV vaccine arranged to elicit a protective immune response against HIV infection and/or disease progression.

In a further aspect of the invention, there is provided a purified or isolated peptide consisting of or consisting essentially of or comprising an amino acid

sequence selected from the group consisting of: GTMLLGMLM (SEQ ID No. 13); CSAAENLWV (SEQ ID No. 14); PTDPNPQEI (SEQ ID No. 15); VTENFNMWK (SEQ ID No. 16); VTEEFNMWK (SEQ ID No. 17); VTINCTRPY (SEQ ID No. 18); RIGPGQAFY (SEQ ID No. 19); PIGLGQALY (SEQ ID No. 20); FNCGGEFFY
5 (SEQ ID No. 21); ISNYTDIIY (SEQ ID No. 22); YTDIIYSLI (SEQ ID No. 23); YTDIIYNLI (SEQ ID No. 24); LVNRVRQGY (SEQ ID No. 25); FILIAARTV (SEQ ID No. 26); RLGWEGLKY (SEQ ID No. 27); LLDTTAIAV (SEQ ID No. 28); LLDTIAIAV (SEQ ID No. 29); GTDRVIEVV (SEQ ID No. 30); WTDRVIEIG (SEQ ID No. 31); and GTDRVIEIV (SEQ ID No. 32) (Table 3). As discussed herein, these
10 peptides correspond to regions of the env protein that are linked to a protective immune response. Accordingly and as discussed herein, such peptides can be used as a component of an HIV vaccine arranged to elicit a protective immune response against HIV infection and/or disease progression.

In another aspect of the invention, there is provided a set of purified or
15 isolated peptides which consisting of or consisting essentially of a naturally occurring variant of any one of the resistance-linked peptides, that is, having an amino acid sequence as set forth in any one of SEQ ID Nos 1-32. Exemplary examples of such variants are provided in the accompanying table. As discussed herein, these peptides can be used as a component of an HIV vaccine arranged to
20 elicit a protective immune response against HIV infection and/or disease progression.

Accordingly, in another embodiment of the invention, there is provided a method of preparing a reagent for immunizing or inoculating an individual against an HIV infection comprising mixing one or more of the resistance-linked peptides
25 as set forth in any one of SEQ ID Nos 1-32 with a suitable adjuvant and/or excipient and/or carrier.

As will be appreciated by one skilled in the art, the above-described peptides can also be used to monitor immune response or aid in HIV clinical care and treatment. Specifically, an individual producing antibodies against a
30 resistance-linked peptide is producing a protective immune response.

In another aspect of the invention, there is provided a reagent comprising:

a peptide consisting of or consisting essentially of an amino acid sequence as set forth in SEQ ID Nos. 1-32 or a naturally occurring variant thereof;

at least one MHC class I molecule; and
a detectable label.

For example, the reagent may comprise an MHC tetramer, which are reagents consisting of four MHC class I molecules bound to the HIV peptide of interest, linked together by a detectable label or agent, for example but by no means limited to a fluorescently labeled streptavidin molecule. An exemplary example of such a construct is shown in Figure 4. These reagents will bind specifically to the TCR of all T cells that recognize that particular MHC/peptide complex. This is a very powerful tool for precise, easy, and rapid enumeration of antigen-specific T cells. It has been shown that the HLA class I tetramers can bind with sufficient avidity to HIV-specific $CD8^+$ T cells to allow their detection by flow cytometry. MHC tetramers have been used extensively to visualize antigen-specific T-cell immunity in humans and in animal model systems. Since the discovery of MHC-restricted recognition of peptide antigens by T cells, a main aim has been to visualize disease- and vaccine-induced T-cell immunity. We have shown in the provided examples that the tetramers of the A*0101- NSSKVSQNY and B*0702-SPRTLNAWV/B*0702-APRKKGCWK can be used to monitor such immune responses (Figures 2-3). These tetramers bind to the intended CD8 T cell population expressing MHC class I at a frequency of <1%, which is typical for tetramer specific populations. By using a multicolor flow cytometry panel, along with CD3 and CD8 to gate out populations of interest, we also costained with CD62L and CD45RA. These markers can be used to phenotypically define distinct subsets of T cells, including Naive (CD62L+ CD45RA+), Effector (CD62L-/CD45RA+/-) and Central memory cells (CD62L+/CD45RA-). These various subsets have distinct functional and phenotypic profiles. The tetramers will be used to define antigen specific populations of interest and using multiparametric flow cytometry we will be able to assess potential differences in these populations in terms of both functionality (which includes the ability to proliferate, excrete effector cytokines and capacity for cell killing) and phenotype (including memory, Tcell exhaustion, apoptosis, survival, an costimulatory expression).

Referring to Tables 2 and 4, a number of epitopes recognized by B*0702 are provided. These include gag epitopes IVQNAQGQM (SEQ ID No. 33); SPRTLNAWV (SEQ ID No. 34); TPQDLNMML (SEQ ID No. 35); IVGGHQAAM

(SEQ ID No. 36); RLRPGGKKK (SEQ ID No. 37); GPIPPGQMR (SEQ ID No. 38); TPQEQIGWM (SEQ ID No. 39); VRMYSPVSI (SEQ ID No. 40); YVDRFFKTL (SEQ ID No. 41); RALGPGATL (SEQ ID No. 42); GPGATLEEM (SEQ ID No. 43); GPGHKARVL (SEQ ID No. 44); GPSHKARVL (SEQ ID No. 45); KARVLAEAM
5 (SEQ ID No. 46); QANANTAIM (SEQ ID No. 47); QAQQPNVMM (SEQ ID No. 48); QVNGNTAIM (SEQ ID No. 49); QVQHTNIMM (SEQ ID No. 50); ATNANAAIM (SEQ ID No. 51); AIMMQRGNF (SEQ ID No. 52); NIMMQRGNF (SEQ ID No. 53); NIMMQRSNF (SEQ ID No. 54); NVMMQRSNF (SEQ ID No. 55); APRKKGCWK (SEQ ID No. 56); WPSSKGRPG (SEQ ID No. 57); RPGNFPQSR (SEQ ID No. 58);
10 FPQSRPEPT (SEQ ID No. 59); APPAEIFGM (SEQ ID No. 60); YPLVSLKSL (SEQ ID No. 61); and env epitopes APRGPDRPE (SEQ ID No. 62); APTKAKRRV (SEQ ID No. 63); APTRAKRRV (SEQ ID No. 64); CPKVSFEPI (SEQ ID No. 65); CPKVTFEPI (SEQ ID No. 66); FILIAARTV (SEQ ID No. 67); GLRIVFAVL (SEQ ID No. 68); GLRLGWEGL (SEQ ID No. 69); GLRRGWEAL (SEQ ID No. 70);
15 GPCKNVSTV (SEQ ID No. 71); GQRICRAIL (SEQ ID No. 72); IGLGQALYT (SEQ ID No. 73); ILIAARIVE (SEQ ID No. 74); IPIHYCAPA (SEQ ID No. 75); IPRRIRQGL (SEQ ID No. 76); IRPVVSTQL (SEQ ID No. 77); IVQRACRAV (SEQ ID No. 78); KPCVKLTPL (SEQ ID No. 79); KPVVSTQLL (SEQ ID No. 80); LALDKWASL (SEQ ID No. 81); LGRRGWEAL (SEQ ID No. 82); LIAARTVEL
20 (SEQ ID No. 83); LINCNTSAI (SEQ ID No. 84); LPAPRGPDR (SEQ ID No. 85); LPCRIKQII (SEQ ID No. 86); MRDNWRSEL (SEQ ID No. 87); NAKNIIVQL (SEQ ID No. 88); QARQLLSGI (SEQ ID No. 89); QGRDRSIRL (SEQ ID No. 90); QGRGRSIRL (SEQ ID No. 91); QLQARILAV (SEQ ID No. 92); QLQARVLAV (SEQ ID No. 93); RAIGLGAMF (SEQ ID No. 94); RAVGIGAVF (SEQ ID No. 95);
25 RICRAILNI (SEQ ID No. 96); RIRQGLERA (SEQ ID No. 97); RLRDFILIA (SEQ ID No. 98); RLRDLILIA (SEQ ID No. 99); RLVNGFLAL (SEQ ID No. 100); RLVNGFSAL (SEQ ID No. 101); RLVSGFLAL (SEQ ID No. 102); RPNNNTRKS (SEQ ID N0. 103); RPVVSTQLL (SEQ ID No. 104); RPYNNTRQR (SEQ ID No. 105); RVIEIGQRI (SEQ ID No. 106); RVLAVERYL (SEQ ID No. 107);
30 RVMGIQRNC (SEQ ID No. 108); RVRGIKRNY (SEQ ID No. 109); RVRGIQRNC (SEQ ID No.110); RVRQGYSPL (SEQ ID No. 111); SFNITTEVR (SEQ ID No. 112); SIRLVNGFL (SEQ ID No. 113); SIRLVSGFL (SEQ ID No. 114); SPLSFQTHT (SEQ ID No. 115); SPLSFQTLI (SEQ ID No. 116); SPLSFQTLL

(SEQ ID No. 117); TPIGLGQAL (SEQ ID No. 118); VPVWKDAET (SEQ ID No. 119); VPVWKEANT (SEQ ID No. 120); VPVWKEATT (SEQ ID No. 121); VQRACRAIL (SEQ ID No. 122); VQRACRAVL (SEQ ID No. 123); and YALFYRLDV (SEQ ID No. 124).

5 As discussed above, while the HLA-A*0101 is associated with resistance to HIV-1 infection, HLA-B*0702 with susceptible to HIV-1 infection through the study of highly exposed sexworkers in the Pumwani sexworkers cohort. Women who are A*0101 positive are more likely to be HIV-1 uninfected under highly exposed conditions, whereas women who are B*0702 positive are more likely to be infected 10 in the Pumwani sexworker cohort. Furthermore, HIV-1 infected B*0702 positive Caucasians also have higher viral load and rapidly progress to AIDS. Since A*0101 positive women are more likely to be uninfected under the highly exposed conditions and the A*0101 only recognizes a few and unique epitopes in HIV-1 gag and env protein, the protective immune responses correlate to fewer epitopes, 15 narrow in spectrum, specific in location of the epitopes. On the other hand, B*0702 positive women are more likely to be HIV-1 positive and once infected are more likely to have higher viral load and rapidly progress to AIDS and B*0702 recognize many epitopes with high affinity, low off-rate in HIV-1 *gag* and env proteins, thus accounting for the unprotective immune response or immune failure or harmful 20 immune responses correlated to many epitopes and wide spectrum. The correlates of protective or bad immune response to HIV-1 infection provides a guide to develop and test effective vaccines for HIV-1 and the tools, such as tetramers constructed with the specific alleles and peptides can be used to monitor the effective of the vaccination. The specific peptides can be used as components 25 of peptide-based vaccines.

In another aspect of the invention, there is provided a reagent comprising:

a peptide consisting of or consisting essentially of an amino acid sequence selected from the group consisting of any one of SEQ ID Nos. 33-124;

at least one MHC class I molecule; and

30 a detectable label.

Accordingly, in another embodiment of the invention, there is provided a method of preparing a reagent for immunizing or inoculating an individual against an HIV infection comprising mixing one or more of the resistance-linked peptides

as set forth in any one of SEQ ID Nos 1-32 or natural variant thereof with a suitable adjuvant and/or excipient and/or carrier with the proviso that the reagent is free of peptides having an amino acid sequence as set forth in any one of SEQ ID Nos. 33-124.

5 As can be seen, the B*0702 epitopes as components of tetramers can be used to monitor failed vaccine responses, or to identify immunologic failure in terms of patient care and treatment. That is, Individuals recognizing 'bad' epitopes may need different clinical care compared to those recognizing 'good' immune responses, as discussed herein.

10 Accordingly, the inventors believe that this represents a novel strategy for HIV-1 vaccines and treatment. As discussed above, the HIV vaccine should include only a narrow spectrum of peptides, such as the A*0101 epitope that is located at the protease cleavage site of p17/p24, in the critical site of env N-terminal and/or any one of the peptides as set forth in SEQ ID Nos. 1-32. Immune 15 responses generated against these epitopes or multiple copies of these epitopes can be expected to provide sufficient protection. The B*0702 epitopes should be avoided in HIV-1 vaccine and measures that can neutralize or block the immune recognition of B*0702 epitopes should be used in conjunction of the HIV-1 vaccination.

20 While not wishing to be bound to a particular theory or hypothesis, the inventors note that since HIV-1 cannot efficiently infect and replicate in resting T cells, immune activation by recognition of multiple epitopes with high affinity and low off-rates will provide more targets and conditions for HIV-1 infection and replication. The large number and wide spectrum of *gag* peptides recognized by 25 B*0702 will result in a wider spectrum of T cell activation and therefore provide many more targets and conditions for HIV-1 infection and replication. By blocking or limiting recognition of multiple B*0702 epitopes, thus limit targets and conditions for HIV-1 infection and replication while targeting responses to a single allele demonstrated to be protective in epidemiologic studies (A0101).

30 For example, antibodies or other means known in the art could be used in the vaccine to neutralize and/or block B*0702 epitopes, thereby avoiding generating unfavorable immune responses.

Thus, the instant invention represents a departure from conventional thinking. Specifically, it was previously believed that strong immunogenic responses to any region of a foreign peptide indicated a productive immune response. However, the research described herein indicates that that is not the case with HIV. Specifically, a strong but non-productive or non-protective immune response may actually promote the spread of the virus within the host. Furthermore, a vaccine which is a mixture of protective and non-protective epitopes may face the same problem albeit to a lesser extent. That is, the presence of highly immunogenic, non-protective alleles may result in over-stimulation of the immune system thereby promoting spread of the virus despite the presence of the protective epitopes. Accordingly, in one aspect of the invention there is provided the use of the specific resistance-linked peptides as set forth in any one of SEQ ID Nos. 1-32 or combinations thereof for the inoculation of an individual or for the immunization of an individual against an HIV infection with the proviso that said reagent does not include or comprise a peptide as set forth in any one of SEQ ID Nos. 33-124.

In another aspect of the invention, there is provided a method for preparing a medicament for use in inoculation or immunization of an individual in need of such treatment, for example, an individual who has contracted or is at risk of infection by the human immunodeficiency virus comprising: mixing one or more of the resistance-linked peptides as set forth in any one of SEQ ID Nos 1-32 or natural variant thereof with a suitable adjuvant and/or excipient and/or carrier.

As discussed above, in other embodiments, the resistance-linked peptides (SEQ ID Nos. 1-32) and/or the susceptibility-linked peptides (SEQ ID Nos. 33-124) or natural variants thereof may be incorporated into a panel or chip or other such support for the monitoring or assessing of the immune response of an individual to a vaccination or treatment for HIV infection or for providing an overview of possible disease progression in an individual. Specifically, by using such a panel, one can determine whether the immune system of the individual will be largely protective or susceptible to the HIV infection.

Furthermore, as will be appreciated by one of skill in the art, this concept can be expanded to other HLA epitopes and peptides and additional targets can be identified. Clearly this demonstrates that a strategy to identify protective HLA

alleles in large cohort studies and then using commercial systems to identify epitopes presented by these alleles could aid HIV vaccine development. Specifically, by studying immune and genetic correlates of cohorts with well defined phenotypes to HIV-1, such as HIV-1 resistant sexworkers in Pumwani
5 sexworkers cohort, we can identify HLA alleles that are significantly associated with resistance or susceptibility to HIV-1 infection and identify their epitopes which in turn will contribute to the development of HIV vaccines. We have identified multiple HLA class I and class II alleles that are associated with resistance or susceptibility to HIV-1 infection in the Pumwani Sexworker cohort (see Table 1) in
10 addition to A*0101 and B*0702. We expect that epitopes of the alleles can also be used as components of peptide-based vaccines or tetramers to monitor immune responses for vaccine development and monitor immune responses after vaccination.

We are conducting ELISPOT and CSFE/ cytokine flow cytometry assays, as
15 well as tetramer studies to characterize, both phenotypically and functionally, immunological responses to these epitopes. In the meantime, we are also exploring ways, such as generating antibodies, interfering peptides or other approaches that can neutralizing the B*0702 epitopes or block and/or interfere bad and broad spectrum of immune activation.

20 Additionally, we have tested the binding properties of A*0101 to epitope NSSKVSQNY variants that present in the consensus sequences of major HIV-1 subtypes and shown that the recognition of A*0101 to the consensus sequence variants of major HIV-1 subtypes (Table 2). This would suggest that the application of the invention to different HIV-1 subtypes. We could presume (as would any
25 skilled in 'the art' that variants of this peptide that can be presented by A0101 or other protective HLA types could also be used for this strategy. This would include naturally occurring variants of NSSKVSQNY (Table 2) as well as any peptides that may be longer or shorter and still capable of being presented by A0101 class I epitope that still contain the NSSKVSQNY (SEQ ID No. 6) or its variants motif or
30 an amino acid sequence as set forth in any one of SEQ ID Nos. 1-32.

Methods

Study subjects.

This study was conducted among 880 women enrolled in the Pumwani Sex Worker Cohort established in 1985 in Nairobi, Kenya to study risk factors for HIV-1 infection and disease[5]. Of 880 women from the Pumwani Sex Worker Cohort 132 women were classified as HIV-1-resistant based on the criteria that they remained HIV seronegative and PCR negative in the cohort for at least 3 years while continuing in sex work[2] and still negative at the time of this study[6]. The 132 women who were classified as resistant were all enrolled in the cohort before 1999 with an average follow-up time of 9.6 +/- 4.3 years. The detailed description of cohort design and follow-ups can be found elsewhere. This study has been approved by the Ethics Committee of the University of Manitoba and the Ethics and Research Committee of Kenyatta National Hospital. Informed consent was obtained from all women enrolled in the study.

HLA typing.

A high resolution sequence-based HLA typing method was used to type HLA class I genes and a two-step sequence-based method was used to type DRB genes[24, 25]. Ambiguous allele combinations were resolved by sequencing with sequence-specific primers. Since only exon 2 and exon 3 of class I genes are involved in the peptide binding grove, we did not attempt to resolve class I alleles that differ beyond exon 2 and exon 3. DRB genes were screened by a first round of sequence analysis, and high resolution typing was carried out for the DRB1*01 group that was previously associated with resistance to HIV-1 infection. We also analysed multiple samples at different time points from the same subjects whenever available to eliminate or minimize sample mix-ups that are difficult to avoid in a large cohort study that has continued for such a long period of time. Three independent quality control procedures for HLA typing were established to ensure accuracy. HLA class I genes and DRB1*01 and DRB1*1503 subgroups are typed to the four-digit resolution and subsequently reduced to either a two-digit classification in order to compare with previous published results, or grouped into supertypes. All homozygotes were classified based on the highest resolution (four-digit) typing possible for the peptide-binding regions of the HLA genes (exon 2 and 3 for HLA-A, -B and –C, exon 2 for DRB).

Statistical analysis.

Analysis was performed with SPSS for Windows 13.0 statistical analysis program package. Standard univariate methods such as crosstabs and the Chi-square test were used to explore the relationship between binary outcomes and explanatory variables. Multivariate logistic regression was used to explore the relationship between time-to-event data and explanatory variables. Both Kaplan-Meier plots with the log rank test and Cox's proportional hazards regression were used for survival analysis.

HIV-1 gag polyprotein epitope screen with iTopia Epitope Discovery system

Overlapping peptides were designed in sequences of 9 amino acid residues overlapping by 8, to span the entire HIV-1 gag polyprotein region. A total of 609 peptides were synthesized. A*0101 and B*0702 iTopia kits were purchased from Beckman Coulter and the peptides were screened following protocols provided by the supplier. Once positive binding was identified, affinity and off-rate assays were conducted with the iTopia epitope discovery system. The affinity and off-rate of the peptide binding by the A*0101 and B*0702 were determined.

Confirmation of epitope recognition and immunogenicity using ELISPOT and CSFE proliferation assay with patient PBLs

A major correlates of protection in HIV infection and the rate of disease progression are HIV-specific CTL responses. To determine whether HLA-restricted HIV peptides identified using the iTopia® system are indeed relevant *in vivo*, we will test the responsiveness of peripheral blood mononuclear cells (PBMC) isolated from HIV-infected patients with the appropriate HLA type in the Pumwani sex-worker cohort and the mother-to child transmission cohort using peripheral mononuclear cells (PBMC) isolated using a combination of IFNγ ELISPOT, intracellular cytokine staining and multicolour flow cytometry-based CFSE proliferation assay. Further functional and phenotypic characterization utilizing Class I tetramers (in further collaboratin with Beckman-Coulter) can then be conducted using 10-colour flow cytometry in our facilities in Nairobi. This comprehensive analysis will allow us to determine both the functional and memory

phenotype of immune responses generated to these identified peptides and allow a more accurate qualitative and quantitative analysis of the anti-viral cellular immune response...

5

Appendix

iTopia® Epitope Discovery System

The challenge in selecting the "right" immunogenic proteins is a major obstacle to the development of cell-mediated vaccines. Some progress has been made using algorithmic identification, peptide elution and cell-based binding assay techniques,
10 but success has been impeded by method-specific issues such as laborious lab work, low throughput, poor reproducibility or restriction to only the most commonly studied MHC types. The iTopia® Epitope Discovery System was recently developed by Beckman Coulter to identify, rapidly and reproducibly, epitopes for specific HLA alleles.

15 The fundamental units of the iTopia® system are assembled tetramers—consisting of properly folded complexes of specific MHC molecules, beta 2 microglobulin and placeholder peptides—which are bound to both streptavidin-coated microplate wells and a fluorescent-labelled antibody designed to recognize only a properly folded peptide/MHC complex. Overlapping peptides spanning the
20 protein of interest or protein region of interest are synthesized. A binding assay is performed for each of the test peptides by introducing a buffer designed to unfold and dissociate the MHC and placeholder peptide in the microtitre well. The placeholder peptide and beta 2 microglobulin are washed away, leaving the unfolded MHC bound to the reaction well. A peptide from the synthesized library
25 and additional beta 2 microglobulin are added to each well and incubated in a buffer designed to promote refolding of the complex. A fluorescent-labelled antibody designed to recognize only a properly folded peptide/MHC complex is added to each well. This step identifies test peptides that bind to the MHC and warrant additional analysis in order to characterize binding affinity and rate of
30 dissociation. Peptides that do not bind to the MHC are clearly identified and eliminated from further study. The iTopia® system allows rapid identification of pathogen epitopes for specific HLA allele(s) without using live cells and pathogens.

Functional studies and confirmation of epitopes can then be performed using live cells on a much smaller scale.

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be
5 made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.

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Table 1. HLA genotypes associated with resistance or susceptibility to HIV infection

HLA genotypes associated with resistance to HIV infection				
	p value	Odds ratio (95% CI)	*p* value*	*IRR*# (CI95%)
DRB1*01	0.003	2.0 (1.28-3.39)	0.0026	1.4 (1.1-1.8)
DRB1*010101	0.016	2.6 (1.16-5.61)		
DRB1*010201	0.019	1.9 (1.10-3.15)		
A*01	0.016	1.7 (1.11-2.78)	0.0033	1.4 (1.1-1.8)
A*0101	0.049	1.6 (0.97-2.73)		
A*6802 (1985-1992)	0.016	1.9 (1.12-3.34)	-	-
A*6802 (1993-2001)	0.515	-	-	-
A2 supertype (1985-1992)	0.002	2.0 (1.27-3.14)	-	-
A2 supertype (1993-2001)	0.164	-	-	-
B*1303	0.049	4.4 (0.88-22)	-	-
B*151701	0.013	3.4 (1.2-9.6)	0.024	1.8 (0.98-3.56)
B*180101	0.004	2.5 (1.3-4.7)	-	-
B*1807	0.004	13.3 (1.37-128)	0.036	3.1 (0.85-25.5)
B*4101	0.010	4.5 (1.3-15.7)	-	-
B*470101	0.011	1.5 (1.3-15.6)	-	-
B*5702	0.004	2.7 (1.3-5.5)	0.017	1.6 (1.0-2.5)
CW*070101	0.006	1.7 (1.2-2.6)	0.0002	1.4 (1.2-1.8)
CW*070401	0.002	2.8 (1.4-5.6)	0.040	1.4 (0.95-2.24)
HLA genotypes associated with susceptibility to HIV infection				
DRB1*1503-DRB5*010101	0.0002	0.30 (0.15-0.58)	0.0007	0.69 (0.55-0.87)
A*2301	0.029	0.42 (0.19-0.93)	-	-
A*6601	0.026	0.39 (0.17-0.92)	-	-
B*4201	0.043	0.53 (0.28-0.99)	-	-
Cw*020204	0.035	0.47 (0.23-0.96)	0.01	0.71(0.53-0.95)
B*070201	0.035	0.38 (0.14-1.1)	0.0018	0.57(0.40-0.83)
B7 supertype	0.011	0.57 (0.37-0.88)	0.0035	0.59 (0.41-0.88)

-The results presented are the genotypes that contain one or two copies of the indicated allele.
-The alleles in **bold** face are alleles that were identified in previous studies[9].
*: The *p* value is the exact test of time to seroconversion.
#: Incident rate ratio.

Table 2. A*0101 and B*0702 Epitopes in HIV-1 gag protein						
allele	peptide sequence	AA no in gag	gag protein	% of positive control	Affinity	Half-Life
					ED50	T1/2
A*0101	GTEELRSLF	71-79	p17	20.8	2.958E-04	1.662
	KTGTEELKS	69-77	p17	20.3		2.856
	TTEGCQQIM	53-61	p17	26.2	1.696E-05	1.536
	YVDRFFKTL	296-304	p24	33.1	4.906E-05	1.860
	NSSKVSQNY	124-132	p17	70.6	1.211E-05	0.995
	SSSKVSQNY	124-132	p17	82.2	5.13E-06	0.773
	NSSQVSQNY	124-132	p17	73.9	5.02E-06	0.931
	NSSKVSQNY	124-132	p17	76.7	6.11E-06	1.298
	HSNQVSQNY	124-132	p17	69.9	1.43E-05	0.385
	HSSQVSQNY	124-132	p17	83.7	4.30E-06	1.122
	NSSQVSRNY	124-132	p17	76.6	1.21E-05	0.184
	SSSQVSQNY	124-132	p17	85.1	5.18E-06	0.589
B*0702	IVQNAQGQM	134-142	p24	38.8	4.134E-04	1.602
	SPRTLNAWV	148-156	p24	113.3	1.611E-06	1.345
	TPQDLNMML	180-188	p24	36.9	8.115E-05	1.358
	IVGGHQAAM	190-198	p24	46.4	4.489E-05	1.674
	RLRPGGKKK	20-28	p17	32.4	7.829E-05	1.538
	GPIPPGQMR	221-229	p24	45.9	4.843E-04	1.378
	TPQEQIGWM	242-250	p24	44.8	2.601E-05	1.644
	VRMYSPVSI	274-282	p24	41.6	1.689E-04	1.604
	YVDRFFKTL	296-304	p24	60.0	4.373E-05	1.465
	RALGPGATL	335-343	p24	52.2	1.491E-05	1.370
	GPGATLEEM	338-346	p24	35.1	1.784E-05	1.364
	GPGHKARVL	355-363	p24	93.1	4.797E-06	1.230
	GPSHKARVL	355-363	p24	91.6	3.135E-05	1.444
	KARVLAEAM	359-367	p24-p2	66.5	8.08E-06	1.103
	QANANTAIM	369-377	p2	46	1.36E-05	1.551
	QAQQPNVMM	369-377	p2	46.2	1.92E-05	0.374
	QVNGNTAIM	369-377	p2	46.3	2.28E-05	1.625
	QVQHTNIMM	369-377	p2	46.1	2.55E-05	0.169
	ATNANAAIM	370-378	p2	37.4	2.08E-05	0.082
	AIMMQRGNF	375-383	p2-p7	91.6	3.45E-06	0.518
	NIMMQRGNF	375-383	p2-p7	73.4	9.72E-06	0.043
	NIMMQRSNF	375-383	p2-p7	73	9.31E-06	0.057
	NVMMQRSNF	375-383	p2-p7	70.2	8.96E-06	0.174
	APRKKGCWK	406-414	p7	145.3	8.962E-07	1.040
	WPSSKGRPG	430-438	p1	81.0	2.709E-05	1.472
	RPGNFPQSR	443-451	p1-p6	37.3	5.371E-05	1.519
	FPQSRPEPT	447-455	p1-p6	55.6	1.496E-05	1.431
	APPAEIFGM	456-464	p6	57.6	1.745E-05	1.157
	YPLVSLKSL	483-491	p6	75.5	2.269E-05	1.372

Table 3 A*0101 epitopes in HIV-1 env					
peptide sequence	AA no in env	env domains	percent of positive control	affinity	Half-Life
				ED50	T1/2
GTMLLGMLM	17-25	SIGNAL	54.0	1.747E-05	0.131
CSAAENLWV	27-35	SIGNAL-C1	35.0	2.449E-05	0.226
PTDPNPQEI	75-83	C1	44.0	3.736E-05	1.321
VTENFNMWK	88-96	C1	98.2	8.193E-06	0.540
VTEEFNMWK	88-96	C1	89.2	9.955E-06	0.902
VTINCTRPY	294-302	V3	79.2	8.426E-06	0.449
RIGPGQAFY	310-318	V3	64.3	1.080E-04	1.884
PIGLGQALY	310-318	V3	40.4	1.016E-04	3.400
FNCGGEFFY	378-386	C3	32.2	8.832E-06	2.234
ISNYTDIIY	638-646	GP41	76.5	8.751E-06	0.449
YTDIIYSLI	641-649	GP41	124.5	2.107E-06	0.958
YTDIIYNLI	641-649	GP41	129.9	2.733E-06	1.491
LVNRVRQGY	707-715	GP41	31.5	5.014E-05	2.124
FILIAARTV	777-785	GP41	40.4	1.903E-05	5.116
RLGWEGLKY	797-805	GP41	85.8	7.895E-06	0.390
LLDTTAIAV	824-832	GP41	73.7	5.618E-06	0.658
LLDTIAIAV	824-832	GP41	43.7	1.656E-06	0.680
GTDRVIEVV	835-843	GP41	99.0	9.374E-06	1.705
WTDRVIEIG	835-843	GP41	60.5	1.105E-05	0.529
GTDRVIEIV	835-843	GP41	86.2	1.119E-05	1.457

Table 4-1 B*0702 epitopes in HIV-1 env					
peptide sequence	AA no in env	env domains	percent of positive control	affinity	Half-Life
				ED50	T1/2
APRGPDRPE	726-734	GP41	68.7	2.628E-05	1.212
APTKAKRRV	500-508	C5	95.2	7.088E-06	1.154
APTRAKRRV	500-508	C5	100.2	2.905E-06	1.050
CPKVSFEPI	207-215	C2	37.5	4.928E-05	0.876
CPKVTFEPI	207-215	C2	30.1	9.672E-05	0.916
FILIAARTV	777-785	GP41	68.9	1.318E-05	1.368
GLRIVFAVL	697-705	GP41	45.3	5.569E-07	1.006
GLRLGWEGL	697-705	GP41	48.5	3.325E-05	1.498
GLRRGWEAL	795-803	GP41	97.9	4.540E-06	0.821
GPCKNVSTV	239-247	C2	43.9	1.026E-05	0.862
GQRICRAIL	843-851	GP41	59.1	1.465E-06	1.157
IGLGQALYT	311-319	V3	35.7	5.110E-05	1.845
ILIAARIVE	779-787	GP41	71.5	5.951E-06	0.947
IPIHYCAPA	215-223	C2	58.5	1.893E-06	0.904
IPRRIRQGL	853-861	GP41	180.2	4.975E-07	1.609
IRPVVSTQL	253-261	C2	49.6	1.985E-05	1.017
IVQRACRAV	842-850	GP41	70.7	2.285E-06	1.075
KPCVKLTPL	116-124	C1	64.2	3.345E-06	1.204
KPVVSTQLL	254-262	C2	45.0	9.149E-05	1.192
LALDKWASL	664-672	GP41	35.8	2.573E-05	0.323
LGRRGWEAL	795-803	GP41	52.9	6.066E-06	1.137
LIAARTVEL	779-787	GP41	68.0	1.387E-06	0.981
LINCNTSAI	195-203	V2-C2	39.3	6.507E-06	0.635
LPAPRGPDR	724-732	GP41	50.9	2.448E-05	1.671
LPCRIKQII	418-426	V4	75.9	5.185E-06	1.300
MRDNWRSEL	478-486	C5	27.4	3.074E-05	1.593
NAKNIIVQL	282-290	C2	30.6	4.080E-06	1.621
QARQLLSGI	543-551	GP41	69.4	9.675E-06	1.061
QGRDRSIRL	743-751	GP41	37.7	2.901E-05	1.709
QGRGRSIRL	743-751	GP41	76.4	9.379E-06	1.292
QLQARILAV	578-586	GP41	35.1	1.360E-05	1.418
QLQARVLAV	578-586	GP41	32.0	1.215E-05	1.493

Table 4-2 B*0702 epitopes in HIV-1 env					
peptide sequence	AA no in env	env domains	percent of positive control	affinity	Half-Life
				ED50	T1/2
RAIGLGAMF	514-522	GP41	80.8	5.112E-06	1.166
RAVGIGAVF	514-522	GP41	78.3	1.720E-06	1.124
RICRAILNI	845-853	GP41	45.2	4.865E-06	1.100
RIRQGLERA	856-864	GP41	36.9	1.926E-05	1.411
RLRDFILIA	773-781	GP41	36.9	6.528E-06	1.445
RLRDLILIA	773-781	GP41	38.3	4.790E-06	1.288
RLVNGFLAL	750-758	GP41	35.5	1.175E-05	1.353
RLVNGFSAL	750-758	GP41	41.4	8.288E-06	1.343
RLVSGFLAL	750-758	GP41	51.6	1.767E-06	1.233
RPNNNTRKS	300-308	V3	59.0	3.723E-05	1.406
RPVVSTQLL	254-262	C2	78.1	1.743E-05	1.198
RPYNNTRQR	300-308	V3	86.2	3.241E-05	1.096
RVIEIGQRI	838-846	GP41	39.7	1.489E-05	1.333
RVLAVERYL	582-590	GP41	32.0	2.687E-05	1.593
RVMGIQRNC	2-10	SIGNAL	58.7	1.173E-05	0.443
RVRGIKRNY	2-10	SIGNAL	57.0	1.658E-05	0.278
RVRGIQRNC	2-10	SIGNAL	97.3	2.692E-06	1.208
RVRQGYSPL	710-718	GP41	107.2	1.563E-06	1.519
SFNITTEVR	157-165	V2	98.3		1.063
SIRLVNGFL	748-756	GP41	90.8	6.341E-06	0.962
SIRLVSGFL	748-756	GP41	97.0	3.499E-06	1.251
SPLSFQTHT	716-724	GP41	50.8	4.525E-05	0.497
SPLSFQTLI	716-724	GP41	55.4	2.369E-05	0.750
SPLSFQTLL	716-724	GP41	70.0	2.872E-05	1.013
TPIGLGQAL	309-317	V3	33.9	2.217E-05	1.334
VPVWKDAET	41-49	C1	32.7	4.101E-05	0.719
VPVWKEANT	41-49	C1	37.9	3.763E-05	0.593
VPVWKEATT	41-49	C1	44.8	1.293E-05	0.793
VQRACRAIL	843-851	GP41	80.3	1.797E-06	0.927
VQRACRAVL	843-851	GP41	81.6	1.055E-06	1.041
YALFYRLDV	172-180	V2	34.4	4.814E-05	0.506

Table 5. HIV subtype sequence variants at gag A*0101 epitope location

HIV subtypes	Consensus and variations
CON_OF_CONS	NSSKVSQNY
M.group.anc	DSSQVSQNY
A1.anc	NSSKVSQNY
B.anc	NSSQVSQNY
C.anc	DNGKVSQNY
CONSENSUS_A1	NSSKVSQNY S..N.....
A2	TGSSSSQNY ..N......
B	NSSQVSQNY H.N...... KNNP.....
C	DKGKVSQNY .R..D.... .D....... AD.K.....
D	NSSQVSQNY NN....... DN....... S........
F	ADKGVSQNY .E....... ...E..... VS.......
G	NSSQVSQNY S........ ..NP..... ..N......

HIV subtypes	Consensus and variations
H	KDNKVSQNY ..S.I....
J	DNSQVSQNY
K	ADKGVSQNY
CONSENSUS_01_AE	SSSKVSQNY
CONSENSUS_02_AG	ATGSSSQNY
CONSENSUS_03_AB	SSSKVSQNY
CONSENSUS_04_CPX	GSSNVSQNY
05	NSSQASQNY
CONSENSUS_06_CPX	NSSNLSQNY
CONSENSUS_07_BC	ADGKVSQNY
CONSENSUS_08_BC	AEEKVSQNY
09	NNSKVSQNY
CONSENSUS_10_CD	NSSQVSQNY
CONSENSUS_11_CPX	NSSKVSQNY
CONSENSUS_12_BF	ADKGVSQNY
13	NSNQVSQNY
CONSENSUS_14_BG	NNSQASQNY
15	SSSTVSHNY
16	TGSSGSQNY
18	SSSKVSQNY
19	HSSQVSQNY
O	SARQTGQNY ....A.... .P...S...

CLAIMS

1. A method of preparing a reagent for inoculating an individual against an HIV infection comprising mixing at least one resistance-linked peptide having an amino acid sequence as set forth in any one of SEQ ID Nos 1-32 or natural variant thereof with a suitable adjuvant, excipient or carrier.

2. The method according to claim 1 with the proviso that the reagent is free of peptides having an amino acid sequence as set forth in any one of SEQ ID Nos. 33-124.

3. The method according to claim 1 wherein the peptide has an amino acid sequence as set forth in any one of SEQ ID Nos. 1-12.

4. The method according to claim 1 wherein the peptide has an amino acid sequence as set forth in any one of SEQ ID Nos. 6-12.

5. The method according to claim 1 wherein the peptide has an amino acid sequence as set forth SEQ ID No. 1 or a natural variant thereof.

6. The method according to claim 1 wherein the peptide has an amino acid sequence as set forth in SEQ ID No. 6 or a natural variant thereof.

7. A reagent comprising:

a peptide consisting of or consisting essentially of an amino acid sequence as set forth in SEQ ID Nos. 1-32 or a naturally occurring variant thereof;

at least one MHC class I molecule; and

a detectable label.

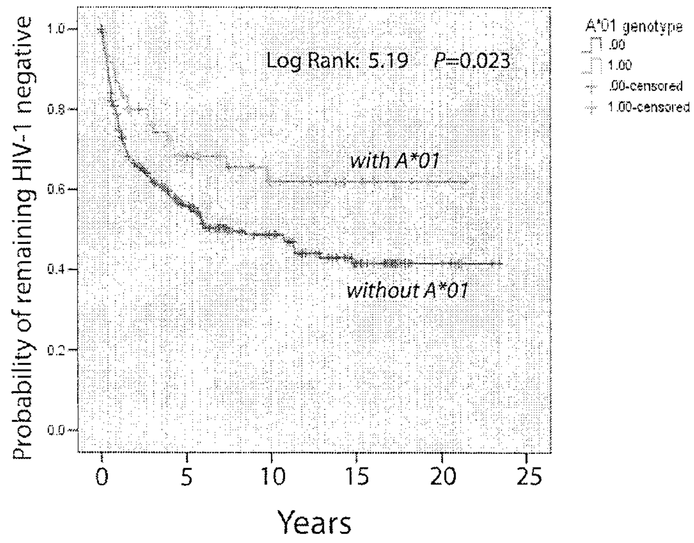
8. A reagent comprising:

a peptide consisting of or consisting essentially of an amino acid sequence selected from the group consisting of any one of SEQ ID Nos. 33-124;

at least one MHC class I molecule; and

a detectable label.

A



B

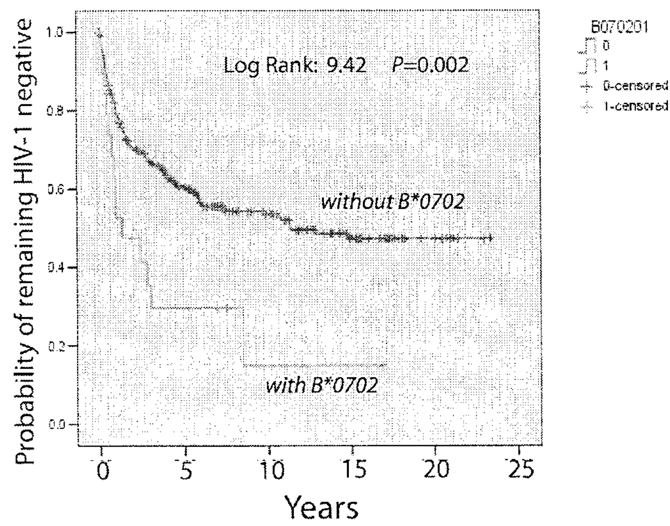
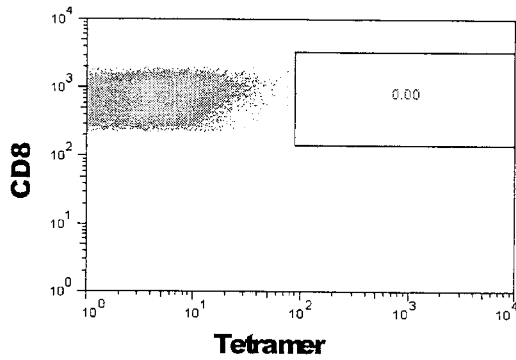


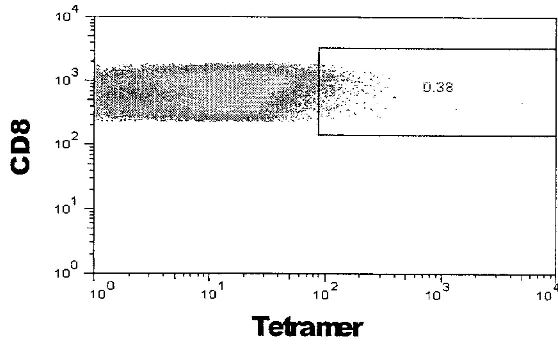
Figure 1

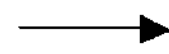
ML 2003 A01 no tetramer (All other staining is included)



*These are gated on CD3+CD8+ T cells. This background was similar for all samples

ML 2003 A01 with NSSKVSQNY Tetramer 7ul





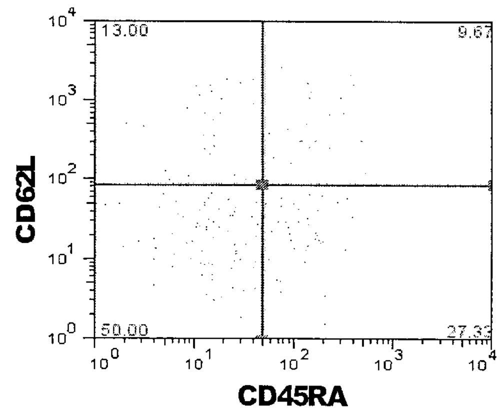
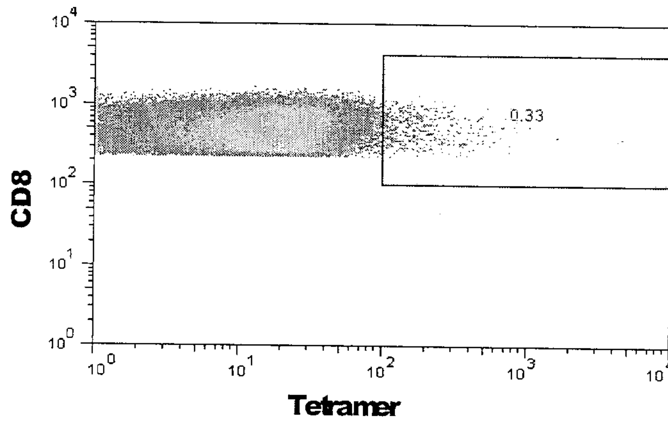
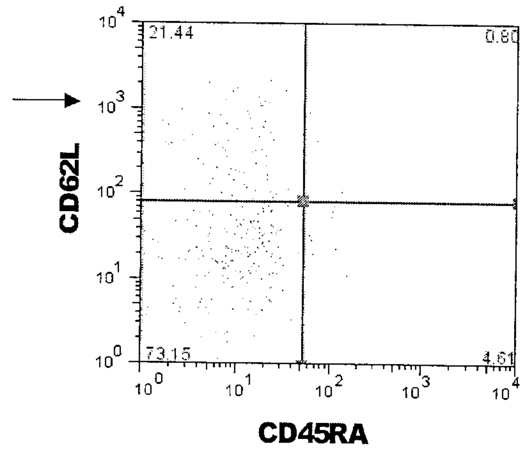


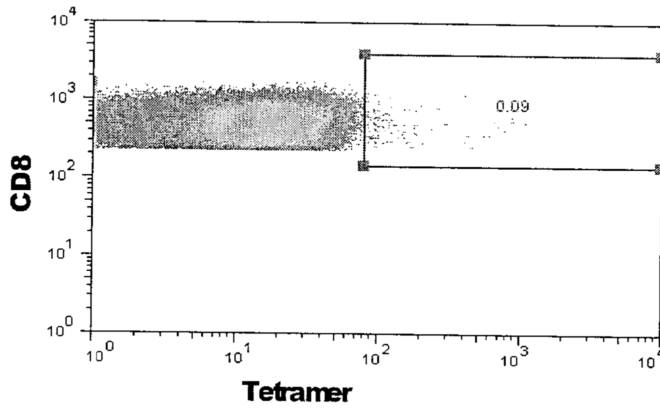
Figure 2

ML 1957 B07, APRKKGCWK 7 ul





ML 1957 B07, SPRTLNAW 7 ul



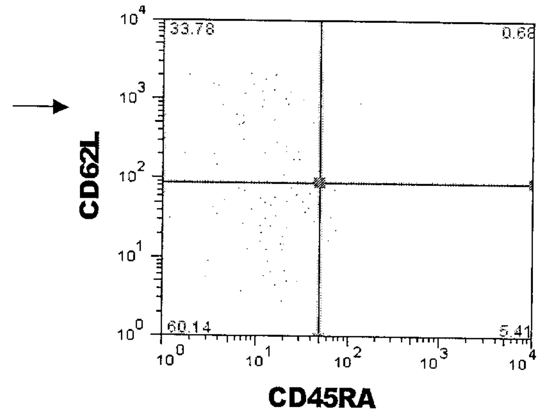


Figure 3

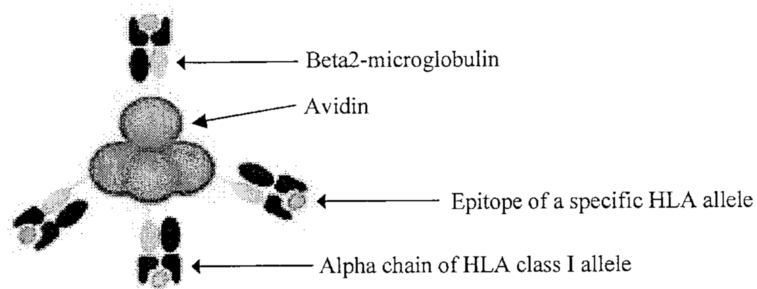


Figure 4