Fitness Constraints on Immune Escape from HIV: Implications of Envelope as a Target for Both HIV-Specific T Cells and Antibody

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Abstract: Sterilising immunity against HIV-1 infection, whilst ideal, appears an unrealistic vaccination goal in the short term. More achievable is slowing the progression to disease and decreasing transmission by mounting strong T cell and neutralising antibody responses to maintain low viral loads. However, in both acute and chronic infection, mutant virus is selected to escape both arms of the adaptive immune system. Each mutation away from wildtype virus likely incurs at least some reduction in replicative capacity ("fitness") of the virus. Rapid reversion to wildtype of some immune escape mutations upon transmission, suggests fitness costs may be significant. HIV-1 Envelope is unique in that it is subject to both neutralising antibody and cell-mediated immune responses. Although Envelope is variable between strains, considerable serial pressure and mutational escape from both neutralising antibody and cytotoxic T lymphocyte attack may result in impaired structure and function. This could ultimately be exploited in HIV vaccine design.

Keywords: HIV-1, envelope, escape, vaccine, fitness, T cell, neutralising antibody.

INTRODUCTION

Untreated, severe immunodeficiency causes death from opportunistic infections on average 10 years after initial HIV infection. However, spectacular damage is done to the immune system within the first 10-14 days of infection. Memory CD4+ T lymphocytes within the gut mucosa are exquisitely sensitive to infection and deletion during acute HIV infection [50]. In the well-studied SIV-macaque model of HIV infection, up to 60% of all gut memory CD4+ T cells were SIV-infected as early as 10 days post infection, and 4 days later, 80% of these cells were eliminated [54]. Host immune responses can partially control viremia in acute infection, although many immune responses select viral immune escape mutations [40, 65]. Understanding and modulating the early virological and immunological events set in motion by acute HIV-1 infection is likely to be critical for long term control of viremia and prevention of immunodeficiency without drug therapy [13, 39, 66].

ADAPTIVE IMMUNE CONTROL OF HIV-1

Immediately following the exponential growth of virus during acute infection, an approximate 2 $\log_{10}$ reduction in viral levels typically ensues [22] (Fig. **1**).

T lymphocytes (both CD4+ T helper cells and CD8+ cytotoxic T lymphocytes (CTL)) and neutralising antibodies (NAb) are now well recognised as the critical adaptive immune responses partially controlling HIV. HIV-1 specific CD8+ CTL responses, rather than NAb responses, correlate with falls in acute viremia prior to seroconversion [9, 47]. CTL responses during early HIV infection, particularly to the HIV-1 Envelope (Env) protein, have been associated with a slower progression of disease [57]. A contrasting view on this phenomenon suggests that the viral decrease immediately following acute HIV infection could be a result of the massive reduction of infectable cellular targets, rather than the immune response [63]. However, CD8+ T cell depletion of SIV-infected macaques results in a brisk increase in SIV viremia [41, 74], suggesting a direct role for CD8+ T cells. Activated CD8+ T cells also release many soluble factors, shown to suppress HIV-1 activity, including chemokines which bind CCR5 and inhibit HIV-1 entry [19]. The relative roles of direct cytolytic killing verses release of soluble factors by CD8+ T cells in the control of viremia is controversial [24], but recent analysis suggest indirect mechanisms may predominate [2].

Virus-specific CD4+ T helper cell responses are intimately linked with the development of CD8+ responses [43]. Unfortunately, activated memory CD4+ T cells are preferentially infected, and ultimately destroyed by HIV-1. HIV-1 specific T cells are even more readily infected by HIV-1 than those not specific for the virus [27]. This process undermines the long-term immune control of this virus [72].

Several human and non-human primate passive transfer studies have corroborated the importance of NAb to HIV-1 Env [37, 53, 55, 80]. Trkola and colleagues recently showed that IV administration of a cocktail of NAb to HIV-1 infected patients after cessation of antiretroviral therapy (ART) resulted in a slower rebound of viremia in a subset of patients [79].

Antibody-dependent cellular cytotoxicity (ADCC) activity has also been linked to control of SIV in macaques [34]. There is also *in vitro* evidence in samples from HIV-infected humans that ADCC activity could have strong antiviral activity [31]. Mutational escape from Env ADCC epitopes has yet to be addressed, but it is well established that NK cells are the main effector cells in ADCC [1] and that NK cell-mediated ADCC is dysfunctional in HIV patients [28].

IMMUNE ESCAPE

HIV-1 employs several methods to avoid detection by the human immune system. The HIV-1 regulatory protein Nef,

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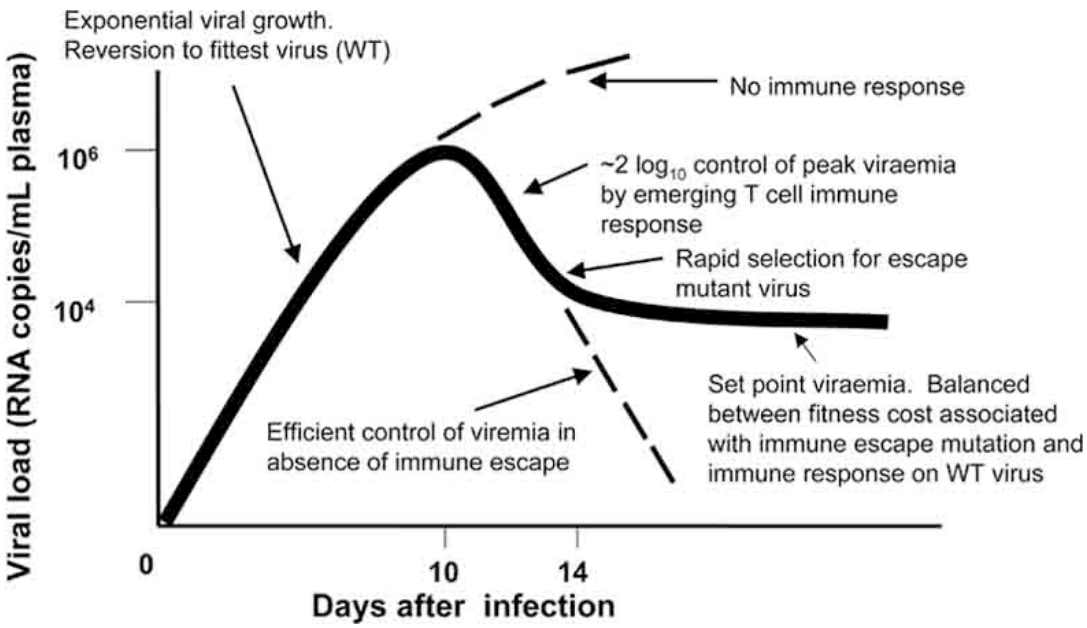

Fig. (1). Schematic diagram of partial control of viremia during acute HIV infection. Potential scenarios of the absence of effective immunity such as by CD8 T cell depletion [51], and effective immune responses such as those that are not readily escaped during acute infection [38, 44] are shown by the dashed and dotted lines respectively.

not only hinders MHC-I progress towards the cell surface and recognition by CTL, but also results in internalisation and endosomal degradation of many MHC-I molecules [21, 75]. MHC class I deficiency should lead to targeting by NK cells, however, HIV-1 allows normal expression of HLA-C and HLA-E which inhibits NK cells [20, 61].

Mutational escape from T cell immunity in HIV was first demonstrated in 1991 [65]. With an unreliable reverse transcriptase and incompetent repair mechanisms [12], the high rate of HIV-1 replication provides numerous opportunities for the Darwinian selection of valuable mutations [5]. High rates of genetic recombination between strains and subtypes of HIV-1 compound problems of immune escape [17, 49]. CD8+ T cell escape probably accounts for a considerable proportion, if not most of all viral diversity [4, 56].

Numerous studies have shown that CTL escape occurs at dominant T cell epitopes derived from Env [10, 42] and all other HIV-1 proteins. Indeed, escape is considered a "hallmark of acute infection" [59]. Typical T cell escape mutations cause the presented epitope to be sufficiently different as to be no longer bound by the presenting MHC class I, or recognisable by the TCR of the specific CTL [69].

Escape from CD4+ T cells has been shown in mice infected with the RNA virus lymphocytic choriomeningitis virus [18], suggested by a "directional change" in viral sequence [64]. This phenomenon of CD4+ T cell escape has not yet been described in HIV models, despite numerous reports of SIV and HIV-specific CD4 T cells [8, 73]. A recent study found that viral escape was not linked to poor HIV-1 Env-specific Th responses in the patients studied [52]. Two reasons might account for this. Firstly, the CD4+ response may not be effective. This appears unlikely, as the potent CD8+ immune response usually mounted in acute infection requires CD4+ T cell help [43]. Secondly, the CD4+ T cells are not generally cytolytic; mounting direct, selective pressure. Consequently, it may not be necessary for the virus to mutate at CD4 T cell epitopes having already evaded the immune response through mutation at CD8 epitopes.

Env is unique among the HIV genes in being targeted by both NAb and T cell responses. Structural investigations of the surface Env protein gp120 have elicited 4 distinct faces: a non-neutralising and a neutralising face, a variable face and a silent face [48]. Changes are wrought in the Env depending on whether it is in its liganded or unliganded state. Refolding of gp120 on binding CD4 will present multiple, different antigenic molecules to a host's humoral immune system [16]. A major hope for a broadly effective NAb target would be antigens within the neutralising face [83], but it, like the silent and variable faces is subject to an "evolving glycan shield", where selected mutations in Env-directed sugar packing prevent attachment by NAb [82]. Highlighting serial NAb escape at Env epitopes, *in vivo* studies have shown that sera from HIV infected subjects could neutralize past viral quasispecies, but not the concurrent or future variants of the virus [70]. The vast array of mutations HIV-1 Env incurs to keep ahead of these rapidly evolving NAb responses [70, 82] likely incurs at least modest, but serial fitness costs.

FITNESS COST OF VIRAL ESCAPE

There is a growing appreciation that immune pressure to mutate away from wildtype (WT) virus usually elicits an appreciable reduction in the comparative replicative capacity ("fitness") of the mutant virus. The fitness impact of mutations was first studied in detail following acquisition of drug resistant mutations [71], many of which result in impaired fitness during *in vitro* competition assays, a delay in disease and reversion to WT sequence following withdrawal of drug pressure [58]. Viral escape is well documented from the popular nucleoside reverse

transcriptase inhibitor lamivudine [11], but a consequent decrease in viral fitness has now also been established [81].

Heterosexual transmission is largely responsible for the AIDS pandemic, yet actual transmission rates per act of sexual intercourse are low [67]. Subtype C HIV-1 strains transmitted during acute infection can have shorter variable regions in Env [25]. This should reduce the number of N-linked glycosylation sites and enable easier access to receptor sites on the recipient's immune cells. However, this also renders the virus highly sensitive to NAb. Contrasting results were found in a study of homosexual transmission of HIV-1 subtype B, where transmission of a viral quasispecies that was more susceptible to the recipient's NAb occurred infrequently [33]. Whether this contrast is due to mode of transmission, the subtype of the virus or the diversity of quasispecies in the source is unclear [33]. The "transmission" of neutralisation sensitive HIV-1 subtype C strains [25] could also be interpreted as reversion of NAb escape variants to WT during acute infection (as in Fig. **2C** where MHC matching is not relevant since this is a humoral response). Note that reversion of T cell escape mutations can be very rapid – completed by 14 days post infection [30], well before the vast majority of HIV-1 infected subjects are identified and have samples taken for genetic analysis. Thus the identified "transmitted" strain may contain substantial numbers of reversion mutations of the escaped virus.

Recently, the costs of T cell and possibly NAb immune escape have become clearer. CD8+ T cell immune escape variants generally revert to WT upon passage to a new host that cannot respond to the same epitope, both in macaques with SIV/SHIV infection [30, 32] and humans with HIV-1 infection [3]. A reduced SIV viral load occurs in pigtail macaques which respond to the immunodominant Gag epitope KP9 [77], even in animals where the virus has escaped this epitope [78]. SIV/SHIV escape mutants can eventually cause disease, however this is likely to take longer in comparison to WT virus infection effectively controlled by CTL [7].

It has been relatively difficult until recently to quantify the fitness impact of T cell escape mutations *in vivo.* However, the impact of viral mutations can be calculated by the rate of reversion back to WT *in vivo*, in the absence of CTL pressure, when virus is transmitted between hosts not sharing MHC alleles [30] (Fig. **2B**). Those mutations that prove more costly in terms of viral replication should revert rapidly back to WT when immune pressure is lifted, whilst those with minimal impact will revert slowly [45]. This is a simplistic view of T cell escape mutations and their fitness cost, since any fitness reductions may be alleviated to some extent by compensatory mutations elsewhere in the genome [35]. Interestingly, transient reversion and re-escape is likely to ensue when escape mutant (EM) virus is transmitted to a host that eventually generates a T cell response at this epitope [45] (Fig. **2C**).

CONSTRAINTS ON IMMUNE ESCAPE

Reversion of immune escape variants to WT upon transmission, in the absence of immune pressure, implies a fitness cost of the immune escape mutation. That is, the amino acid change made by the escape mutation results in some functional deficit to the protein, commonly through the resultant change in secondary structure [76]. However, HIV is not infinitely malleable. Indeed, escape from most T cell responses follow highly defined escape motifs, even at the nucleotide level [46]. This suggests that the common escape motifs result in the best balance of high immune escape and low fitness cost.

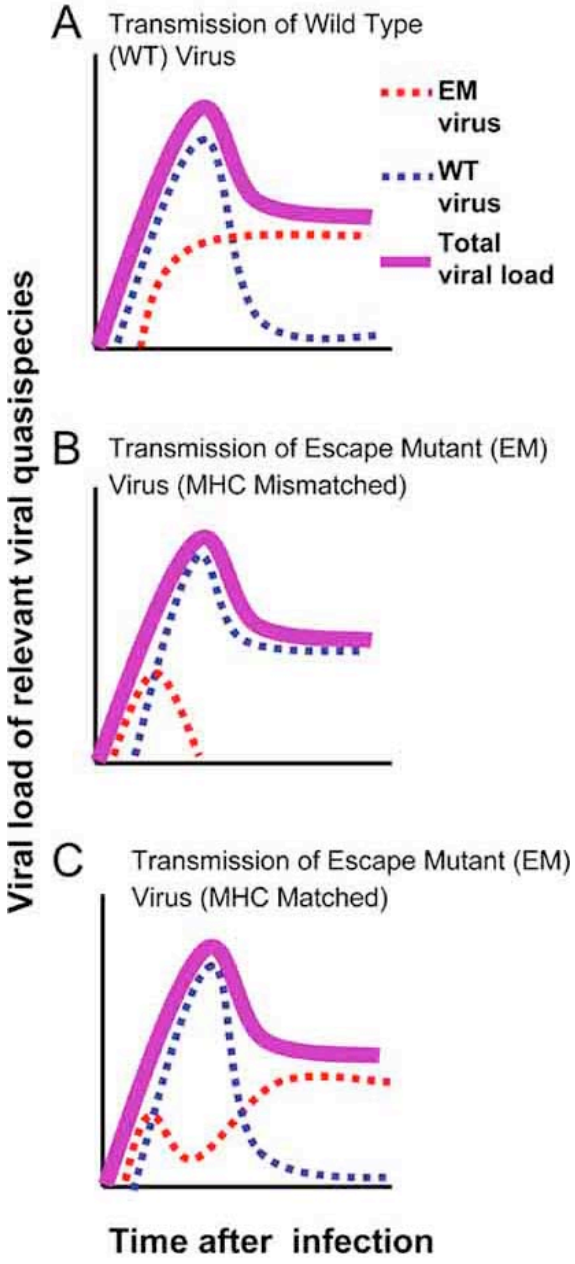

Fig. (2). A. Emergence of T cell escape mutant (EM) variant as a result of the CD8+ T cell response during acute infection with WT virus. The EM virus dominates subsequent virus population.

B. Transmission of EM virus to an MHC-mismatched recipient which cannot generate a T cell response to the relevant epitope results in reversion to WT and disappearance of the EM population.

C. Transmission of EM virus to an MHC-matched recipient which can generate a T cell response to the WT epitope. Since no HIV-specific T cells are present at the time of transmission, initial reversion of the EM virus to the WT occurs (similar to B). However, as a T cell response is generated, re-escape to EM virus occurs (as in A).

As more escape mutations are acquired, a progressive loss of fitness would be expected to ensue, assuming only partial compensation of the fitness deficit incurred by the initial mutations. However, only a limited number of T cell epitopes are recognised by any one host. Hosts generating T cell responses that recognise broader arrays of epitopes, particularly if some are targeted at conserved regions, are likely to impart a greater fitness cost to the virus as escape ensues at most, or all epitopes. This is broadly supported by the finding that subjects with greater numbers of different MHC class I alleles (heterozygous at each of the HLA-A, B and C loci), control HIV viremia more effectively than homozygotes [15]. Only rare cases have been reported where viremia is controlled by a narrowly focused CD8 T cell response [29].

ENVELOPE AS A TARGET FOR BOTH T CELLS AND NAb

Env is a highly variable protein, therefore intuitively not an attractive target for generating T cell immunity. Unexpectedly perhaps, several macaque studies have now demonstrated a significant additional benefit in control of SIV or SHIV viremia when Env genes are added to T cell-based vaccine regimes [6, 26, 68]. This occurs despite any clear induction of NAb by the vaccines to the challenge virus. Our own recent studies have also demonstrated significant protection from B clade Env-expressing T cell based DNA/fowlpox virus (FPV) prime/boost regimes despite a heterologous B clade SHIV challenge [23]. This occurred despite the Env genes being deleted in the middle third of the protein so no NAb could be induced prior to challenge. Interestingly, this level of protection was substantially less when an AE Env gene was expressed by the DNA/FPV vaccine, and the same B clade SHIV challenge (Kent *et al*. unpublished data). Only modest cross-type recognition of Env-specific T cell epitopes (2 of 10 Env epitopes were recognised by both subtype B and AE derived peptides) was generated in that study (Peut *et al*. unpublished data). Further, Musey *et al.* showed that Env-specific CTL during acute HIV-1 infection correlated with reduced viral load [57]. Taken together, these studies suggest Env-specific T cells may be useful despite the heterogeneity of this protein.

In the successful macaque studies cited above, Env expressing vaccines produced significant (and likely helpful) levels of NAb to the heterologous challenge viruses. This response was generated early after challenge and was much higher and broader than in control animals. The ability to generate these NAb reflects, in part, the lack of significant, early immunosuppression of these primarily CXCR4-utilising SHIV challenges in the vaccines. None-the-less, the virus now faces dual pressure at the Env protein from both T cells and NAb.

The forces of both CD8+ T cells and NAb during acute infection are highly likely to generate immune escape variants. Escape from Env-specific CD8 T cells in humans has previously been documented [10, 42] and we have recently identified common escape mutations at Env-specific CD8 T cell epitopes in vaccinated and SHIV-challenged pigtailed macaques (Peut *et al*. unpublished data). Defining escape motifs from particular NAb responses has been less well studied, although it undoubtedly exists [70, 82]. Trkola and colleagues showed that infusion of defined, broadly reactive NAb resulted in biological NAb escape in some subjects [79]. Haigwood and colleagues recently reported the evolution of N-linked glycosylation mutants [36].

For adequate function, Env must be a highly conformational, flexible protein. We speculate that there are likely to be significant constraints on the ability of Env to mutate away from broadly targeted CD8+ T cell and NAb responses and still maintain functional integrity of the protein. The fitness deficit incurred with mutations away from both parts of the adaptive immune response may partially explain the encouraging outcomes associated with Env-expressing T cell – based S/HIV vaccine regimes.

Ultimately, using knowledge on which particular sets of Env-specific T cells and NAb impart the greatest "fitness barrier" to overcome if escape ensues, could have a significant effect on rational HIV vaccine design. We are currently grappling with defining both T cell and NAb escape in macaques that control SIV or SHIV replication well. Some of these highly escaped viruses are now being reintroduced to naïve macaques to study their rate of reversion to WT, and thereby ameliorate their *in vivo* fitness cost [45]. Such data should guide the selection of antigens to induce immune responses which incur the highest fitness cost of escape.

CONCLUSION

With the remarkable genetic plasticity of HIV-1, it is possible that an untreated patient has every possible viral genome circulating through their body [60]. There are, however, structural and functional constraints to mutation levels in response to immune pressure [4]. Compensatory mutations can partially ameliorate these constraints [44, 62], but may incur their own fitness costs. We hypothesise that as the virus mutates away from immune responses, it incurs a fitness cost that will, at least in some subjects, slow the course of the disease. Env is the only HIV-1 protein targeted by both NAb and cellular arms of the adaptive immune system. Focusing research on Env, its structural biology, function and NAb interactions [14] as well as the epitopes presented to effector T cells could favourably influence vaccine design.

ABBREVIATIONS

ADCC = Antibody-dependent cellular cytotoxicity

ART = Antiretroviral therapy

CTL = Cytotoxic T lymphocyte

EM = Escape mutant

Env = Envelope

FPV = Fowlpox virus

HLA = Human leukocyte antigen

MHC = Major histocompatibility complex

NAb = Neutralising antibody

NK = Natural killer cells

SHIV = Simian human immunodeficiency virus

SIV = Simian immunodeficiency virus

TCR = T cell receptor

Th = T helper

WT = Wildtype

REFERENCES

[1] Ahmad A, Menezes J. (1996). Antibody-dependent cellular cytotoxicity in HIV infections. Faseb Journal. 10: 258-266.

[2] Alfano M, Poli G. (2005). Role of cytokines and chemokines in the regulation of innate immunity and HIV infection. Molecular Immunology. 42: 161-182.

[3] Allen TM, Altfeld M, Yu XG, O'Sullivan KM, Lichterfeld M, Le Gall S, John M, Mothe BR, Lee PK, Kalife ET, Cohen DE, Freedberg KA, Strick DA, Johnston MN, Sette A, Rosenberg ES, Mallal SA, Goulder PJR, Brander C, Walker BD. (2004). Selection, transmission, reversion of an antigen-processing cytotoxic T-lymphocyte escape mutation in human immunodeficiency virus type 1 infection. Journal of Virology. 78: 7069-7078.

[4] Allen TM, Yu XG, Kalife ET, Reyor LL, Lichterfeld M, John M, Cheng M, Allgaier RL, Mui S, Frahm N, Alter G, Brown NV, Johnston MN, Rosenberg ES, Mallal SA, Brander C, Walker BD, Altfeld M. (2005). *De novo* generation of escape variant-specific CD8(+) T-cell responses following cytotoxic T-lymphocyte escape in chronic human immunodeficiency virus type 1 infection. Journal of Virology. 79: 12952-12960.

[5] Altman JD, Feinberg MB. (2004). HIV escape: there and back again. Nature Medicine. 10: 229-230.

[6] Amara RR, Smith JM, Staprans SI, Montefiori DC, Villinger F, Altman JD, O'Neil SP, Kozyr NL, Xu Y, Wyatt LS, Earl PL, Herndon JG, McNicholl JM, McClure HM, Moss B, Robinson HL. (2002). Critical role for Env as well as Gag-Pol in control of a simian-human immunodeficiency virus 89.6P challenge by a DNA prime/recombinant modified vaccinia virus Ankara vaccine. Journal of Virology. 76: 6138-6146.

[7] Barouch DH, Kunstman J, Kuroda MJ, Schmitz JE, Santra S, Peyerl FW, Krivulka GR, Beaudry K, Lifton MA, Gorgone DA, Montefiori DC, Lewis MG, Wolinsky SM, Letvin NL. (2002). Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature. 415: 335-339.

[8] Betts MR, Ambrozak DR, Douek DC, Bonhoeffer S, Brenchley JM, Casazza JP, Koup RA, Picker LJ. (2001). Analysis of total human immunodeficiency virus (HIV)-specific CD4(+) and CD8(+) T-cell responses: Relationship to viral load in untreated HIV infection. Journal of Virology. 75: 11983-11991.

[9] Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MBA. (1994). Virus-specific CD8+ cytotoxic T lymphocyte activity associated with control of viremia in primary Human-Immunodeficiency-Virus type-1 infection. Journal of Virology. 68: 6103-6110.

[10] Borrow P, Lewicki H, Wei XP, Horwitz MS, Peffer N, Meyers H, Nelson JA, Gairin JE, Hahn BH, Oldstone MBA, Shaw GM. (1997). Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nature Medicine. 3: 205-211.

[11] Boucher CAB, Cammack N, Schipper P, Schuurman R, Rouse P, Wainberg MA, Cameron JM. (1993). High-level resistance to (-) Enantiomeric 2'-Deoxy-3'-Thiacytidine *in vitro* is due to one amino-acid substitution in the catalytic site of Human-Immunodeficiency-Virus Type-1 reverse-transcriptase. Antimicrobial Agents and Chemotherapy. 37: 2231-2234.

[12] Brander C, Walker BD. (2003). Gradual adaptation of HIV to human host populations: good or bad news? Nature Medicine. 9: 1359-1362.

[13] Burton DR, Desrosiers RC, Doms RW, Feinberg MB, Gallo RC, Hahn B, Hoxie JA, Hunter E, Korber B, Landay A, Lederman MM, Lieberman J, McCune JM, Moore JP, Nathanson N, Picker L, Richman D, Rinaldo C, Stevenson M, Watkins DI, Wolinsky SM, Zack JA. (2004). Public health - A sound rationale needed for phase III HIV-1 vaccine trials. Science. 303: 316-316.

[14] Burton DR, Desrosiers RC, Doms RW, Koff WC, Kwong PD, Moore JP, Nabel GJ, Sodroski J, Wilson IA, Wyatt RT. (2004). HIV vaccine design and the neutralizing antibody problem. Nature Immunology. 5: 233-236.

[15] Carrington M, Nelson GW, Martin MP, Kissner T, Vlahov D, Goedert JJ, Kaslow R, Buchbinder S, Hoots K, O'Brien SJ. (1999). HLA and HIV-1: Heterozygote advantage and B*35-Cw*04 disadvantage. Science. 283: 1748-1752.

[16] Chen B, Vogan EM, Gong HY, Skehel JJ, Wiley DC, Harrison SC. (2005). Structure of an unliganded simian immunodeficiency virus gp120 core. Nature. 433: 834-841.

[17] Chin MPS, Rhodes TD, Chen JB, Fu W, Hu WS. (2005). Identification of a major restriction in HIV-1 intersubtype recombination. Proceedings of The National Academy of Sciences of the United States of America. 102: 9002-9007.

[18] Ciurea A, Hunziker L, Martinic MMA, Oxenius A, Hengartner H, Zinkernagel RM. (2001). CD4(+) T-cell-epitope escape mutant virus selected *in vivo*. Nature Medicine. 7: 795-800.

[19] Cocchi F, Devico AL, Garzinodemo A, Arya SK, Gallo RC, Lusso P. (1995). Identification of Rantes, Mip-1-Alpha, Mip-1-Beta as the major HIV-suppressive factors produced by CD8(+) T-cells. Science. 270: 1811-1815.

[20] Cohen GB, Gandhi RT, Davis DM, Mandelboim O, Chen BK, Strominger JL, Baltimore D. (1999). The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. Immunity. 10: 661-671.

[21] Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. (1998). HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. Nature. 391: 397-401.

[22] Daar ES, Moudgil T, Meyer RD, Ho DD. (1991). Transient high levels of viremia in patients with primary Human-Immunodeficiency-Virus Type-1 infection. New England Journal of Medicine. 324: 961-964.

[23] Dale CJ, De Rose R, Stratov I, Chea S, Montefiori DC, Thomson S, Ramshaw IA, Coupar BEH, Boyle DB, Law M, Kent SJ. (2004). Efficacy of DNA and fowlpox virus priming/boosting vaccines for simian/human immunodeficiency virus. Journal of Virology. 78: 13819-13828.

[24] Davenport MP, Ribeiro RM, Perelson AS. (2004). Kinetics of virus-specific CD8(+) T cells and the control of human immunodeficiency virus infection. Journal of Virology. 78: 10096-10103.

[25] Derdeyn CA, Decker JM, Bibollet-Ruche F, Mokili JL, Muldoon M, Denham SA, Heil ML, Kasolo F, Musonda R, Hahn BH, Shaw GM, Korber BT, Allen S, Hunter E. (2004). Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science. 303: 2019-2022.

[26] Doria-Rose NA, Ohlen C, Polacino P, Pierce CC, Hensel MT, Kuller L, Mulvania T, Anderson D, Greenberg PD, Hu SL, Haigwood NL. (2003). Multigene DNA priming-boosting vaccines protect macaques from acute CD4(+)-T-cell depletion after simian-human immunodeficiency virus SHIV89.6P mucosal challenge. Journal of Virology. 77: 11563-11577.

[27] Douek DC, Brenchley JM, Betts MR, Ambrozak DR, Hill BJ, Okamoto Y, Casazza JP, Kuruppu J, Kuntsman K, Wolinsky S, Grossman Z, Dybul M, Oxenius A, Price DA, Connors M, Koup RA. (2002). HIV preferentially infects HIV-specific CD4(+) T cells. Nature. 417: 95-98.

[28] Fauci AS, Mavilio D, Kottilil S. (2005). NK cells in HIV infection: Paradigm for protection or targets for ambush. Nature Reviews Immunology. 5: 835-842.

[29] Feeney ME, Tang Y, Roosevelt KA, Leslie AJ, McIntosh K, Karthas N, Walker BD, Goulder PJR. (2004). Immune escape precedes breakthrough human immunodeficiency virus type 1 viremia and broadening of the cytotoxic T-lymphocyte response in an HLA-B27-positive long-term-nonprogressing child. Journal of Virology. 78: 8927-8930.

[30] Fernandez CS, Stratov I, De Rose R, Walsh K, Dale CJ, Smith MZ, Agy MB, Hu SL, Krebs K, Watkins DI, O'Connor DH, Davenport MP, Kent SJ. (2005). Rapid viral escape at an immunodominant simian-human immunodeficiency virus cytotoxic T-lymphocyte epitope exacts a dramatic fitness cost. Journal of Virology. 79: 5721-5731.

[31] Forthal DN, Landucci G, Phan TB, Becerra J. (2005). Interactions between natural killer cells and antibody Fc result in enhanced antibody neutralization of human immunodeficiency virus type 1. Journal of Virology. 79: 2042-2049.

[32] Friedrich TC, Dodds EJ, Yant LJ, Vojnov L, Rudersdorf R, Cullen C, Evans DT, Desrosiers RC, Mothe BR, Sidney J, Sette A, Kunstman K, Wolinsky S, Piatak M, Lifson J, Hughes AL, Wilson

N, O'Connor DH, Watkins DI. (2004). Reversion of CTL escape-variant immunodeficiency viruses *in vivo*. Nature Medicine. 10: 275-281.

[33] Frost SDW, Liu Y, Pond SLK, Chappey C, Wrin T, Petropoulos CJ, Little SJ, Richman DD. (2005). Characterization of human immunodeficiency virus type 1 (HIV-1) envelope variation and neutralizing antibody responses during transmission of HIV-1 subtype B. Journal of Virology. 79: 6523-6527.

[34] Gomez-Roman VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, Florese R, Robert-Guroff M. (2005). Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. Journal of Immunology. 174: 2185-2189.

[35] Goulder PJR, Watkins DI. (2004). HIV and SIVCTL escape: Implications for vaccine design. Nature Reviews Immunology. 4: 630-640.

[36] Haigwood N, Stamatatos L, Doria-Rose NA, Blay W. 2004. N-linked glycosylation sites undergo predictable patterns of variation within constrained regions of HIV-1 Envelope (abstract 179), AIDS Vaccine 2004, Lausanne.

[37] Haigwood NL, Montefiori DC, Sutton WF, McClure J, Watson AJ, Voss G, Hirsch VM, Richardson BA, Letvin NL, Hu SL, Johnson PR. (2004). Passive immunotherapy in simian immunodeficiency virus-infected macaques accelerates the development of neutralizing antibodies. Journal of Virology. 78: 5983-5995.

[38] Hel Z, Nacsa J, Tryniszewska E, Tsai WP, Parks RW, Montefiori DC, Felber BK, Tartaglia J, Pavlakis GN, Franchini G. (2002). Containment of simian immunodeficiency virus infection in vaccinated macaques: Correlation with the magnitude of virus-specific pre- and postchallenge CD4(+) and CD8(+) T cell responses. Journal of Immunology. 169: 4778-4787.

[39] Hel Z, Venzon D, Poudyal M, Tsai WP, Giuliani L, Woodward R, Chougnet C, Shearer G, Altman JD, Watkins D, Bischofberger N, Abimiku A, Markham P, Tartaglia J, Franchini G. (2000). Viremia control following antiretroviral treatment and therapeutic immunization during primary SIV251 infection of macaques. Nature Medicine. 6: 1140-1146.

[40] Horton H, Vogel T, O'Connor D, Picker L, Watkins DI. (2002). Analysis of the immune response and viral evolution during the acute phase of SIV infection. Vaccine. 20: 1927-1932.

[41] Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, Blanchard J, Irwin CE, Safrit JT, Mittler J, Weinberger L, Kostrikis LG, Zhang LQ, Perelson AS, Ho DD. (1999). Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. Journal of Experimental Medicine. 189: 991-998.

[42] Jones NA, Wei XP, Flower DR, Wong ML, Michor F, Saag MS, Hahn BH, Nowak MA, Shaw GM, Borrow P. (2004). Determinants of human immunodeficiency virus type 1 escape from the primary CD8(+) cytotoxic T lymphocyte response. Journal of Experimental Medicine. 200: 1243-1256.

[43] Kalams SA, Buchbinder SP, Rosenberg ES, Billingsley JM, Colbert DS, Jones NG, Shea AK, Trocha AK, Walker BD. (1999). Association between virus-specific cytotoxic T-lymphocyte and helper responses in human immunodeficiency virus type 1 infection. Journal of Virology. 73: 6715-6720.

[44] Kelleher AD, Long C, Holmes EC, Allen RL, Wilson J, Conlon C, Workman C, Shaunak S, Olson K, Goulder P, Brander C, Ogg G, Sullivan JS, Dyer W, Jones I, McMichael AJ, Rowland-Jones S, Phillips RE. (2001). Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses. Journal of Experimental Medicine. 193: 375-385.

[45] Kent SJ, Fernandez CS, Dale CJ, Davenport MP. (2005). Reversion of immune escape HIV variants upon transmission: insights into effective viral immunity. Trends in Microbiology. 13: 243-246.

[46] Kijak GH, Currier JR, Tovanabutra S, Cox JH, Michael NL, Wegner SA, Birx DL, McCutchan FE. (2004). Lost in translation: Implications of HIV-1 codon usage for immune escape and drug resistance. Aids Reviews. 6: 54-60.

[47] Koup RA, Safrit JT, Cao YZ, Andrews CA, McLeod G, Borkowsky W, Farthing C, Ho DD. (1994). Temporal association of cellular immune-responses with the initial control of viremia in primary Human-Immunodeficiency-Virus Type-1 syndrome. Journal of Virology. 68: 4650-4655.

[48] Kwong PD, Doyle ML, Casper DJ, Cicala C, Leavitt SA, Majeed S, Steenbeke TD, Venturi M, Chaiken I, Fung M, Katinger H, Parren P, Robinson J, Van Ryk D, Wang LP, Burton DR, Freire E, Wyatt R, Sodroski J, Hendrickson WA, Arthos J. (2002). HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. Nature. 420: 678-682.

[49] Levy DN, Aldrovandi GM, Kutsch O, Shaw GM. (2004). Dynamics of HIV-1 recombination in its natural target cells. Proceedings of The National Academy of Sciences of the United States of America. 101: 4204-4209.

[50] Li Q, Duan L, Estes JD, Ma Z-M, Rourke T, Wang Y, Reilly C, Carlis J, Miller CJ, Haase AT. (2005). - Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. Nature. 434: 1148-1152.

[51] Madden LJ, Zandonatti MA, Flynn CT, Taffe MA, Marcondes MCG, Schmitz JE, Reimann KA, Henriksen SJ, Fox HS. (2004). CD8+cell depletion amplifies the acute retroviral syndrome. Journal of Neurovirology. 10: 58-66.

[52] Malhotra U, Holte S, Zhu TF, Delpit E, Huntsberry C, Sette A, Shankarappa R, Maenza J, Corey L, McElrath MJ. (2003). Early induction and maintenance of Env-specific T-helper cells following human immunodeficiency virus type 1 infection. Journal of Virology. 77: 2663-2674.

[53] Mascola JR, Sambor A, Beaudry K, Santra S, Welcher B, Louder MK, VanCott TC, Huang Y, Chakrabarti BK, Kong WP, Yang ZY, Xu L, Montefiori DC, Nabel GJ, Letvin NL. (2005). Neutralizing antibodies elicited by immunization of monkeys with DNA plasmids and recombinant adenoviral vectors expressing human immunodeficiency virus type 1 proteins. Journal of Virology. 79: 771-779.

[54] Mattapalli JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. (2005). Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature. 434: 1093-1097.

[55] Montefiori DC, Pantaleo G, Fink LM, Zhou JT, Zhou JY, Bilska M, Miralles GD, Fauci AS. (1996). Neutralizing and infection-enhancing antibody responses to human immunodeficiency virus type 1 in long-term nonprogressors. Journal of Infectious Diseases. 173: 60-67.

[56] Moore CB, John M, James IR, Christiansen FT, Witt CS, Mallal SA. (2002). Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level. Science. 296: 1439-1443.

[57] Musey L, Hughes J, Schacker T, Shea T, Corey L, McElrath MJ. (1997). Cytotoxic-T-cell responses, viral load, disease progression in early human immunodeficiency virus type 1 infection. New England Journal of Medicine. 337: 1267-1274.

[58] Nijhuis M, Deeks S, Boucher C. (2001). Implications of antiretroviral resistance on viral fitness. Current Opinion in Infectious Diseases. 14: 23-28.

[59] O'Connor DH, Allen TM, Vogel TU, Jing PC, DeSouza IP, Dodds E, Dunphy EJ, Melsaether C, Mothe B, Yamamoto H, Horton H, Wilson N, Hughes AL, Watkins DI. (2002). Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. Nature Medicine. 8: 493-499.

[60] Overbaugh J, Bangham CRM. (2001). Selection forces and constraints on retroviral sequence variation. Science. 292: 1106-1109.

[61] Peterlin BM, Trono D. (2003). Hide, shield and strike back: How HIV-infected cells avoid immune eradication. Nature Reviews Immunology. 3: 97-107.

[62] Peyerl FW, Barouch DH, Yeh WW, Bazick HS, Kunstman J, Kunstman KJ, Wolinsky SM, Letvin NL. (2003). Simian-human immunodeficiency virus escape from cytotoxic T-lymphocyte recognition at a structurally constrained epitope. Journal of Virology. 77: 12572-12578.

[63] Phillips AN. (1996). Reduction of HIV concentration during acute infection: Independence from a specific immune response. Science. 271: 497-499.

[64] Phillips RE, Harcourt GC, Price DA. (2001). CD4(+) T cells: The great escape. Nature Medicine. 7: 777-778.

[65] Phillips RE, Rowlandjones S, Nixon DF, Gotch FM, Edwards JP, Ogunlesi AO, Elvin JG, Rothbard JA, Bangham CRM, Rizza CR, McMichael AJ. (1991). Human-Immunodeficiency-Virus genetic-variation that can escape cytotoxic T-cell recognition. Nature. 354: 453-459.

[66] Picker LJ, Watkins DI. (2005). HIV pathogenesis: The first cut is the deepest. Nature Immunology. 6: 430-432.

[67] Piot P, Bartos M, Ghys PD, Walker N, Schwartlander B. (2001). The global impact of HIV/AIDS. Nature. 410: 968-973.

[68] Polacino PS, Stallard V, Klaniecki JE, Pennathur S, Montefiori DC, Langlois AJ, Richardson BA, Morton WR, Benveniste RE, Hu SL. (1999). Role of immune responses against the envelope and the core antigens of simian immunodeficiency virus SIVmne in protection against homologous cloned and uncloned virus challenge in macaques. Journal of Virology. 73: 8201-8215.

[69] Price DA, West SM, Betts MR, Ruff LE, Brenchley JM, Ambrozak DR, Edghill-Smith Y, Kuroda MJ, Bogdan D, Kunstman K, Letvin NL, Franchini G, Wolinsky SM, Koup RA, Douek DC. (2004). T cell receptor recognition motifs govern immune escape patterns in acute SIV infection. Immunity. 21: 793-803.

[70] Richman DD, Wrin T, Little SJ, Petropoulos CJ. (2003). Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proceedings of The National Academy of Sciences of the United States of America. 100: 4144-4149.

[71] Rooke R, Tremblay M, Soudeyns H, Destephano L, Yao XJ, Fanning M, Montaner JSG, Oshaughnessy M, Gelmon K, Tsoukas C, Gill J, Ruedy J, Wainberg MA. (1989). Isolation of drug-resistant variants of HIV-1 from patients on long-term Zidovudine therapy. Aids. 3: 411-415.

[72] Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, Walker BD. (1997). Vigorous HIV-1-specific CD4(+) T cell responses associated with control of viremia. Science. 278: 1447-1450.

[73] Sarkar S, Kalia V, Murphey-Corb M, Montelaro RC. (2002). Detailed analysis of CD4(+) Th responses to envelope and Gag proteins of simian immunodeficiency virus reveals an exclusion of broadly reactive Th epitopes from the glycosylated regions of envelope. Journal of Immunology. 168: 4001-4011.

[74] Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Dalesandro M, Scallon BJ, Ghrayeb J, Forman MA, Montefiori DC, Rieber EP, Letvin NL, Reimann KA. (1999). Control of viremia in simian immunodeficiency virus infection by CD8(+) lymphocytes. Science. 283: 857-860.

[75] Schwartz O, Marechal V, LeGall S, Lemonnier F, Heard JM. (1996). Endocytosis of major histocompatibility complex class I molecules is induced by the HIV-1 Nef protein. Nature Medicine. 2: 338-342.

[76] Sewell AK, Price DA, Oxenius A, Kelleher AD, Phillips RE. (2000). Cytotoxic T lymphocyte responses to human immunodeficiency virus: Control and escape. Stem Cells. 18: 230-244.

[77] Smith MZ, Dale CJ, De Rose R, Stratov I, Fernandez CS, Brooks AG, Weinfurter J, Krebs K, Riek C, Watkins DI, O'Connor DH, Kent SJ. (2005). Analysis of pigtail macaque major histocompatibility complex class I molecules presenting immunodominant simian immunodeficiency virus epitopes. Journal of Virology. 79: 684-695.

[78] Smith MZ, Fernandez CS, Chung A, Dale CJ, De Rose R, Lin J, Brooks AG, Krebs KC, Watkins DI, O'Connor DH, Davenport MP, Kent SJ. (2005). The pigtail macaque MHC class I allele Mane-A*10 presents an immundominant SIV Gag epitope: identification, tetramer development and implications of immune escape and reversion. Journal of Medical Primatology. 34: 282-293.

[79] Trkola A, Kuster H, Rusert P, Joos B, Fischer M, Leemann C, Manrique A, Huber M, Rehr M, Oxenius A, Weber R, Stiegler G, Vcelar B, Katinger H, Aceto L, Gunthard HF. (2005). Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nature Medicine. 11: 615-622.

[80] Veazey RS, Shattock RJ, Pope M, Kirijan JC, Jones J, Hu QX, Ketas T, Marx PA, Klasse PJ, Burton DR, Moore JP. (2003). Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. Nature Medicine. 9: 343-346.

[81] Wei X, Liang C, Gotte M, Wainberg MA. (2003). Negative effect of the M184V mutation in HIV-1 reverse transcriptase on initiation of viral DNA synthesis. Virology. 311: 202-212.

[82] Wei XP, Decker JM, Wang SY, Hui HX, Kappes JC, Wu XY, Salazar-Gonzalez JF, Salazar MG, Kilby JM, Saag MS, Komarova NL, Nowak MA, Hahn BH, Kwong PD, Shaw GM. (2003). Antibody neutralization and escape by HIV-1 (vol 422, pg 307, 2003). Nature. 423: 197-197.

[83] Wyatt R, Kwong PD, Desjardins E, Sweet RW, Robinson J, Hendrickson WA, Sodroski JG. (1998). The antigenic structure of the HIV gp120 envelope glycoprotein. Nature. 393: 705-711.

Received: November 10, 2005 **Revised: December 8, 2005** **Accepted: December 9, 2005**