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Host Factors Involved in Low Susceptibility to HIV Infection

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Abstract

There are individuals who despite being exposed to HIV, in some cases repeatedly and over long periods, remain without HIV seroconversion. These individuals are called HIV-exposed seronegatives, and have been reported among commercial sex workers, men having sex with men, injection drug users, hemophiliacs who received contaminated blood preparations, healthcare workers with accidental percutaneous exposure to HIV-infected blood, infants born to HIV-infected mothers, and individuals having HIV-positive heterosexual partners. Genetic and immunological mechanisms have been involved in the production of this resistance to HIV acquisition. Genetic factors have been linked to genes encoding chemokine receptors and their natural ligands as well as genes of the major histocompatibility complex. Immunological factors are grouped within both innate and adaptive immunity. The study of HIV-exposed seronegatives provides a unique opportunity to understand the mechanisms of natural protection against HIV infection. The better understanding of this protection may lead to novel preventive and immunotherapeutic approaches, including vaccines. **(AIDS Rev. 2011;13:30-40)**

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Key words

HIV. ESN. Resistance. Genetic factors. Immune response.

Introduction

There is a considerable variation in susceptibility to HIV infection. Despite multiple and repeated unprotected exposure to HIV, some individuals remain HIV seronegative[1]. These exposed but seronegative individuals (ESN) include commercial sex workers[2], men having sex with men[3], injection drug users[4], hemophiliacs who received contaminated blood products[5], healthcare workers with accidental percutaneous exposure to infected blood[6], infants born to HIV-infected mothers[7], and individuals with HIV-infected heterosexual partners[8-10]. There is no clear explanation for this low susceptibility to HIV infection. Studies of these ESN individuals have documented a number of probable host factors, including coreceptor susceptibility and innate and adaptive immunity[11]. However, none of these has identified a single genetic or immunological mechanism to explain this phenomenon fully.

The ESN individuals represent a model to study potential host factors associated with viral control, which can be used for the design of immunotherapeutic agents for the control of infection in infected subjects. This review will focus on the genetic and immunological factors that have been associated with the phenomenon of low susceptibility to infection observed in different cohorts of ESN individuals.

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Genetic factors involved in low susceptibility to HIV infection

Human leukocyte antigen polymorphisms and killer immunoglobulin-like receptors

Human leukocyte antigen (HLA) genes are highly polymorphic loci encoding for cellular surface molecules

that present antigens to T-cells. The efficiency of this process is different between individuals because of the high number of allelic variants of HLA class I (A, B, C) and class II (DR, DQ, DP) genes. In viral infections, HLA class I process and present antigens to $CD8^+$ T-cells, which subsequently eliminate infected cells by direct lysis or by cytokine production. Killing of infected cells by $CD8^+$ T-cells, following recognition of HIV epitopes presented by HLA-I molecules, is fundamental for immune control of HIV, and mutation of the presented epitopes allows HIV to escape this control[12]. In this context, a particular HLA class I allele may be deleterious or beneficial to HIV infection, depending on the ability of the virus to escape recognition by this allele and on the fitness of escape mutant. Specific HLA class I alleles, by differential HIV epitope binding, influence the effectiveness of the immune response and, as a consequence, HIV progression[13].

The role of HLA alleles in the development of resistance to infection has been described in healthy newborns of HIV-infected mothers and in sexually exposed seronegative subjects. Sharing of HLA alleles between mothers and newborns or maternal HLA class I homozygosity both increased the risk of mother-to-child transmission[14,15]. The HLA homozygosity decreases the number of HLA alleles, thus decreasing the number of different epitopes presented and the extent of mutation needed to escape presentation. However, not all homozygotes are at a disadvantage since, in the case of protection from AIDS, homozygosity for HLA-Bw4-bearing B alleles is associated with a significant advantage[16].

Regarding supertypes of HLA molecules, characterized by functional and structural similarity, MacDonald, et al. found an association between the HLA A2/6802 supertype molecule and reduced perinatal HIV infection risk[17], as well as an association of this same supertype with a decreased risk of seroconversion in commercial sex workers[18]. Other authors found that the presence of HLA-Bw4 alleles in HIV-infected men was associated with a decreased risk of male-to-female HIV transmission, which suggests that these alleles reduce the infectivity of HIV[19]. Tang, et al., in an analysis of a group of 429 heterosexual discordant couples, found that several HLA class I variants in index partners were associated with contrasting rates and incidence of HIV transmission; in particular the A*30-Cw*B-57 haplotype in the index partner was the most favorable marker of HIV non-transmission[20]. More recently it has been shown that HLA-B polymorphisms that affect the rate of progression to AIDS may also alter HIV infectivity, but they did not appear to protect against infection in a cohort of ESN female partners from HIV-infected subjects[21].

Non classical HLA class I (HLA-G and HLA-E) can act as powerful modulators of the innate immune response through interaction with natural killer (NK) cells. A group of researchers showed that the HLA-E*0103 allele alone or the combination of the $HLA\text{-}E^G$ homozygote and HLA-G*0105N heterozygote genotypes had a decreased risk of HIV infection in African women compared with women carrying neither genotype[22].

Regarding HLA class II molecules, which have a key role in presenting antigens to $CD4^+$ T-cells, Hardie, et al. reported several HLA-DP genotypes associated with HIV susceptibility in a group of sex workers. Among these, the DPA1*010301 genotype was the most associated with HIV resistance and a slower rate of seroconversion[23]. Another study investigating the role of HLA-DQ genes in the differential susceptibility to HIV infection in an ESN sex workers cohort found an association of DQB1*0603 and DQA1*010201-DQB1*0603 haplotype with resistance in this group. The authors emphasize the importance of the HLA-DQ genes in the natural resistance to HIV infection[24].

Human leukocyte antigen class I molecules are ligands for the killer immunoglobulin-like receptors (KIR). The KIR-repertoire is preferentially expressed at the surface of NK cells and regulates their activation status through inhibitory or activating signaling. Certain KIR/HLA combinations have been found to protect against HIV disease progression. A study analyzing KIR/HLA interaction in group of ESN African sex workers suggested that resistance to HIV infection is associated with inhibitory KIR in the absence of their HLA ligand[25]. Ravet, et al. found that the presence of some activating KIR genes (or the absence of the inhibitory KIR3DL1) could contribute to the increased level of NK cell activation in ESN[26]. Homozygosity for the activating NK receptor KIR3DS1 was recently reported to contribute to heightened NK cell function in ESN individuals and to their relative resistance to HIV infection compared to individuals with HIV primary infection[27]. Another study from the same group showed that the co-expression of KIR3DL1*h and HLA-B*57, which has been associated with a reduced risk of progression to AIDS, also was associated with a reduced risk of infection in ESN subjects[28].

Chemokines and their receptors

Chemokines are chemoattractant cytokines produced by a variety of cell types including T-cells,

macrophages, NK cells, B-cells, and masts cells. These molecules are involved in cell trafficking and immunomodulation of inflammation and immune responses. In some cases their receptors serve as entry of pathogens into target cells to establish infection. The binding characteristics of chemokine receptors are determined by a specific gene that encodes for the receptor. In certain instances mutations or polymorphisms affect the binding characteristics of the receptor, and the existence of these polymorphisms has been associated with the phenomenon of resistance to infection and with the rate of progression in infected patients, which make sense, considering that some of these receptors are used preferentially by the HIV (primarily CCR5 and CXCR4). Multiple polymorphic variations have been described in the gene encoding CCR5 receptor. Of these, the CCR5-Δ32 variant, a natural deletion of 32 bases that introduce a premature stop codon resulting in a truncated protein product, has been the most extensively studied[29]. This deletion in homozygous state provides completely restricted HIV entry[29] and delayed disease progression in heterozygote patients[30]. However, the infrequent inheritance of homozygosity for the CCR5-Δ32 allele cannot explain the majority of persons who are repeatedly exposed to HIV but remain seronegative. A previous study has shown that CCR5-Δ32 heterozygosity and the CCR5 promoter polymorphism –2459 A/G genotype combination offers an advantage in resistance to HIV sexual transmission in a group of ESN subjects[31].

Another coreceptor used by HIV strains is CCR2, but this is uncommon. A polymorphism at position 64 of the CCR2 gene (CCR2-64I) has also been shown to be important in the transmission and progression of HIV disease. One study suggests that people who are homozygous for the CCR2-64I allele have some level of natural resistance to the sexual transmission of HIV[32].

Variations in the genes encoding for chemokines have also been studied in different populations in relation to susceptibility to HIV infection. The genetic polymorphisms of chemokines MIP-1α (CCL3), MIP-1β (CCL4), RANTES (CCL5), and MIP-1αP (CCL3L1) may be implicated in HIV acquisition and disease progression. Single nucleotide polymorphisms (SNP) in the MIP-1α gene are found at higher frequencies in ESN individuals compared with HIV-infected subjects[33]. In a study performed on the MACS cohort, the RANTES-403A polymorphism was identified as a risk factor for HIV transmission and as a protective factor for HIV progression[34]. It has been suggested that because CCL3L1 is a potent ligand of CCR5, its copy number and thus its expression might influence entry of the virus. González, et al. showed that CCL3L1 secretion levels are directly associated with gene copy number and inversely related to CCR5 expression on $CD4^+$ T-cells in a cohort of HIV-infected and -uninfected individuals of different ethnicities. The authors indicated that copy number variations in CCL3L1 could be associated with susceptibility to HIV infection[35]. These results are consistent with a study of HIV-infected pregnant women, which found a strong association between higher infant CCL3L1 gene copies and reduced susceptibility to HIV in the absence of maternal nevirapine[36]. In contrast, more recently other authors found no significant effect of CCL3L1 copy number variation on HIV-infection and argued that natural variation in CCL3L1 gene copies does not appear to have any important effect on the control of HIV[37].

The genetic factors associated with low susceptibility to HIV infection are summarized in table 1.

Immunological factors involved in low susceptibility to HIV infection

Innate immunity

Only a few studies have addressed the role of innate immunity in the low susceptibility to HIV in ESN. It has been reported that the abundant presence of macrophages, dendritic cells, and NK cells in the mucosa could be involved in protection against HIV infection[38]. Scott-Algara, et al. have shown that NK cells producing interferon-γ (INF-γ), tumor necrosis factor-α (TNF-α), and chemokines CCL3, CCL4, and CCL5 were increased in a cohort of ESN intravenous drug users (IDU), either after *in vitro* activation or without stimulation compared to HIV-infected IDU, suggesting that NK cells contribute to the protection against HIV infection[39]. Other authors found, in a group of sexually HIV-exposed seronegative subjects, a significantly higher expression of IFN-γ by NK cells compared with healthy controls and suggest that IFN-γ production by cells of the innate immune system may play a major role in natural resistance to HIV infection[40].

A role for HIV-specific NK cell responses in the prevention of maternal-infant HIV transmission has been suggested. A recent study has shown that mothers and infants who have NK cells that respond to HIV peptides are substantially less likely to transmit and acquire infection, respectively[41].

Table 1. Genetic factors involved in the low susceptibility to HIV infection in HIV-exposed seronegative subjects

Gene variants	References
Human leukocyte antigen (HLA)	
HLA-I discordance	14, 15
HLA supertype A2/6802	17
HLA-Bw4	19
HLA-A*30-Cw*B-57 haplotype	20
Combination of the HLA-E^G homozygote and HLA-G*015N heterozygote genotypes	22
HLA-E^G (the HLA-E*0103 allele)	22
HLA-DPB*3001	23
HLA-DPA*010301	23
KIR3DL1 in the absence of HLA-Bw4	25
KIR3DS1 homozygotes	27
Co-expression of KIR3DL1*h/*y and HLA-B*27	28
Chemokine coreceptors and their ligands	
CCR5Δ32 in homozygous state	29
Combination of CCR5-2459 A/G y CCR5Δ32	31
CCR264I in homozygous state	32
Polymorphisms in β-chemokines	33, 34
Increased copy number of CCL3L1	35

Defensins have also been involved in low susceptibility to HIV infection in ESN subjects. The number of α-defensin-producing $CD8^+$ T-cells is augmented both in the genital and in the peripheral blood of ESN, and α-defensin mRNA is also increased in peripheral blood mononuclear cells (PBMC) and in cervical biopsies of ESN individuals compared to healthy controls[42]. In contrast, Levinson, et al. showed that despite significant HIV inhibitory activity, cervicovaginal levels of α-defensins and the cathelicidin LL-37 were associated with increased HIV acquisition, and suggested that this perhaps is due to the existence of sexually transmitted bacterial infections inducing the production of these defensins[43]. Regarding β-defensins, mainly produced by epithelial cells, it has been demonstrated that vaginal mucosa of ESN subjects had higher levels of human β-defensin mRNA than seropositive individuals[44]. Ricci, et al. found that in children, some β-defensin-1 SNP had a protective role against HIV infection and suggest a critical role of innate immunity in pediatric HIV infection[45].

A study performing transcriptome and proteome analyses on T-cells and plasma from ESN individuals, their HIV-infected partners, and healthy controls reported that compared to HIV-positive and healthy controls, activated T-cells from ESN subjects overproduce several proteins involved in the innate immune response, principally those including high levels of peroxiredoxin II, NK-enhancing factor B and interleukin-22 (IL-22), a cytokine involved in the production of acute-phase proteins[46].

Chemokines and other proteins with antiviral activity have also been investigated in different cohorts of ESN. High levels of secretory leukocyte protease inhibitor (SLPI) in vaginal secretions of HIV-infected mothers has been associated with reduced HIV transmission

during childbirth and higher saliva levels in infants with reduced HIV acquisition through breastfeeding. However, a study in serodiscordant men having sex with men (MSM) couples showed no association of SLPI levels with neutralizing activity of saliva in ESN partners. The neutralizing activity was instead associated with increased levels of β-chemokines (CCL2, CCL4, CCL5, CCL11)[47]. Iqbal, et al. found that the cervicovaginal levels of CCL5 were increased tenfold in ESN sex workers compared with HIV-unexposed controls[48]. In contrast, Kault, et al. demonstrated that increased genital levels of RANTES could reflect an increased HIV susceptibility, as indicated by the number of HIV-susceptible target cells present in the cervical mucosa[49]. When a proteomics approach was used to characterize mucosal proteins potentially associated with HIV resistance, the antimicrobial protein elafin/trappin-2 as a novel innate immune factor was found elevated in a group ESN women, and the prospective association of elevated cervicovaginal levels of this protein with protection from HIV acquisition was then confirmed in a independent cohort of high-risk female sex workers[50].

The C-type lectins, DC-SIGN (expressed primarily on dendritic cells) and DC-SIGNR (DC-SIGN-related molecule) function as trans-receptors for HIV. It has been shown that genetic variants in these molecules have a significant effect on protection against HIV infection in ESN subjects[51-53]. However, the role of these variations in protecting from HIV infection has yet not been fully elucidated.

The innate immune response mediated by human cellular restriction factors

Recent reports have suggested that some ESN individuals have low T-cell permissiveness to HIV infection *in vitro*[54,55]. In recent years several host factors have been described that can restrict HIV replication and thereby block the establishment of infection after entry into the host cell. The discovery of intracellular proteins with innate antiviral activity has led to consideration of the possibility that these proteins may be involved in protection against HIV infection in ESN individuals. The APOBEC3G protein is a host antiviral factor. It is a cytidine deaminase that induces G→A hypermutation in newly synthesized retroviral DNA, resulting in instability of the nascent viral transcripts or lethal mutations[56]. A recent study has suggested that exposure to HIV may trigger APOBEC3G expression in PBMC in the absence of infection in ESN subjects. Additionally, cessation of exposure is associated with decreased APOBEC3G expression[57]. Biasin, et al. demonstrated that high expression of APOBEC3G is significantly increased in peripheral PBMC (mainly monocytes) and in cervical tissue of ESN subjects, both basal and after stimulation with IFN-α, compared with a group of HIV-unexposed individuals. High expression of this protein correlated with a reduced *in vitro* susceptibility of PBMC to infection with a R5 HIV strain[58].

Another intracellular antiviral factor, tripartite interaction motif 5α (TRIM5α), restricts the replication of some retroviruses through its interaction with the viral capsid protein, leading to abortive infection. One study of the effects of TRIM5α polymorphisms on HIV infection was conducted in a cohort of HIV-infected and -uninfected MSM. The authors found that TRIM5α haplotype containing R136Q mutation exhibited increased frequency among HIV-infected subjects compared with ESN[59]. In contrast, a second study found an apparently protective effect of R136Q in a different cohort of ESN subjects. The different ethnic origins and the different routes of exposure might explain these contradictory results[60]. More recently it has been found that high expression of TRIM5α is associated with reduced susceptibility to HIV-1 infection[61].

Adaptive immunity

Humoral immune response

Humoral responses encompass a family of B-cell secreted, antigen-specific immunoglobulins (IgA, IgD, IgE, IgG, and IgM) that show differential localization and functions[62]. Among the different immunoglobulin subtypes, the most relevant in the settings of HIV transmission are IgA and IgG. While IgA is the predominant immunoglobulin found in mucosa (genital tract and gut) and colostrum, which are relevant locations for HIV transmission, IgG is the main isotype found in serum and provides systemic protection. Both isotypes may also contribute to the protection of the fetus or newborn, as in some cases IgG is able to cross placental barriers and, along with IgA, be secreted in colostrum[63]. Although the exact contribution of humoral responses in progression to AIDS is still unclear[64], the effective pressure of humoral responses on HIV evolution has been clearly demonstrated[65]. Therefore, several studies have also addressed the role of humoral responses in protection against HIV infection in ESN. According to the group of ESN analyzed and

the mechanisms of transmission involved, several immunoglobulin subtypes and specificities have been analyzed. Among these, anti-gp41 IgA and anti-CD4/gp120 complex IgG were reported as better predictors of HIV exposure[66].

By definition, ESN are seronegative, and this fact rules out the development of an overt, systemic, IgG-based anti-HIV response (seroconversion) leading the initial characterization of ESN to cellular immunity bases (see below)[67,68]. In fact, initial evidence for a role of humoral immunity in protecting exposed individuals did not come from the analysis of HIV-specific responses but from data on autoantibodies, whose detection is usual in HIV-positive individuals[69,70]. Autoantibodies against CD4 were identified in sexually exposed ESN in 1996[71]. Although these antibodies were unable to block the binding of gp120 to CD4, they efficiently inhibited HIV-induced membrane fusion, potentially interrupting transmission[71]. Further work expanded the range of autoantibodies involved in protection against HIV transmission, describing the higher prevalence of anti-HLA[72,73] and anti-CCR5 autoantibodies in ESN[74,75]. The link between the existence of autoantibodies and exposure to HIV is still unclear; however, their protective role in vertical, sexual, and systemic exposures has been highlighted by analyzing geographically separate cohorts of newborns[76], heterosexuals, or intravenous drug users[66].

Despite the seronegative status of ESN, the existence of adaptive HIV-specific humoral responses has been widely studied. In fact, a very interesting report described the presence of systemic anti-HIV IgG in seronegative hemophiliacs repeatedly exposed to HIV[77]. Although these responses were low, transient, and mainly focused on gag antigens, this observation confirmed the elicitation of systemic responses against HIV by repeated inoculums, a phenomenon that could also occur at mucosal level during sexual or postpartum HIV transmission. Focusing on mucosal immunity, HIV-specific IgA responses have been described in European ESN heterosexual cohorts[8] and in a Vietnamese ESN IDU cohort[66] and for HIV-2 infection in an African heterosexual ESN cohort[78]. Noteworthy, Nguyen, et al. describe the presence of anti-HIV antibodies as the main factor of protection from sexual HIV transmission in a Cambodian cohort[79]. Most of these analyses were performed using plasma or serum samples, in which IgA represents a small proportion of total immunoglobulins. However, analysis of mucosal secretions has widely confirmed these initial observations: HIV-specific IgA has been found in saliva of HIV-1-exposed African infants[80] or European MSM[81], and seminal fluid and urethral swabs from European heterosexual ESN[8]. Furthermore, systemic IgG has been found in ESN that recognizes cryptic CD4 epitopes exposed by the binding of gp120 to CD4 in different cohorts[66]. Several factors have been associated with the elicitation of specific humoral responses in ESN, such as the route of exposure to HIV and viral load of infected partners[81]. Despite the failure to detect consistent HIV-specific responses reported in nonhuman primate models of HIV transmission[82], further development of these models could help in defining these factors. Recently it has been reported, using infant macaques, that the presence of suboptimal non-protective quantities of neutralizing antibodies before oral SHIV challenge could enhance the development of a protective neutralizing humoral response[83]. These results may have important repercussions in the context of vertically exposed newborns.

Cellular immune response

HIV-specific cellular response has been studied in ESN subjects, evaluating cytokine and chemokine production following HIV-specific antigen stimulation. Many authors suggest that HIV-specific cytotoxic T-lymphocyte response is fundamental for resistance to HIV infection. The presence of HIV-specific $CD8^+$ T-cell response and its functional activity mediated by IFN-γ production was detected in a group of ESN sex workers from Kenya in both cervical mucosa[84] and peripheral blood[85]. HIV-specific $CD8^+$ T-cell responses have been also demonstrated in ESN sexual partners of HIV-infected individuals[86]. Similar results were found in a group of ESN who had parenteral exposure to high HIV viremia[87]. Moreover, both parenteral and mucosal exposure to HIV can elicit HIV-specific $CD8^+$ T-cell responses with similar characteristics[88]. A recent study demonstrated that HIV exposure through oral sex is sufficient to induce systemic HIV-specific T-cell responses in some ESN individuals[89]. Non-cytotoxic $CD8^+$ T-cell anti-HIV response has also been found in ESN individuals, suggesting that non-cytotoxic $CD8^+$ T-cell-mediated mechanisms maybe play an important role in resistance to HIV infection[90].

Regarding the specificity of the $CD8^+$ T-cell immune responses, some researchers have found that $CD8^+$ T-cells from ESN individuals recognize different epitopes than those recognized by lymphocytes from

HIV-infected patients, and the maintenance of response in ESN subjects appears to be dependent on persistent HIV exposure[91-93]. Erickson, et al. showed a multifunctional response of $CD8^+$ T-cells able to lyse cells presenting cognate epitopes in ESN homosexual men. These results suggest that low susceptibility to HIV infection in ESN individuals may be due to the quality rather than quantity of the cellular immune response[94].

Several reports have shown the relevance of $CD4^+$ T-cells in the induction and maintenance of the host immune response against HIV in ESN individuals. In two independent studies, ESN individuals have shown significantly increased HIV-specific $CD4^+$ T-cell responses compared with HIV-unexposed subjects, as determined by IL-2 production following stimulation with HIV peptides[95,96]. Other results demonstrated spontaneous and antigen-induced chemokine production in ESN partners of HIV-infected individuals[97]. HIV-specific, IFN-γ producing, $CD4^+$ peripheral lymphocytes were present in a group of ESN men compared to HIV-infected and unexposed individuals[8,9,98]. In contrast, another study reported IFN-γ production by HIV-specific $CD4^+$ T-cells from ESN subjects, but at lower levels compared to HIV-positive subjects[2].

Most studies of HIV-specific immune response conducted in ESN have used cross-sectional designs. Thus, the interpretation of results in terms of a protective role of immune responses on resistance to HIV infection is often difficult. In this regard, the demonstration of HIV-specific immune responses cannot be considered as a proof of resistance to infection as it can only reflect exposure to the virus. Some authors consider that the presence of HIV-specific T-cell responses in ESN subjects may be associated with repeated sexual exposure to HIV[2,9] rather than resistance to HIV infection. In a group of 24 serodiscordant couples we observed a high prevalence of HIV-specific T-cell responses in ESN subjects with low HIV exposure (frequent unprotected sexual intercourse with HIV-infected partner with plasma HIV RNA < 50 copies/ml) compared to ESN subjects with high HIV exposure (frequent unprotected sexual intercourse with HIV-infected partner with plasma HIV RNA > 50 copies/ml). The results of this study suggest that low but persistent HIV exposure is able to induce virus-specific T-cell response in a high proportion of ESN individuals and support that real virus exposure occurs, even in conditions of maximal viral suppression under antiretroviral therapy in the infected partner[99].

To elucidate if the HIV-specific immune response is really implicated in the resistance to HIV infection or if its presence in ESN subjects is merely a consequence of viral exposure, longitudinal studies are necessary. Only a few works have analyzed the prospective significance of HIV-specific immune responses in ESN subjects and the results are controversial. Makedonas, et al. showed that ESN subjects who developed HIV-specific effector responses were at reduced risk for seroconversion compared with ESN who did not develop this type of immunity[4]. Other authors observed that genital HIV-neutralizing IgA and systemic HIV-specific proliferative responses were prospectively associated with maintenance of seronegative status in a group of ESN sex workers compared to sex workers without HIV-specific immune response[100]. Although all these observations support the hypothesis that HIV-specific immune responses play an important role in the prevention of HIV infection, Kaul, et al. found that cytotoxic T-lymphocytes were associated with recent HIV exposure in ESN sex workers, but not prospectively associated with protection in a multivariable model that included HIV exposure and duration of sex work[101]. Moreover, in another study by the same group it was found that late seroconversion may occur in ESN sex workers despite preexisting HIV-specific $CD8^+$ T-cell responses and considered that this seroconversion could be related to the waning of HIV-specific $CD8^+$ responses due to reduced antigenic exposure[102]. Interestingly, a recent study found that the presence of a previous broad HIV-specific $CD8^+$ T-cell response in an ESN subject was not able to prevent the subsequent acquisition of HIV infection, even though the infecting viruses were not particularly distant from the virus that may have elicited the initial response[103].

The immunological factors associated with low susceptibility to HIV infection are summarized in table 2.

Other immunological abnormalities in HIV-exposed but seronegative individuals

Different studies have analyzed the phenotype of T-lymphocytes in ESN subjects, focusing on cellular activation and maturation stage of T-cells. A reduction in the proportion of naive T-cells and an increase of memory lymphocytes has been detected in several studies on different cohorts of ESN subjects[10,104,105]. This phenomenon was explained as a result of viral exposure in ESN subjects and indicated that this exposure

Table 2. Immunological factors involved in low susceptibility to HIV infection in HIV-exposed seronegative subjects

Immunological factors	References
Innate immunity	
High activity of NK cells	38, 39
HIV-specific NK cell responses	41
Increased production of α-defensins by T-cells	42
Higher levels of human β-defensins mRNA	44
Increased levels of IL-22	46
Elevated levels of elafin/trappin-2	50
High expression levels APOBEC3G mRNA	57
High expression of TRIM5α	61
Adaptive immunity	
Autoantibodies against CD4+ T-cells	71
Anti-HLA and anti-CCR5 antibodies	73, 74, 75
Systemic anti-HIV IgG	77
HIV-specific IgA responses in mucosa and saliva	66, 79, 80, 81
Increased production IFN-γ by mucosal and blood CD8+ T-cells	84, 85, 86, 93
Recognition of peculiar epitopes	91
Polyfunctional activity from HIV-specific CD8+ T-cells	94
High proportion of CD4+ producing IFN-γ and IL-2	8, 9, 95, 96, 98

NK: natural killer; TRIM: tripartite interaction motif; IL: interleukin; IFN: interferon.

is enough to modulate the memory/naive ratio[105]. Others authors found that ESN subjects had high levels of systemic and mucosal immune activation compared with healthy unexposed controls and suggested that active HIV infection is not required for T-cell activation[106,107]. Suy, et al. found that the changes in memory and activated T-cells in ESN subjects were directly correlated with plasma viral load of their HIV-infected partners, whereas changes in naive and $CD4^+CD28^+$ T-cells were inversely related with viral load of the HIV-infected partners[10]. A study on discordant couples conducted in our laboratory showed high levels of activation in various subpopulations of $CD4^+$ and $CD8^+$ T-lymphocytes in ESN individuals similar to those found in HIV-infected partners, suggesting that these immune alterations are a consequence of exposure to virus and not necessarily a phenomenon associated with resistance to infection[99]. In contrast, these results were not confirmed by other studies that found low levels of T-cell activation in ESN subjects, suggesting that this phenomenon may contribute to the protection against HIV acquisition in these individuals[108,109]. Card, et al. found reduced frequencies of T-cell activation in a group of ESN sex workers and elevated frequencies of regulatory T (T_{reg}) cells compared to control subjects, suggesting that T_{reg} cells may contribute to HIV resistance by minimizing the pool of cells susceptible to infection[110].

A more recent study showed that different patterns of sexual behavior could help explain the discrepancy observed in the abovementioned studies. The authors found lower levels of $CD4^+$ T-cell activation in ESN

subjects compared to control subjects; however, this appeared to be associated with a high degree of condom use among ESN subjects compared to control subjects. Also, markedly higher levels of T-cell activation in women compared with men, irrespective of sexual behavior, were observed. These results question the relevance of low-level CD4 T-cell activation in resistance to HIV infection and underscore the need to take into account gender and sexual behavior characteristics of ESN subjects when analyzing correlates of protective immunity[111].

Conclusion

Several studies have highlighted the role played by host factors in the low susceptibility to HIV infection in ESN individuals. However, most of these factors might be a consequence of exposure to the virus instead of resistance to infection. Future studies using large cohorts of ESN followed over time are necessary to elucidate the real impact of all these genetic and immunological factors in the probability of seroconversion. This may have important implications in the design of future immune-based therapeutic interventions aimed at controlling or preventing HIV infection.

References

1. Rowland-Jones S, McMichael A. Immune responses in HIV-exposed seronegatives: have they repelled the virus? Curr Opin Immunol. 1995;7:448-55.
2. Jennes W, Vuylsteke B, Borget M, et al. HIV-specific T helper responses and frequency of exposure among HIV-exposed seronegative female sex workers in Abidjan, Cote d'Ivoire. J Infect Dis. 2004;189:602-10.
3. Hladik F, Desbien A, Lang J, et al. Most highly exposed seronegative men lack HIV-1-specific, IFN-gamma-secreting T cells. J Immunol. 2003;171:2671-83.
4. Makedonas G, Bruneau J, Lin H, Sekaly R, Lamothe F, Bernard NF. HIV-specific CD8 T-cell activity in uninfected injection drug users is associated with maintenance of seronegativity. AIDS. 2002;16:1595-602.
5. Salkowitz J, Purvis S, Meyerson H, et al. Characterization of high-risk HIV-1 seronegative hemophiliacs. Clin Immunol. 2001;98:200-11.
6. Clerici M, Levin J, Kessler H, et al. HIV-specific T-helper activity in seronegative health care workers exposed to contaminated blood. JAMA. 1994;271:42-6.
7. Hygino J, Lima P, Filho R, et al. Altered immunological reactivity in HIV-1-exposed uninfected neonates. Clin Immunol. 2008;127:340-7.
8. Lo Caputo S, Trabattoni D, Vichi F, et al. Mucosal and systemic HIV-1-specific immunity in HIV-1-exposed but uninfected heterosexual men. AIDS. 2003;17:531-9.
9. Promadej N, Costello C, Wernett M, et al. Broad HIV-specific T cell responses to conserved HIV proteins in HIV-seronegative women highly exposed to a single HIV-infected partner. J Infect Dis. 2003;187:1053-63.
10. Suy A, Castro P, Nomdedeu M, et al. Immunological profile of heterosexual highly HIV-exposed uninfected individuals: predominant role of CD4 and CD8 T-cell activation. J Infect Dis. 2007;196:1191-201.
11. Miyazawa M, Lopalco L, Mazzotta F, et al. The 'immunologic advantage' of HIV-exposed seronegative individuals. AIDS. 2009;23:161-75.
12. Goulder P, Watkins D. Impact of MHC class I diversity on immune control of HIV replication. Nat Rev Immunol. 2008;8:619-30.
13. Carrington M, O'Brien S. The influence of HLA genotype on AIDS. Annu Rev Med. 2003;54:535-51.
14. Mackelprang R, John-Stewart G, Carrington M, et al. Maternal HLA homozygosity and mother-child HLA concordance increase the risk of vertical transmission of HIV-1. J Infect Dis. 2008;197:1156-61.
15. MacDonald K, Embree J, Njenga S et al. Mother-child class I HLA concordance increases perinatal HIV type 1 transmission. J Infect Dis. 1998;177:551-6.
16. Flores-Villanueva P, Yunis E, Delgado J, et al. Control of HIV-1 viremia and protection from AIDS are associated with HLA-Bw4 homozygosity. Proc Natl Acad Sci USA. 2001;98:5140-5.
17. MacDonald K, Embree J, Nagelkerke N, et al. The HLA A2/6802 supertype is associated with reduced risk of perinatal HIV type 1 transmission. J Infect Dis. 2001;183:503-6.
18. MacDonald K, Fowke K, Kimani J, et al. Influence of HLA supertypes on susceptibility and resistance to HIV type 1 infection. J Infect Dis. 2000;181:1581-9.
19. Welzel T, Gao X, Pfeiffer R, et al. HLA-B Bw4 alleles and HIV-1 transmission in heterosexual couples. AIDS. 2007;21:225-9.
20. Tang J, Shao W, Yoo Y, et al. Human leukocyte antigen class I genotypes in relation to heterosexual HIV type 1 transmission within discordant couples. J Immunol. 2008;181:2626-35.
21. Gao X, O'Brien T, Welzel T, et al. HLA-B alleles associate consistently with HIV heterosexual transmission, viral load, and progression to AIDS, but not susceptibility to infection. AIDS. 2010;24:1835-40.
22. Lajoie J, Hargrove J, Zijenah L, Humphrey J, Ward B, Roger M. Genetic variants in non-classical major histocompatibility complex class I human leukocyte antigen (HLA)-E and HLA-G molecules are associated with susceptibility to heterosexual acquisition of HIV-1. J Infect Dis. 2006;193:298-301.
23. Hardie R, Knight E, Bruneau B, et al. A common human leucocyte antigen-DP genotype is associated with resistance to HIV-1 infection in Kenyan sex workers. AIDS. 2008;22:2038-42.
24. Hardie R, Luo M, Bruneau B, et al. Human leukocyte antigen-DQ alleles and haplotypes and their associations with resistance and susceptibility to HIV-1 infection. AIDS. 2008;22:807-16.
25. Jennes W, Verheyden S, Demanet C, et al. Cutting edge: resistance to HIV-1 infection among African female sex workers is associated with inhibitory KIR in the absence of their HLA ligands. J Immunol. 2006;177:6588-92.
26. Ravet S, Scott-Algara D, Bonnet E, et al. Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. Blood. 2007;109:4296-305.
27. Boulet S, Sharafi S, Simic N, et al. Increased proportion of KIR3DS1 homozygotes in HIV-exposed uninfected individuals. AIDS. 2008; 22:595-9.
28. Boulet S, Kleyman M, Kim J, et al. A combined genotype of KIR3DL1 high expressing alleles and HLA-B*57 is associated with a reduced risk of HIV infection. AIDS. 2008;22:1487-91.
29. Liu R, Paxton W, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell. 1996;86:367-77.
30. Buseyne F, Janvier G, Teglas J, et al. Impact of heterozygosity for the chemokine receptor CCR5 32-bp-deleted allele on plasma virus load and CD4 T lymphocytes in perinatally HIV-infected children at 8 years of age. J Infect Dis. 1998;178:1019-23.
31. Hladik F, Liu H, Speelmon E, et al. Combined effect of CCR5-Delta32 heterozygosity and the CCR5 promoter polymorphism -2459 A/G on CCR5 expression and resistance to HIV type 1 transmission. J Virol. 2005;79:11677-84.
32. Louisirirotchanakul S, Liu H, Roongpisuthipong A, et al. Genetic analysis of HIV-1 discordant couples in Thailand: association of CCR2 64I homozygosity with HIV-1-negative status. J Acquir Immune Defic Syndr. 2002;29:314-15.
33. Modi W, Lautenberger J, An P, et al. Genetic variation in the CCL18-CCL3-CCL4 chemokine gene cluster influences HIV Type 1 transmission and AIDS disease progression. Am J Hum Genet. 2006;79:120-8.
34. McDermott D, Beecroft M, Kleeberger C, et al. Chemokine RANTES promoter polymorphism affects risk of both HIV infection and disease progression in the Multicenter AIDS Cohort Study. AIDS. 2000;14: 2671-8.
35. Gonzalez E, Kulkarni H, Bolivar H, et al. The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. Science. 2005;307:1434-40.
36. Kuhn L, Schramm D, Donninger S, et al. African infants' CCL3 gene copies influence perinatal HIV transmission in the absence of maternal nevirapine. AIDS. 2007;21:1753-61.
37. Urban T, Weintrob A, Fellay J, et al. CCL3L1 and HIV/AIDS susceptibility. Nat Med. 2009;15:1110-12.
38. Lehner T, Wang Y, Pido-Lopez J, Whittall T, Bergmeier L, Babaahmady K. The emerging role of innate immunity in protection against HIV-1 infection. Vaccine. 2008;26:2997-3001.
39. Scott-Algara D, Truong L, Versmisse P, et al. Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. J Immunol. 2003;171:5663-7.
40. Montoya C, Velilla P, Chougnet C, Landay AL, Rugeles M. Increased IFN-gamma production by NK and CD3+/CD56+ cells in sexually

HIV-1-exposed but uninfected individuals. Clin Immunol. 2006;120: 138-46.
41. Tiemessen C, Shalekoff S, Meddows-Taylor S, et al. Cutting Edge: Unusual NK cell responses to HIV-1 peptides are associated with protection against maternal-infant transmission of HIV-1. J Immunol. 2009; 182:5914-18.
42. Trabattoni D, Caputo S, Maffeis G, et al. Human alpha defensin in HIV-exposed but uninfected individuals. J Acquir Immune Defic Syndr. 2004;35:455-63.
43. Levinson P, Kaul R, Kimani J, et al. Levels of innate immune factors in genital fluids: association of alpha defensins and LL-37 with genital infections and increased HIV acquisition. AIDS. 2009;23:309-17.
44. Zapata W, Rodriguez B, Weber J, et al. Increased levels of human beta-defensins mRNA in sexually HIV-1 exposed but uninfected individuals. Curr HIV Res. 2008;6:531-8.
45. Ricci E, Malacrida S, Zanchetta M, Montagna M, Giaquinto C, De Rossi A. Role of beta-defensin-1 polymorphisms in mother-to-child transmission of HIV-1. J Acquir Immune Defic Syndr. 2009;51:13-19.
46. Misse D, Yssel H, Trabattoni D, et al. IL-22 participates in an innate anti-HIV-1 host-resistance network through acute-phase protein induction. J Immunol. 2007;178:407-15.
47. Hasselrot K, Bratt G, Duvefelt K, Hirbod T, Sandstrom E, Broliden K. HIV-1 exposed uninfected men who have sex with men have increased levels of salivary CC-chemokines associated with sexual behavior. AIDS. 2010;24:1569-75.
48. Iqbal S, Ball T, Kimani J, et al. Elevated T cell counts and RANTES expression in the genital mucosa of HIV-1-resistant Kenyan commercial sex workers. J Infect Dis. 2005;192:728-38.
49. Kaul R, Rebbapragada A, Hirbod T, et al. Genital levels of soluble immune factors with anti-HIV activity may correlate with increased HIV susceptibility. AIDS. 2008;22:2049-51.
50. Iqbal S, Ball T, Levinson P, et al. Elevated elafin/trappin-2 in the female genital tract is associated with protection against HIV acquisition. AIDS. 2009;23:1669-77.
51. Rathore A, Chatterjee A, Sivarama P, Yamamoto N, Dhole T. Role of homozygous DC-SIGNR 5/5 tandem repeat polymorphism in HIV-1 exposed seronegative North Indian individuals. J Clin Immunol. 2008; 28:50-7.
52. Wichukchinda N, Kitamura Y, Rojanawiwat A, et al. The polymorphisms in DC-SIGNR affect susceptibility to HIV type 1 infection. AIDS Res Hum Retroviruses. 2007;23:686-92.
53. Liu H, Hwangbo Y, Holte S, et al. Analysis of genetic polymorphisms in CCR5, CCR2, stromal cell-derived factor-1, RANTES, and dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin in seronegative individuals repeatedly exposed to HIV-1. J Infect Dis. 2004;190:1055-8.
54. Truong L, Luong T, Scott-Algara D, et al. CD4 cell and CD8 cell-mediated resistance to HIV-1 infection in exposed uninfected intravascular drug users in Vietnam. AIDS. 2003;17:1425-34.
55. Saez-Cirion A, Versmisse P, Truong L, et al. Persistent resistance to HIV-1 infection in CD4 T cells from exposed uninfected Vietnamese individuals is mediated by entry and post-entry blocks. Retrovirology. 2006;3:81.
56. Chiu Y, Greene W. Multifaceted antiviral actions of APOBEC3 cytidine deaminases. Trends Immunol. 2006;27:291-7.
57. Vazquez-Perez J, Ormsby C, Hernandez-Juan R, Torres K, Reyes-Teran G. APOBEC3G mRNA expression in exposed seronegative and early stage HIV infected individuals decreases with removal of exposure and with disease progression. Retrovirology. 2009;6:23.
58. Biasin M, Piacentini L, Lo Caputo S, et al. Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G: a possible role in the resistance to HIV of HIV-exposed seronegative individuals. J Infect Dis. 2007;195:960-4.
59. Speelmon E, Livingston-Rosanoff D, Li S, et al. Genetic association of the antiviral restriction factor TRIM5alpha with HIV type 1 infection. J Virol. 2006;80:2463-71.
60. Javanbakht H, An P, Gold B, et al. Effects of human TRIM5alpha polymorphisms on antiretroviral function and susceptibility to HIV infection. Virology. 2006;354:15-27.
61. Sewram S, Singh R, Kormuth E, et al. Human TRIM5alpha expression levels and reduced susceptibility to HIV-1 infection. J Infect Dis. 2009;199:1657-63.
62. Brandtzaeg P. Mucosal immunity: induction, dissemination, and effector functions. Scand J Immunol. 2009;70:505-15.
63. Van de Perre P. Transfer of antibody via mother's milk. Vaccine. 2003;21:3374-6.
64. Doria-Rose N, Klein R, Daniels M, et al. Breadth of HIV-specific neutralizing activity in sera: clustering analysis and association with clinical variables. J Virol. 2010;84:1631-6.
65. Richman D, Wrin T, Little S, Petropoulos C. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc Natl Acad Sci USA. 2003;100:4144-9.
66. Lopalco L, Barassi C, Paolucci C, et al. Predictive value of anti-cell and anti-HIV humoral responses in HIV-1-exposed seronegative cohorts of European and Asian origin. J Gen Virol. 2005;86:339-48.
67. Ahearne P, Matthews T, Lyerly H, White G, Bolognesi D, Weinhold K. Cellular immune response to viral peptides in patients exposed to HIV. AIDS Res Hum Retroviruses. 1988;4:259-67.
68. Clerici M, Giorgi J, Chou C, et al. Cell-mediated immune response to HIV type 1 in seronegative homosexual men with recent sexual exposure to HIV-1. J Infect Dis. 1992;165:1012-19.
69. Borghi M, De Santis C, Barcellini W, et al. Autoantibodies against beta 2-microglobulin-free HLA antigens in AIDS patients. J Acquir Immune Defic Syndr. 1993;6:1114-19.
70. Callahan L, Roderiquez G, Mallinson M, Norcross M. Analysis of HIV-induced autoantibodies to cryptic epitopes on human CD4. J Immunol. 1992;149:2194-202.
71. Burastero S, Gaffi D, Lopalco L, et al. Autoantibodies to CD4 in HIV type 1-exposed seronegative individuals. AIDS Res Hum Retroviruses. 1996;12:273-80.
72. Beretta A, Weiss S, Rappocciolo G, et al. HIV-1-seronegative injection drug users at risk for HIV exposure have antibodies to HLA class I antigens and T cells specific for HIV envelope. J Infect Dis. 1996;173:472-6.
73. Lopalco L, Pastori C, Cosma A, et al. Anti-cell antibodies in exposed seronegative individuals with HIV type 1-neutralizing activity. AIDS Res Hum Retroviruses. 2000;16:109-15.
74. Ditzel H, Rosenkilde M, Garred P, et al. The CCR5 receptor acts as an alloantigen in CCR5Delta32 homozygous individuals: identification of chemokine and HIV-1-blocking human antibodies. Proc Natl Acad Sci USA. 1998;95:5241-5.
75. Lopalco L, Barassi C, Pastori C, et al. CCR5-reactive antibodies in seronegative partners of HIV-seropositive individuals down-modulate surface CCR5 in vivo and neutralize the infectivity of R5 strains of HIV-1 in vitro. J Immunol. 2000;164:3426-33.
76. Lopalco L, Magnani Z, Confetti C, et al. Anti-CD4 antibodies in exposed seronegative adults and in newborns of HIV type 1-seropositive mothers: a follow-up study. AIDS Res Hum Retroviruses. 1999;15:1079-85.
77. Tenenbaum S, Morris C, Alexander S, McFerrin H, Garry R, Leissinger C. Evidence of HIV exposure and transient seroreactivity in archived HIV-negative severe hemophiliac sera. J Virol. 2005;2:65.
78. Lizeng Q, Nilsson C, Sourial S, et al. Potent neutralizing serum immunoglobulin A (IgA) in HIV type 2-exposed IgG-seronegative individuals. J Virol. 2004;78:7016-22.
79. Nguyen M, Pean P, Lopalco L, et al. HIV-specific antibodies but not t-cell responses are associated with protection in seronegative partners of HIV-1-infected individuals in Cambodia. J Acquir Immune Defic Syndr. 2006;42:412-19.
80. Farquhar C, VanCott T, Bosire R, et al. Salivary HIV-1-specific immunoglobulin A in HIV-1-exposed infants in Kenya. Clin Exp Immunol. 2008;153:37-43.
81. Hasselrot K, Bratt G, Hirbod T, et al. Orally exposed uninfected individuals have systemic anti-HIV responses associating with partners' viral load. AIDS. 2010;24:3-43.
82. Letvin NL, Rao S, Dang V, et al. No evidence for consistent virus-specific immunity in simian immunodeficiency virus-exposed, uninfected rhesus monkeys. J Virol. 2007;81:12368-74.
83. Ng C, Jaworski JP, Jayaraman P, et al. Passive neutralizing antibody controls SHIV viremia and enhances B cell responses in infant macaques. Nat Med. 2010;16:1117-19.
84. Kaul R, Plummer F, Kimani J, et al. HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. J Immunol. 2000;164:1602-11.
85. Alimonti J, Kimani J, Matu L, et al. Characterization of CD8 T-cell responses in HIV-1-exposed seronegative commercial sex workers from Nairobi, Kenya. Immunol Cell Biol. 2006;84:482-5.
86. Skurnick J, Palumbo P, De Vico A, et al. Correlates of non-transmission in US women at high risk of HIV type 1 infection through sexual exposure. J Infect Dis. 2002;185:428-38.
87. Missale G, Papagno L, Penna A, et al. Parenteral exposure to high HIV viremia leads to virus-specific T cell priming without evidence of infection. Eur J Immunol. 2004;34:3208-15.
88. Makedonas G, Bruneau J, Alary M, et al. Comparison of HIV-specific CD8 T-cell responses among uninfected individuals exposed to HIV parenterally and mucosally. AIDS. 2005;19:251-9.
89. Perez C, Hasselrot K, Bratt G, Broliden K, Karlsson A. Induction of systemic HIV-1-specific cellular immune responses by oral exposure in the uninfected partner of discordant couples. AIDS. 2010;24:969-74.
90. Furci L, Lopalco L, Loverro P, et al. Non-cytotoxic inhibition of HIV-1 infection by unstimulated CD8+ T lymphocytes from HIV-exposed-uninfected individuals. AIDS. 2002;16:1003-8.
91. Kaul R, Dong T, Plummer F, et al. CD8(+) lymphocytes respond to different HIV epitopes in seronegative and infected subjects. J Clin Invest. 2001;107:1303-10.
92. Kaul R, Rowland-Jones S, Kimani J, et al. New insights into HIV-1 specific cytotoxic T-lymphocyte responses in exposed, persistently seronegative Kenyan sex workers. Immunol Lett. 2001;79:3-13.

93. Kebba A, Kaleebu P, Rowland S, et al. Distinct patterns of peripheral HIV-1-specific interferon-gamma responses in exposed HIV-1-seronegative individuals. J Infect Dis. 2004;189:1705-13.
94. Erickson A, Willberg C, McMahan V, et al. Potentially exposed but uninfected individuals produce cytotoxic and polyfunctional HIV type 1-specific CD8(+) T-cell responses which can be defined to the epitope level. Clin Vaccine Immunol. 2008;15:1745-8.
95. Fowke K, Kaul R, Rosenthal K, et al. HIV-1-specific cellular immune responses among HIV-1-resistant sex workers. Immunol Cell Biol. 2000;78:586-95.
96. Pallikkuth S, Wanchu A, Bhatnagar A, Sachdeva R, Sharma M. HIV gag antigen-specific T-helper and granule-dependent CD8 T-cell activities in exposed but uninfected heterosexual partners of HIV type 1-infected individuals in North India. Clin Vaccine Immunol. 2007;14: 1196-202.
97. Suresh P, Wanchu A, Bhatnagar A, Sachdeva R, Sharma M. Spontaneous and antigen-induced chemokine production in exposed but uninfected partners of HIV type 1-infected individuals in North India. AIDS Res Hum Retroviruses. 2007;23:261-8.
98. Eyeson J, King D, Boaz M, et al. Evidence for Gag p24-specific CD4 T cells with reduced susceptibility to R5 HIV-1 infection in a UK cohort of HIV-exposed-seronegative subjects. AIDS. 2003;17:2299-311.
99. Restrepo C, Rallon N, del Romero J, et al. Low-level exposure to HIV induces virus-specific T cell responses and immune activation in exposed HIV-seronegative individuals. J Immunol. 2010;185:982-9.
100. Hirbod T, Kaul R, Reichard C, et al. HIV-neutralizing immunoglobulin A and HIV-specific proliferation are independently associated with reduced HIV acquisition in Kenyan sex workers. AIDS. 2008; 22:727-35.
101. Kaul R, Rutherford J, Rowland-Jones S, et al. HIV-1 Env-specific cytotoxic T-lymphocyte responses in exposed, uninfected Kenyan sex workers: a prospective analysis. AIDS. 2004;18:2087-9.
102. Kaul R, Rowland-Jones S, Kimani J, et al. Late seroconversion in HIV-resistant Nairobi prostitutes despite pre-existing HIV-specific CD8+ responses. J Clin Invest. 2001;107:341-9.
103. Liu Y, Woodward A, Zhu H, et al. Preinfection HIV-specific cytotoxic T lymphocytes failed to prevent HIV type 1 infection from strains genetically unrelated to viruses in long-term exposed partners. J Virol. 2009;83:10821-9.
104. Tran H, Chartier L, Troung L, et al. Systemic immune activation in HIV-1-exposed uninfected Vietnamese intravascular drug users. AIDS Res Hum Retroviruses. 2006;22:255-61.
105. Schenal M, Lo Caputo S, Fasano F, et al. Distinct patterns of HIV-specific memory T lymphocytes in HIV-exposed uninfected individuals and in HIV-infected patients. AIDS. 2005;19:653-61.
106. Biasin M, Caputo S, Speciale L, et al. Mucosal and systemic immune activation is present in HIV-exposed seronegative women. J Infect Dis. 2000;182:1365-74.
107. Jennes W, Sawadogo S, Koblavi-Deme S, et al. Cellular HIV-protective factors: a comparison of HIV-exposed seronegative female sex workers and female blood donors in Abidjan, Cote d'Ivoire. J Infect Dis. 2003;187:206-14.
108. Begaud E, Chartier L, Marechal V, et al. Reduced CD4 T cell activation and in vitro susceptibility to HIV-1 infection in exposed uninfected Central Africans. Retrovirology. 2006;3:35.
109. Koning F, Otto S, Hazenberg M, et al. Low-level CD4+ T cell activation is associated with low susceptibility to HIV-1 infection. J Immunol. 2005;175:6117-22.
110. Card C, McLaren P, Wachihi C, Kimani J, Plummer F, Fowke K. Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of CD4(+)CD25(+)FOXP3(+) regulatory T cells. J Infect Dis. 2009;199:1318-22.
111. Camara M, Dieye T, Seydi M, et al. Low-level CD4+ T cell activation in HIV-exposed seronegative subjects: influence of gender and condom use. J Infect Dis. 2010;201:835-42.