

Determinants of Protection among HIV-Exposed Seronegative Persons: An Overview

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Both clinical experience and a growing medical literature indicate that some persons who have been exposed to human immunodeficiency virus (HIV) infection remain uninfected. Although in some instances this may represent good fortune, cohorts of uninfected persons have been reported who are considered at high risk for infection. In these cohorts a variety of characteristics have been proposed as mediating protection, but to date only the 32–base pair deletion in the chemokine (C-C motif) receptor 5 gene, which results in complete failure of cell surface expression of this coreceptor, has been associated with high-level protection from HIV infection. With this in mind, there are probably many other factors that may individually or in combination provide some level of protection from acquisition of HIV infection. Because some of these factors are probably incompletely protective or inconsistently active, identifying them with confidence will be difficult. Nonetheless, clarifying the determinants of protection against HIV infection is a high priority that will require careful selection of high-risk uninfected cohorts, who should undergo targeted studies of plausible mediators and broad screening for unexpected determinants of protection.

As outlined by articles throughout this supplement, there have been numerous reports of persons who were presumably exposed to human immunodeficiency virus (HIV) infection yet who remained uninfected. In light of the progressive spread of the HIV epidemic and the failure to date of most of our prevention strategies, it is increasingly important to evaluate potential deter-

minants of this protection. This stated, it may be very difficult to identify these determinants, for reasons we will outline in this brief overview.

Why do some presumably HIV-exposed persons seem to remain uninfected? Because only a small minority of sexual exposures to persons who are HIV infected result in transmission of infection [1], undoubtedly some exposed persons who remain uninfected have just been fortunate. One could nonetheless divide the universe of potentially protective mechanisms into those characterized by diminished intrinsic susceptibility to infection, protective adaptive immune defenses, protective innate immune defenses, and another category that we will call protection mediated by incidental events.

As we start to explore these potential determinants of protection in various cohorts, several caveats are in order. First, the risks for HIV acquisition and factors

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that might prevent acquisition may vary according to the route of transmission (eg, parenteral versus mucosal) and, among mucosal transmissions, may vary according to the nature of the mucosal surface (vaginal, rectal, penile, oral, or neonatal oral) [1, 2]. As a possible example of this, the -336T polymorphisms in promoter for the dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) seem to be associated with modest relative protection against parenteral but not mucosal acquisition of HIV infection [3].

Which factors may protect these persons? At the outset we must recognize the possibilities (if not probabilities) that multiple factors contribute to protection from infection, protection by any single factor may be only partial, and combinations of factors may collaborate to provide higher-level protection from infection [4]. Complicating this exploration even more is the recognition that protection may not be consistent over time, affected by recognized factors such as hormonal environment [5] or other factors not yet fully understood [4, 6], so that an individual apparently protected for 1 period of high-risk exposure may, for a variety of reasons, lose that relative protection at other times. Thus, it will not be an easy task to identify mechanisms that may protect against acquisition of infection.

ABSENCE OF HIV CORECEPTOR CHEMOKINE (C-C MOTIF) RECEPTOR 5 AND HIGH-LEVEL RESISTANCE TO HIV ACQUISITION

Somewhere, perhaps in Europe, several thousand years ago [7, 8], a 32-base pair deletion in the open reading frame for the chemokine (C-C motif) receptor 5 (CCR5) was passed on in the germ line and concentrated in the population. This mutation results in a frame shift, such that several downstream amino acids differ from the wild-type sequences until a premature stop codon terminates translation. Thus, a truncated nonfunctional protein is generated. Persons homozygous for this mutation are highly protected from HIV infection [9–11]. The haplotypic structure suggests that this mutation has occurred only once in human history, but the allele frequency for *CCR5* Δ 32 ranges from 5% to 15% in European populations; currently, the allele is in an apparent Hardy-Weinberg equilibrium, indicating that at least in recent times there was no major survival advantage or disadvantage to any of the 3 possible *CCR5* genotypes. However, with an allele frequency as high as 15% or more, there may have been powerful selection pressure for its persistence in humans. Plague and poxviruses have been accused of providing this selection pressure, but to date, no compelling evidence has been presented to support these possibilities. Likewise, there is no indication to date that there has been a remote interaction between Europeans and HIV or a related lentivirus. Relative protection may be conferred by the heterozygous state, particularly when accompanied by other polymorphisms in the other *CCR5* allele [12, 13]. As noted

above, protection may be relative and additive. Several reports have suggested that predicted variation in (or sensitivity to [14]) levels of β -chemokine ligands that can block or sequester *CCR5* may also determine the relative risk [15] or protection [16] from acquisition of HIV infection. To date, however, only complete absence of surface *CCR5* expression has been shown capable of providing high-level protection from HIV infection.

INNATE DEFENSES AGAINST HIV ACQUISITION

Innate defenses play critical roles in early defense against microbial invasion and must hold small enemies at bay, either blocking their tissue entry entirely or attenuating the magnitude of their growth until adaptive defenses can provide a targeted antimicrobial defense. Conceivably, variation in their expression or function could contribute to heterogeneous risks for HIV acquisition. Thus, the physicochemical characteristics of mucus and the intercellular barriers to fluid and particulate transport protect against tissue penetration of viruses. Variation in the characteristics of these elements might play a role in protection against HIV acquisition (reviewed in [17]). These surfaces also contain antimicrobial defense molecules, such as defensins, that have antiretroviral activity [18] and are variably expressed in human populations, with gene repeat frequencies ranging from 2 to 12 in any single individual [19]. Type I interferons comprise a large family of innate antiviral defense molecules that can be induced by a variety of microbial elements, including viruses and their genetic sequences [20]. Type I interferons bind to a common receptor expressed on numerous cell types and activate hundreds of cellular genes, including genes encoding certain proteins with antiviral activity [21], but at mucosal sites of challenge with the simian immunodeficiency virus, the kinetics of interferon induction may be too delayed to provide sufficient protection from experimental infection [22]. It is not unreasonable to propose that there may be population variability in the induction, regulation, and activities of such a complex defense network. To this point, a polymorphism near the gene for a type III interferon (interferon- λ 3) has been found to be linked to variability in the outcome of hepatitis C virus infection, both spontaneously and with treatment using a type I interferon plus ribavirin [23, 24].

The regulation of the activities of cellular RNase L (up-regulation) and eukaryotic initiation factor 2a (down-regulation) by type I interferons paralyzes translation of both cellular and viral RNAs [20]. Type I interferons also can activate other cellular elements, such as APOBEC3G, tripartite motif protein 5 α (TRIM5 α), and tetherin, that more selectively restrict the replication of lentiviruses, such as HIV. The importance of APOBEC3G and tetherin in antiviral defense is underscored by the existence of HIV proteins, specifically viral infectivity factor (VIF) and viral protein U (VPU), respectively, that block their

antiviral activities [25, 26]. Although human TRIM5 α does not block HIV replication very effectively, certain nonhuman primate homologues do [27], and small changes in the human sequences result in potent restriction of HIV replication [28]. In 1 small study, higher levels of TRIM5 α RNA were found in unseparated peripheral blood mononuclear cells from high-risk persons who escaped HIV infection than in cells from persons who did not escape infection [29]. A single-nucleotide polymorphism in the APBOEC3G gene (C49693T) has been associated in 1 study with an increased risk for HIV acquisition [30]. It is conceivable that other genetic variants in this restriction factor confer some degree of protection from infection. Do some persons apparently protected from HIV acquisition have intracellular restriction factors or combinations of restriction factors that are more highly expressed, more effective, or relatively resistant to the activities of their viral inhibitors? This remains an area worthy of further investigation. At the same time, knock-down studies have identified hundreds of cellular elements that are used for HIV propagation [31]. Whereas different studies have not always identified the same elements, it is plausible that differences in the expression or function of ≥ 1 of these elements also affect relative resistance to HIV infection.

Natural killer (NK) cells are capable of lysing viral infected cells, particularly cells that have diminished expression of class I major histocompatibility complex, a defense strategy used by many viruses, including HIV, to escape recognition by cytolytic T cells [32]. Epidemiologic analyses indicate that genetic differences in the expression of particular killer immunoglobulin receptors, typically expressed on NK cells, in conjunction with their human leukocyte antigen ligands determine differential courses of HIV infection [33]. Furthermore, elevated NK cell activity, along with elevated *KIR3DS1* transcript expression, has been associated with reduced risk of infection in a cohort of intravenous drug users, suggesting that these innate effector cells may play a role in preventing parenteral HIV acquisition [34, 35]. Is it possible that heterogeneities in NK cell regulation determine relative protection from HIV infection?

ADAPTIVE IMMUNITY AND PROTECTION FROM HIV INFECTION

Several groups [36–39], though not all, have found circulating T cells recognizing HIV peptides in exposed seronegative persons, and it has been proposed that these adaptive defenses might confer some level of protection against HIV acquisition. To our mind, this is a difficult proposal to support, because vaccine studies in nonhuman primates have often generated more robust T cell responses than the responses found in these exposed seronegative humans [40–42], yet these more robust responses have not succeeded in protecting challenged animals from infection. On the other hand, the high-level viral exposure

designed to infect all challenged animals in experimental settings may overwhelm modest immune responses that might protect against the lower levels of virus exposure that at-risk human subjects typically experience. This stated, it is our opinion that these systemic responses, when found, probably represent evidence of exposure to HIV rather than determinants of protection.

Exposed seronegative persons by definition do not have systemic antibodies reactive with HIV proteins. Several groups, however, have identified HIV-reactive antibodies in genital secretions of exposed seronegative subjects that in some settings have had neutralizing activity [43]. However, antibodies recognizing viral surface proteins could plausibly provide some level of protection, for example, by blocking the free movement of virus in mucosal fluid (Tom Hope, personal communication).

ROLE OF ENVIRONMENTAL DETERMINANTS IN DRIVING PROTECTIVE MECHANISMS

There is reasonable evidence that environmental factors may determine risk for or protection against HIV acquisition. Thus, ulcerative infections of the genitourinary tract are associated with increased risk of HIV acquisition [44]. Likewise, bacterial vaginosis is associated with increased risk of infection [45], and it is not clear whether this is a consequence of inflammation, replacement of the lactobacilli that are thought to be protective, or both. Because different colonizing microbes can differentially activate the spectrum of innate immune receptors that are widely distributed throughout the mucosal epithelium and on host defense cells, it is not unreasonable to propose that the microbiome at mucosal sites could plausibly but temporally and inconsistently determine the relative susceptibility to and protection from HIV infection. This proposal can be tested, but not simply, and if correct it will complicate efforts to sort out the determinants of HIV protection. Likewise, some viruses, including certain ubiquitous herpesviruses, may attenuate HIV replication and could conceivably mediate some level of protection (reviewed in [46]).

FOCUSING ON EXPOSED SERONEGATIVE SUBJECTS

As noted above, the risk of HIV acquisition with any mucosal exposure is estimated to be low, so calculating the magnitude of risk among exposed seronegative individuals is difficult. This adds another layer of complexity to studies aimed at identifying protective factors among exposed seronegative subjects; including in the study population persons who are just “lucky” and who do not possess the putative protective elements dilutes the power of the study to be informative. For this reason, it is very important to study individuals who are demonstrably at high risk for HIV infection yet remain uninfected, a group we

prefer to call “high-risk seronegative” (HRSN), but in line with recent consensus will call “HIV-exposed seronegative” (HESN).

RISK OF HIV INFECTION IN MEN WITH HEMOPHILIA

During the early years of the AIDS epidemic, HIV infection was broadly transmitted to the hemophilic population through administration of pooled concentrates of plasma-derived clotting factor VIII and clotting factor IX [47, 48]. Each vial of concentrate was derived from pooled plasmas of thousands of donors throughout the United States who donated before the AIDS agent was identified and before the blood supply could be protected through donor screening. By accessing infusion records from participants in the National Cancer Institute–sponsored multicenter hemophilia cohort study, Kroner et al were able to demonstrate that the risk of HIV acquisition was directly related to the type (higher with factor VIII than with factor IX) and intensity of exposure to these concentrates [49].

Among persons who were moderately or heavily treated before 1985, the risk of HIV infection was ~95%. We elected to study the 5% of these heavily treated men who did not acquire HIV infection despite intensive exposure. We found that 7 of these 43 HESN hemophiliacs were homozygous for the *CCR5Δ3* mutation, an estimated 16-fold concentration of this uncommon genotype that validates identification of this cohort as HESN [50]. An additional 7 were heterozygous for this allele, compatible with findings in larger cohort studies suggesting that partial or low-level protection from HIV infection might be conferred by the *CCR5Δ3* heterozygote state [12, 13].

So what are the other potential contributors to protection? In unpublished work, we did not find evidence of T cell reactivity with HIV antigens, and we could not find neutralizing antibodies in serum. The density of CCR5 was not low either on circulating CD4⁺ T cells or on circulating monocytes. Expression of CCR5-binding β -chemokines macrophage inflammatory protein (MIP) 1 α , MIP-1 β , and regulated on activation, normal T cell expressed and secreted (RANTES) chemokine was normal in several culture conditions; their cells were not demonstrably resistant to infection with CCR5-tropic HIV in vitro [50]. However, an interesting characteristic that distinguished these patients from unselected healthy control subjects not at risk for HIV infection was that their cells were less readily activated by exposure to multiple concentrations of lectin (phytohemagglutinin) and interleukin 2 [50].

This quiescent phenotype seems to be a consistent observation among HESN subjects, because both gay men who did not seroconvert in the Amsterdam cohort study had fewer activated cells than did gay men who later seroconverted [51], and similar findings were reported among HESN subjects in the Pumwani sex cohort [52]. HIV more readily infects activated cells that typically express higher levels of chemokine

receptors, which not only can serve as coreceptors for HIV but also may serve to amplify the initial rounds of infection at mucosal sites [53]. Activated T cells may also express less active forms of endogenous inhibitors of HIV replication, such as APOBEC3G [54]. It is therefore plausible to propose that activation state could be a factor that determines relative risk or protection from HIV acquisition. Careful study of activation phenotypes and the functional consequences of activation in circulating blood cells and at mucosal sites of HIV exposure is clearly warranted.

FUTURE DIRECTIONS

Aside from the well-defined protection from infection that is provided by the *CCR5Δ32* homozygous state, there are numerous proposed and plausible determinants of protection against HIV acquisition, but none has been clearly proved. Identifying these factors is a high priority, because provision or induction of these elements could prove useful in HIV prevention strategies. Identifying these factors will also be challenging, because protection could be partial, inconsistent, relative, and overcome with sufficient challenge or other alteration in susceptibility. Important protective factors also might provide protection against HIV acquisition at some routes but not others. Thus, it is especially important that cohort studies assembled to explore the prevalence of these potentially protective factors be homogeneous at the start and rigorously defined to include only persons at the highest risk for HIV acquisition.

SUMMARY

There is compelling epidemiologic evidence supporting the existence of additional factors that protect persons from acquisition of HIV infection. Identifying these factors is a high scientific priority, but for the reasons outlined above, this will not be a simple task. Success in this endeavor will require the establishment of cohorts of persons unambiguously at high risk for HIV infection but who have nonetheless escaped infection. Effective recruitment of these cohorts will require attention to local and social sensitivities. To these cohorts must be applied targeted studies of plausible protective mediators, as well as genetic screens to dissect other determinants of protection that are not yet suspected.

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