Outcomes of a National Institute of Allergy and Infectious Diseases Workshop on Understanding HIV-Exposed but Seronegative Individuals

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Abstract

The fascinating conundrum that some individuals who are exposed to HIV in ways that would make viral transmission highly likely, yet are able to remain uninfected, has been appreciated for many years. As early as the late 1980s, reports of such individuals began appearing in the HIV/AIDS literature. Despite the critical importance of understanding possible mechanisms of natural HIV resistance for developing effective prevention strategies, numerous obstacles have prevented this essential area of scientific exploration from moving forward. The Workshop held on July 8–9, 2010 and supported by the Office of AIDS Research (OAR), the National Institute of Allergy and Infectious Diseases (NIAID), and the National Institute on Drug Abuse (NIDA) at the NIH hosted 200 participants and utilized the expertise of 42 AIDS researchers as invited speakers, session chairs, and discussion leaders for presentations and breakout sessions in an attempt to remove some of those obstacles. Accomplishments of the participants included developing a consensus for a new general term for the field, HIV-exposed seronegative (HESN), while recognizing the necessity to identify and utilize secondary descriptive criteria such as exposure level, risk group, duration of seronegativity, or natural resistance. Three key questions for future research were also identified by the group: (1) What is different in HESN versus those who get infected? (2) What is the immune response in HESN and is it just a marker of exposure or a correlate of protection? (3) What are the HESN host factors that help HESN resist infection? This report briefly summarizes the presentations, and describes future directions for addressing these questions and challenges.

Introduction

A “WORKSHOP ON HIV-EXPOSED AND RESISTANT” was convened July 8–9, 2010 by the Division of AIDS (DAIDS) of the National Institute of Allergy and Infectious Diseases (NIAID) in partnership with the Office of AIDS Research (OAR) and the National Institute on Drug Abuse (NIDA). The primary purpose of this workshop was to review the current status of the field, and to identify scientific gaps (Table 1). It brought together a diverse group of experienced AIDS investigators some of whom have been in this field for a long time, and others who have not been involved in the field, but who could bring critically relevant expertise to the current challenges and opportunities. The workshop included presentations and two major breakout sessions to permit participants to brainstorm on a number of thorny issues that have plagued the field since its inception. This meeting was also

aimed at continuing the progress made at the International Symposium on Natural Immunity to HIV (ISNIH) <http://www.icid.com/isnih/> held in November 2009 in Winnipeg^{1,2} and exploring the next steps to determine if there are specific mechanism(s) explaining natural HIV protection in individuals exposed to HIV who remain seronegative or demonstrate resistance to infection. This workshop had three goals:

1. Develop specific definitions of “high exposure” and “uninfected or HIV resistant”;
2. Identify assays that would be most useful for studies to elucidate mechanisms of natural resistance, assays that would benefit from standardization, and new assays that need to be developed; and
3. Brainstorm on key questions that are most important to answer as well as novel, out-of-the-box ideas for moving the field forward.

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TABLE 1. FIFTEEN QUESTIONS AND ISSUES POSED BY PARTICIPANTS PRIOR TO THE WORKSHOP

Definitions, assays, and quantitation

How do we define “exposed uninfected”?

To what are highly exposed seronegative (HESN) individuals exposed?

Can the field arrive at a consensus on EU definition and choosing common assays?

Mucosal emphasis and immune aspects

What mucosal parameters are distinctly different in the resistant population compared with exposed and seroconverted populations?

Which types of inhibitory antibodies can mediate protection, and what systemic or mucosal concentrations are needed? To establish correlates of protective immunity at mucosal surfaces.

What is the role of innate mucosal immunity in resistance to HIV infection?

Is there really an immunologic basis for resistance to HIV infection?

The field needs to come to grips with the fact that conventional approaches will not permit the identification of correlates of immune protection and that there might be different mechanisms to get protection from infection. Integrated approaches are critical.

Nature of the host’s immune reaction at the first encounter with the foreign virus if there is no preexisting immunity.

What immune mechanism protects highly exposed seronegative women from infection in spite of HIV exposure? Are these women able to make a local antibody response to viral fragments that can lead to ADCC in the genital tract?

Do the strong T cell responses to the HIV proteome correlate to the protection from HIV-1 infection and/or disease progression?

Genetic aspects, models, cohorts

Genetic association studies of large populations of HIV-exposed and resistant subjects and controls that focus on genes involved in the innate immune response and immune activation pathways (e.g., TLR, IRF, HLA, KIR).

What are the main genetic correlates that confer resistance?

Development of cohorts large enough to address correlates of natural immunity to HIV.

During the on-line registration for the Workshop participants were asked to submit their most important question that the HESN field needs to answer. During the Workshop participants were invited to vote on the set of questions received. This table represents the questions receiving the most votes.

While the field has lacked a shared name for individuals who are exposed to HIV and remain seronegative or demonstrate resistance to infection, there was consistent discussion throughout the two days that such a name was urgently needed. The group in the end agreed to recommend HESN or HIV-Exposed Seronegative as the general term for the field, while recognizing that it does not address all aspects of the phenomenon. It was also accepted that future meetings would develop additional terms that would more specifically define these individuals identified within specific HIV-1 transmission categories. These additional terms will identify critical secondary descriptive criteria such as exposure levels, duration of seronegativity or natural resistance to HIV-1 infection, and mode of transmission. The general HESN term will be used throughout this summary.

The first part of the workshop was devoted to presentations and discussions in breakout sessions of challenges inherent with different modes of transmission and populations being studied: Commercial Sex Workers, People with Hemophilia, Discordant Couples, Intravenous Drug Users, and Mother-to-Child Transmission. The second part of the workshop focused on what is known about mechanisms, and tools used and those that are needed to move from observations on association to an understanding of causation.

To begin the meeting and link this workshop to the previous symposium Dr. Frank Plummer (Public Health Agency of Canada) summarized the outcomes of the ISNIH conference. At that meeting, participants supported the idea of generating a consensus descriptive name and definition for identifying naturally HIV-resistant subjects. The group also proposed to develop tools to quantitate risk of exposure to HIV, share protocols, and standardize methods that would allow comparison of data between different studies and cohorts.

The formation of working groups to take an active role in moving the consortium concept forward was also endorsed. Following Dr. Plummer, Dr. Gene Shearer (National Cancer Institute) in the charge to the workshop participants reiterated the need for researchers in the field to collaborate and pool data, share samples, and standardize their methodologies.

Part One: Defining “Exposed” and “Uninfected”

This section of the workshop, devoted to challenges inherent with different modes of transmission, was opened by Dr. Bruce Walker (Ragon Institute of MIT, MGH, and Harvard) who acknowledged two fundamental issues in the field: reaching consensus on a definition of HIV-resistant subjects and reproducibility of results. He described a blinded study of 61 discordant couples in Zambia performed by his group. They found that these multiply exposed, uninfected individuals did not have any detectable HIV-specific CD8 T cell responses in blood using ELISpot and ICS assays, demonstrating that examining blood for HESN markers may not be the right place to look in all situations. Dr. Walker proposed that the creation of a specimen repository of persons with a consensus definition of HESN would address these two gaps and have a significant impact on future progress in HESN research. To begin to address this, Dr. Walker proposed that 5–10 HESN individuals and 5–10 controls should be identified and cells collected by leukapheresis. Mucosal tissue samples from different sites including the lower gastrointestinal tract and genital biopsies would also be acquired. The samples would be characterized using state-of-the-art techniques, and then expanded and transferred to an HESN-specific specimen repository. Blinded samples would then be

made available to the research community through an application process to a steering committee.

HESN cohort: commercial sex workers

Dr. Rupert Kaul (University of Toronto) defined HESN female sex workers in the Nairobi cohort as individuals who did not become infected despite self-reported risk and clear infection pressure, documented through HIV incidence data in their non-HESN sex worker peers. The major obstacle to defining an HESN cohort, in the commercial sex worker or any context, is that transmission is a rare event and so persistent HIV seronegativity is the norm rather than the exception. The HIV incidence rate in female sex workers is very variable: in the early days of the Pumwani cohort the HIV incidence rate was 50%,³ but this has fallen over time. Dr. Kaul urged that the definition of baseline risk must be supported, if possible, by a careful collection of longitudinal epidemiological data. In addition, confounding variables complicate our ability to define the mucosal immune correlates of HIV resistance. By definition, HESN sex workers have frequent unprotected sex: not only does semen induce immunological changes in the mucosa, but genital infections can also cause profound genital immune alterations. Therefore, a careful definition of cohort HIV epidemiology and coinfection status is a key component of any HESN study.

HESN cohort: people with hemophilia

Dr. Michael Lederman (Case Western Reserve University) stated that quantifying risk of exposure is difficult, and analysis of risk is complicated by the prevalence of infection among contacts. Because of these difficulties Dr. Lederman has chosen hemophiliacs to study HIV resistance. Some individuals with hemophilia were infected with HIV through transfusion of contaminated concentrates of pooled plasma from thousands of U.S. donors before HIV was identified and the blood supply was protected. Infusion records make it possible to identify which individuals received a high intensity of exposure.^{4,5} His group has attempted to determine the protective mechanisms that defend against HIV-1 infection. In their cohort, 43 hemophiliacs were at an estimated 95% risk for HIV acquisition, but remain uninfected; seven were homozygous for the 32 base pair deletion in the CCR5 gene; the mechanism of protection in the other 36 individuals remained uncertain.⁶ Lymphocytes of these individuals were less readily activated to proliferate than were lymphocytes of healthy controls. There was also a significantly lower frequency of alloreactive antibodies in serum of the HESN hemophiliacs than among those who later became infected. Sorting out the plausible determinants of decreased *in vivo* susceptibility to HIV infection is challenging, and Dr. Lederman cautioned that a rigorous definition of "high risk" for infection is needed. Broad genetic screens complemented by targeted functional studies will be needed to identify the factors essential to establishing the HESN phenotype in HESN.

HESN cohort: discordant couples

Dr. Jairam Lingappa (University of Washington) presented a risk model to quantify HIV-1 exposure based on epidemiological variables associated with risk of HIV-1 transmission in an HESN cohort of 3408 heterosexual discordant couples derived from a recently completed longitudinal clinical trial.

The epidemiological variables included in this model are plasma HIV RNA, circumcision status, gender of uninfected partner, age of both infected and uninfected partners, and frequency of unprotected sex. Using multivariable modeling they obtained the hazard ratio for each of these factors and used the model coefficients to translate this into a risk score. Consistent with previous studies, the factor with the greatest impact on HIV-1 transmission risk was plasma HIV-1 RNA level of the HIV-1-infected partner.⁷ Based on viral sequence data from both partners in transmitting couples, individuals who were enrolled as HIV-1 uninfected were stratified into three categories: linked infection (virus transmitted from an enrolled HIV-1-infected partner), unlinked infection (virus transmitted by a third partner), and never infected (no transmission). Using these definitions he found that the median risk scores for partners who went on to develop linked infection differ significantly from the distributions for unlinked infections ($p < 0.001$) and uninfected individuals ($p < 0.001$). There was no significant difference between risk scores for unlinked and uninfected partners ($p = 0.3$). This model needs to be validated in the other HESN cohorts. A simpler model using only plasma HIV viral load also yielded statistically significant differences between linked and unlinked or uninfected individuals. Further work is being done to assess whether this HIV-1 risk score analysis among HIV-1-serodiscordant couples can provide insights that are more broadly applicable to other HESN cohorts.

HESN cohort: intravenous drug users

Dr. Don Des Jarlais (Beth Israel Medical Center) focused on injecting drug users (IDUs). In IDU populations with high rates of syringe sharing HIV incidence can be very high with a risk at, or greater than, 10/100 person-years. However, in some populations with moderate rates of syringe sharing HIV incidence may be extremely low, under 0.5/100 person-years.⁸ These data suggest the possibility of an innate immune factor that protects against HIV infection at moderate rates of syringe sharing among whom both the HIV transmission rate and prevalence are high. In collaboration with Dr. Jay Levy (University of California, San Francisco) his group found that CD8 T cells from exposed but uninfected IDUs can suppress HIV replication in infected CD4 T cells without killing the cells (J. Levy and D.C. Des Jarlais, unpublished data). This activity appears to be primarily mediated through secretion of an unidentified CD8 cell factor, producing a cell-mediated noncytotoxic antiviral response (CNAR). As with other populations of exposed but HIV-seronegative persons, there are complex methodological issues and ethical factors in conducting this research.

HESN cohort: mother-to-child transmission

Dr. Grace Aldrovandi (Children's Hospital of Los Angeles) reviewed the literature on "HIV-exposed or resistant" in mother-to-child transmission (MTCT). HIV transmission in MTCT can occur during gestation, intrapartum, or through breast milk. In this group, the timing of when virus is transmitted during pregnancy can be made based on when the virus is first detected in the infant, as well as by the genotypic lineage of the virus found in the mother and child. In some cases protection in this cohort can be attributed to the CCR5 $\Delta 32$ deletion. Possession of HLA A2/6802 supertype alleles is associated with a reduction of risk for HIV acquisition in the

first 6 months of life but not afterward.⁹ In these children having a reduction of risk for HIV acquisition multiple immunological changes have been observed in addition to HIV-specific T cell responses, including changes in T cell subsets, cytokine and chemokine production, and maturation factors among others. These immune changes may protect the children from HIV acquisition. There was discussion about reports that show these children can suffer from immune abnormalities that result in growth and developmental problems.^{10,11}

HESN: animal models

No animal model has been established to study the phenomenon of HESNs. Dr. Jeff Lifson and Dr. Jake Estes described the usefulness of the SIV model to understand the immunological mechanisms that may intervene to protect against HIV acquisition. In monkeys, the timing of the infection, the dose, route, number of exposures, and transmitted virus can all be precisely controlled. Intrarectal or intravaginal routes of infection can result in “abortive” infection. Dr. Lifson cautioned that negative data had limited interpretive value. Dr. Estes described the early host innate responses following intravaginal challenge that ultimately result in CD4⁺ T cell recruitment to fuel viral expansion at the portal of entry in addition to highlighting the usefulness, as well as the limitations, of the intravaginal route of SIV infection in macaques.¹²

Part Two: Identification of Mechanisms of Natural Resistance to HIV-1

This part of the workshop explored assays currently being used by the field to define cohorts, and to understand the mechanisms behind observed associations.

Behavioral instruments for determining exposure

Dr. Sarah Fidler (Imperial College of London) described the efforts by the Center for HIV/AIDS Vaccine Immunology (CHAVI) to develop a clinical definition of the “exposed, uninfected” phenotype. In the CHAVI002 cohort they studied HESN HIV resistance factors in men who have sex with men (MSM) in the UK and heterosexual discordant couples in Uganda. For this study they developed a sexual behavioral questionnaire that collected specific details of frequency of sex acts over variable time periods. These data were used in a novel mathematical “risk algorithm” based on published data from HIV-discordant couple studies, to quantify HIV exposure risks and enable longitudinal comparisons within and between individuals and between sites. Dr. Fidler emphasized that for this approach it is vital to enroll the appropriate control (HIV-uninfected unexposed) population(s) for comparison. The control cohort must be engaged in a similar frequency of sexual activity as the test population, be in a monogamous relationship, and be free of STI in the preceding year. One of the caveats of the questionnaire is that bias in reporting techniques can mislead the risk score.

Proteomic approaches

Using surface-enhanced laser desorption ionization mass spectrometry (SELDI-MS) Dr. Blake Ball (Public Health Agency of Canada) and his group have identified a serine antiprotease, elafin/trappin-2, from genital tracts of HIV-resistant Kenyan sex workers that is associated with protec-

tion against HIV acquisition.¹³ *In vitro*, this protein has been shown to quell HIV's ability to infect cells.¹⁴ Using alternate proteomic platforms such as 2D-DIGE and 2D LC-FT-MS approaches they further identified several additional serine proteases (serpins) to be overexpressed at the level of the female genital tract including Serpin B1, B3, 4, and B13.^{15,16} These serpins and other antiproteases may target early infection events by reducing immune activation, inhibiting HIV-1 infection locally, maintaining epithelial integrity, and/or controlling infected cell population expansion long enough for adaptive responses to become active.

Cytotoxic T lymphocytes in mucosa and blood

Dr. Julie McElrath (University of Washington) reflected on lessons learned from the HESN MSM cohort study in Seattle. Enrichment of the heterozygosity for the CCR5-delta 32 and CCR5 promoter -2459 A to G genotype combination was observed, in association with a lower density of CCR5 expression on CD4⁺ T cells and CD14⁺ monocytes.¹⁷ Although HIV-specific T cell responses can be detected intermittently in peripheral blood and the mucosa from these high-risk men, these responses usually do not persist.

T cell responses

Dr. Andrew McMichael (University of Oxford) described T cell responses in HESNs in the CHAVI002 cohort. In both MSMs and heterosexual discordant couples, HIV-specific CD4 T cell responses were detected. Dr. McMichael uses a cultured ELISpot assay using interleukin (IL)-2 that permits expansion of cells 10- to 20-fold prior to testing for immune response. Their assay shows good correlation with other standard ELISpot assays but is more sensitive. They observed that HESN-exposed seronegatives and HUSN-unexposed seronegatives have had CD4 T cell responses specific for HIV peptides. An interesting observation is that both HESN and HUSN individuals appear to be responding to the same HIV peptides, suggesting that these peptides are immunodominant in the early affected patients because they are a preexisting memory of responses, which may be cross-reactive to these epitopes. HESN persons have significantly stronger and more persistent responses over time than control unexposed uninfected (UU) donors. T cell responses were found only when they used the highly sensitive cultured ELISpot assay, and responses were found for CD4 T cells and not CD8 T cells. A possible reason for the difference between T cell subsets is that CD4 T cells require less antigen for priming and less stringent peptide selection for HLA class II. McMichael's group is conducting similar assays in the placebo arm of a VaxGen study to determine whether CD4 T cell responses detectable before HIV exposure affect outcome of subsequent infection.

Mucosal proteins

Dr. Georgia Tomaras (Duke University) and colleagues determined HIV envelope antibody responses in a cohort of HESNs. Previous studies have found mucosal anti-HIV IgA in HESN, whereas others have failed to detect such antibodies. Dr. Tomaras measured HIV-specific antibody responses in seminal plasma and cervical lavage samples from the study participants in CHAVI002. HIV-specific IgA antibodies can be

detected in some HESN subjects. Mucosal Ab epitopes recognized in HESNs include the 2F5 MPER epitope, gp140, gp120, and gp41 proteins.

Innate immunity

Dr. Persephone Borrow's (University of Oxford) group defined the innate immune characteristics of CHAVI002 participants, studying systemic levels of cytokines, chemokines, and other factors and the activation state and functions of circulating DC, NK, and NKT subsets. Their findings indicated that innate immune responses in HESNs appear to be in a state of chronic low-level activation. Many of the changes in innate response in HESNs were similar to alterations in innate responses observed during chronic HIV infection.

Genetics

Dr. David Goldstein (Duke University) shed light on genomic analysis in three ongoing studies to find determinants that influence HIV acquisition: hemophiliacs (CHAVI014) in North America, Japan, and Europe; heterosexual couples in Malawi; serodiscordant couples in Africa; and MSMs in the United States. In the Malawi cohort, no genetic factors have been found as of yet using a genome-wide association study (GWAS). A GWAS, however, is known to adequately represent common variants (5–50% minor allele frequency) and is not so efficient at representing more rare variants. This suggests that finding the variants that influence acquisition may depend on sequencing-based approaches able to detect rarer gene variants.

Alloimmunity aspects

Dr. Gene Shearer (National Cancer Institute) discussed alloimmune aspects of HESN. Studies reported that *in vitro* allostimulation activates peripheral blood mononuclear cells (PBMCs) to produce the RNase, eosinophil-derived neurotoxin,¹⁸ and APOBEC3G (A3G) by memory T cells.¹⁹ Also, uninfected partners of sexual discordant couples (HESN) produce CCR5 antibody that downmodulates CCR5 expression and inhibits *in vitro* replication of CCR5 HIV strains²⁰ and upregulates A3G²¹ in blood and cervical tissue compared to controls.

Recommendations and Future Directions

Definitions

Since the first observations of individuals who appeared to be highly exposed to HIV, but remained seronegative or uninfected,^{22–27} a plethora of definitions and nomenclature describing these individuals and the field have proliferated. These have included Exposed Seronegative (ESN), Highly Exposed Persistently Seronegative (HEPS), Exposed Uninfected (EU), and HIV Resistant (HR). Participants at this workshop had the following concerns about previously used nomenclature: the term “uninfected” was objectionable because some subjects might be infected; “highly” was objectionable because of an absence of consensus on what “high” means (i.e., is it high viral load or high number of times or length of exposure); and “resistant” was objectionable because this is very difficult to define.

The workshop participants agreed on a general term for the field: HIV-Exposed Seronegative or HESN and recommended

its use as much as possible whenever appropriate. It was also clear that the majority of participants thought that additional definitions were required to clarify the risk of exposure for each route of transmission, and whenever possible the specific level of exposure. Investigators studying mother-to-child transmission took exception to the word seronegative because HIV-exposed children have been shown to develop an antibody-based immune response.

Assays and methodology for defining exposure and measuring natural resistance

The HESN subjects are critically important to the HIV prevention field. But regardless of many years of work there remains little mechanistic understanding of these subjects or their meaning for HIV prevention beyond the homozygous CCR5 delta 32 deletion. Data presented at the Workshop showed a variety of sporadic immune responses and some novel host factors as HIV defense agents. Many of these studies were limited because they examined blood samples to the exclusion of other tissue compartments and secretions. Better quantitative characterization of exposure and biomarkers of exposure must be a high priority. The “exposure score” in discordant couples offered by Dr. Jai Lingappa (University of Washington) is a good example of what is needed to allow groups to be compared directly. He proposed a specific method for defining the risk of HIV exposure. This model develops a risk score for each cohort, and for discordant couple cohorts would include the following variables: the frequency of unprotected anogenital sex, index case plasma viral load, durability of partnership, as well as other factors such as circumcision.

There was also considerable discussion during the sessions and breakout groups about the importance of emphasizing the identification of mechanism(s), and not simply cataloging associations with the phenomenon. It was suggested that a study design to identify a protective biomarker must be enriched in HESN, establish durability over time, be confirmed in different cohorts, and demonstrate plausibility and mechanism *in vitro*, or in an animal model. Dr. Myron Cohen (University of North Carolina) suggested that for this purpose a drug discovery paradigm would be desirable, if not essential. What are the critical steps to prove that a defensive factor(s) is causative? Can a defensive factor be turned into an HIV prevention product?

The lack of an animal model for the HESN phenomenon was viewed by participants as a gap in the tools available for the field. Although there are limitations with all animal models, studies could be developed with known levels of exposure. Results could assist human cohort studies in which exposure must be based on a risk score.

Most important questions

Workshop participants identified the following questions that need to be addressed to move the field forward.

- What mucosal parameters are distinctly different in the resistant population compared with exposed in the seroconverted populations?
- What is the nature of the host's immune response at the first encounter with the virus if there is no preexisting immunity?

- What host features (proteins/expression patterns/genes) or viral features are responsible for resistance?

Participants recognized that mechanisms of resistance may vary for sexual, mother-to-child, intravenous drug use (IVDU), and blood transmission in people with hemophilia. Studies should be conducted on well-defined HESN and non-HESN controls using standardized assays and independently confirmed by other investigators using shared specimens and/or cohorts. It is hoped that future meetings will include reports aimed at identifying answers to these questions.

Future directions

A second International Symposium on Natural Immunity to HIV (ISNIH) is being planned by investigators at the Public Health Agency of Canada in partnership with the University of Manitoba that is expected to take place in June 2011. It is anticipated that this Conference will continue to expand on the theme of collaboration that was emphasized in this Workshop and at the November 2009 ISNIH Conference in Winnipeg.

Working Groups have been established by Canadian investigators to explore the current obstacles to understanding the HESN phenomenon: Epidemiology (definitions and tools to assess resistant phenotypes); Data Cataloging and Collection (cohort size, structure, and specimens); SOP Standardization and Protocol exchange (identify/develop the best SOPs and protocols); Legal and Ethical (MTAs and IRB approvals); and Immunologic Correlates of Protection and Genetic Correlates of Protection (two groups to decide on new research priorities and the expansion of existing studies in the fields of immunology and genetics, respectively). Please contact Dr. Adrienne Meyers (Adrienne.Meyers@phac-aspc.gc.ca) if you are interested in participating in these working groups.

Investigators involved in these studies should benefit by engaging researchers outside of the HIV/AIDS field, and establishing linkages for collaboration and utilization of specimens from the NIAID Epidemiology Cohorts (MACS, WIHS, WITS, IeDEA, and CNICS), acute infection studies, and the HIV/AIDS Clinical Trials Networks (<http://www.niaid.nih.gov/topics/hivaids/research/pages/clinicalresearch.aspx>).

The Division of AIDS at NIAID remains supportive of meritorious, unsolicited applications to identify mechanisms responsible for individuals who are HIV-Exposed Seronegative (HESN). There are ongoing discussions to determine other mechanisms that the NIH may be able to utilize to provide assistance to this field.

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References

1. Cohen J: AIDS vaccine research. HIV natural resistance field finally overcomes resistance. *Science* 2009;326:1476–1477.
2. Fowke K and Meyers A (Eds.): Natural immunity to HIV infection. *J Infect Dis* 2010;202(Suppl 3):S327–S328.
3. Fowke KR, Nagelkerke NJ, Kimani J, *et al.*: Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. *Lancet* 1996;348:1347–1351.
4. Menitove JE, Aster RH, Casper JT, *et al.*: T-lymphocyte subpopulations in patients with classic hemophilia treated with cryoprecipitate and lyophilized concentrates. *N Engl J Med* 1983;308:83–86.
5. Lederman MM, Ratnoff OD, Evatt BL, and McDougal JS: Acquisition of antibody to lymphadenopathy-associated virus in patients with classic hemophilia (factor VIII deficiency). *Ann Intern Med* 1985;102:753–757.
6. Salkowitz JR, Purvis SF, Meyerson H, *et al.*: Characterization of high-risk HIV-1 seronegative hemophiliacs. *Clin Immunol* 2001;98:200–211.
7. Lingappa JR, Hughes JP, Wang RS, *et al.*: Estimating the impact of plasma HIV-1 RNA reductions on heterosexual HIV-1 transmission risk. *PLoS One* 2010;5:e12598.
8. Des Jarlais DC and Friedman SR: HIV infection among intravenous drug users: Epidemiology and risk reduction. *AIDS* 1987;1:67–76.
9. MacDonald KS, Embree JE, Nagelkerke NJ, *et al.*: The HLA A2/6802 supertype is associated with reduced risk of perinatal human immunodeficiency virus type 1 transmission. *J Infect Dis* 2001;183:503–506.
10. Miyamoto M, Pessoa SD, Ono E, *et al.*: Low CD4+ T-cell levels and B-cell apoptosis in vertically HIV-exposed non-infected children and adolescents. *J Trop Pediatr* 2010;56:427–432.
11. Slogrove AL, Cotton MF and Esser MM: Severe infections in HIV-exposed uninfected infants: clinical evidence of immunodeficiency. *J Trop Pediatr* 2010;56:75–81.
12. Li Q, Estes JD, Schlievert PM, *et al.*: Glycerol monolaurate prevents mucosal SIV transmission. *Nature* 2009;458:1034–1038.
13. Iqbal SM, Ball TB, Levinson P, *et al.*: Elevated elafin/trappin-2 in the female genital tract is associated with protection against HIV acquisition. *AIDS* 2009;23:1669–1677.
14. Ghosh M, Shen Z, Fahey JV, Cu-Uvin S, Mayer K, and Wira CR: Trappin-2/Elafin: A novel innate anti-human immunodeficiency virus-1 molecule of the human female reproductive tract. *Immunology* 2009;129:207–219.
15. Burgener A, Boutilier J, Wachhi C, *et al.*: Identification of differentially expressed proteins in the cervical mucosa of HIV-1-resistant sex workers. *J Proteome Res* 2008;7:4446–4454.

16. Burgener A, Sainsbury J, Plummer FA, and Ball TB: Systems biology-based approaches to understand HIV-exposed uninfected women. *Curr HIV/AIDS Rep* 2010;7:53–59.
17. Hladik F, Liu H, Speelman E, *et al.*: Combined effect of CCR5-Delta32 heterozygosity and the CCR5 promoter polymorphism –2459 A/G on CCR5 expression and resistance to human immunodeficiency virus type 1 transmission. *J Virol* 2005;79:11677–11684.
18. Rugeles MT, Trubey CM, Bedoya VI, *et al.*: Ribonuclease is partly responsible for the HIV-1 inhibitory effect activated by HLA alloantigen recognition. *AIDS* 2003;17:481–486.
19. Jeffrey P, Wang Y, Seidl T, Babaahmady K, Vaughan R, and Lehner T: The effect of allogeneic in vitro stimulation and in vivo immunization on memory CD4(+) T-cell APOBEC3G expression and HIV-1 infectivity. *Eur J Immunol* 2009;39:1956–1965.
20. 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;164:3426–3433.
21. 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;195:960–964.
22. Horsburgh CR, Ou C-Y, Holmberg SD, Schochetman G, Jaffe H, Lifson, AR, and Rutherford, GW: Correspondence, Human immunodeficiency virus type 1 infection in homosexual men who remain seronegative for prolonged periods. *N Engl J Med* 1989;321:1678–1681.
23. Imagawa DT, Lee MH, Wolinsky SM, *et al.*: Human immunodeficiency virus type 1 infection in homosexual men who remain seronegative for prolonged periods. *N Engl J Med* 1989;320:1458–1462.
24. Pan LZ, Sheppard HW, Winkelstein W, and Levy JA: Lack of detection of human immunodeficiency virus in persistently seronegative homosexual men with high or medium risks for infection. *J Infect Dis* 1991;164:962–964.
25. Clerici M, Giorgi JV, Chou CC, *et al.*: Cell-mediated immune response to human immunodeficiency virus (HIV) type 1 in seronegative homosexual men with recent sexual exposure to HIV-1. *J Infect Dis* 1992;165:1012–1019.
26. Cheynier R, Langlade-Demoyen P, Marescot MR, *et al.*: Cytotoxic T lymphocyte responses in the peripheral blood of children born to human immunodeficiency virus-1-infected mothers. *Eur J Immunol* 1992;22:2211–2217.
27. Ranki A, Mattinen S, Yarchoan R, *et al.*: T-cell response towards HIV in infected individuals with and without zidovudine therapy, and in HIV-exposed sexual partners. *AIDS* 1989;3:63–69.

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