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Mucosal and Plasma IgA from HIV-1-Exposed Uninfected Individuals Inhibit HIV-1 Transcytosis Across Human Epithelial Cells

Claudia Devito,* Kristina Broliden,‡ Rupert Kaul,† Lennart Svensson,‡ Kari Johansen,‡ Peter Kiama,‡ Joshua Kimani,‡ Lucia Lopalco,§ Stefania Piconi,§ Job J. Bwayo,† Francis Plummer,†∥ Mario Clerici,∥ and Jorma Hinkula‡

HIV-1-specific IgA has been described in the genital tract and plasma of HIV-1 highly exposed, persistently seronegative (HEPS) individuals, and IgA from these sites has been shown to neutralize HIV-1. This study examines the ability of IgA isolated from HEPS individuals to inhibit transcytosis across a tight epithelial cell layer. A Transwell system was established to model HIV-1 infection across the human mucosal epithelium. The apical-basolateral transcytosis of primary HIV-1 isolates across this mucosal model was examined in the presence and the absence of IgA isolated from the genital tract, saliva, and plasma of HEPS individuals enrolled in both a sex worker cohort in Nairobi, Kenya, and a discordant couple cohort in Italy. In the absence of IgA, HIV-1 primary isolates were actively transported across the epithelial membrane and were released on the opposite side of the barrier. These transcytosed HIV-1 particles retained their ability to infect human mononuclear cells. However, IgA purified from the mucosa and plasma of HEPS individuals was able to inhibit HIV-1 transcytosis. Inhibition was seen in three of six cervicovaginal fluid samples, five of 10 saliva samples, and three of six plasma samples against at least one of the two primary HIV-1 isolates tested. IgA from low risk, healthy control subjects had no inhibitory effect on HIV-1 transcytosis. The ability of mucosal and plasma IgA to inhibit HIV-1 transcytosis across the mucosal epithelium may represent an important mechanism for protection against the sexual acquisition of HIV-1 infection in HEPS individuals.

*Department of Clinical Virology, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden; ‡Department of Medical Microbiology, University of Nairobi, Nairobi, Kenya; †Department of Clinical Virology, Swedish Institute for Infectious Disease Control, Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden; §Immunobiology of HIV Unit, San Raffaele Scientific Institute, Milan, Italy; ∥Division of Infectious Diseases, L. Sacco Hospital, Dip-Lita Viaba, Milan, Italy; and ‡Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada

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2 Address correspondence and reprint requests to Dr. Kristina Broliden, Department of Clinical Virology, F68, Karolinska Institute, Huddinge University Hospital, S-141 86 Stockholm, Sweden. E-mail address: kristina.broliden@viruslab.hs.sll.se

3 Current address: Chair of Immunology, University of Milan, Via GB Grassi 74, 20157 Milan, Italy.

4 Abbreviations used in this paper: HEPS, highly exposed, persistently seronegative; CFV, cervicovaginal fluid; NSI, nonsyncytium-inducing; SI, syncytium-inducing.

of this epithelium (12, 13). In elegant studies, Bomsel et al. (14) have shown that HIV-1 particles were internalized by the epithelium after contact of HIV-1-infected cells with the apical surface of an epithelial cell line. The virus crossed the epithelial tight barrier without infecting the epithelium itself, using transcytosis. The transcytosed particles could subsequently infect host submucosal mononucleated target cells, indicating a new mechanism for virus spread in vivo. Furthermore, this transcellular pathway was blocked by anti-HIV envelope protein dIgA or pIgM purified from HIV-1-infected individuals (15). We now show that IgA from HEPS individuals is also able to block transcytosis of HIV-1 primary isolates across a model of the tight epithelial barrier, suggesting a novel mechanism by which these individuals may be protected against mucosal acquisition of HIV-1.

Materials and Methods

Study subjects

Female sex workers were enrolled through a dedicated sex worker clinic in the Pumwani area of Nairobi, Kenya (codes 320-1803). Despite behavioral counseling and condon provision, it is estimated that these women have a minimum of 6 unprotected sexual exposures to HIV-1/year. Women are classified as HIV-1 resistant if they are seronegative at enrollment and remain both seronegative and PCR negative during at least 3 years of follow-up while continuing in sex work (16). Sex workers included in the present study were enrolled during the 1999 annual clinic resurvey, when a standard questionnaire was completed, a physical examination performed, and blood and mucosal samples collected.

Italian exposed uninfected heterosexual partners of HIV-1-infected individuals were also enrolled in the study (codes N13-N30). This cohort of couples, discordant for HIV IgG in serum, has previously been described (7). All the HIV-IgG-positive partners were undergoing combined antiretroviral therapy. No correlations were detected between lack of transmisson and CD4 counts, plasma viral load, or CDC stage of the seropositive partner. All the couples reported regular aprotinin intake in vanguard intercourse during the last 3 years. The study subjects were selected from the two cohorts on the basis of available mucosal and plasma samples. IgA purified from mucosal and plasma specimens from healthy low risk HIV-1-seronegative Kenyan (n = 7), Italian (n = 10), and Swedish (n = 6) individuals were used as controls.

Ethical approval was obtained from University of Nairobi’s scientific ethical review committee, the H. L. Sacco ethical review committee, and from the Karolinska Institute ethical committee, respectively.

Clinical samples

Cervicovaginal fluid (CVF) and plasma were collected as previously described (10). Saliva was collected by different techniques in the two cohorts. For Kenyan female sex workers saliva was collected directly into a sterile 50-ml tube and frozen at −80°C (18). For Italian exposed uninfected heterosexual partners of HIV-1-infected individuals (codes 10), and Swedish (n = 6) individuals were used as controls.

Inhibition of transcytosis was calculated as follows: (1 − (absorbance value of the p24 Ag content in sample/absorbance value of the p24 Ag content without sample)) × 100.

Recombinant p24 Ag

The virus stock was from CA-CaCo-2 cell line (19). The supernatants from viral cultures were concentrated by ultracentrifugation and dialyzed extensively against PBS. The IgA-depleted fractions contained no HIV-1-neutralizing activity (see Footnote 5). An ELISA was used for quantification of purified IgA (20), and IgA purified from pooled normal human colostrum (Sigma, Life Technologies, Paisley, U.K.) was used as standard to perform the quantification.

Transcytosis assay

To measure transcytosis, the cDNA pIgR-transfected human intestinal epithelial cell line CaCo-2 was used (21). The cell cultures were performed on Transwell nitrocellulose filters (0.4-μm pore size filter, Costar, Cambridge, MA) in MEM containing 10% inactivated FCS (Life Technologies) during 8–10 days, until the cell lines formed tight monolayer cultures. One milliliter of medium per well was exchanged at the basolateral side, and 0.5 ml/well was exchanged at the apical side every 2 h. The tightness of the epithelial cells was measured as electrical resistance and was >400 ohm/cm² (range, 420–630) at the start of analysis with apical HIV-1 transmisson or basolateral IgA addition or sampling as described previously (22, 23). Transepithelial electrical resistance was measured with a Millicell ERS resistance apparatus (Millipore, Bedford, MA). To further control the tightness of the epithelial cell barrier, passage of recombinant p24 Ag (p24 Ag) was measured.

IgA purified from samples from the HEPS or low risk control individuals (20–30 μg from plasma, 4–18 μg from saliva, and 0.3–7.9 μg from CVF, respectively) was added to the basolateral side of the Transwell chambers. Due to the small amount of mucosal material available from each subject, a lower concentration had to be used compared with plasma.

Films of/cellulose membranes of were collected from the apical side after 0, 1, 2, 4, 6, 12, 24, 48, and 72 h. At 1 and 2 h, 1 ml of the basolateral and apical medium, respectively, was collected and analyzed for p24 Ag content, and medium was collected for HIV-1 isolation. HIV-1 isolation was performed by coculture of 1 ml of 10⁶ PHA-stimulated PBMCs in a 24-well plate for 14 days. To analyze the epithelial cells for content of infectious virus, the apical side of the chambers was washed three times for 5 min each time with serum-free medium and once with 1% trypsin-containing PBS without Ca²⁺ and Mg²⁺ to remove cell surface-bound virus. Adding 1 ml of MEM/10% FCS completed the trypsinization procedure. Cells were spun down, and virus isolation was performed from the cell pellet as described above.

Inhibition of transcytosis was calculated as follows: (1 − (absorbance value of the p24 Ag content in sample/absorbance value of the p24 Ag content without sample)) × 100.

Results

Model system

A Transwell model system was used to assess the ability of IgA to inhibit the transcytosis of HIV-1. This system consisted of two chambers separated by a nitrocellulose filter on which a tight monolayer of epithelial cells (CaCo-2) was allowed to grow. The lower chamber, filled with medium, represented the basolateral (mucosal) side to which Abs were added. The upper chamber, filled with medium, represented the apical (luminal) side to which HIV-1-infected PBMCs were added (Fig. 1). Transepithelial electrical resistance was measured to ensure that the membrane was tight. The tightness of the epithelial cells was further confirmed by the lack of passage of recombinant p24 Ag. The Ag was added to the apical side and could not be detected in the basolateral medium in any of the consecutive samples collected during 48 h. In the
absence of a cell layer on the filter, the p24 Ag was detectable in the lower chamber within a few hours (data not shown).

Transcytosis of purified IgA

To evaluate the efficiency and kinetics of IgA transcytosis, purified IgA samples representing HEPS individuals were added to the basolateral side of the epithelial cell layer. Altogether, IgA purified from 10 CVF samples and eight plasma samples from the female sex workers and from seven saliva samples from the exposed HIV-1 IgG-seronegative partners were used to evaluate the efficiency of IgA transcytosis. When IgA purified from CVF and saliva were quantified on the apical side after 12–24 h, 5.5–16.8% (median, 9.2%) and 10.3–13% (median, 10.2%), respectively, of the Abs were recovered. Corresponding values for plasma were 1–2% (median, 1.7%). The percentage of transcytosed IgA was calculated as the ratio of detectable IgA in the apical medium and the total amount of added IgA in the basolateral medium at a given time point. The kinetics of Ab transcytosis across the cell layer is shown for one of the samples (N18) and, for comparison, a human anti-HIV-1 mAb of the IgM class (Fig. 2). The highest IgA concentrations were seen at 24 and 48 h, respectively. IgG Abs were not transcytosed across the epithelial cell layer (data not shown).

Transcytosis of HIV-1

The efficacy of HIV-1 transcytosis was evaluated by applying cell-free virus or different numbers of HIV-1-infected PBMCs to the apical side of the Transwell system. When using cell-free virus, the efficacy of transcytosis was poor (data not shown). When applying either $1 \times 10^6$ or $5 \times 10^5$ PBMCs/well (four wells of each concentration), the electrical resistance decreased in two of the eight wells after 6 h. This reduction in electrical resistance indicates that the tightness of the epithelial cell barrier had been damaged and
therefore could not be used for measuring transcytosis. However, when applying $1 \times 10^5$ HIV-1-infected PBMCs the electrical resistance remained stable (>400 ohm) for >36 h in 10 of 10 wells, so this concentration of PBMC was used in all the following experiments. Furthermore, electrical resistance was measured in all wells to ensure that the cell membrane remained intact. If the resistance was <400 ohm/cm², transcytosis was not evaluated.

The efficacy of HIV-1 transcytosis across the epithelial model system was measured by adding PBMCs infected with either of the two HIV-1 primary isolates to the apical side and measuring the HIV p24 Ag concentration in the basolateral medium at different time points. HIV p24 Ag was detected within 1 h (Fig. 3A). These transcytosed viral particles were shown to retain their infectious capacity by coculture of the basolateral medium with fresh PBMCs (medium was collected 12 h post-HIV-1 challenge; Fig. 3B).

p24 Ag was never detected in the CaCo2 cells when uninfected PBMCs were tested in a parallel control culture (data not shown).

$Inhibition of HIV-1 transcytosis by IgA$

Having established the efficiency and kinetics of HIV-1 and IgA transcytosis, the ability of IgA to inhibit HIV-1 transcytosis was examined. Purified IgA samples from the saliva of the two HEPS individuals from the Italian discordant couple cohort (N16 and N18) and one Italian low risk uninfected control were added to the basolateral medium 6 h before addition of HIV-1-infected PBMCs to the apical medium. The ability of this IgA to block HIV-1 transcytosis across the mucosal epithelium was examined in our experimental model by testing the apical medium, epithelial cells, and basolateral medium for the presence of infectious virus. In case N16, infectious HIV-1 could not be detected in either epithelial cells or basolateral medium, indicating a complete inhibition of HIV-1 (NSI and SI strains) transcytosis by the IgA (Fig. 4A). IgA purified from saliva from case N18 completely inhibited transcytosis of the HIV-1 isolate of the SI phenotype, whereas inhibition of the NSI phenotype was less efficient (Fig. 4B). In contrast, IgA purified from the low risk uninfected control individual did not inhibit transcytosis of HIV-1, with high levels of infectious virus detected in the basolateral medium (Fig. 4C). Recombinant gp160 Ag inhibited the blocking capacity by >80% in two of three IgA samples in the HIV-1 transcytosis assay (Fig. 4D).

These observations were extended to include IgA purified from genital tract (CVF), saliva, and plasma of Kenyan HEPS sex workers and from saliva of individuals from the Italian discordant couple cohort (Table I). IgA was added to the basolateral side of the epithelial cell layer and was preincubated for 6 h, after which HIV-1-infected PBMCs were added to the apical side. HIV-1 p24 Ag was quantified in the basolateral medium of these experimental wells and compared with the p24 Ag content in control wells without IgA.

$FIGURE 4.$ Inhibition of HIV-1 transcytosis by IgA. Purified IgA samples from saliva of three individuals were added to the basolateral medium 6 h before addition of HIV-1-infected cells to the apical medium. In this way the ability of the Abs to block HIV-1 transcytosis could be measured. Two primary HIV-1 isolates of different phenotypes (NSI and SI) were tested in parallel. A, Subject N16 (HEPS individual). B, Subject N18 (HEPS individual). C, Low risk uninfected control individual. D, Recombinant gp160 Ag (HTLVIIIB/Lai, clade B) inhibited the effect of IgA purified from saliva of subject 1260. In this example, the results of the HIV-1 isolate (SI) 6794 are shown. Apical medium, cell suspensions, and basolateral medium, respectively, were cocultured with fresh human PBMCs for 7 days, after which HIV-1 p24 Ag concentrations (picograms per milliliter) were measured (values given in the figures).
In this way the ability of IgA to inhibit HIV-1 transcytosis was expressed as a percentage. IgA purified from the CVF of three of six Kenyan HEPS sex workers could inhibit at least 67% of HIV-1 (NSI phenotype) transcytosis. When an HIV-1 virus of the SI phenotype was used, only one of four of the CVF IgA samples inhibited transcytosis. Salivary IgA from four of 10 of HEPS individuals could inhibit at least 67% of the HIV-1 transcytosis of an NSI isolate, and three of nine could inhibit the HIV-1 transcytosis of an SI isolate. This ability of HEPS individuals from the discordant couple cohort (N13-N30) was limited and was not seen in the saliva of Kenyan sex workers (no. 320-1785). Corresponding figures for plasma IgA from sex workers were three of six for both NSI and SI isolates (Table I). No inhibition of HIV-1 transcytosis was seen in IgA purified from the CVF, saliva, and plasma of low risk uninfected controls (Table I).

### Discussion

Many HEPS individuals show signs of HIV-specific systemic and mucosal cellular immune responses as well as the presence of HIV-specific IgA Abs (5–7, 10, 11, 26, 27). We recently reported that these IgA Abs could neutralize HIV infection of mononuclear cells (11) (see Footnote 5). In the present report we show for the first time that IgA from some of these individuals is able to inhibit transcytosis of primary HIV-1 isolates across a human epithelial lining. This activity was seen in IgA purified from both systemic and mucosal compartments in two different cohorts of HEPS individuals. The first cohort consisted of Kenyan HIV-1 IgG-seronegative female sex workers, and the second cohort consisted of Italian HIV-1 IgG-seronegative heterosexual partners of HIV-1-infected individuals. The IgA-mediated inhibition of HIV-1 transcytosis in these HEPS individuals may contribute to their apparent resistance to HIV infection.

Mucosal IgA able to inhibit HIV-1 epithelial transcytosis was found in the genital tract of half the Kenyan sex workers tested in this study. IgA-mediated neutralization of HIV-1 (11) and CD8+ lymphocyte responses to HIV-1 CTL epitopes (28) have been previously described in the genital tract of this cohort. The finding of IgA-mediated inhibition of HIV-1 transcytosis therefore suggests that these highly exposed, seronegative women may have developed an array of mucosal immune defenses to protect against sexual HIV-1 acquisition. It is of interest that IgA with the ability to inhibit HIV-1 transcytosis was not found in the saliva of these sex workers, because oral sex is quite uncommon in the sex worker cohort (29), while IgA purified from the saliva of seronegative subjects in the Italian discordant couple cohort was often able to inhibit transcytosis. However, it is also possible that this is a reflection of the different methods used to collect saliva specimens in the two cohorts.

This study used an experimental system that mimicked natural HIV-1 infection, in which the first steps of infection involve contact between secretions containing HIV-infected cells or cell-free virus and the mucosal luminal surface (14, 30). The mucosal surfaces have a covering of either pluristratified epithelial cells (in the vagina, exocervix, prepuce, and anus) or a simple epithelial monolayer (in the rectum, endocervix, and intestine). The epithelial cells are not infected themselves, but due to their polymeric Ig receptor function and polarization they can actively transport (transcytose) molecules such as dimeric IgA and HIV-1 primary isolates to the submucosal layer (31). In this study the human intestinal polarized epithelial cell line CaCo-2 was grown on a filter in a two-chamber (Transwell) culture system (22) in which the investigator had independent access to both apical and basolateral medium (14, 15, 32). The culture conditions allowed the cells to form a tight barrier and optimized for polarity development. The upper chamber thus represented the apical (luminal) side, and the lower chamber represented the basolateral (mucosal) side of the epithelium. In this model, both IgA Abs and HIV-1 were readily transcytosed across the polarized epithelial cell layer by active transportation when tested in separate experiments. The HIV-1 particles that were transcytosed remained infectious, as shown by coculture experiments with mononuclear cells. Within this epithelial model, IgA purified from the mucosal and systemic compartments of HEPS individuals was able to completely or partially inhibit HIV-1 epithelial transcytosis in about 50% of the IgA fractions purified from CVF, saliva and plasma samples when tested against at least one of two primary HIV-1 isolates. The main part of the inhibition of HIV-1 transcytosis was seen in IgA purified from the CVF, saliva, and plasma of low risk uninfected controls (Table I).

### Table I. IgA-mediated inhibition of HIV-1 transcytosis across a human epithelial cell layer

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sample</th>
<th>HIV-1 Primary Isolate (phenotype) Inhibition of Transcytosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6727 (NSI)</td>
</tr>
<tr>
<td>HEPS Individuals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>CVF</td>
<td>49</td>
</tr>
<tr>
<td>1250</td>
<td>CVF</td>
<td>0</td>
</tr>
<tr>
<td>1700</td>
<td>CVF</td>
<td>65</td>
</tr>
<tr>
<td>1705</td>
<td>CVF</td>
<td>69</td>
</tr>
<tr>
<td>1707</td>
<td>CVF</td>
<td>93</td>
</tr>
<tr>
<td>1803</td>
<td>CVF</td>
<td>84</td>
</tr>
<tr>
<td>No. of samples &gt;67%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N13</td>
<td>Saliva</td>
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</tr>
<tr>
<td>N16</td>
<td>Saliva</td>
<td>91</td>
</tr>
<tr>
<td>N18</td>
<td>Saliva</td>
<td>66</td>
</tr>
<tr>
<td>N23</td>
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</tr>
<tr>
<td>320</td>
<td>Saliva</td>
<td>30</td>
</tr>
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</tr>
<tr>
<td>1705</td>
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<td>19</td>
</tr>
<tr>
<td>1785</td>
<td>Saliva</td>
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<td>No. of samples &gt;67%</td>
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<td></td>
</tr>
<tr>
<td>320</td>
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</tr>
<tr>
<td>1803</td>
<td>Plasma</td>
<td>92</td>
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<td>No. of samples &gt;67%</td>
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<td></td>
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<tr>
<td>183</td>
<td>Plasma</td>
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</tr>
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<tr>
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<tr>
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</tr>
<tr>
<td>188</td>
<td>Plasma</td>
<td>26</td>
</tr>
<tr>
<td>No. of samples &gt;67%</td>
<td></td>
<td>0/6</td>
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</table>

transcytosis by the IgA Abs was shown to be at the intracellular level rather than on the apical or basolateral side. The frequency with which IgA from HEPS individuals is able to inhibit HIV-1 epithelial transcytosis must be examined in larger studies, as must the ability of IgA to inhibit transcytosis of differing HIV-1 clades.

Although IgA isolated from the mucosa and plasma of half the HEPS subjects studied was unable to inhibit transcytosis, this inhibition could have been underestimated in the mucosal samples for several reasons. Firstly, a lower mucosal IgA concentration was used in the assay compared with plasma IgA, and the collection of mucosal material is less standardized than blood sampling. These factors make comparison of results from mucosal and systemic sites difficult. Furthermore, IgA present in genital secretions may be degraded by IgA proteases, such as those produced by Neisseria gonorrhoeae. For this reason, only sex workers with no laboratory or clinical evidence of gonorrhoea were enrolled in this study. Finally, the jacalin used for purification of IgA mainly binds IgA1, and Abs of the IgA2 subclass may have been missed. These technical difficulties together with different epitope specificities in mucosal vs systemic IgA (33) may explain why in some cases the same individual had divergent IgA responses in the different body fluids. To better elucidate functional IgA responses in the different body compartments an expanded study would need to include IgA epitope specificities and subclass distributions as well as quantitation of the secretory component (S-IgA).

This study examined the ability of HEPS IgA to inhibit the epithelial transcytosis of two phenotypically distinct HIV-1 primary isolates, an NS1 and an SI isolate from HIV-1 clade B. Transcytosis of both isolates was inhibited by HEPS IgA. Interestingly, the female sex workers who could block transcytosis of these isolates had probably been exposed to HIV-1 clade A and D isolates, which are the dominant subtypes in Nairobi (34). This suggests that IgA Abs in this HEPS population may be broadly cross-reactive and are perhaps specific for conserved epitopes of HIV-1. These epitopes may not be the same as those involved in neutralization of HIV-1 infection in PBMCs, because some samples that were able to inhibit transcytosis were not able to neutralize HIV infection of PBMCs and vice versa (see Footnote 5). It has been suggested that IgA from HEPS individuals recognize several conserved epitopes on HIV-1 gp41 that differed from epitopes recognized by HIV-1-positive individuals (35). Furthermore, passive immunotherapy with IgG mAbs against the HIV-1 gp120 CD4 binding region and the conserved gp41-neutralizing epitope ELDKWS protected rectally simian HIV-challenged monkeys (36). Having secretory IgA directed against these epitopes could well result in protection from infection. Combining Abs against these epitopes together with Abs against the HIV-1 cellular coreceptor CCR5 (37) may function synergistically, reducing the amount of Abs necessary for obtaining a protective immunity.

It is also likely that other factors contribute to HIV-1 resistance in the HEPS subjects, such as cell-mediated immunity and chemokine repertoires (38, 39). However, to date no environmental factors (including condom use, number of sexual contacts, and sexually transmitted diseases), or viral or genetic factors have been found to correlate with protection against HIV-1 infection in these cohorts (16). When looking at the HIV IgG discordant couples, it cannot be ruled out that HIV viral load in semen/cervicovaginal secretions, HIV phenotype and/or genotype, or host genetic factors may have contributed to the lack of observed HIV-1 transmission.

In contrast, the cohort of Kenyan sex workers has remained uninfected despite presumed exposure to many variants of HIV-1 and to individuals with varying viral loads. Therefore, at least in the latter group, it seems likely that the phenomenon of HIV-1 resistance is mediated by host factors, rather than to qualitative or quantitative viral factors. Although HIV-1 has not been detected in the exposed individuals, it seems unlikely that induction of these immune responses could have occurred without at least transient viral replication. Reduction of at-risk sex within the discordant couple cohort has been followed by a drop in HIV-specific IgA titers, indicating that HIV-specific immunity in exposed uninfected subjects could be dependent on a continuous exposure (10).

A combination of different immune mechanisms is probably necessary for protection against HIV-1 infection. By defining different functional mechanisms of IgA interactions with the virus on the mucosal surface we hope to contribute to the development of an efficient vaccine against HIV that would stimulate mucosal humoral immunity.

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References


