Neutralization of Human Immunodeficiency Virus Type 1 (HIV-1) Mediated by Parotid IgA of HIV-1-Infected Patients

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Infection with human immunodeficiency virus type 1 (HIV-1) has been shown to elicit a serum antibody response with neutralizing activity against T cell line–adapted HIV strains and primary HIV-1 isolates. Mucosal surfaces are the primary route of HIV-1 infection. Evidence is presented here for the presence of HIV-neutralizing antibodies in secretions. Infection of mucosal cells with HIV stimulates systemic and mucosal immune responses and results in the generation of neutralizing antibodies. Serum IgG and IgA neutralize HIV-1MN infection of susceptible T cell lines; serum IgG inhibits more effectively. Mucosal IgA purified from parotid saliva of HIV-1–seropositive individuals could neutralize both a T cell line–adapted strain and a primary isolate. The neutralizing activity of IgA was not directed against the anti–third-variable-loop or the anti-ELDKWA epitope. Thus, the specificity of mucosal IgA for HIV-1 neutralization epitopes remains to be determined and may provide insight into development of a mucosal vaccine.

The immune system can be divided into 2 functionally independent compartments: systemic and mucosal. The development of human immunodeficiency virus (HIV) vaccines has primarily targeted the systemic immune response, which has a limited value for the prevention of mucosally contracted diseases [1]. The predominant mode of HIV transmission occurs at the mucosal surface of genital tracts in both sexes by contact with HIV-infected secretions, such as semen [2] and cervicovaginal fluid [3]. The induction of an effective mucosal immune response is of primary importance to protect against HIV infection. Most antibodies produced in human mucosae are secretory IgA (sIgA). sIgA has been shown to act as a first line of defense against infection by preventing pathogen adherence to epithelial walls, neutralizing intracellular microorganisms within epithelial cells, and binding and removing these foreign agents in the lamina propria. This results in limiting systemic exposure and virus spreading [4]. Many groups, including ours, have shown evidence for the presence of antibodies to HIV in external secretions of HIV–1–positive patients. The sIgA and IgG antibodies to HIV-1 have been detected in various body fluids, including parotid saliva, cervicovaginal secretions, semen, breast milk, bronchoalveolar lavage fluids, and intestinal fluids [5–11]. Neutralizing antibodies are frequently found in serum from HIV-1–infected patients and may have a protective effect. Some investigators have found that high serum-neutralizing antibody titers are strongly associated with an asymptomatic state [12]. These antibodies are often, but not always, found in long-term nonprogressor patients and are usually associated with low virus load [13]. Serum IgA antibodies isolated from HIV-1–infected individuals were also reported to be capable of neutralizing [14, 15] and enhancing HIV infection of CD4+ lymphocytes in vitro [16]. Recently, specific anti–HIV-1 sIgA has been detected at mucosal sites of most uninfected HIV-negative partners of infected individuals (discordant couples) for HIV infection [17]. Moreover, intracellular neutralization of HIV transcytosis across epithelial barriers by anti-HIV envelope protein dimeric IgA or IgM was clearly shown [18]. In this study, we show that mucosal IgA elicited by natural infection such as parotid saliva IgA in HIV-positive individuals does have a neutralizing effect in vitro on T cell line–adapted (TCLA) and primary isolates.

Materials and Methods

Subjects. Serum specimens and parotid saliva samples were collected from 92 adults with sexually or drug-transmitted HIV-1 infection (men : women ratio, 73 : 19; mean age, 36.1 years; range, 18–61 years). The study population was categorized on the basis of disease stage. It consisted of 60 otherwise healthy, asymptomatic, HIV-positive patients (CDC stage II), 14 HIV-positive patients with persistent generalized lymphadenopathy (CDC stage III), and 18 patients with ≥1 AIDS-defining illnesses (CDC stage IV). A control group of 30 healthy, HIV-negative volunteers matched for age and
sex was included in the study [15]. The production of saliva was stimulated by a drop of green lemon applied to the lateral side of the tongue. The volume of parotid saliva obtained from each patient varied between 1 and 2 mL. Parotid saliva was collected ≥1 h after a meal by use of a flexible catheter introduced into the parotid duct (Centracath ORX, Vygon, France). The concentration of albumin, measured by nephelometry, was used to evaluate the extent of parotid saliva contamination by blood proteins during the sampling process. Subjects with an albumin concentration value greater than the controls' mean value ± 2 SD were excluded from the study. Accordingly, 17 parotid saliva samples were also discarded from the present study. Sera and parotid saliva were stored as aliquots at −80°C until use. The patient groups were also categorized according to their numbers of circulating CD4¹ ⁰ T cells: 25 patients had >500 cells/mm³; 25 patients had 200–500 cells/mm³; and 25 patients had <200 cells/mm³.

**ELISA for total IgA and IgG.** Total IgA and IgG levels in serum and parotid saliva were determined by ELISA as described elsewhere [19]. Concentrations of serum IgA and IgG were extrapolated from reference curves generated by assaying dilutions of a pool of serum specimens from 100 healthy blood donors whose IgA and IgG concentrations had been determined by immunonephelometric technique. Parotid saliva IgA concentrations were extrapolated from a curve established with dilutions of purified colostrum sIgA standard (Cappel, Cochranville, PA).

**ELISA for specific antibody reactivity to HIV-1.** The presence of IgA antibodies specific to HIV in serum and parotid saliva was tested by use of a commercially available kit (Rapid Elavia Mixt; Diagnostics Pasteur, Marne-la-Coquette, France), as described elsewhere [11]. Samples with optical densities (ODs) greater than the cutoff serum values were considered positive for either serum or saliva antibodies. Because IgG may compete with specific IgA binding, serum and parotid saliva samples were systematically absorbed with lyophilized sheep antiserum to human IgG (RF-Absorbant; Behringwerke AG, Marburg, Germany) before testing for the presence of IgA [19].

**Determination of antibody reactivity to HIV-1 proteins by immunoblot.** To determine the specificity of anti-HIV IgA to various viral proteins, including the surface membrane gp120, HIV-1–preblotted nitrocellulose strips (NewLav blot; Diagnostics Pasteur) were incubated overnight at 4°C with unfractionated and IgG-depleted parotid saliva, with a final dilution ratio of 1 : 2 in milk buffer [19]. After washes, bound immunoglobulins were probed with peroxidase-conjugated goat polyclonal antiserum to human α or γ chains (Cappel) diluted 1 : 100 in milk buffer, which was applied for 1 h at room temperature with gentle shaking. After washes, 2 mL of 0.1 M Tris-saline containing 0.05% (w/v) diaminobenzidine (Sigma, St. Louis) and 0.03% (v/v) H₂O₂ was added, and color reaction was allowed to develop for a few minutes. The bands were read visually by 2 independent observers.

**Affinity purification of IgA and IgG.** Saliva contains factors that inhibit infection with HIV-1 in vitro. One of these factors was recently identified as the secretory leukocyte protease inhibitor, a salivary protein that was reported to block HIV infectivity of monocytes and primary T cells at physiologic concentrations [20]. To avoid the complication that the use of diluted saliva would impose on our experiments, we used affinity chromatography to purify immunoglobulins. Purification of IgG from serum and parotid saliva was performed by use of protein G–sepharose chromatography (Sigma). IgA was purified by use of affinity-purified goat anti-human IgA (Jackson Immunoresearch Laboratories, West Grove, PA) immobilized through its carbohydrates on Carbolink Gel (Pierce, Rockford, IL). Isolated IgG and IgA were dialyzed successively against PBS and RPMI and then were concentrated (Centriprep 30; Amicon, Beverly, MA), sterilized by 0.22-µm–pore-size filtration, and analyzed for immunoglobulin content by use of ELISA. IgG was 99%–100% pure; IgA was 98%–100% pure.

We analyzed some samples by use of gel filtration chromatography (Sephacryl S-300 SF; Pharmacia, Uppsala, Sweden) to determine whether the parotid IgA was monomeric (possibly serum derived), polymeric (locally produced), or a combination of both. In all samples, purified IgA was exclusively polymeric and thus was produced locally, as was expected, because every sample containing albumin was systematically removed from the study.

**In vitro HIV-1 neutralization assays.** Unfractionated serum and purified IgG and IgA samples obtained from serum and parotid saliva were assayed for their ability to neutralize HIV. Duplicate samples of 100 µg of serum IgA or IgG (an amount previously used by Kozlowski et al [15]) or 1 µg of serum IgA or sIgA in 50 µL of culture medium were incubated for 1 h (IgG) or 2 h (IgA and sIgA) at 37°C with 50 µL of 100 TCID₅₀ HIV-1MN or non syncytium inducing (NSI) primary isolate clade B. The latter HIV-1 isolate was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institutes of Health, and from Dr. G. Melchers’s HIV-1 Isolate Panel. To perform the neutralization assays, we used target cells consisting either of phytohemagglutinin (PHA)– and IL-2–activated peripheral blood mononuclear cells (PBMC) or of MT4 cells. Antibody–virus conjugates were added to cell suspensions (100 µL; 5 × 10⁶ cells/mL) for 1 h at 37°C. Conjugates with HIV MN were applied to MT4 cells, and NSI conjugates were applied to PBMC. PBMC were obtained from buffy coats from healthy HIV-negative donors; they were cultured for 3 days in 10% fetal calf serum (FCS)-enriched RPMI 1640 in the presence of 2 µg/mL PHA. A 50% reduction of RT activity was considered significant.

**ELISA determination of IgA reactivity to HIV peptides.** Neutralizing IgAs were tested for their specific reactivity to the third-variable-loop (V3) region and to the ELDKWA epitope in the gp41 region. Microwell plates (Immulon II; Dynatech Laboratories, Chantilly, France) were coated overnight at 4°C with 5 µg/mL of V3MN amino acid (aa) sequence 303–333 or with 5 µg/mL of gp41MN aa 659–675 containing ELDKWA peptide (kindly provided by the Agence Nationale de Recherche sur le SIDA) in carbonate buffer. After washes, plates were saturated with 0.05% PBS-Tween and 10% FCS for 2 h at 37°C. After washes, IgG-depleted, IgA-containing samples were diluted as appropriate and incubated for 2 h at 37°C; a subsequent 1-h incubation step was performed with peroxidase-conjugated goat anti–α chain antibody (Cappel). The enzymatic reaction was allowed to develop with orthophenylene-diamine and was stopped by the addition of 0.1 N HCl. Reading was done at 492 nm. Results were expressed as OD per milligram of parotid saliva IgA. The positive control antibodies in this assay consisted of specific monoclonal antibodies raised against aa IHI-
Table 1. Characteristics of the 15 human immunodeficiency virus type 1 (HIV-1)-infected patients with IgA directed to env products in parotid saliva.

<table>
<thead>
<tr>
<th>Subject/ Sex</th>
<th>Age, years</th>
<th>CD4+ T cells/ mm$^3$</th>
<th>CDC status</th>
<th>sIgA, mg/mL</th>
<th>sIgA to HIV-1$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F</td>
<td>25</td>
<td>890 A1</td>
<td>100.5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2/M</td>
<td>28</td>
<td>653 A1</td>
<td>118.4</td>
<td>++</td>
<td>+</td>
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<tr>
<td>3/M</td>
<td>25</td>
<td>512 A1</td>
<td>80.6</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>4/M</td>
<td>53</td>
<td>502 A1</td>
<td>116.8</td>
<td>++</td>
<td>+</td>
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<tr>
<td>5/M</td>
<td>44</td>
<td>468 B2</td>
<td>83.3</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6/M</td>
<td>26</td>
<td>413 B2</td>
<td>52.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7/M</td>
<td>33</td>
<td>381 B2</td>
<td>164.9</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>8/M</td>
<td>61</td>
<td>324 B2</td>
<td>109.8</td>
<td>+</td>
<td>–</td>
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<tr>
<td>9/M</td>
<td>35</td>
<td>290 B2</td>
<td>201.7</td>
<td>+</td>
<td>–</td>
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<tr>
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<tr>
<td>11/F</td>
<td>26</td>
<td>222 B2</td>
<td>84.8</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>12/M</td>
<td>34</td>
<td>187 B3</td>
<td>309.5</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>13/F</td>
<td>33</td>
<td>144 B3</td>
<td>123.9</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>14/F</td>
<td>46</td>
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<td>++</td>
<td>–</td>
</tr>
<tr>
<td>15/M</td>
<td>32</td>
<td>54 C3</td>
<td>96.6</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE. CDC status, CDC 1993 classifications of AIDS: M, male; F, female; sIgA, secretory IgA.

$^*$ Reactivity of saliva IgA to HIV, studied by Western blot analysis and determined by 2 observers. ++, high reactivity; +, low reactivity; –, no reactivity.

The ability of purified serum immunoglobulins to protect cells from HIV infection was tested in a cell-free assay. Infection of cells by HIV could be inhibited by 100 μg of IgG obtained from all 15 HIV patients tested. However, at the same concentration, only 11 (73%) of 15 IgA samples were able to neutralize HIV infection of cells (figure 1). No correlation between neutralizing activity and CD4+ T cell counts could be found. In contrast, IgA from patients 5 and 7 increased the viral infectivity, suggesting that antibodies in these individuals have somehow facilitated HIV infection.

Neutralizing capacities of antibodies from parotid saliva. Before testing the neutralizing capacity of salivary IgA, we verified that IgA preparations did not contain factors that inhibit infection with HIV-1 in vitro. Such effect was not shown in any purified samples from controls.

The ability of 1 mm of IgA from parotid saliva to protect MT4 cells from HIVMN infection was tested in parallel with that of serum IgA (figure 2). Of the 15 parotid saliva specimens assayed, 7 effectively neutralized HIV infection. At the same concentration, 7 of 15 serum samples of IgA inhibited HIV infection. It is noteworthy that there was no concordance between the neutralizing ability of IgA isolated from the parotid saliva and from the serum in the same patients. Two patients had neutralizing IgA in saliva but not in serum, whereas 2 other patients had neutralizing IgA in serum but not in saliva. No correlation with CD4+ T cell counts was found.

We were able to study primary HIV isolate neutralization with IgA antibodies from parotid saliva in only 4 of the 7 patients because there was not enough immunoglobulin to study the others (figure 3). Of 4 parotid saliva samples that

Figure 1. Results of cell-free human immunodeficiency virus type 1 (HIV-1) neutralization assay testing the ability of 100 μg of purified IgG or IgA to inhibit HIV-1MN infection of MT4 cells and thus neutralize HIV-1 infection. Also shown is the mean inhibition observed in 6 HIV-1-seronegative controls. Results are expressed as the percentage of infection inhibition, compared with control cultures, without antibodies. The 15 patients were classified according to decreasing CD4+ T cell counts. The values on the y axis represent the percentage of viral infection over that observed in control cultures.
Results of cell-free human immunodeficiency virus type 1 (HIV-1) neutralization assay testing the ability of 1 μg of IgA purified from serum and parotid saliva to inhibit HIV-1\textsubscript{MN} infection of MT4 cells and thus neutralize HIV-1 infection. Also shown is the mean inhibition observed in 6 HIV-1-seronegative controls. The results are expressed as the percentage of infection inhibition, compared with control cultures, without antibodies. The 15 patients were classified according to decreasing CD4\textsuperscript{T} T cell counts.

Anti-V3 and gp41 epitope activities of IgA from parotid saliva. We then examined whether there was a relationship between the neutralizing capacity of parotid saliva and the specific recognition of the V3 antigenic region. This was addressed by means of a specific ELISA using an HIV-1\textsubscript{MN} gp120 V3 peptide as capture antigen (figure 4). Although some parotid saliva IgA exhibited an anti-MN V3 activity, this was not correlated with an increased ability to inhibit infection of MT4 cells by HIV-1\textsubscript{MN} isolate. Furthermore, none of the saliva samples with neutralizing IgA recognized the ELDKWA peptide, and only 4 of the 15 serum IgA samples were specific to this peptide (results not shown). This suggests that the ability of IgA to neutralize HIV infection was likely due to the presence of antibodies directed to viral protein determinants outside the tested regions, such as the V3 loop and the ELDKWA peptide.

Discussion

Mucosae are the major port of entry for HIV into an organism after contact with infected cells or with free virion present in sexual secretions of infected subjects [22]. The objective of this study was to determine whether natural HIV infection can induce the production of IgA antibodies with HIV-1 neutralizing activity at mucosal sites.

We confirmed previous data showing that serum anti-HIV IgA from HIV-infected patients possesses neutralizing activity [14, 15]. Half of the samples tested had serum IgA that could neutralize free HIV virions. Purified serum IgG was more effective in neutralizing HIV-1 infection than was an equal concentration of IgA. In addition, samples of purified IgA from 2 patients exhibited a higher viral infection level than did control IgA, suggesting that, at least at some concentrations, IgA may facilitate HIV infection. One study suggested that this effect could be mediated by FcaR [16]. In this study, antibody-dependent enhancement of promyelomonocytic cell line infection resulted from receptor-mediated endocytosis of FcaR-bound antibody-HIV complexes. In our study, we used a lymphocytic cell line that does not express FcaR. In this case, antibody-dependent enhancement is likely to occur by another mechanism. IgA could induce a conformational change in the HIV envelope that might increase infectivity [23]. Another mechanism could involve complement-mediated, antibody-dependent enhancement of HIV-1 infection. HIV-IgA immune complexes could be coated by C3 fragments; the interaction between virus-bound C3 and CR2 (CD21) has been reported elsewhere to enhance HIV-1 infection [24].

An important purpose of the present work was to examine the neutralization of HIV-1 by mucosal IgA antibodies. This approach was constrained by the limited volumes and concentrations of immunoglobulin available from parotid saliva. However, we were able to show that parotid saliva IgA can inhibit HIV infection with either a TCLA strain or a primary isolate.

![Figure 2](image1)

**Figure 2.** Results of cell-free human immunodeficiency virus type 1 (HIV-1) neutralization assay testing the ability of 1 μg of IgA purified from serum and parotid saliva to inhibit HIV-1\textsubscript{MN} infection of MT4 cells and thus neutralize HIV-1 infection. Also shown is the mean inhibition observed in 6 HIV-1-seronegative controls. The results are expressed as the percentage of infection inhibition, compared with control cultures, without antibodies. The 15 patients were classified according to decreasing CD4\textsuperscript{T} T cell counts.

![Figure 3](image2)

**Figure 3.** Results of cell-free human immunodeficiency virus type 1 (HIV-1) neutralization assay testing the ability of 1 μg of IgA purified from serum and parotid saliva to inhibit HIV-1 primary isolate infection of peripheral blood mononuclear cells and thus neutralize HIV-1 infection. Also shown is the mean inhibition observed in 4 HIV-1-seronegative controls. The results are expressed as the percentage of infection inhibition, compared with control cultures, without antibodies. The patients were classified according to decreasing CD4\textsuperscript{T} T cell counts.
Also shown is the mean level in 6 HIV-1 seronegative controls. The values given are optical density per milligram. We observed that the primary HIV-1 isolate was more resistant to neutralization than was the TCLA strain [25]. When we compared serum IgA and parotid saliva IgA in the same individuals, we did not find any correlation between the abilities of serum and parotid IgA to mediate neutralization of HIV. This confirms that mucosal immune responses are distinct from systemic immune responses [26]. Although comparisons of the neutralizing potency of serum and secretory IgA are difficult because the proportions of HIV-specific antibodies are different [27], mucosal IgA seemed to be more efficient than serum IgA in neutralizing HIV-1 infection. This could be explained by the polymeric structure of the mucosal secretory IgA [28]. We and others have reported that, despite the elevated levels of local IgA, the anti-HIV specificity, as determined in Western blot assays, was predominantly observed in the IgG isotype in parotid saliva and other secretions [11, 27]. Because of the very low level of IgG found within parotid saliva, quantities of purified IgG sufficient for in vitro assays could be obtained from 3 patients only; all 3 had IgG with substantial neutralizing activity against HIV (data not shown).

Neutralizing antibody activity is generally directed at a limited number of epitope clusters on the HIV surface glycoproteins gp120 and gp41. The neutralizing antibodies generated are mainly targeted at linear determinants in V3 [20, 29]. In our study, the reactivity against this peptide did not correlate with the ability of IgA antibodies to neutralize HIV. The ability of mucosal IgA to ELDKWA to block HIV transcytosis has been reported elsewhere [18]. No reactivity against the ELDKWA epitope of gp41 could be found in IgA from parotid saliva. We propose that the mechanism implicated in the HIV neutralization described in the present study is a synergistic mechanism dependent on the presence of antibodies directed against >1 viral determinant [30]. A more accurate way to characterize these epitopes would be to absorb successively the parotid IgA with different peptides to determine whether the neutralizing activity is still present. We are currently testing for the specificity of mucosal IgA to other HIV peptides in envelope glycoproteins. The identification of these peptides would promote the development of an effective mucosal vaccine against HIV.

Many groups are developing HIV vaccine strategies. One strategy is to elicit a mucosal immune response to an HIV vaccine [31, 32]. Although cellular immunity represents a crucial component of biological responses to HIV infection, there is a need to identify antibodies capable of protecting an individual against HIV-1 and other virus infections. Antibodies are essential for the elimination of free virus particles and for reduction of the magnitude of the infectious inoculum. Secretory IgA is the first immunological barrier at the mucosal level against infection. It blocks the infection of mucosae by pathogens, and this protective role has been shown in various viral infections [33]. Indeed, an important role for antibodies in the prevention of viral infections has been described in studies of HIV-1 infection in humans and chimpanzees. In these studies, antibodies are involved in many processes, including preventing, delaying, and decreasing the extent of HIV-1 infection [34, 35]. In a recent study of heterosexual couples discordant for HIV status, Mazzoli et al. showed that HIV-exposed, seronegative individuals had HIV-specific IgA in urine and in vaginalwash samples [17]. Furthermore, the development of mucosal simian immunodeficiency virus (SIV)–specific IgA was observed in SIV-vaccinated macaques resisting an intrarectal challenge with live infectious SIV [36]. Secretory IgA induced in mice by oral immunization with a multicomponent peptide vaccine were also capable of neutralizing HIV [37].

Epithelial barriers covering the mucosal surface in contact with cell-free or cell-associated HIV differ in their cellular organization: some are composed of a stratified squamous epithelium (e.g., the vagina), whereas others are covered by a single epithelial cell layer (e.g., the rectum). In vitro, primary HIV-1 isolates were found to cross epithelial cells from the apical side via transcytosis and to infect mononuclear cells at the basolateral side without infecting epithelial cells themselves [22, 38]. Hocini et al. have shown the ability of colostrum sIgA and IgG to block HIV-1 transcytosis [28]. Moreover, transcytosis of primary HIV isolates is blocked intracellularly by dimeric IgA or IgM [18].

In conclusion, we have shown that mucosal IgA is able to neutralize HIV. This may be an important finding for the development of a mucosal HIV vaccine. However, because this was an in vitro study, it may be difficult to determine whether immunoglobulin-mediated neutralization of TCLA or primary HIV-1 isolates of PBMC will have some protective effect in preventing mucosal HIV-1 infection in vivo. Other cell types may be targets for initial infection or adherence of HIV. Because natural infection usually results from the exposure of mucosae to virus-infected cells in secretions, more work needs to be done on understanding how to prevent the contamination by HIV.
infected cells. HIV-1 has been shown to infect epidermal Langerhans cells and dendritic cells in vitro [39]; these cells are present in simple and stratified epithelia [40]. Acute SIV vaginal infection of rhesus macaques resulted in an initial infection of dendritic cells within the lamina propria beneath mucosal epithelium [41]. The use of dendritic cell and primary mucosal isolates in a neutralization assay would be useful in estimating the protective effect of mucosal antibodies in natural infection against infected cells and in testing for the mucosal immunogenicity of a candidate HIV-1 vaccine.

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References


