Human Immunodeficiency Virus (HIV)–Specific IgA and HIV Neutralizing Activity in the Serum of Exposed Seronegative Partners of HIV-Seropositive Persons

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The presence and activity of human immunodeficiency virus (HIV)–speciﬁc antibodies were analyzed in the sera of 15 sexually exposed seronegative persons who had systemic HIV-speciﬁc cell-mediated immunity and IgA-mediated mucosal immunity and in their HIV-infected partners. The HIV-positive subjects had HIV-speciﬁc serum IgG and IgA; the seronegative persons had HIV-speciﬁc serum IgA in the absence of IgG. Testing of the seronegative persons 1 year after the interruption of at-risk sex showed that no IgG seroconversion had occurred and that HIV-speciﬁc IgA serum concentrations had declined. Serum from the HIV-exposed seronegative persons was analyzed for the ability to neutralize primary HIV-1 isolates. Neutralizing activity was detected in 5 of 15 sera and in 2 cases was retained by serum-puriﬁed IgA. Thus, the immunologic picture for resistance to HIV infection should include HIV-speciﬁc cell-mediated immunity as well as HIV-speciﬁc IgA-mediated mucosal and systemic immunity.

Materials and Methods

Populations studied. We examined 15 HIV-seropositive subjects and their HIV-seronegative partners who were previously included in a study on HIV-speciﬁc mucosal immunity. Serologic status was determined by EIA (HIV 1/2 + 3rd generation ELAs; Abbott Laboratories, Abbott Park, IL) and Western blot (New Lav Blot I; Sanofi Pasteur, Paris, France). ESPs were repeatedly HIV seronegative by culture and RNA virus load methods. Inclusion criterion for ESPs was a history of multiple unprotected sexual episodes (with the same HIV-seropositive partner) for \( > 2 \) years. Of the 15 HIV-seropositive partners, 8 were exposed to HIV via in-

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Written informed consent was obtained from all patients before enrollment. The protocol was approved by the Research Ethics Committee, Ospedale Luigi Sacco, Milan.

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travenous drug use and 7 sexually (5 bisexuals, 2 heterosexuals).

Serologic analysis was determined on fresh samples by EIAs (Abbott HIV I/II + 3rd generation). IgA antibodies were detected by modified EIAs (HIV-1 EIA; Calypse Biomedical, Berkeley, CA) based on a recombinant HIV-1 envelope protein. The procedures were done as follows: 100 µL of fresh serum specimens and 25 µL of sample buffer were added to the wells and incubated for 1 h at 37°C; 100 µL of a goat anti-human IgA (α chain) (final dilution 1 : 500; Binding Site, London, UK) was added to the wells and incubated for 1 h at 37°C; 100 µL of p-nitrophenyl phosphate was added to all wells and incubated for 30 min at 37°C; the reaction was terminated by a stop solution; and absorbance (optical density [OD]) values were determined by spectrophotometer. Positive- and negative-control sera were utilized in the assays. Positive samples were retested and were considered positive only when they were repeatedly and consistently reactive. Values shown are arithmetic means of two different assays on the same sample.

Virologic analyses. For virus titration, we determined ID₅₀ of peripheral blood mononuclear cells (PBMC). Virus was diluted 5-fold beginning with 1 - 5. Each dilution (150 µL) was added to 6 wells of a microtiter plate (Nunc, Roskilde, Denmark) containing 10⁵ resting PBMC in 75 µL of medium, incubated for 2 h, washed, and resuspended in medium containing phytohemagglutinin (PHA) and 10 U/mL recombinant interleukin-2 (IL)-2. After 7 days, samples were analyzed for HIV p24. ID₅₀ titers were defined as the reciprocal of the virus dilution resulting in 50% positive wells of a microtiter plate (Nunc, Roskilde, Denmark) containing sera from the same donor, PHA, and IL-2. p24 concentrations were determined on fresh samples by EIAs (Abbott HIV I/2). ID₅₀ titers were calculated as the reciprocal of the virus dilution resulting in 50% positive wells of a microtiter plate (Nunc, Roskilde, Denmark) containing sera from the same donor, PHA, and IL-2. p24 concentrations were determined on fresh samples by EIAs (Abbott HIV I/2).

Discussion

We previously reported the presence of HIV-specific mucosal IgA in HIV-1-infected subject's urine samples. Analysis of systemic immunity in the same persons confirmed the presence of HIV-specific type 1 cytokine-producing Th lymphocytes in peripheral blood, whereas no differences were observed in CC production or in CCR5 expression [7]. Those results suggested that correlates of immune protection include the compartmentalized activation of IgA.
of both cell-mediated and humoral immunity. To analyze the possibility that serum IgA could also be present in ESPs, we screened 15 donors. This hypothesis was confirmed as we observed HIV-specific IgA, in the absence of IgG, in sera of ESPs. Thus, exposure to HIV can result in a typical seroconversion, characterized by the generation of IgG and followed by productive infection, immunodeficiency, and disease or in an IgA-restricted seroconversion that might not be associated with active infection or disease.

HIV serostatus was determined in all HIV-infected persons and their partners by standard EIA and Western blot techniques. IgA was not detected by the routine EIA, even though this method is theoretically capable of detecting all immunoglobulin classes (the kit was adequate for detection of IgM and IgG but not of IgA) [11]. This discrepancy might be secondary to the fact that the sensitivity of this kit for IgA could be lower than that for other immunoglobulin classes. HIV-infected persons were always positive and their partners were consistently negative, confirming that these methods are adequate to detect the presence of HIV infection accompanied by IgG-based se-
roconversion. However, these data suggest that routine EIAs are not adequate for screening persons at-risk for HIV infection in whom exposure to HIV results in selective production of IgA.

Why an IgA-inducing exposure to HIV may not be associated with disease is unclear, and two different explanations can be envisioned. A typical seroconversion (i.e., development of IgG and IgA) could be associated with viral replication and disease progression because the concentration of IgA observed is lower or, alternatively, because the presence of IgG may play a potentially deleterious role [12]. The first possibility is partially supported by the fact that IgA concentrations were higher in ESPs than in HIV-seropositive partners. More intriguing is the possibility that IgG production could exert a negative role, allowing disease progression to occur. Support for this hypothesis stems from diverse experimental observations.

First, immunocomplexes (ICs) formed between IgG and HIV are bound to the surface of monocytes/macrophages by different receptors [13]; this favors IC uptake and monocyte/macrophage infection. Because the receptors are specific for the γ chain but do not recognize the α chain [13], IC formed between IgA and HIV will not be taken up by monocytes/macrophages and infection of these cells should not be enhanced by IgA. Second, during HIV infection, HIV is localized on follicular dendritic cells (FDCs) in the lymph nodes [14, 15] and is bound on the surface of FDCs in IgG-formed ICs [15]. However, it is not known whether the trapping of infectious HIV on FDCs would be possible upon the generation of ICs with IgA. Third, IgG but not IgA activate complement. Complement activation allows deposition of Clq on the HIV surface and activation to C3b, which bind to complement receptors (reviewed in [16]). This sequence of events favors HIV infection of complement receptor–positive cells including macrophages/monocytes and FDCs.

Condom use and a reduction of at-risk sex was followed by a drop in HIV-specific IgA titers. These results suggest that HIV-specific immunity in ESPs could be dependent on a continuous exposure and are in concordance with results obtained when we analyzed other cohorts of ESPs (reviewed in [2]). That cessation of exposure results in down-modulation of a specific immune response indicates that such immune response is capable of controlling and preventing virus spread.

In conclusion, our results suggest that immune protection against HIV infection is secondary to the activation of multiple and partially unknown mechanisms that include the presence of potent HIV-specific cell-mediated immunity in peripheral blood, mucosal immunity in the genital tract, and IgA in plasma.

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