CONCISE COMMUNICATIONS

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

EIA detection of HIV-specific antibodies. Serologic status was determined on fresh samples by EIA as (Abbott HIV II + 3rd generation). IgA antibodies were detected by modified EIA (HIV-1 EIA; Calypte 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 10⁶ resting PBMC in 75 µL of medium, incubated for 2 h, washed, and resuspended in medium containing phytomenegallitin (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 (Reed-Muench calculation). ID₅₀ for each assay ranged from 1/10 to 1/50.

For the virus neutralization assay, we added 2 x 10⁶ resting PBMC [10] to microplate wells containing 75 µL of medium containing heat-inactivated ESP sera, which was incubated for 1 h before addition of 75 µL of virus diluted at two different ID₅₀. Cultures were then incubated for 2 h, washed, and resuspended in medium containing sera from the same donor, PHA, and IL-2. p24 concentrations were determined on day 7.

Immunoglobulin purification. Cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) was coupled with rabbit anti-human IgA (2 µg/mL; Sigma, St. Louis). We incubated 3 mL of serum at room temperature for 15 min with columns containing 800 µL of Sepharose and anti-IgA. After the columns were washed, IgA was eluted with 0.2 M glycine/NaCl; and the solution was neutralized with 1 M Tris, pH 11. Purified immunoglobulins were concentrated on Ultrafree-15 Biomax 30 membranes (30-kDa cutoff; Millipore, Bedford, MA). Immunoglobulins were also purified by the procedure on sheep anti-human IgA Sepharose-conjugated columns to exclude the presence of cross-reactive material. IgA was then used in the neutralization assay. IgA from sera of 4 healthy donors was affinity purified and used as negative controls.

Results

Serologic analyses of 15 ESPs and their HIV-seropositive sex partners. By routine EIA, all 15 HIV-infected subjects were positive, but no ESPs or healthy controls were positive (data not shown). Thus, the method used for routine screening was adequate to detect HIV IgG seroconversion. This test detects HIV-specific IgG and IgM, but its ability to detect IgA is not established [11]. Because HIV-specific IgA in the absence of IgG was present in the urine of ESPs [7], we tested all sera with the same kit that permitted detection of urinary IgA (Calypte) and used an anti-α chain-specific (IgA) antisera in the second step. Results (figure 1, upper panels) showed the presence of HIV-specific IgA in all ESP sera and in sera of 14 of 15 HIV-infected partners. Mean IgA titer was higher in the ESPs than in their partners (mean ODs: ESPs, 2.49; HIV-infected partners, 1.97; P = .07). The same samples were tested with an anti-γ chain–specific antisera. Results confirmed the presence of serum IgG in all HIV-infected partners but not in the ESPs. No antibodies were detected in 16 low-risk controls by either assay.

Immunologic analyses after interruption of at-risk sex. Sera of 11 ESPs and 10 of their partners were retested by EIA 1 year after the first examination (counseling was offered; at-risk sex was reportedly not practiced; all couples used condoms). In all but 1 retested serum sample, HIV-specific IgA declined (the exception was in an ESP; this couple reported routine use of condoms). The mean serum IgA OD was 2.49 at the first time point and 0.76 after 1 year of condom use (P > .001; figure 1, lower panels).

HIV suppression by sera and purified IgA of ESPs and controls. Sera from ESPs were tested for neutralizing activity against two primary HIV-1 isolates (HIV-1si and HIV-1si SI phenotype) on control PBMC from a healthy donor. The isolates were tested at high (49–100 TCID₅₀/75 µL) or low (25–45 TCID₅₀/75 µL) virus input. At low input, reduction of viral growth was observed in sera of 5 of 15 ESPs (figure 2A). Modulation of HIV infectivity was not observed in sera of 16 healthy donors (data not shown). Similar results were obtained with both isolates.

Neutralizing sera were purified by affinity column, and acid-eluted fractions were tested for neutralizing activity of an HIV-1 primary virus (HIV-1si, SI phenotype) at a low viral input (20 TCID₅₀/75 µL). Reduction of infectivity was associated with the IgA fraction in 2 of 5 cases in which neutralization by whole serum was observed (figure 2B). No neutralizing activity was observed in affinity column–purified IgA from sera of 4 healthy controls (data not shown).

Discussion

We previously reported the presence of HIV-specific mucosal IgA in HIV-seronegative sexually exposed heterosexual partners of HIV-infected subjects [7]. 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
Figure 1. Top, HIV-specific IgG and IgA in sera of HIV-infected partners, exposed seronegative persons (ESPs), and low-risk healthy controls. Modified EIA and anti-α chain–specific (IgA) antisera was used in second step. Bottom, reduction in titers of HIV-specific serum IgA in ESPs 1 year after first time point examined and after counseling was offered. A, Optical densities (ODs) (HIV-specific IgA) at beginning of study; B, ODs (HIV-specific IgA) 1 year after interruption of at-risk sex. Each symbol indicates a serum specimen from a different ESP.

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 C1q 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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References