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Differences in Humoral Responses to the p24 Antigen between Ethiopian and Swedish Human Immunodeficiency Virus Type 1-Infected Patients May Suggest Influences from a T-Helper 2-Like Phenotype

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The levels of human immunodeficiency virus type 1 p24 antibodies and p24 antigen among 256 Ethiopians and Africans and 86 Swedes were compared. The elevated levels of total anti-p24 immunoglobulin G (IgG) and anti-p24 IgG4 among the Ethiopian and African patients suggest influences from T-helper-cell type 2-like responses.

Some aspects of infection with human immunodeficiency virus type 1 (HIV-1) differ between African and European or American patients. African HIV-1-infected patients often show lower levels of p24 antigen and higher levels of p24-specific antibodies in serum than those of Western patients (1, 9, 10). We were interested in characterizing in greater detail the variables that may correlate with these differences between African and Swedish HIV-1-infected patients.

A total of 347 samples were analyzed. Samples from Ethiopia were classified as asymptomatic (EA [$n = 53$]) or symptotic (ES [$n = 116$]) based on Centers for Disease Control and Prevention-World Health Organization clinical case definitions adopted for Ethiopian patients (1992, AIDS Control Program, Ministry of Health, Addis Ababa, Ethiopia). All Ethiopian samples were confirmed positive for HIV-1 antibodies with enzyme immunoassays (EIAs; Wellcome Diagnostics, Darford, United Kingdom; and Abbott Laboratories, Chicago, Ill.) and Western blotting (Du Pont Medical Products, Boston, Mass.). Another 92 HIV-1-positive samples were obtained from other central and East African countries, and 86 samples were from confirmed-positive Swedish asymptomatic (SA [$n = 39$]) and symptotic (SS [$n = 47$]) patients. Twenty-four samples negative for HIV antibodies served as negative controls.

HIV-1 serotyping was performed exactly as described in reference 19 by determination of the relative avidity to seven 15-amino-acid-long peptides corresponding to the V3 loop of HIV-1 gp120, subtypes A to E. In total, the HIV-1 V3 serotype could be estimated in 294 (85%) samples: 165 samples (98%) were Ethiopian (subtype B, $n = 1$; C $n = 164$; not typeable, $n = 4$), 62 (67%) were other African (subtype A, $n = 11$; B $n = 9$; C, $n = 41$; D, $n = 1$, and not typeable, $n = 30$), and 67 (78%) were Swedish ($n = 86$; subtype A, $n = 1$; B, $n = 59$; C, $n = 7$; not typeable, $n = 19$).

HIV-1 p24 antigen levels in serum were determined for 163 samples (EA, $n = 42$; ES, $n = 42$; SA, $n = 36$; and SS, $n = 43$) with the Du Pont HIV-1 p24 TSA enzyme-linked immunosorbent assay (Du Pont Medical Products, Boston, Mass.) according to the manufacturer's recommendations. To detect immune-complexed p24 antigen, each serum sample was boiled on a 100°C heating block for 5 to 7 min prior to analysis (19). Samples with an absorbance greater than twice the mean of the negative control were considered positive as suggested by the manufacturer.

A recombinant p17-p24 fusion protein (rp17/24) expressed and purified from *Escherichia coli* was kindly provided by D. L. Peterson, Virginia Commonwealth University, Richmond). All EIAs for the detection of human and murine rp17/24-specific antibodies were performed with rp17/24 according to previously described protocols (18, 20). In brief, 0.5 μg of rp17/24 per ml was passively adsorbed to 96-well microtiter plates. Plates were blocked for 2 h with 2% bovine serum albumin in phosphate buffered saline solution. Serum samples, diluted 1:100 and 1:1,000 in phosphate-buffered saline containing 0.75% bovine serum albumin, 0.05% Tween 20, and 2% goat serum, were incubated on the plates for 45 min at 37°C. Antibodies were indicated by an incubation at 37°C for 45 min with alkaline phosphatase-labelled goat anti-human IgG (A3150; Sigma, St. Louis, Mo.). The plates were developed by the addition of *p*-nitrophenyl phosphate (Sigma) for 30 min.

For the analysis of immunoglobulin G (IgG) subclasses specific for rp17/24, serum samples were incubated on the plates for 1 h at 37°C. Human IgG subclasses were indicated by monoclonal antibodies to IgG1 (clone HP6001; Sigma), IgG2 (clone HP6014; Sigma), IgG3 (clone HP6050; Sigma), and IgG4 (clone HP6025; Sigma). Bound monoclonal antibodies were indicated by peroxidase-conjugated rabbit anti-mouse Ig (P260; Dako AS, Roskilde, Denmark) for 1 h at 37°C, followed by the addition of *o*-phenylenediamine (Sigma) for 30 min. The cutoffs in all antibody EIAs were set at the mean value of the eight negative samples plus three times their standard deviation.

Detection of p24 antigen after heat dissociation of immune complexes confirmed previous observations (1) of lower levels of p24 antigenemia among the 84 Ethiopian patients compared to those among the 79 Swedes (Fig. 1). We found p24 antigen in a majority (23 of 42) of the HIV-1 infected Ethiopians in the

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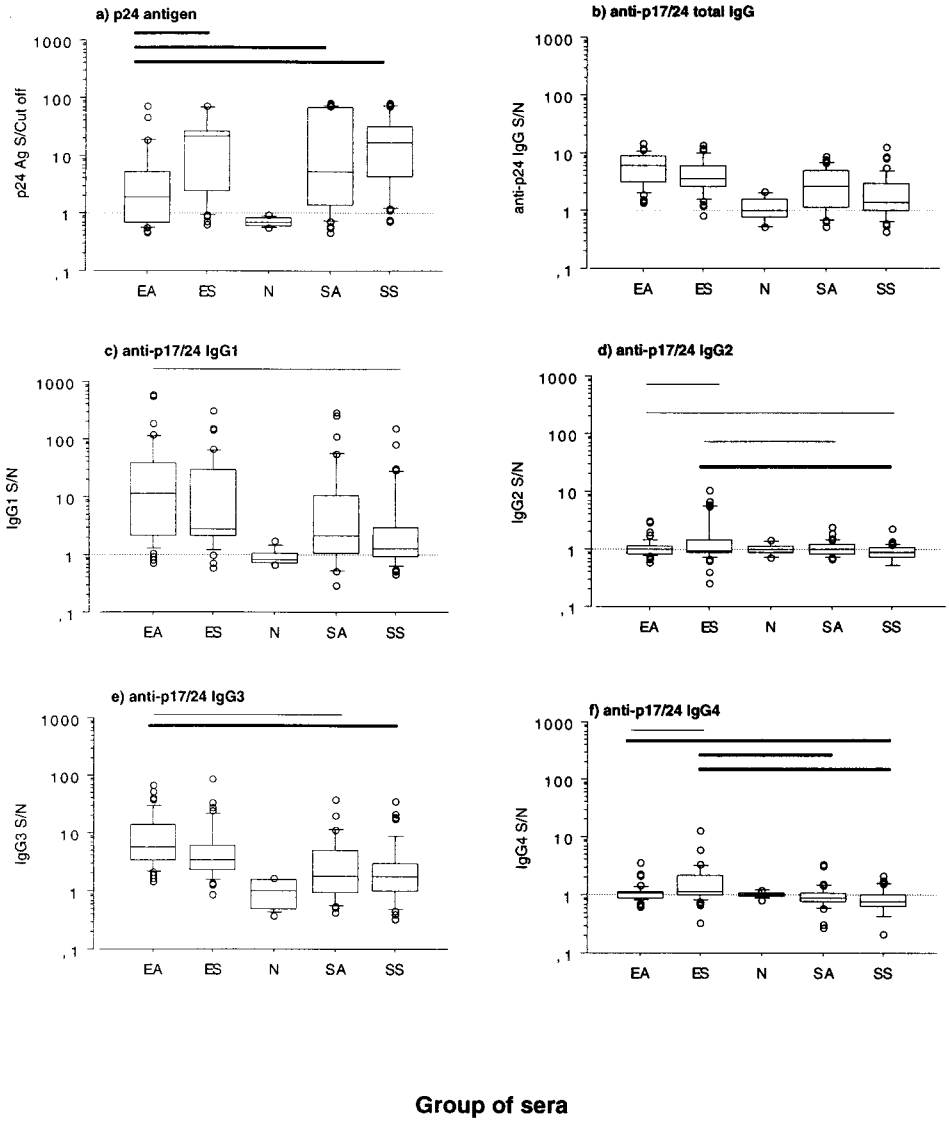

FIG. 1. Levels of p24 antigen (a) and p17/24 total IgG (b), IgG1 (c), IgG2 (d), IgG3 (e), and IgG4 (f) in serum samples from 42 EA, 42 ES, 36 SA, and 43 SS HIV-1-infected patients. Also given are the results from 24 HIV-1-negative Swedish samples (N). Values are given as the sample to cutoff ratio (S/Cut off [a]) or the S/N ratio (b to f). Thin and thick horizontal bars indicate differences at significance levels of $P < 0.05$ and $P < 0.01$, respectively.

asymptomatic phase of the disease. However, the mean p24 sample-to-negative (S/N) ratios in the EIAs were lower among the asymptomatic Ethiopians than those among the Swedes (EA, 6.6 ± 13.3; SA, 27.7 ± 31.8; $P < 0.0001$; ES, 24.2 ± 24.3; SS, 24.7 ± 25.9; not significant, Student's *t* test).

A total of 259 (75%) of the 347 human HIV-1-positive serum samples were reactive by the rp17/24 EIA. In detail, 45 of 53 (85%) asymptomatic and 94 of 116 (81%) symptomatic patients from Ethiopia, and 78 of 92 (85%) African patients had p24 antibodies. In contrast, 23 of 39 (59%) asymptomatic and 19 of 47 (40%) symptomatic Swedish patients had p24 antibodies. We confirmed the previously observed elevated anti-p24 IgG S/N levels in the Ethiopians (EA, 5.4 ± 2.9; SA, 3.5 ± 2.8; $P < 0.01$, Student's *t* test; ES, 5.4 ± 2.9; SS, 2.2 ± 1.9; $P < 0.001$, Student's *t* test) and Africans (7.5 ± 5.59) compared to those in the Swedes (1, 10). The level of antibodies to HIV-1 rp17/24 was neither proportional to the level of p24 antigen present in serum (analysis of variance) nor differed between HIV-1 subtypes (Fig. 2).

The distributions of members of the IgG subclass specific for the rp17/24 protein were compared for the 163 Ethiopian and Swedish patients that were analyzed for p24 antigen (Fig. 1). IgG1 was higher among the EA samples than among the SS samples (Fig. 1). The anti-rp17/24 IgG2 S/N ratios were highest among the ES samples compared to those of the other three groups (Fig. 1). The IgG3 S/N values to anti-rp17/24 were higher among the EA samples than those of the SA and SS samples (Fig. 1). Interestingly, the anti-rp17/24 IgG4 S/N ratios were highest among the ES samples compared to those of all other groups (Fig. 1).

It has been suggested that the balance between T-helper 1 (Th1) and Th2-like responses during HIV-1 may affect the viral replication and the progression of the disease (2, 5, 7, 15, 16). We found two factors among the Ethiopian patients which

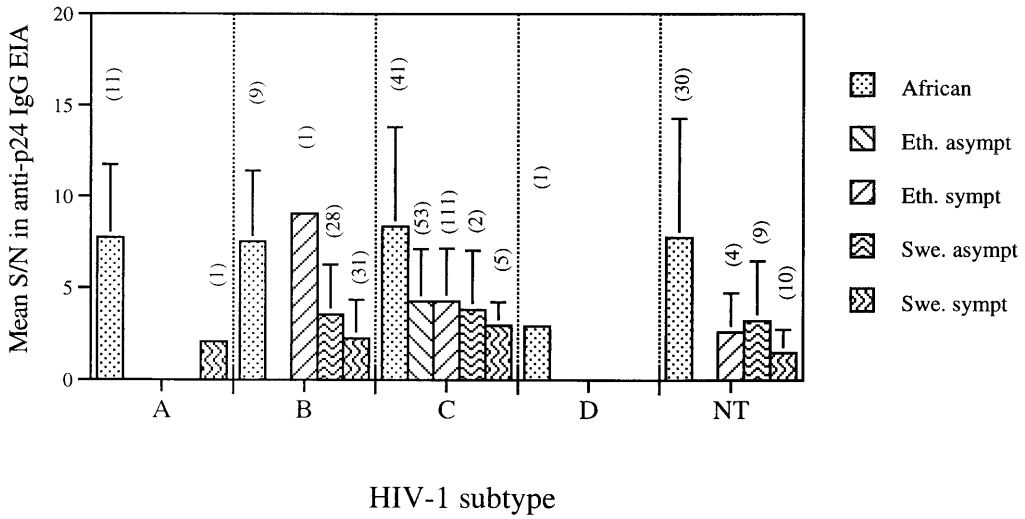

FIG. 2. Relationship between S/N ratios in the p17/24 antibody EIA and the HIV-1 V3 subtypes A, B, C, and D and samples that could not be typed by V3 peptide serology (NT). Vertical bars indicate mean S/N ratios, and the numbers within parentheses indicate the number of samples used for the calculations for each bar. Eth., Ethiopian; Swe., Swedish; asympt, asymptomatic; sympt, symptomatic.

are suggestive of Th2-like responses. First, it is well documented that p24-specific antibody levels are higher among African patients than among HIV-1-infected patients in the western world. Strong humoral responses have, in murine models, been proposed to correlate with a Th2-like cytokine profile (6, 8, 12–14). However, with respect to humans, this is still largely unknown. Second, rp17/24 IgG4 levels were highest among the Ethiopians with symptomatic HIV-1 infection. As of today, IgG4 and IgE are the only human Ig isotypes which are known to be positively regulated by Th2-like cytokines such as interleukin 4 (6, 8, 12, 13). It is known from murine systems that parasitic infections may induce Th2-like responses with a preferential production of IgE (3, 4, 11, 17). Thus, the infectious spectra in the African continent may offer one explanation for the possible Th2-like responses in the African HIV-1-infected patients described herein.

REFERENCES

1. **Ayehune, S., A. Sönnerberg, B. Desta, H. Kefene, D. Zewdie, S. Britton, and Ö. Strannegård.** 1992. Relationship between cell free viremia, antigenemia and antibody levels in HIV-1 infected Ethiopian patients. AIDS **6:**651–657.
2. **Barker, E., C. E. Mackewicz, and J. A. Levy.** 1995. Effects of TH1 and TH2 cytokines on CD8+ cell response against human immunodeficiency virus: implications for long-term survival. Proc. Natl. Acad. Sci. USA **92:**11135–11139.
3. **Demant, P., M. Lipolodova, and M. Svobodova.** 1996. Resistance to *Leishmania major* in mice. Science **274:**1392.
4. **Erard, F., M. T. Wild, J. A. Garcia-Sanz, and G. Le Gros.** 1993. Switch of CD8 T cells to noncytolytic CD8−CD4− cells that make Th2 cytokines and help B cells. Science **260:**1802–1805.
5. **Feinberg, B. M.** 1996. Changing the natural history of HIV disease. Lancet **348:**239–246.
6. **Fujieda, S., K. Zhang, and A. Saxon.** 1995. IL-4 plus CD40 monoclonal antibody induces human B cells gamma subclass-specific isotype switch: switching to gamma 1, gamma 3, and gamma 4, but not gamma 2. J. Immunol. **155:**2318–2328.
7. **Graziosi, C., G. Pantaleo, K. R. Gantt, J. P. Fortin, J. F. Demarest, O. J. Cohen, R. P. Sekaly, and A. S. Fauci.** 1994. Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals. Science **265:**248–252.
8. **Jabara, H. H., R. Loh, N. Ramesh, D. Vercelli, and R. S. Geha.** 1993. Sequential switching from mu to epsilon via gamma 4 in human B cells stimulated with IL-4 and hydrocortisone. J. Immunol. **151:**4528–4533.
9. **Janvier, B., F. Mallet, V. Cheynet, P. Dalbon, G. Vernet, M. J. Besnier, P. Choutet, A. Goudeau, B. Mandrand, and F. Barin.** 1993. Prevalence and persistence of antibody titers to recombinant HIV-1 core and matrix proteins in HIV-1 infection. J. Acquired Immune Defic. Syndr. **6:**898–903.
10. **Kaleebu, P., C. R. Popov, D. Callow, E. Katabira, F. Mubiru, B. Biryahwaho, S. Sempala, C. Gilks, R. Brindle, J. B. O. Were, and J. Weber.** 1991. Comparative humoral responses to HIV-1 p24 gag and gp120 env in subjects from east Africa and UK. AIDS **5:**1015–1019.
11. **Kemp, M., T. G. Theander, and A. Kharazmi.** 1996. The contrasting roles of CD4(+) T cells in intracellular infections in humans—leishmaniasis as an example. Immunol. Today **17:**13–16.
12. **King, C. L., and T. B. Nutman.** 1993. IgE and IgG subclass regulation by IL-4 and IFN-gamma in human helminth infections. Assessment by B cell precursor frequencies. J. Immunol. **151:**458–465.
13. **Kotowicz, K., and R. E. Callard.** 1993. Human immunoglobulin class and IgG subclass regulation: dual action of interleukin-4. Eur. J. Immunol. **23:**2250–2256.
14. **Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman.** 1986. Two types of murine helper T cell clones. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. **136:**2348–2357.
15. **Romagnani, S., G. Del Prete, R. Manetti, A. Ravina, F. Annunziato, M. De Carli, M. Mazzetti, M. P. Piccinni, M. M. D'Elios, P. Parronchi, et al.** 1994. Role of Th1/Th2 cytokines in HIV infection. Immunol. Rev. **140:**73–92.
16. **Romagnani, S., and E. Maggi.** 1994. Th1 versus Th2 responses in AIDS. Curr. Opin. Immunol. **6:**616–622.
17. **Sabin, E. A., M. I. Araujo, E. M. Carvalho, and E. J. Pearce.** 1996. Impairment of tetanus toxoid-specific Th1-like immune responses in humans infected with *Schistosoma mansoni*. J. Infect. Dis. **173:**269–272.
18. **Sällberg, M., P. Pushko, I. Berzinsh, V. Bichko, P. Sillekens, M. Noah, P. Pumpens, E. Grens, B. Wahren, and L. O. Magnius.** 1993. Immunochemical structure of the carboxy-terminal part of hepatitis B e antigen: identification of internal and surface-exposed sequences. J. Gen. Virol. **74:**1335–1340.
19. **Schüpbach, J., M. Flepp, D. Pontelli, Z. Tomasaki, R. Lüthy, and J. Böni.** 1996. Heat mediated immune complex dissociation and enzyme-linked immunosorbent assay signal amplification render p24 antigen detection in plasma as sensitive as HIV-1 RNA detection by polymerase chain reaction. AIDS **10:**1085–1090.
20. **Sherefa, K., A. Sönnerborg, J. Steinbergs, and M. Sällberg.** 1994. Rapid grouping of HIV-1 infection in subtypes A to E by V3 peptide serotyping and its relation to sequence analysis. Biochem. Biophys. Res. Commun. **205:**1658–1664.