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Evidence for a Shift from a Type I Lymphocyte Pattern with HIV Disease Progression

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Summary: Whether a shift from a type I (cell mediated) immune profile occurs with progressive HIV-related immune dysfunction is a matter of heated debate. We analyzed data for 333 HIV antibody-positive (HIV^+) and -negative (HIV^-) hemophilic children/adolescents, to examine whether the relationships among immunologic parameters and vaccine-related serology supported a shift with advancing HIV infection. In stepwise logistic regression analysis of HIV^+ children's data, anergy to a panel of delayed hypersensitivity skin test antigens was positively associated with serum immunoglobulin A (IgA) levels ($p = 0.012$) and $CD8^+$ cell counts ($p = 0.021$) and negatively associated with $CD4^+$ cell counts ($p = 0.002$). Modeling supported anergy as a positive correlate of log IgA level ($p = 0.046$) and $CD4^+$ lymphocyte count as a negative correlate, for HIV^+ participants only ($p < 0.0001$). For mumps, the proportion of vaccinated HIV^+ participants with protective IgG antibody titers was higher among those with $CD4^+$ lymphocyte counts <200 cells/mm^3 ($p = 0.058$). For HIV^+ participants <14 years of age, this same trend was seen for measles and rubella, but was not seen in any age group for bacterial vaccine antigens. The intercorrelations among skin test anergy, $CD4^+$ lymphocyte counts, serum IgA levels, and viral vaccine antigen-related serologic titers for HIV^+ participants are consistent with an association between progressive HIV-related immune dysfunction and a predominance of type II (humoral immunity) or Type 0 (mixed immunity), relative to type I, lymphocyte profiles. **Key Words:** HIV—T cells—Lymphocyte subsets.

Three interregulatory subpopulations of $CD4^+$ ymphocytes can be defined on the basis of their ssociated cytokine profiles and functions, rather han any known surface antigens. There is currently debate about whether human immunodeficiency virus (HIV), which infects $CD4^+$ cells, has profound effects upon the balance among these $CD4^+$ subpopulations (1–7). Clones of murine and, less often, of human $CD4^+$ T lymphocytes may produce one or another of two distinct patterns of cytokines. These patterns identify two interregulatory $CD4^+$ T lymphocyte subsets, differing in their effector response to infectious organisms. $T_{helper\ 1}$ (T_H1) lymphocytes are associated with interleukin-2 (IL-2), interferon-γ, and lymphotoxin. These cells facilitate a type I response pattern, i.e., cell-mediated immunity,

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needed for optimal responsiveness to intracellular organisms and viruses (including HIV), skin test delayed hypersensitivity reactions, and clearance of organisms from infected cells. $T_{helper\ 2}$ lymphocytes (T_H2) are also $CD4^+$ and are associated with IL-4, IL-5, IL-6, and IL-10. These cells preferentially facilitate a type II response pattern, i.e., T cell–dependent humoral immunity, including immunoglobulin class switching from immunoglobulin M (IgM) to IgG4, IgA, and IgE and antibody production (8,9). A Type II response is needed for optimal responsiveness to most extracellular bacteria and some intestinal helminth parasites. Findings from one murine study suggest that IgA synthesis may be further facilitated by a mixture of T_H1 and T_H2 cytokines (9); these could be provided by either a combination of T_H1 and T_H2 cells or a third $CD4^+$ cell type. This third type of $CD4^+$ T_{helper} lymphocyte, termed T_H0, can be cloned from humans; it produces a composite T_H1/T_H2 cytokine pattern (2,5). Recent reports suggest that in addition to these $CD4^+$ subpopulations, $CD8^+$ lymphocytes may sometimes produce T_H2-like cytokine patterns (2,6,10). Thus, rather than the terms "T_H1, T_H2, and T_H0 lymphocytes," more generic terminology is increasingly used: i.e., type I, type II, and type 0 immune profiles.

Some studies have suggested that, with progression of HIV-associated disease, the T cell immune profile switches from a predominance of type I to type 0 or II. This suggestion is based on a shift, with disease progression, from type I to type II patterns of in vitro cytokine production by cultured peripheral blood lymphocytes (PBL) or cloned $CD4^+$ lymphocytes of infected individuals (1). Additional evidence includes signs of HIV-specific cell mediated immunity in healthy, HIV-exposed but seronegative individuals, loss of in vitro type I responsiveness to influenza and HIV antigens with progressive HIV disease, and production of type II cytokine profiles by in vitro mitogen-stimulated PBL of HIV-infected persons (1). However, other researchers have found no shift in cytokine profiles, T-helper clone distributions, or soluble CD23 (a marker of cytokine IgE regulation) with disease progression (4,11). Others have suggested that destruction of T_H2, not T_H1, cells occurs early in HIV infection and that, in some HIV-infected individuals, $CD8^+$ T cells produce type II cytokines (2,3,5,6,10).

Resolution of this issue has important implications for HIV disease treatment and vaccine development. Some researchers feel that effective treatment and immunization prophylaxis will require the return or elicitation of a type I response pattern to HIV (12,13). Indeed, since these lymphocyte subpopulations are interregulated through various cytokines, attempts to restore Type I predominance with cytokine manipulation are already under way (12). Because all cited studies have relied on in vitro or ex vivo systems, we examined in vivo data from an HIV-infected cohort for evidence for or against a shift from a type I to a type II or 0 pattern with progressive HIV immune dysfunction.

PARTICIPANTS AND METHODS

The Hemophilia Growth and Development Study includes 333 hemophilic children and adolescents from 14 collaborating hemophilia treatment centers across the United States (14), 207 of whom were HIV antibody-positive (HIV^+) at enrollment (median age 13.2 years, range 6–19 years) and 126 who were HIV antibody-negative (HIV^-) (median age 10.2 years, range 7–19 years).

Lymphocyte subpopulations were quantitated using commercial monoclonal antibodies and direct two-color immunofluorescence flow cytometry following whole blood lysis. The baseline evaluation included quantitation of serum IgG, IgA, and IgM and assessment of serologic responses to diphtheria, tetanus, measles, mumps, rubella, and *Hemophilus influenzae* type B (Hib). Individuals were considered vaccinated if they had received the following: one dose of the pertinent vaccine, for measles, mumps, rubella, and Hib, and ≥3 doses of vaccines, for tetanus and diphtheria. Delayed hypersensitivity skin testing was done on 175 HIV^+ and 112 HIV^- participants, using shared lots of the Multitest CMI (cell-mediated immunity) panel (Merieux Institute, Miami, FL, U.S.A.), distributed centrally from the Centers for Disease Control and Prevention. Anergy is defined herein as an absence of erythema and induration to all seven antigens in the CMI panel, as read at 48 h after application. One anergic subject with a positive response to glycerin was excluded from all delayed hypersensitivity analyses. Full details of participant characteristics and all immunologic assessments, including skin testing and methods used for serologic testing, are provided elsewhere (14). Individuals who had received intravenous gammaglobulin (IVIg) in the previous 28 days were not included in serologic analyses or IgG assessments (n = 9).

The Wilcoxon rank sum test was used to assess differences in lymphocyte counts and serologic titers by anergy status. The proportions of persons with $CD4^+$ lymphocyte counts of <200 versus ≥200 cells/mm^3 having titers indicative of prior exposure to vaccine antigens were compared, with stratification by age using the Mantel-Haenszel χ^2 test. Stepwise multiple linear regression was used to identify variables associated with log transformed IgA levels. Stepwise logistic regression was used to identify variables associated with anergy. A result was considered significant if the two-sided p value was <0.05.

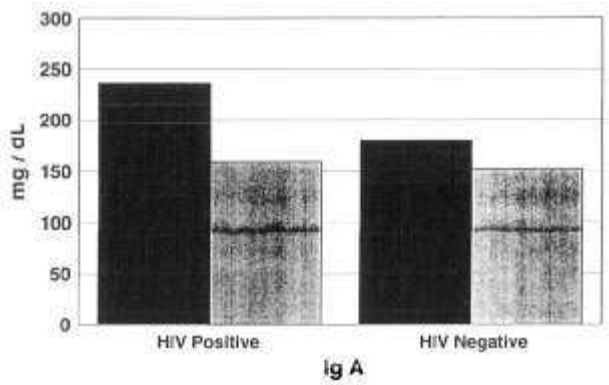
RESULTS

The majority of HIV^+ participants (71%) were anergic to all skin test antigens; the proportion a

ergic did not vary significantly by age. HIV^+ participants who were anergic had lower median $CD4^+$ lymphocyte counts (anergic, 332 cells/mm^3; reactive, 514 cells/mm^3; n = 176, p = 0.0003) and higher median levels of serum IgA (236 mg/dl vs. 159 mg/dl; n = 177, p = 0.0004) (Fig. 1). The differences in serum IgM were of only borderline significance (anergic, 148 mg/dL; reactive, 128 mg/dL; n = 177, p = 0.063). $CD8^+$ lymphocyte counts did not differ (p = 0.693), nor did serum IgG levels (p = 0.366).

Only 28% of HIV^- participants were anergic. In the HIV^- group, anergic participants (n = 31) had a higher median level of serum IgA than nonanergic participants (n = 81) (anergic, 180 mg/dL; reactive, 152 mg/dL; p = 0.002) (Fig. 1). They tended to have higher median levels of serum IgG (anergic, 1256 mg/dl; reactive, 1,195 mg/dl; p = 0.101) and IgM (anergic, 139 mg/dL; reactive, 106 mg/dL; p = 0.053). For HIV^- participants, there were no significant differences by skin test reactivity status in median $CD4^+$ lymphocyte counts (anergic, 836 cells/mm^3; reactive, 922 cells/mm^3; p = 0.234) or $CD8^+$ lymphocyte counts (anergic, 669 cells/mm^3; reactive, 720 cells/mm^3; p = 0.398).

For HIV^+ participants, stepwise modeling of anergy with IgA level, $CD4^+$ lymphocyte count, and $CD8^+$ lymphocyte count as potential predictors resulted in a final three-variable model: anergy was positively associated with IgA level (p = 0.012) and $CD8^+$ lymphocyte count (p = 0.021), and negatively associated with $CD4^+$ lymphocyte count (p = 0.002). It should be noted that $CD8^+$ lymphocyte counts, considered alone, did not differ for anergic and reactive HIV^+ participants. The significant association between anergy and $CD8^+$ lymphocyte counts became apparent only when $CD4^+$ lymphocyte counts were taken into consideration analytically. To illustrate: median $CD8^+$ lymphocyte counts were 752 cells/mm^3 for 38 anergic and were 675 cells/mm^3 for 20 reactive HIV^+ participants with $CD4^+$ lymphocyte counts in the range of 200–500 cells/mm^3. Median $CD8^+$ lymphocyte counts were 1,256 cells/mm^3 for 47 anergic and were 948 cells/mm^3 for 27 reactive HIV^+ participants with $CD4^+$ lymphocyte counts $\geq$500 cells/mm^3. (Only four of 44 participants with lymphocyte counts of <200 cells/mm^3 were reactive.)



G. 1. Median serum IgA levels, by skin test reactivity and V status. Black bars, anergic; gray bars, reactive.

A similar stepwise procedure performed on data for the HIV^- participants resulted in only one predictor of anergy: serum IgA level (p = 0.001). Unlike the results of the HIV^+ group, $CD4^+$ and $CD8^+$ lymphocyte counts were not significant predictors of anergy after accounting for serum IgA level in HIV^- participants.

Stepwise modeling was also used to identify correlates of log IgA level, using anergy, $CD4^+$ lymphocyte count, and $CD8^+$ lymphocyte as potential predictors. For HIV^+ participants, this procedure resulted in a final two-variable model: log $CD4^+$ lymphocyte was negatively associated (partial correlation of 0.382, p < 0.0001) and anergy was positively associated (partial correlation of 0.140, p = 0.046) with log IgA level. For HIV^- participants, only anergy was a significant predictor of log IgA level (correlation of 0.285, p = 0.002). Log $CD4^+$ lymphocyte count was not a significant predictor (p = 0.145).

The relationship between titers indicative of prior exposure to various vaccine antigens and $CD4^+$ lymphocyte count (<200 vs. $\geq$200 cells/mm^3) of vaccinated HIV^+ participants was also examined.

The proportion of participants with serum IgG titers indicative of prior exposure to mumps was higher for those with low CD4 lymphocyte counts (n = 46) compared to those with higher $CD4^+$ lymphocyte counts (n = 133) (Fig. 2). For measles and rubella, a similar relationship was found for participants under 14 years of age (although not generally significant) (measles: n = 33 for 6–9 years old, n = 75 for 10–13 years old; rubella, n = 33 and n = 73) (Fig. 2). This relationship was not observed for the bacterial vaccine antigens: tetanus (p = 0.565), diphtheria (p = 0.444), and Hib (p = 0.608).

DISCUSSION

It is biologically plausible that progressive HIV infection would be associated with a shift from a

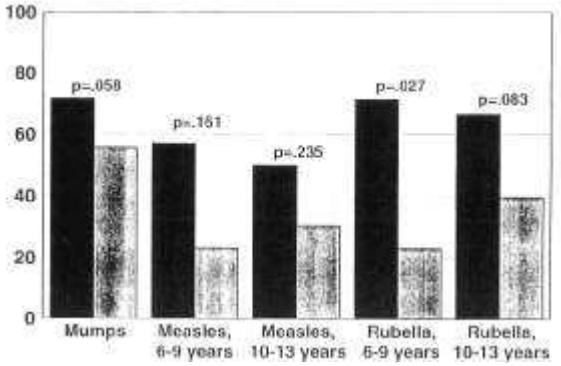


FIG. 2. Proportion of HIV-positive, vaccinated participants with protective titers to viral vaccine antigens by $CD4^+$ lymphocyte counts. Black bars, $CD4^+ < 200$; gray bars, $CD4^+ \geq 200$.

type I pattern, since this fits the pattern seen in successful versus unsuccessful immune responses to other intracellular pathogens such as *Mycobacterium tuberculosis* or *M. leprae*. In these infections, a type I response leads to successful control or elimination of established disease, but a type II pattern does not. In the case of HIV, an initial type II response—especially one associated with IgA antibody production—might be helpful in preventing viral transmission through mucosal surfaces, i.e., preventing the infection of an individual exposed to HIV through sexual contact with an HIV-infected partner. However, once HIV infection has occurred and the virus is established within an individual's lymphocyte/macrophage population, a type II response pattern would be ineffective in clearing infection. Thus, in established HIV infection, a type II response pattern might be associated with progressive immune dysfunction, albeit not necessarily causally. A shift toward a type II pattern in advancing HIV infection is also consistent with the frequently documented anergy and occasionally reported elevations in serum IgE (10,15–18) and eosinophilia (19) in HIV-infected persons.

Despite the intuitive appeal of the shift concept, evidence for it is sparse and not reproducible from one laboratory to another. Indeed, within the last year three laboratories have published strikingly different results and conclusions. Based on in vitro antigen responsiveness and cytokine profiles of peripheral lymphocytes of HIV-infected persons, Clerici et al. (1) reported that a shift from type I to type II occurs with advancing disease. Graziosi et al. (4) measured the ex vivo cytokine mRNA production of lymph node cells of infected persons and found no evidence to support the shift hypothesis. Maggi et al. (2) and Romagnani et al. (3) examined the cytokine profiles of T cell clones from HIV^+ and HIV^- persons, as well as HIV replication rates in cytokine profile-typed T cell clones. They reached a more complex conclusion, i.e., that (a) a shift to type 0 may be favored in response to recall antigens, but a shift to type II does not occur, (b) HIV preferentially infects T_H2 and T_H0 cells, not T_H1 cells, and (c) some HIV-infected persons have $CD8^+$ T cells producing type 2 cytokines (2, 3,5,10).

Various reports have addressed CD4 lymphocyte counts, skin test reactivity, or immunoglobulin levels in HIV-infected persons, but not the intercorrelations among these parameters. Indeed, only a few studies have dealt with the relationship between skin test reactivity and progressive HIV disease in any quantitative fashion (14,20–22). Similarly, although the effects of HIV infection on in vitro reactivity to recall antigens have been widely reported, the relationship between in vivo vaccine antigen-related serologic titers and CD4 cell counts have not.

The intercorrelations we found among skin test anergy, $CD4^+$ lymphocyte counts, and serum IgA levels of HIV^+ participants and the higher IgG levels to virus vaccine antigens found in those with low $CD4^+$ lymphocyte counts are all consistent with a shift from type I (cell-mediated immunity) to either a type II (antibody-predominant) or "type 0" (mixed) immune profile with progressive HIV-associated disease. Our result concerning a positive association between anergy and $CD8^+$ cell counts is also consistent with reports of type II $CD8^+$ cells in advanced HIV infection (2,5,6,10).

Resolution of this increasingly confusing issue has important pathogenic and therapeutic implications. If a shift does occur, it could be either secondary to or a partial cause of the interpatient variability in immune deterioration found with HIV infection. In either case, cytokine manipulation could facilitate both HIV therapy and vaccine efficacy (12,13). Interestingly, although Clerici et al.'s (12) and Romagnani et al.'s (2) conclusions differ, their therapeutic implications are similar: attempt to stimulate a shift toward a type predominance. Our data provide in vivo evidence supporting the occurrence of a shift from a type immune pattern with HIV-related immune deterioration.

APPENDIX

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