Evaluation of CD4+ T Cell Function In Vivo in HIV-Infected Patients as Measured by Bacteriophage phiX174 Immunization

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Bacteriophage phiX174 immunization was used to measure CD4+ T cell function in vivo in human immunodeficiency virus (HIV)-infected patients across all disease stages. Function was evaluated by measuring the ability of T cells to provide help to B cells in antibody production, amplification, and isotype switching. A total of 33 patients and 10 controls received 3 bacteriophage phiX174 immunizations 6 weeks apart. The patients’ responses regarding bacteriophage-specific total antibody titers and IgG titers were quantitatively and qualitatively inferior to the controls’ responses. Overall, 7 of 33 patients had normal T cell function. Baseline CD4 counts provided the strongest correlation with total antibody and IgG titers. HIV RNA had a weaker association with responses but had some predictive power among patients with a CD4 count >200 cells/µL. Bacteriophage phiX174 immunization seems to be a useful tool for measuring immune function in vivo, which suggests that most HIV-infected patients may have abnormal CD4+ T cell function despite adequate antiretroviral treatment.

The hallmark of human immunodeficiency virus (HIV)—associated immunodeficiency is a decrease in CD4+ T cell numbers and function. A growing body of clinical and laboratory evidence indicates that the immune competence of HIV-infected patients improves when viral replication is suppressed. Several studies have reported that the use of highly active antiretroviral therapy (HAART) is associated with significant increases in CD4+ T cell counts, decline in the incidence of opportunistic illnesses [1, 2], increases in naive and memory CD4+ T cells [3], and decreases in markers of polyclonal activation [4]. Increases in proliferative responses to recall antigens have also been reported [5, 6]; however, with these assays it is difficult to dissociate true immune reconstitution from global increases in CD4+ T cell numbers. Detailed analyses of the T cell repertoire have yielded varying results, with some studies suggesting that the CD4+ T cell increases seen in the context of HAART are polyclonal expansions of the existing repertoire [7], whereas others have yielded data more consistent with the development of a new repertoire [8]. Despite the evidence of immunologic improvement, the degree of functional reconstitution that can be achieved with HAART remains unclear. The enumeration of CD4+ T cells and in vitro functional assays offer little or no information on whether the remaining or rebounding CD4+ T cells and subsets recovering under HAART represent a complete repertoire capable of mounting effective immune responses against foreign antigens in vivo. Hence, methods that better quantify immune function in vivo are needed to fully assess the nature and magnitude of the functional immune reconstitution that can be achieved with therapy that effectively suppresses viral replication.

This study evaluated antibody responses to the T cell–dependent neoantigen bacteriophage phiX174 as a method to assess CD4+ T cell function in vivo. CD4+ T cells provide critical help to B cells in the production of antibodies against T cell–dependent antigens, including stimulatory signals for B cell proliferation, differentiation into immunoglobulin-producing memory B cells, and antibody isotype switching [9]. Immunization with bacteriophage phiX174 has been used extensively to diagnose and monitor primary and secondary immunodeficiencies [10, 11]. Patients with a variety of conditions, including T cell or T/B cell interaction dysfunctions such as adenosine deaminase deficiency [12], X-linked immunodeficiency with hyper IgM and CD40 ligand deficiency [13], major
histocompatibility complex class II deficiency [14], and HIV disease itself [15, 16], have been evaluated with this method. Patients with T cell or T/B cell interaction deficiencies have a characteristic pattern of response to bacteriophage phiX174 immunization, characterized by decreased or absent amplification of antibody titers and limited antibody isotype switching from IgM to IgG after repeated immunizations. These previous in vivo studies have demonstrated that intact CD4+ T cell help is required for the production of bacteriophage-specific antibodies with normal titers and isotypes.

The humoral immune responses to a series of 3 immunizations with bacteriophage phiX174 of HIV-infected patients in various stages of disease, with and without therapeutic intervention, were compared with those of uninfected normal controls. The utility of the method to differentiate patients from controls, as well as the relationship between CD4+ T cell function in vivo and CD4 counts, virus load levels, naive/memory cell subsets, and activation markers, was evaluated.

Subjects and Methods

Patient population. Patients with documented HIV infection and healthy uninfected controls who were at least 18 years old were recruited for this study, which was conducted by the Food and Drug Administration (FDA) and the National Institute of Allergy and Infectious Diseases (NIAID) in Bethesda, MD. Patients were classified into 3 groups according to their CD4 count at baseline (i.e., entry into the study): (1) <200 cells/μL, (2) 200–500 cells/μL, and (3) >500 cells/μL. Patients receiving FDA-approved or Expanded-Access antiretroviral drugs were eligible for enrollment, if they had been following a stable regimen for at least 1 month prior to screening. Patients who were receiving no or suboptimal drug treatment at screening (virus load >500 copies/mL) were offered the opportunity to start or switch to a new treatment regimen and to defer enrollment until 1 month of stable use was completed. Those who refused any or a switch in treatment regimen were allowed to enroll immediately. Changes after enrollment were allowed 2 weeks after the primary immunization. HIV-infected patients were excluded if they had been previously immunized with bacteriophage phiX174, had a current opportunistic infection, had a history of severe atopic conditions, or had used immune-based therapies, other experimental agents, corticosteroids, or any other immunomodulatory drug within 6 months prior to enrollment. Plasma HIV RNA concentrations were determined with the branched DNA signal amplification assay (Chiron, Emeryville, CA), with a lower limit of detection of 500 copies/mL. Determinations of lymphocyte subgroups and surface markers were performed by 1-color or 2-color flow cytometry with monoclonal antibodies, as described elsewhere [7].

Bacteriophage phiX174 immunization and CD4+ T cell function in vivo. Bacteriophage phiX174 was grown in Escherichia coli, harvested, purified, and sterilized, as described elsewhere [10]. The final preparation of bacteriophage contained 10^11 pfu/mL; it was administered intravenously at a standard dose of 2 × 10^9 pfu/kg of body weight on 3 separate occasions 6 weeks apart. Blood samples for antibody titers were collected prior to and at 1, 2, and 4 weeks after each immunization, resulting in a total of 12 time points per patient. Antibody activity was determined by a neutralizing antibody assay and was expressed as the rate of bacteriophage inactivation in vitro (Kv), derived from a standard formula [10]. Neutralizing antibodies resistant to 2-mercaptoethanol were considered to be IgG. The antibody titers (Kv) in normal individuals conformed to lognormal frequency distributions [10].

Statistical methods. Antibody titers and virus load measurements were log transformed. Means were compared between groups, using the 2-sample t test on the log-transformed values. Means for other variables were compared between groups, using the 2-sample t test on the untransformed values. Simple linear regression analyses examined the impact of continuous variables by themselves (for example, CD4 count, virus load) on outcomes (log-transformed total antibody and IgG titers). Multiple linear regression models, relating combinations of covariates to outcome, were also used to assess whether the linear relationships held after adjusting for other factors. Reported P values for all regression models came from the t statistics for the model terms.

Results

Demographics and clinical characteristics. A total of 37 HIV-infected patients (mean age, 38.5 years; 89% male) and 13 HIV-uninfected controls (mean age, 38.3 years; 31% male) volunteered to be immunized. Four patients and 3 normal volunteers had significant deviations from the standard immunization protocol with regard to the timing of immunizations and were excluded from the analyses. Two patients who missed their third immunization contributed with only partial information to the analyses. Three patients met the definition of long-term nonprogressors (LTNPs; i.e., HIV infection had been present for >10 years in the setting of stable CD4 counts and no antiretroviral therapy). HIV-infected patients’ baseline immuno-

Figure 1. Primary, secondary, and tertiary antigen-specific total (IgM + IgG) antibody responses to bacteriophage phiX174 immunization in healthy uninfected controls. Broken lines represent the range for normal responses (geometric mean ± 2 SD), as reported in the literature [17]. Continuous lines represent each individual healthy control. Arrows indicate week of immunization.
Table 1. Participants’ immunologic and virologic characteristics and antiretroviral treatment at baseline.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Evaluable HIV-infected patients (n = 33)</th>
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<tbody>
<tr>
<td>CD4 count at baseline, cells/μL</td>
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<tr>
<td>&lt;200</td>
<td>8</td>
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<td>200–500</td>
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<tr>
<td>&gt;500</td>
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<td>Virus load at baseline, copies/mL</td>
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<td>&lt;500</td>
<td>14</td>
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<tr>
<td>501–10,000</td>
<td>10</td>
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<tr>
<td>&gt;10,000</td>
<td>9</td>
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<tr>
<td>Treatment regimen at baseline</td>
<td></td>
</tr>
<tr>
<td>HAART</td>
<td>17</td>
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<tr>
<td>2-Drug regimen</td>
<td>7</td>
</tr>
<tr>
<td>No treatment</td>
<td>9</td>
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NOTE. HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy. 

a Only 3 of these patients were antiretroviral naive when they started to receive HAART. Median duration of prior use was 10.9 months (range, 1.9–29.5 months). 

b Three of these patients switched to HAART 2–6 weeks after the primary immunization. Median duration of prior use was 7.2 months (range, 1.6–45.9 months). 

a One of these patients started to receive HAART during week 6.

Overall responses to bacteriophage immunization. The 10 controls responded to bacteriophage immunization with the titers and patterns of antibody responses considered to be normal for the general population [17], experiencing robust increases in antibody titers and a switch from IgM to a predominance of IgG with repeated bacteriophage immunizations (figure 1). Responses did not differ between men and women. In contrast, after 3 immunizations with bacteriophage phiX174, responses among patients were quantitatively and qualitatively inferior to those of controls with regard to all outcome measures: mean total antibody titers (765.6 vs. 26.3; \( P = .001 \)), percentage of IgG (77.5% vs. 22.6%; \( P = .001 \)), and mean IgG titers (585 vs. 3.3; \( P = .006 \)). Significant differences between patients and controls were also observed at earlier time points. Responses varied greatly among the 33 patients but were abnormal in 26 of them, with regard to the ability to amplify antibody titers and/or to switch from IgM to IgG. Although 12 patients produced total antibody titers in the normal range after the tertiary immunization (i.e., within 2 SDs of values for the control group [total antibody ≥222]), only 7 of the 12 also had bacteriophage-specific IgG titers in the normal range (IgG ≥123).

Relationship with CD4 count and virus load. Baseline CD4 count provided the strongest correlation with total antibody \( (R = 58\%, \ P < .001) \) and IgG titers. A weaker relationship was observed between HIV RNA levels and responses. These relationships were observed in regression models in which CD4 count and HIV RNA were analyzed separately or jointly. Further analyses evaluated patients in 3 prospectively defined categories for baseline CD4 count (<200, 200–500, or >500 cells/μL) and 2 categories for virus load levels (>500 or ≤500 copies/mL). These analyses indicated that patients with <200 cells/μL at baseline were unlikely to respond normally to bacteriophage immunization, irrespective of virus load levels. Seven of 8 patients with a baseline CD4 count <200 cells/μL had absent or severely depressed responses (median tertiary antibody titers, 1.5; figures 2 and 3). Poor responses were observed even among 3 patients with low virus load levels who had been receiving HAART for >11 months, including a patient whose CD4 cell count had risen from 4 to 153 cells/μL after 22 months of HAART. Similarly, a poor response after a low CD4 count nadir was observed in another patient, whose CD4 count rose from 6 to 205 cells/μL, despite following a 4-drug regimen for 14 months and having virus load levels <500 copies/mL.

Patients with CD4 counts above the threshold of 200 cells/μL had responses that ranged from absent to normal (figures 4–6). In this subset of patients, HIV RNA levels appeared to have some predictive power, as evidenced by patients with lower virus load levels having better immune responses more consistently than those with higher virus load levels (figures 2 and 7). Two of the 3 LTNPs were able to amplify antibody titers to normal levels; the third missed his last immunization but had abnormal antibody titers and isotypes at earlier time points (figure 6).

Relationship with naive and memory subsets and activation markers. The proportion of CD4+ T cells expressing memory (CD45RO+) or naive (CD45RA+) phenotypes and surface activation markers (CD25 and HLA-DR) were evaluated for their potential association with responses to bacteriophage immu-

Figure 2. Peak antigen-specific total (IgM + IgG) antibody titers after tertiary immunization with bacteriophage phiX174 in human immunodeficiency virus–infected patients, according to baseline CD4 count (in cells/μL) and virus load levels (in copies/mL); one patient missed his third immunization, but, since his secondary response (Kv = 560) reached the normal range, it was represented as such; one of the 3 long-term nonprogressors (LTNPs) missed the third immunization and was not represented.

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Immunization with the T cell–dependent neoantigen bacteriophage phiX174 indicated that the majority of patients with HIV infection have evidence of abnormal CD4+ T cell function in vivo. Abnormalities were observed even among patients receiving HAART who had virus load levels ≤500 copies/mL and moderate or high CD4 cell counts. Measurement of humoral responses to bacteriophage phiX174 immunization in vivo clearly distinguished uninfected controls from HIV-infected patients with regard to immune function. Controls exhibited the previously reported patterns of response, characterized by a primary IgM response that was followed by recall or memory amplification of bacteriophage-specific total antibody titers and a switch from IgM to predominantly IgG isotypes with further immunizations [17]. In contrast, only 7 of the 33 HIV-infected patients investigated had normal T cell function according to these criteria. Most patients exhibited abnormalities in amplification of total antibody titers and/or in the ability to switch antibody isotypes from IgM to IgG.

This exploratory study is an attempt to specifically measure CD4+ T cell function in vivo across all stages of HIV disease and virus load levels. CD4 count, as assessed by bacteriophage phiX174 immunization, was the most important predictor of immune function in vivo. Patients with a baseline CD4 count <200 cells/μL had minimal or no evidence of immune function in vivo, regardless of virus load levels. Among those with CD4 counts ≥200 cells/μL, responses ranged from absent to normal, and in this subset, virus load seemed to be of some predictive value. Among several patients whose virus load was >500 copies/mL, total antibody titers progressively declined, rather than undergoing the usual amplification, with repeated immunizations. Such decline could reflect the loss of bacteriophage-specific immunity (i.e., antigen-specific T cells that are destroyed once activated and infected by HIV). Interestingly, patients whose CD4 count increased during the 4-month study period

Discussion

Immunization with the T cell–dependent neoantigen bacteriophage phiX174 indicated that the majority of patients with
may represent increases in mature T cells rather than increases in T cell differentiation, and increases in naive T cells may also be a reflection of peripheral expansion [7]. The rapidity with which certain opportunistic illnesses clear with the initiation of HAART may indicate that these illnesses were occurring in the presence of antigen-specific T cells that were immunosuppressed and unable to mount an adequate immune response due to the presence of virus. In this setting, reduction in levels of virus can lead to rapid improvement in the remaining immune function of pre-existing antigen-specific T cells, at times in the setting of an exaggerated (type IV hypersensitivity) immune response [21]. Thus, the rapid clearing of opportunistic infections or Kaposi’s sarcoma after therapy may be a reflection of the removal of a suppressive effect of HIV infection, presumably related to HIV-associated polyclonal activation, whereas the persistent defect in immune response to bacteriophage immunization reflects the immunodeficiency consequent to HIV infection. The immunosuppressive aspects of this disease appear easily reversible with antiretroviral therapy that controls virus load; the immunodeficient aspects, however, may not be so easily reversed. This dichotomy may help explain why the incidence of Pneumocystis carinii has dropped dramatically, whereas the declines in the incidence of lymphoma are less impressive.

The clinical impact of a low CD4 nadir among recovering advanced patients was recently characterized by Miller et al. [22]. In that study, among patients with a current CD4 count >200 cells/μL, a previous CD4 nadir <50 cells/mm³ remained associated with a higher rate of disease progression and a higher relative hazard for progression than was a CD4 count nadir >150 cells/μL. It is possible that the higher rate of progression in the patients with a low CD4 nadir who were identified by
Miller et al. could be explained by the lack of functional recovery of CD4+ T cells in patients who reach very low CD4 nadirs, as suggested in this study. The lack of antibody amplification observed in patients with advanced disease is indicative of an inadequate generation of memory cells in response to a neoantigen. This observation may explain why improvement in proliferation to recall antigens in vitro with the use of HAART has been reported to occur only in those advanced patients who already had some responsiveness to recall antigens at baseline (i.e., those with pre-existing memory cells [5]). In addition, this suggests that neoantigens may be a useful complement to recall antigens in the evaluation of immune function, since they are not dependent on events that occurred prior to the onset of immunodeficiency.

Given the requirement for CD4+ T cell help, a normal amplification of antibody titers and isotype switching in a given patient constitutes evidence of the presence of functional T cells. However, the reverse (i.e., an abnormal antibody response) may or may not be direct evidence of T cell dysfunction. Intrinsic B cell defects, known to occur in HIV disease, could account for part or even all of the abnormal responses observed in this study. The cause of B cell dysfunction in HIV is not fully understood; however, Wolthers et al. suggest that such B cell defects may originate from improper T cell–B cell interactions, caused by a defective expression of CD40 ligand on CD4+ T cells [23]. Defective CD40 ligand expression results in an inadequate interaction with its CD40 counterpart on B cells, leading to an insufficient expression of CD70 on B cells, which is required for responses to T cell–dependent antigens. Hence, it is conceivable that most abnormalities underlying humoral responses to T cell–dependent antigens in HIV disease, whether manifested as a T or B cell dysfunction, may originate from T cell defects.

In this study, the ability to switch antibody isotypes from IgM to IgG seemed to be more compromised than the ability to amplify antibody titers, and discordance between these 2 parameters was common. Many patients were able to produce and amplify antibodies in reasonable quantities, yet had low isotype switching and, consequently, low IgG titers; although it was less frequent, the reverse was also observed. Such discordance may reflect the complexity of T cell function, which suggests that different tasks performed by T cells may involve specific requirements or abilities, which may be lost or restored independently and may be of different clinical significance. In this regard, antibody isotype switching and, consequently, IgG titers may be of greater clinical relevance than total antibody titers, as inferred from other immunodeficiencies, such as the hyper-IgM syndrome [24]. In that disease, which results from ineffective T cell help to B cells, patients with very low or absent IgG, IgA, and IgE levels and normal-to-increased IgM titers experience recurrent bacterial infections and are usually treated with intravenous immunoglobulins.

HIV RNA and CD4 have been consistently shown to be predictors of HIV disease progression. However, many studies have also repeatedly found that these 2 markers are not complete predictors of clinical outcome and may explain only up to 50% of the variability in clinical responses. This study suggests that immune function in vivo, as measured by bacteriophage phiX174 immunization, might be used in addition to traditional markers to provide information regarding a patient’s status. However, further studies are necessary to evaluate the relationship of this biomarker to prognosis, clinical outcome, and therapy. In addition, methods that measure functional reconstitution might be of value to quantify susceptibility to opportunistic infections and guide the management of prophylactic therapies for individual patients, as well as to aid in the clinical evaluation and development of new therapies for HIV with regard to their impact on the immune system.

References

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