Correlation of HIV-Specific Immunity, Viral Control, and Diversification following Planned Multiple Exposures to Autologous HIV in a Pediatric Population

William Borkowsky,1* Elizabeth J. McFarland,2 Ram Yogev,3 Yonghua Li,1 and Paul Harding2
NYU School of Medicine, New York, New York1; University of Colorado Health Sciences Center, Denver, Colorado2; and Chicago Children’s Memorial Hospital, Chicago, Illinois3

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Repeated controlled exposure to autologous virus was previously shown to result in increased CD8 T lymphocyte response to HIV antigens and accompanying reduction in viremia. We attempted to see if this immunity contributed to virologic control by correlating the immune response with quasispecies envelope diversification, an indicator of immune selection. The greatest diversification was seen in those with the greatest reduction in viremia but was unrelated to the frequency of Env-specific gamma interferon-producing cells. There was a trend toward correlation between the response to multiple HIV antigens and diversification.

We showed previously that a novel antiretroviral medication (ARV) interruption strategy resulted in improved virologic control after multiple carefully graded exposures to autologous virus (3). The strategy utilized periods of ARV alternating with slowly increasing durations off ARV, starting with 3 days and progressing by intervals of 2 days to periods as long as 41 days. We found that the onset of viral control corresponded to the increase in cellular immune responses to HIV. At study end, HIV-specific gamma interferon (IFN-γ) values were 4- to 30-fold higher and plasma HIV RNA levels, during ARV interruption, were 0.32 to 2.10 (median, 1.3) log units lower than during interruptions early in the study. In a subset of individuals who advanced to interruptions of 35 days, there was a correlation between the increase in cellular immunity and the degree of virologic control, but it was not clear whether this observation was merely an association or a direct cause and effect. To address this question, we determined changes in quasispecies diversity, which may result from immunologic pressure, and compared this to the change in the IFN-γ response in each individual.

(The clinical results were published previously.)

MATERIALS AND METHODS

Methodology. The details of the study design and documentation of informed consent have been reported elsewhere (3). In short, the drug interruption (DI) group participated in cycles that alternated the time on and off antiviral medication with interruptions lasting for 2x + 1 days, where x is the cycle number (i.e., cycle 1 had a 3-day DI). At the end of the DI, if plasma HIV was below the level of detection (i.e., <50 copies/ml), the next cycle began. Persistent detectable viremia (i.e., >50 cp/ml) required an additional 28 days of highly active antiretroviral therapy (HAART) and a viral load of <50 cp/ml before the subject entered the next cycle. The change in viral load for each subject was defined as the peak RNA value after DI minus the RNA value after the final DI. Peak viral RNA typically occurred after the 4th to 9th DI, and subsequent plasma RNA levels after DI were lower. HIV plasma RNA levels were quantified using the Amplicor HIV-1 Monitor Test versions 1 and 1.5 (Roche Diagnostics).

Data analysis. Gel images were edited and imaged using the graphics program Adobe Photoshop 3.0 (Adobe Systems Inc., San Jose, CA). In order to quantify the degree of diversification between the first and second specimens in each pair, a scoring system was devised which measured the change in mobility from the baseline bands divided by the migratory distance from the baseline band to the single-stranded DNA band. For each patient, the migration profile of the first time point was subtracted from the profile at the subsequent time point. Both positive and negative differences were summed to determine the absolute difference between the two. Since the total amount of intensity in each lane is normalized to be identical, the diversity quotient measures the change in distribution of signal, which in turn measures the degree of viral diversification between the two time points. Higher total scores indicate greater diversification.

Statistical analysis. The diversification quotient for a given patient was correlated with the log of the number of IFN-γ spots/10^6 PBMC. A regression line...
was assessed for significance using SigmaPlot software. This included nonlinear regression, Cook’s distance, and a power analysis using an alpha of 0.05. The Mann-Whitney rank sum test was used to test differences between those individuals with and without significant reduction in RNA.

RESULTS

Eight patients who completed 13 or more interruptions of antiretrovirals were studied. These are the patients previously reported. Quasispecies migration changes ranged from 0.01 to 0.76 (Fig. 1). As can be seen in Fig. 2a, three of the four largest changes in migration were seen in subjects with the largest reduction in plasma HIV-RNA (0.8 log units). Using traditional criteria for significant decrease in viremia (a 5-fold or 0.7-log-unit change), the 5 individuals with significant decline in viremia had a median migration change of 0.273 (0.035 to 0.778 [25th and 75th quartiles]), while the other 3 individuals without such a decrease in viremia had a median migration change of 0.01 (0.009 to 0.015). These are significantly different (P = 0.036).

To measure CD8 T cell responses, we looked at the frequency of cells expressing IFN-γ in response to HIV epitopes presented by autologous cells infected with recombinant vaccinia vectors at the associated time point. The response to the Env vector ranged from 5 to 6,640/10⁶ PBMC. The total response (the sum of frequencies for each of the 4 vectors [HIV-sum]) ranged from 58 to 12,900/10⁶ PBMC. Three of 8 individuals (PID2, PID4, and PID6) demonstrated large shifts, with migration changes exceeding 0.3 (Fig. 1). The same 3 individuals exhibited HIV-sum responses greater than 10⁵ spots/10⁶ PBMC. However, 2 other individuals with comparable levels of immune response had quasispecies changes of only 0.02 and 0.04. The migration changes were inversely correlated (slope, −0.11) with the frequency of Env responses (Fig. 2b) and positively correlated (slope, 0.13) with HIV-sum responses (Fig. 2c). This variation in response and the small number of individuals studied resulted in insufficient power to prove a statistically significant correlation between the variables.

DISCUSSION

Viral diversification has been attributed to either increased viral replication or immunologic pressure. A strong immunologic response would be expected to restrict viral replication, so that viral diversification might not be evident but, if partially effective, it might result in viral escape, as demonstrated by an evolution of quasispecies. Since our patients were unable to restrict viral replication to <1,000 copies, we expected to see such evolution. Our observation that viral diversification was the greatest in subjects with the best viral suppression (i.e., a reduction of >0.7 log units), is not consistent with higher viral replication driving viral diversification. We had expected that a change in HIV envelope diversity would correlate with the vigor or the Env-specific CD8 response. Although we could not demonstrate a correlation between increased envelope diversity, as measured by the heteroduplex tracking assay (HTA) assay, and enhanced Env-specific IFN-γ responses to a heterologous Env vector, the sum of the responses to all four vectors (i.e., Env, RT, Gag, and Nef) suggested such a correlation, particularly in the 3 subjects with the largest change in viral diversity.
The considerable differences that exist in the envelope sequence presented by our vaccinia vector from sequences expressed by autologous viruses may explain the observed lack of correlation. The frequency of responses to Env for 5 of the 8 subjects was very low. Also, studies comparing CD8 responses to other variable viral epitopes show enhanced responses to autologous peptides relative to heterologous peptides (1). Ideally, vaccinia vectors that expressed autologous Env proteins or the use of short peptides that reflected autologous Env antigens would have been useful to better assess CD8 responses to autologous Env viruses. However, this approach was limited by the financial constraints of the study.

Alternatively, the selection may be due to other immune pressures, such as escape from neutralizing antibody in the envelope region of HIV (15). Neutralizing antibody to autologous virus, a potential cause of viral diversification, could not be tested in our study. This approach was attempted via collaboration with Monogram Biosciences, but many of the viral isolates were unable to be amplified for insertion into their vector.

Lack of correlation of immune pressure with one aspect of cellular immune response (i.e., IFN-γ production) and quasispecies selection is not entirely surprising. Lytic CD8 T cells may be required (13), a response that may be dissociated from IFN-γ release (9). Lu et al. studied vaccination with zinc finger-inactivated autologous virus presented by matured dendritic cells to stimulate HIV-specific immune responses and demonstrated an 80% decrease in viremia, with suppression persisting for as long as a year (11). The response that correlated best with viral suppression was Gag-specific CD8 perforin release. However, the correlation between cellular responses to other HIV antigens (i.e., RT and Gag) with less variation in sequences from autologous virus suggests that cellular immune pressure may have played a role in some of the individuals. Other studies attempting to correlate virologic control in chronically infected individuals with IFN-γ responses to Env antigens have also not found an association, despite the fact that responses to Gag antigens were associated with control (2, 7, 8, 14). Virologic escape may also correlate with responses to other antigens (4). This can explain increased HTA changes seen in some of our subjects with the greatest responses to the 4 antigens. Decreased viremia in those with the largest diversity may also reflect restrained viremia as a consequence of this escape (12). Further studies, involving a larger cohort of individuals, elucidating the relationship between quasispecies’ evolution and the pressure exerted on the virus by the various immune responses, may contribute to a better selection of vaccine candidates.

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REFERENCES

1. Altfeld, M., et al. 2003. Enhanced detection of human immunodeficiency virus type 1-specific T-cell responses to highly variable regions by using peptides based on autologous virus sequences. J. Virol. 77:7330–7340.
2. Borkowsky, W., et al. 2004. Correlation between HIV-specific CD8 gamma interferon production and plasma HIV RNA in perinatally-infected pediatric populations. J. Infect. Dis. 190:722–726.
3. Borkowsky, W., et al. 2008. Structured treatment interruptions (STI) in
HIV-1 infected pediatric populations increases interferon gamma production and reduces viremia. AIDS Res. Hum. Retroviruses 24:401–411.

4. Brumme, Z. L., et al. 2009. HLA-associated immune escape pathways in HIV-1 subtype B Gag, Pol and Nef proteins. PLoS One 4:e6087.

5. Delwart, E. L., et al. 1993. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 env genes. Science 262:1257–1261.

6. Essajee, S. M., et al. 2002. Recombinant glycoprotein vaccines for human immunodeficiency virus-infected children and their effects on viral quasispecies. Clin. Diagn. Lab. Immunol. 9:79–82.

7. Julg, B., et al. 2010. Enhanced anti-HIV functional activity associated with Gag-specific CD8 T-cell responses. J. Virol. 84:5540–5549.

8. Kiepiela, P., et al. 2007. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat. Med. 13:46–53.

9. Kuerten, S., et al. 2008. Dissociated production of perforin, granzyme B, and IFN-gamma by HIV-specific CD8(+) cells in HIV infection. AIDS Res. Hum. Retroviruses 24:62–71.

10. Larsson, M., et al. 1999. A recombinant vaccinia virus based ELISPOT assay detects high frequencies of Pol-specific CD8 T cells in HIV-1-positive individuals. AIDS 13:767–777.

11. Lu, W., L. C. Arraes, W. T. Ferreira, and J. M. Andrieu. 2004. Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. Nat. Med. 10:1359–1365.

12. Martinez-Picado, J., et al. 2006. Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. J. Virol. 80:3617–3623.

13. Migueles, S. A., et al. 2008. Lytic granule loading of CD8+ T cells is required for HIV-infected cell elimination associated with immune control. Immunity 29:1009–1021.

14. Rolland, M., et al. 2008. Broad and Gag-biased HIV-1 epitope repertoires are associated with lower viral loads. PLoS One 3:e1424.

15. Wei, X., et al. 2003. Antibody neutralization and escape by HIV-1. Nature 422:307–312.