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BRIEF COMMUNICATION

Longitudinal analysis of B cell repertoire and antibody gene rearrangements during early HIV infection

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In chronically HIV infected individuals, a number of functional B cell abnormalities have been described. However, the immediate changes that occur in the B cell compartment following viral exposure and how they affect the long-term course of infection are not well understood. We report the longitudinal analysis of B cell repertoires during early infection in untreated and treated individuals receiving highly active antiretroviral therapy (HAART). Analysis was based on IgG heavy chain gene utilization and CDR3 length measurement and relationship with CD4/CD8 counts, viral load, and total serum IgG, and anti-HIV antibodies levels. Repertoires were assessed at baseline and at weeks 2, 4, 12, 24, and 72 after initiation of therapy. The findings indicate a stable peripheral B cell repertoire during the first 72 weeks following infection, particularly in the HAART treated patients. A modest association between B cell repertoire integrity and viremia levels as well as treatment was detected.
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The factors underlying the transition from the asymptomatic state in primary HIV infection to pathogenic immune dysregulation and AIDS have not been entirely elucidated. A role for humoral immunity in this process is supported by the common development of hypergammaglobulinemia in chronically infected individuals, associated with B cell hyperactivation, clonal expansions and deletions, and deficient costimulatory function.[1–6] It is unclear, however, if these abnormalities are the result of viremia and a direct effect of virus on B lymphocytes or, alternatively, mediated by long-term infection-induced changes in T-cell subsets and cytokine production. To assess the early effect of HIV infection on the integrity of the peripheral B cell repertoires, we analyzed the B cell-receptor diversity in a longitudinal cohort of 10 affected individuals who had been treated with HAART immediately after diagnosis and 10 infected individuals who chose not to be treated. The results were compared with observations from uninfected controls. Analysis was based on *IgG* heavy chain gene utilization and CDR3 length measurement (spectratyping),[7] and relationship with CD4/CD8 counts, viral load, and serologic responses.

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Individuals with acute or recent HIV-1 infection were eligible for this study if the initial evaluation indicated that they met one or more of the criteria for recent HIV-1 infection: (1) detectable HIV-1 RNA in blood plasma and a negative or indeterminate Western blot assay for anti-HIV-1 antibodies; (2) a positive enzyme-linked immunosorbent assay (ELISA) with Western blot confirmation within 12 months of a documented negative HIV-1 antibody test; or (3) a sensitive/less sensitive (S/LS) dual ELISA testing optical density ratio of less than 0.75[8,9] with a history compatible with recent HIV infection.[10] The dual enzyme immunoassay (EIA) testing strategy using a standard high sensitivity EIA and an LS EIA takes advantage of the progressive development of HIV antibody response during the initial phase of infection. LS EIA standardized OD values <0.75 are used to define LS EIA nonreactive infected individuals, consistent with infection within the prior 4 months (recent infection or recent seroconvertor as opposed to long-standing infection).[9] At study entry no patient had received antiretroviral therapy. Among the 10 treated cases, the mean age was 33.4 (range 24–42), nine were men and two were from minority groups. The estimated mean-days from seroconversion was 95.7 (range 0–188). For the 10 untreated cases the mean age was 35.3 (range 22–45), all were men, and three were from minority groups. The estimated mean-days from seroconversion was 126.5 (range 97–264). The eight uninfected controls ranged in

age from 23 to 45, included one woman, and seven were White. At study entry, patients had a median HIV-1 RNA level of 282 439 copies/ml and 28 382 copies/ml (treated and untreated group respectively) and a median CD4+ T-cell count of 641 and 519 cells/μl (treated and untreated group respectively). At week 72, patients had a median HIV-1 RNA level of 108 and 90 749 copies/ml (treated and untreated group, respectively) and a median CD4+ T cell count of 960 and 460 cells/μl (treated and untreated group, respectively).

Peripheral blood lymphocytes from all study participants were obtained at baseline and at weeks 2, 4, 12, 24, and 72 and reverse-transcribed RNAs were PCR-amplified with IgVH sequence-specific primers as described (Figure 1).[7] In controls, the observed Gaussian distribution of spectratypes (number of CDR3 bands and intensities) correlates well with the expected allocation of randomly rearranged *VH*, *D*, and *JH* gene segments, indicating that no bias in the CDR3 amplification occurred (data not shown). The mean maximal log spectra type intensities and number of bands at baseline were similar among the groups (controls, treated and untreated subjects, data not shown).

Predictors of global IgVH repertoire distances from composite control profiles are shown for HIV-infected subjects by treatment status in Table 1A. Among treated cases, repertoire distances from controls declined slowly at an average of 0.4% per month, suggesting that under the influence of HAART, repertoires were gradually

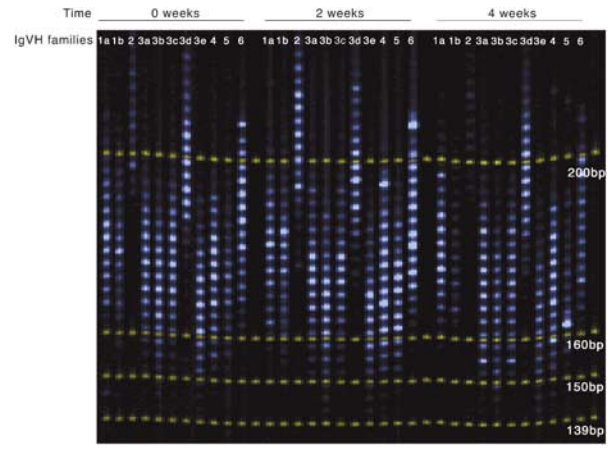

Figure 1 Representative immunoglobulin CDR3 spectratyping. Total RNA was isolated with the Trizol reagent (Life Technologies, Bethesda, MD, USA) from peripheral blood non-CD4+/non-CD8+ lymphocytes. First-strand cDNA was synthesized with the Superscript-II kit (Life Technologies) primed with random hexamers. Oligonucleotide primer sequences have been reported elsewhere.[7] The common IgCγ primer was fluorescently labeled and hot-start PCR performed in 11 separate reactions (one per VH family primer set). A measure of 3 μl PCR products were resolved by electrophoresis in 6% acrylamide denaturing gels on the ABI PRISM 377 Automated DNA Sequencer loaded with an ABI PRISM 672 GENESCAN 2.1 software for fluorescent scanning. Genotyper 2.0 was then used for peak calling. Spectratype data were exported into Excel files for downloading into our data management system.

Table 1 Predictors of distance and intensity measures characterizing the B cell repertoire

Outcome	*Predictor*	*Treated participants*			*Untreated participants*			*P-value for interaction with treatment*
		Estimate	*95% CI*	*P-value*	*Estimate*	*95% CI*	*P-value*	
(A) Log distance from controls	Months since baseline	−0.4	−0.5, −0.2	<0.0001	−0.2	−0.4, 0.0	0.06	0.18
	CD4+ cell percent	−0.1	−0.5, 0.3	0.61	0.6	0.2, 1.0	0.002	0.01
	CD8+ cell percent	−0.2	−0.6, 0.2	0.25	0.6	0.3, 0.9	0.0003	0.001
	Log viral load	0.2	0.0, 0.3	0.01	0.4	0.2, 0.7	0.001	0.12
	Log detuned EIA	0.1	−0.2, 0.3	0.60	−0.1	−0.4, 0.3	0.75	0.57
(B) Log distance from baseline	Months since baseline	0.2	0.0, 0.3	0.05	0.0	−0.2, 0.1	0.66	0.10
	CD4+ cell percent	−0.2	−0.6, 0.3	0.46	0.3	−0.3, 0.8	0.30	0.20
	CD8+ cell percent	−0.1	−0.6, 0.3	0.60	0.4	0.0, 0.8	0.03	0.06
	Log viral load	0.1	−0.1, 0.4	0.24	0.3	0.0, 0.6	0.06	0.40
	Log detuned EIA	−0.2	−0.4, 0.1	0.19	0.4	0.1, 0.7	0.02	0.006
(C) Log-maximal intensity	Months since baseline	0.1	0.0, 0.3	0.07	0.0	−0.2, 0.2	0.74	0.46
	CD4+ cell percent	−0.4	−0.8, 0.0	0.08	0.7	0.3, 1.0	0.001	0.0007
	CD8+ cell percent	−0.3	−0.6, 0.1	0.18	0.6	0.3, 0.9	<0.0001	0.0006
	Log viral load	0.0	−0.2, 0.1	0.80	−0.1	−0.3, 0.2	0.54	0.32
	Log detuned EIA	0.0	−0.2, 0.2	0.85	0.3	0.0, 0.6	0.07	0.19

To assess repertoire divergences, we computed composite profiles for each *IgGVH* family by averaging the intensities at each CDR3 length across spectratypes, including multiple samples over follow-up. These intensities also sum to 100. The distance between each spectratype and the composite profile for that *IgG* family was then computed as described.[14] In addition, we also analyzed the maximal band intensities in each profile as an indirect measure of clonal expansion. Repeated-measures regression models were used to assess the associations of distances and maximal intensities with HIV status, treatment, lab measurements, and time since enrollment in the study.[19] These models take into account the correlation between repeated measures for individuals. To normalize distances, the log transformation was used, with 1 point added to each raw distance before transformation to retain 0 values in the analysis. The log transformation was also used for CD4+ and CD8+ cell numbers, viral load, and antibody levels. All models included random effects for subject and *IgG* family, as well as correlations within subject and *IgG* family that were assumed to depend on the time separating the paired samples. The analysis was performed using Proc Mixed in SAS, Version 8.02 (SAS Institute, Cary, NC, USA). Estimates for months since baseline are unadjusted, and in units of percent change in the outcome per month. Estimates for CD4+ cell percentage, CD8+ cell percentage, and log viral load and detuned assay are in units of percent change in the outcome per 10% increase in the predictor, and are adjusted for all other variables in the table.

becoming more like the control profiles ($P<0.0001$). Among untreated cases, distances from controls appeared to decline somewhat more slowly. In multivariate analysis, distances from control profiles were uncorrelated with either CD4+ or CD8+ cell percent among treated cases, but positively and strongly correlated with both among untreated cases. In addition, distances from control profiles increased with viral load in both groups.

The average distance from baseline increased slightly over time among treated cases, suggesting again the normalization of the repertoire with antiretroviral treatment, but was essentially constant among untreated cases (Table 1B). In multivariate models, distance from baseline increased with CD8+ cell percent; a similar increase in association with viral load was also weakly supported. Distance from baseline increased significantly with detuned assay level among untreated but not among treated cases. The overall good correlation observed between increasing detuned assays levels and spectratyping temporal distances confirm the sensitivity of the method to detect HIV-specific antibody responses.

Lastly, there was weak evidence for a small increase over time in maximal spectratype intensity among treated cases, but among untreated cases maximal intensity was essentially constant (Table 1C). In multivariate analysis, maximal intensity increased with CD4+ cell percent, CD8+ cell percent, and detuned assay level in untreated cases; among treated cases, we found only weak evidence for an inverse association with CD4+ cell percent.

Overall, case–control differences or trends in distance among cases did not vary significantly by *IgGVH* family. This was also evident from visual inspection of spectratype profiles. Modest changes in multiple clonotypes, rather than consistent expansions or deletions, most likely contributed to the perturbations detected in the study group. Associations of the distance and intensity measures with CD4+ and CD8+ cell counts were weaker but in the same direction as with the cell percents shown in Table 1; however, we did not find statistically significant associations with the ratio of the CD4+ and CD8+ cell counts. Results were also qualitatively unchanged after adjustment for minority status.

Total IgG levels were within normal ranges over the course of the study, although they increased modestly but significantly since baseline in untreated individuals (Table 2). Log-distance from baseline, CD4+ and CD8+ cell percents, viral load, and detuned assay results were predictive of antibody levels in univariate analysis. In multivariate analysis, statistically significant predictors of higher antibody levels included lower CD4+ cell percent, higher viral load, and lower detuned assay levels.

This study was designed to assess the effect of infection on the integrity and diversity of the peripheral B cell repertoire very early in the natural history of infection. A population-based analysis detected modest but consistent changes in the expressed repertoires, most notably in untreated HIV infected subjects. Abnormal expression of germline immunoglobulin genes was observed in cancer, autoimmunity and infections, including chronic hepatitis C[11] and measles-associated subacute sclerosing panencephalitis.[12] In trying to identify the variables affecting the integrity of the B cell

Table 2 Predictors of log antibody levels

Model	*Predictor*	*Estimate*	*95% CI*	*P-value*
Univariate	Months since baseline, treated	−0.4	−0.9, 0.1	0.16
	Months since baseline, untreated	0.7	0.0, 1.3	0.05
	Log distance from controls	1.3	−0.7, 3.3	0.2
	Log distance from baseline	−0.3	−0.6, 0.0	0.03
	Log maximal intensity	1.7	−0.6, 3.9	0.14
	CD4+ cell percent	−0.8	−1.1, −0.5	<0.0001
	CD8+ cell percent	0.5	0.2, 0.7	0.0007
	Log viral load	0.5	0.3, 0.7	<0.0001
	Log detuned assay	0.6	0.3, 0.9	<0.0001
Multivariate	Months since baseline, treated	−0.2	−0.6, 0.3	0.46
	Months since baseline, untreated	0.1	−0.7, 0.8	0.82
	Log distance from controls	−0.8	−3.4, 1.9	0.57
	Log distance from baseline	0.2	−0.1, 0.5	0.24
	Log-maximal intensity	1.6	−1.4, 4.6	0.29
	CD4+ cell percent	−0.6	−1.1, −0.1	0.01
	CD8+ cell percent	−0.2	−0.5, −0.1	0.25
	Log viral load	0.3	0.0, 0.5	0.03
	Log detuned assay	0.4	0.1, 0.7	0.02

Specimens were determined to be reactive in licensed high-sensitivity EIA screening and Western blot confirmation assays routinely applied for blood screening in US blood centers. Each sample from enrolled subjects was re-tested for anti-HIV-1 antibody using 3A11 HIV-1 EIA (Abbot laboratories, Chicago, IL, USA) to confirm seropostivity. The same specimens were tested in the three A11 less sensitive (LS/detuned)-EIA protocol as described.[8,9] Results are interpreted based on the standardized OD (SOD) of test samples, which is derived by dividing the mean OD of test sample on the LS-EIA by the mean OD of the CDC supplied assay Calibrator (Cal). Total plasma IgG quantification was performed by single radial immunodiffusion assay using commercially available kits according to the manufacturer's recommended protocol (The Binding Site, San Diego, CA, USA). Estimate for months since baseline are in units of percent change in the outcome per month, and are unadjusted for other covariates in the univariate model. Adjusted estimates for CD4+ cell percentage, log CD8+ cell percentage, log viral load, detuned assay results, and the distance measures are in units of percent change in the outcome per 10% increase in the predictor.

population, we observed that the repertoire dynamics was affected by the viral load and CD4+ and CD8+ T-cell percentages, suggesting a link between effective T-cell-mediated helper and effector functions, and the development of an effective and regulated anti-HIV B cell response. T-cell repertoire perturbations were observed by others and us very early in the natural history of HIV infection.[13,14] Although the clinical consequences of these expansions and deletions of clonotypes remain uncertain, a clonally restricted T-cell antiviral repertoire and activity may impair the ability to recognize a broader range of HIV epitopes and emerging mutant species of the virus. Hence, a main objective of HIV therapy should be to rapidly restore and maintain a diverse T-cell population that will respond promptly to multiple viral epitopes.

A compensatory effect of early HAART on B cell repertoire was detectable in this study. All measures of repertoire normalization were predominantly evident in the treated group, with significance values for interaction between the predictor and repertoire measures detected for CD8+ cell count, viral load, and detuned EIA (Table 1). Previous reports in chronically affected individuals documented that HAART restores defects in B lymphocyte immunity resulting from HIV infection.[15] However, the effect of treatment is partial, and in a substantial number of patients hypergammaglobulinemia is not resolved. As suggested by some authors,[16,17] these persistent B cell abnormalities may result in elevated risks for malignancies and autoimmune diseases. On the other hand, broader neutralization antibody patterns may be characteristic of slow progressors.[18] Long-term follow up is necessary to assess if B cell reconstitution remains incomplete over longer periods of treatment, as well as the effectiveness of early intervention with novel immunotherapeutic protocols and vaccines.

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