Mechanisms of decreasing HIV-1 specific CD8+ T cell activity during progression to AIDS

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Normalisation of the CD4 T-cell receptor repertoire after evolution of syncytium inducing HIV-1 variants.

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Recently, we reported on the dynamics of the T-cell receptor (TCR) diversity during antiretroviral therapy (57). Interestingly, the TCR diversity of naïve (CD45RA+) CD4 cells was as skewed as primed (CD45RO+) cells, which we interpreted as clonal expansions. Earlier it was argued that clonal deletions occur during HIV infection, which lead to perturbations of the TCR repertoire (76,115). In the setting of viral phenotype switch from non syncytium inducing (NSI)- to syncytium inducing (SI)- variants, we found arguments against clonal deletion.

For 2 patients, longitudinal PBMC samples around NSI/SI switch were FACS-sorted in CD4+CD27+CD45RO+ (memory) and CD4+CD27+CD45RO− (naive) subsets. RNA was isolated and 24 TCR-specific PCR reactions per sample were performed. PCR products were analysed on size diversity of the TCR-complementarity determining region 3 (CDR3) by gelelectrophoresis, as published before (57).

As shown in figure 1A, the number of different CDR3 fragment-sizes—indicative for TCR diversity—increased during NSI to SI phenotype switch. The perturbation expressed in percentages, plotted in figure 1B, shows a decrease in the number of clonal expansions. At the first time point analysed, the patients showed approximately 40% compared to 10% perturbation in healthy controls. After SI variants had emerged and could be detected (frequency of cells infected with SI variants were 27 and 175 TCID/10⁶ CD4+ cells for 490 and 1091 resp.), many of the TCR-PCR products showed normalised size distributions. Normalisation of the TCR repertoire can either result from decrease of clonal expansions or from polyclonal replenishment. As CD4 count decreased in both patients, the accompanying normalisation of the repertoire may thus result from disappearance of clonal expansions, allowing lower frequency clones to be detected.

**Figure 1.** A. PCR products of the varying CDR3 regions of T cell receptor VB chain. VB family 1 from patient 1091 is shown during HIV phenotype switch from NSI to SI variants. B. TCRBV perturbations decrease during viral phenotype switch to SI variants. Naive (♀), memory (●), and total CD4 (○) cells decrease in number (dashed lines) as well in clonality (solid lines, large symbols), while viral RNA plasma load increases (solid thin lines).
These findings may implicate that: First, the naive compartment as defined by CD45RO-CD27+, contains expanded clones. Hence, at least in HIV-infection, CD45 and CD27 markers do not properly define naive CD4+ T-cells as in CD8+ T-cells (95). Secondly, it seems that clonal expansions are beneficial. As long as patients are able to maintain antigen driven T helper responses, pathogens are being counter acted. Although TCR repertoires observed in our patients are improved after switch, they may actually lack the capacity to mount essential immune responses. SI variants may kill or inhibit activated T cells before they expand, and restricted expansion may result in an accelerated loss of CD4 cells and faster progression to AIDS (116). Finally, we observed that seemingly deleted CDR3 sizes reappeared despite ongoing infection. Since there are no reasons to assume that polyclonal thymic T-cell renewal is increased, it is most likely that the re-emerging clones and their CDR3 sizes were present before. This means that CDR3 perturbations are not indicative of clonal depletions, as previously suggested (76,115). We conclude that perturbations visible in the repertoire are reflecting previous immune activation rather than immunodeficiency.