HIV-1 sensitivity to neutralization: biological and molecular studies

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Summary
Primary HIV-1 variant are relatively neutralization resistant, hereby optimally adapted to replication in the presence of antibodies (Abs) \textit{in vivo}. Adaptation to growth in T cell lines coincides with the selection of an envelope configuration with increased sensitivity to neutralization by Abs and agents directed against the CD4-binding site. The presence \textit{in vivo} of neutralizing Abs does suggest however that the relevant epitopes for neutralization are being exposed to the immune system. The underlying mechanism for these changes in envelope configuration as well as the induction of antibodies with different sensitivity are largely unknown.

It is generally believed that during acute infection cytotoxic T lymphocytes are responsible for clearance of the virus. During virus latency, Abs develop that in general do not neutralize autologous virus but do recognize monomeric envelope proteins. Only 1 to 2 years after infection and mainly in long-term nonprogressors broadly neutralizing Abs develop. In chapter 2 the conformation specificity of Abs induced during primary infection was studied by precipitation of metabolically labeled gpl20 during pulse chase experiments. Only mature, properly folded gpl20 was recognized by early Abs, and these were relatively strain specific since they could neutralize a primary CCR5-dependent isolate but not the CXCR4-utilizing T cell line adapted (TCLA) IIIB virus. After 3 months, Abs with specificity for immature gpl20 folding intermediates were detected, which preceded the appearance of Abs able to neutralize the TCLA IIIB virus. These observations suggest that Ab-induced clearance and neutralization of HIV-1 occurs early in infection and that soon thereafter Abs arise with increased specificity for biologically irrelevant gpl20 epitopes.

Infection initiates the production of strain specific Abs which drive the development of virus escape mutants. Since infection of an individual is established by primary neutralization resistant HIV the efficacy of humoral immunity is limited. In chapter 3 we studied the clinical significance of primary HIV-1 neutralization resistance by analyzing the HIV-1 variants that were isolated longitudinally from a laboratory worker who progressed to AIDS within 8 years after accidental infection with the TCLA neutralization sensitive IIIB strain. Viruses re-isolated 3 and 7 years after the infection showed an increased resistance to neutralization. The isolate obtained after 3 years was resistant to neutralizing agents directed against epitopes outside the CD4-binding site. The virus re-isolated around the time of disease development was completely resistant, especially to soluble CD4 and the IgG1b12 MAb, which is well known for its potency to neutralize many primary isolates.

The reversal towards a neutralization resistant phenotype in the accidentally infected laboratory worker coincided with 38 amino acid mutations, among which a change of the highly conserved 370 Glutamate (Glu) to an Alanine (Ala). During prolonged \textit{in vitro} culture, this Ala containing variant mutated back to a Glu at position 370, thereby creating again a neutralization sensitive phenotype (chapter 4). Chimeric molecular clones revealed that together with the 370 residue, mutations in the variable loops 1 and 2 determined resistance to neutralization. This suggested that the Ala370 variant was best adapted to growth \textit{in vivo}, whereas in the absence of neutralizing Abs \textit{in vitro} the Glu370 variant was most favorable in the HIV-IIIB like envelope structure.
Disease development in chimpanzees inoculated with TCLA or primary isolates is very rare, which might suggest that in these animals this is not related to neutralization. However, as described in chapter 5, *in vivo* and *in vitro* passage of a primary virus through chimpanzee PBMC generated a phenotype that was neutralization sensitive to CD4-binding site directed agents. In addition, an HIV-IIIB isolate obtained from a chimpanzee after 10 years of asymptomatic infection could still be neutralized by these agents and autologous serum, but not by Abs directed against other envelope epitopes. This suggested that increased sensitivity to CD4-binding site directed neutralization of HIV-1 after passage through chimpanzee cells may in part contribute to the long-term asymptomatic HIV-1 infection in experimentally infected chimpanzees.

Adaptation of primary viruses to replication in chimpanzee PBMC resembles phenotypic changes observed after passage through T cell lines. In chapter 6, we studied how these neutralization sensitive variants develop or are selected from the virus quasispecies. Amino acid alterations observed in gp120 of the H9 passaged progeny of parallel cultures of split inocula of biological HIV-1 clones were generally identical, but differed between unrelated isolates. Synonymous mutations of passaged progeny were also identical, which suggested that T cell line passage is not associated with accumulation of novel mutations, but rather with selection of a virus variant that pre-existed in primary HIV-1 infected PBMC.

In chapter 7 our observations are discussed in the perspective of recent developments in AIDS research.