The 'Triple Study': viral dynamics and immune reconstitution in HIV-1 infection during potent antiretroviral therapy
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In the **Introduction**, HIV and the pathogenesis of the immune dysfunction it causes are described. There is an enormous turnover of infected cells and virus, which has been estimated to $10^{10}$ virions per day in an infected individual, irrespective of disease stage. A number of markers can be used to estimate disease prognosis and evaluate the effects of antiretroviral therapy. The peripheral CD4$^+$ T-cell counts and the plasma HIV-RNA levels are the most important.

A number of drugs inhibiting viral replication has become available over the past years. There are basically three classes, inhibiting two different viral proteins: nucleoside analogue and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, respectively) and protease inhibitors (PIs). When used as mono- or double therapy, the suppression of viral replication is only temporary, as the virus usually becomes resistant to the drugs. When PIs and two NNRTIs were first combined starting late 1995, it was hoped that viral replication could be suppressed to a minimal level and that the occurrence of resistance mutations to all drugs simultaneously would be prevented. It would then become possible to study to what extent the immune damage would be reversible. As lymphoid tissue (LT) contains 98% of CD4$^+$ T lymphocytes in the body and 100 to 10,000 times as much virus as the peripheral blood, it is important to study the effects of therapy in the LT.

In the following chapters, short- and long-term virological and immunological studies as a part of the Triple Study are described. Thirty-three subjects received potent antiretroviral therapy consisting of a triple combination with the PI ritonavir (RTV) and the NRTIs zidovudine (ZDV) and lamivudine (3TC). Half the subjects started with RTV monotherapy for the first three weeks and then added ZDV and 3TC, while the other half started with all drugs simultaneously.

In **Chapter II** results of the first 24 weeks of the study are described. The triple combination resulted in a strong suppression of the plasma HIV-RNA levels, to below the lower cutoff level of the assay used, in the majority of subjects. Furthermore, in LT an HIV-RNA decline comparable to that in plasma was seen. CD4$^+$ T-cell counts increased with more than $100\times10^6$ cells/L. Adverse events were considerable, mostly gastro-intestinal or malaise, which caused eight (24%) subjects to withdraw from the study during the first 24 weeks. No significant long-term differences were seen between the two treatment groups.

The kinetics of the anti-viral effects in LT were studied in detail using in situ hybridization, as described in **Chapter III**. Not only the number of productively infected mononuclear cells (MNCs), but also follicular dendritic cell (FDC)-associated virus declined rapidly. After 24 weeks, however, some MNCs containing a few HIV-RNA copies could still be detected. Also, viral DNA was still present.

In **Chapter IV** the dynamics of viral decline upon initiation of therapy are described. During the first three weeks, HIV-RNA and the number of
productively infected cells (T*') declined rapidly, with a slightly stronger decline in the group starting with the full triple combination. In this group, T*' declined more rapidly in subjects with higher baseline HIV-RNA levels.

Immune reconstitution in peripheral blood during the first 36 weeks of therapy is described in Chapter V, including the different patterns of naive and memory T-cell repopulation. Immediately following therapy a strong increase in CD4+ memory cells was observed. No further increase in memory cells was observed after the first three weeks of therapy. The increase was inversely correlated to the baseline CD4+ T-cell counts and suggested initial redistribution of trapped memory cells from the LT. Naive CD4+ T cells increased more slowly but persistently, as did naive CD8+ T cells. With the increase in CD4+ T-cell numbers, the T-cell function, calculated per T-cell, rapidly increased as well.

CD4+ T-cell repopulation in LT is described in Chapter VI. Before treatment, the extent of CD4+ T-cell depletion compared to uninfected controls was less in LT than in the peripheral blood. In LT, the fraction of naive CD4+ T cells was decreased, whereas the fractions of proliferating- and apoptotic CD4+ T cells were increased compared to the uninfected controls. Upon treatment the total number of CD4+ T cells in the LT increased, with a relatively stronger increase in naive cells. The fraction of proliferating cells normalized, while the fraction of apoptotic cells remained elevated.

Longer term immune reconstitution in 19 subjects, who remained on potent therapy for two years, is described in Chapter VII. During the first 72 weeks CD4+ T-cell counts increased, largely because of a slow but sustained naive cell increase. This increase was considerably lower in subjects with low baseline naive CD4+ T-cell counts. Between week 72 and 108 in half the participants CD4+ T-cell counts did not further increase or even declined. T-cell function increased and CD8+ T-cell activation decreased rapidly, but had not reached normal levels in all participants after 2 years of therapy.

In Chapter VIII, the evaluation of a second generation NASBA-based assay, the NucliSens assay with a lower cutoff of 400 (2.60 log10) copies/mL is described. The assay evaluated in clinical samples from the Triple Study in comparison to the RT-PCR-based Amplicor assay and the bDNA-based Quantiplex assay. The three assays correlated fairly well with each other, with slightly higher Amplicor and lower Quantiplex results. Furthermore, three ultrasensitive protocols were evaluated in constructed panels of samples with known HIV-RNA concentrations. The lower cutoff level of the three protocols were 100 (2.00 log10), 40 (1.60 log10) and 10 (1.00 log10) copies/mL, respectively. The first and last protocols were also tested in samples of subjects participating in clinical trials.

Virus-specific and general antibody responses during potent antiretroviral therapy and relationships between B-cell and T-cell immunity are described in Chapter IX. Both anti-p24 and anti-gp120 antibody titers and total IgG concentrations responded to the decline in the amount of virus with therapy. In subjects who failed therapy, antibodies rebounded back to baseline, following the pattern of the HIV-RNA levels. Significant correlations were found between changes in virus specific antibodies and total- and memory
CD8$^+$ T-cell counts, probably both reflecting a decline in the amount of viral antigen.

In the Discussion and conclusion, the results of the Triple Study are put in perspective with other studies on potent antiretroviral therapy. The extent of the suppression of viral replication is discussed, as it is believed that full suppression is often not achieved. Whereas lymphoid tissue has been shown to be readily reached by the therapy, other reservoirs that may prevent viral eradication might persist. Immune reconstitution occurs to a considerable extent, with the restriction described in Chapter VII. However, HIV-specific T-cell immunity does not improve. New developments in antiretroviral therapy and treatment strategies are discussed. These include the search for regimens that are easier to adhere to and the possibilities to use the immune system to control the virus. Furthermore, long-term side-effects of the drugs are studied.