The 'Triple Study': viral dynamics and immune reconstitution in HIV-1 infection during potent antiretroviral therapy
Notermans, D.W.

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CHAPTER IX

BOTH ANTI-HIV-1 SPECIFIC AND TOTAL ANTIBODY LEVELS DECLINE IN RESPONSE TO SUPPRESSION OF VIREMIA WITH ANTIRETROVIRAL THERAPY AND ARE PARALLELED BY CHANGES IN CIRCULATING CD8+ MEMORY T CELLS


Submitted for publication
Next to a profound T-cell immunodeficiency, HIV-1 infection induces activation and dysfunction of B cells, resulting in hypergammaglobulinemia.

We studied the effect of potent antiretroviral therapy on antibody titers to the viral proteins gp120 and p24 and on total IgG concentrations, as well as on T-cell subsets. Three groups were studied: a successfully treated group, untreated controls and subjects with virological failure after several months of successful therapy.

All antibodies declined in the successfully treated group, while they remained relatively stable in the untreated group and rebounded with the HIV-RNA levels in the therapy failure group. Changes in virus-specific antibodies, but not in total IgG concentrations, correlated with changes in CD8+ T cells, CD8+ memory T cells, and CD8+ T-cell activation, but not with changes in HIV-RNA levels or CD4+ T-cell counts.

In conclusion, with potent antiretroviral therapy both anti-viral antibody titers and non-specific hypergammaglobulinemia decline as a result of diminished virus specific and general activation of the immune system. The relationship of the virus-specific antibodies with changes in CD8+ T-cell subsets suggests that both reflect the decline in viral antigen load with antiretroviral therapy.

INTRODUCTION

Next to a profound T-cell immunodeficiency, infection with the human immunodeficiency virus (HIV) causes activation and dysfunction of B cells. B-cell activation leads to hypergammaglobulinemia, which is partially due to the production of virus-specific antibodies and partially reflects a polyclonal activation. Despite the elevated immunoglobulin levels, B cells respond poorly to mitogens in vitro. In vivo, CD4+ T-helper cell dependent antibody production upon vaccination is limited in subjects with low CD4+ T-cell counts.

Among the virus-specific antibodies are antibodies against the core protein p24 and the envelope glycoproteins gp120 and gp41, which appear within a few weeks to months following infection with HIV. In a considerable number of individuals, p24 antibodies will disappear over the course of the disease and circulating p24-antigen becomes detectable, which is prognostic for the development of AIDS. Patients who rapidly progress to AIDS have lower or no detectable titers of p24 antibodies compared to patients who do not or only slowly progress. In contrast, antibodies to gp120 and gp41 remain relatively stable over the course of the infection and there is no distinction in gp120 antibody titers between fast progressors and long-term non-progressors. Immune-complexing of p24 antibodies by p24-antigen and a difference in CD4+ T-helper cell dependence for production of antibodies to the two different antigens, could both explain the difference in patterns between the core and envelope antibodies.
Chapter IX. Antibody titers during anti-HIV therapy

Current antiretroviral therapy including a protease inhibitor leads to a strong suppression of viral replication, reflected by a rapid and sustained decline of HIV-1 RNA levels in the peripheral blood and lymphoid tissue. In response, CD4+ T-cell counts increase, the antigen-specific and non-specific T-cell function improves and T-cell activation decreases. However, immune reconstitution is a slow process and is not complete in many subjects after two years of therapy.

The effects of potent antiretroviral therapy on B-cell activation and HIV-specific antibodies have not been very well documented. One study has reported a rapid decline in general and virus-specific immunoglobulin-producing cells and a slow decline in total IgG, anti-gp120 and anti-p24 antibodies with the use of antiretroviral therapy. Long-term data were only reported from a very limited number of subjects.

We studied the effects of potent antiretroviral therapy on hypergammaglobulinemia and anti-gp120 and anti-p24 titers by comparing successfully treated subjects with both untreated controls and subjects who failed on treatment. We have shown that all antibody levels decline after successful suppression of HIV-RNA levels with antiretroviral therapy and have related this to other parameters of restoration of the immune system in response to the changing viral burden.

METHODS AND MATERIALS

Subjects
The effects of antiretroviral therapy were studied in 13 previously untreated, HIV-1 infected participants in an open label triple combination study, who had a strong suppression of viral replication for at least one-and-a-half years. All subjects used the protease inhibitor (PI) ritonavir and two nucleoside analogue reverse transcriptase inhibitors (NRTI), zidovudine and lamivudine. Ten of the 13 successfully treated subjects were followed for 84 weeks, one for 60 weeks and two for 52 weeks.

Controls were 13 untreated HIV-1-infected subjects, who were retrospectively chosen by matching their baseline HIV-RNA levels and CD4 T-cell numbers with those of the treated subjects. The controls remained untreated during a period of at least 84 weeks.

The treatment failure group consisted of eight retrospectively selected subjects, who had been treated with various PI-containing combination regimens, resulting in a temporal suppression of HIV-RNA levels to below the assay cutoff level, with subsequent virological failure. The suppression needed to be sustained for at least four months after start of therapy and followed by a rapid increase in HIV-RNA levels to at least 0.5 log10 copies/mL from baseline. The subjects had a median duration of unquantifiable HIV-RNA of 36 weeks (range 16 to 52) and a median total follow-up of 72 weeks (range 48 to 84).
Anti-gp120 titers

Anti-gp120 titers were determined by antigen capture enzyme-linked immunosorbent assay (ELISA). In brief, gp120 from HIV-1 isolate 451 (ABL, Inc., Kensington, MD) was diluted in a sample diluent (SD; phosphate buffered saline (PBS) containing 20% normal goat serum and 1% Triton X-100) and captured onto a solid phase (Greiner plates; Frickenhausen, Germany) via adsorbed mouse antibody M90-2C6 (ABL, Inc., Kensington, MD) to a conformational epitope of gp120. In each assay, after washing away unbound gp120 with 0.05% Tween 20 in PBS (PBST), serum samples were titrated by eight serial threefold dilutions (initial dilution of 1:1000) in SD. Bound antibody was detected by peroxidase-labeled mouse anti-human IgG and tetramethylbenzidine peroxidase (TMB) as substrate. The optical densities (ODs) were measured at 450 nm on an ELISA reader (Reader 510, Organon Teknika, Boxtel, The Netherlands).

Anti-gp120 titers were defined as the log_{10} serum dilution giving an OD of 1.000. For this, the dilutions were plotted against the measured OD and the dilution giving an OD of 1.000 was calculated by regression estimation of the curve. Samples from one subject taken at different moments were always analyzed at the same time, (preferably) in the same microtiter plate.

Anti-p24 titers

Anti-p24 titers were determined by ELISA. In brief, recombinant p24 from HIV-1 isolate RF was coated to a solid phase (Greiner plates; Frickenhausen, Germany). Initially, each serum sample was tested at a single dilution of 1:100 in SD supplemented with 10% of normal human serum (NHS). Subsequently, clearly positive samples were titrated by eight serial threefold dilutions (initial dilution of 1:100). Bound antibody was detected by peroxidase-labeled recombinant p24 and tetramethylbenzidine peroxidase (TMB) as substrate. The optical densities (ODs) were measured and anti-gp24 titers defined as described for the anti-gp120 titers. Samples negative or borderline-positive in the 1:100 dilution were assigned titers of 1.5.

Total IgG levels

Total IgG levels were measured by nephelometry, according to the manufacturers protocol, using a BNA nephelometer, Behring, Breda, The Netherlands. In our hospital, the normal range is 7 to 16 g/L.

P24-antigen concentration

P24-antigen concentration was measured with a commercially available sandwich solid phase enzyme immunoassay, HIVAG-1 Monoclonal Kit (Abbott Laboratories, Abbott Park, IL), according to the manufacturers protocol.

HIV-RNA levels

In the successfully treated group, HIV-RNA levels were measured with the NucliSens assay (Organon Teknika BV, Boxtel, The Netherlands; lower
cutoff level (LCL) of 400 (2.60 log_{10} copies/mL), and if results were below
the lower cutoff, samples were remeasured with an ultra-sensitive protocol
adaptation, the Ultra-NucliSens, which has a LCL of 100 (2.00 log_{10})
copies/mL.\textsuperscript{[28]}

In the control- and treatment failure groups, HIV-RNA levels were
measured with the assay available for routine patient care at that time,
which was usually the NucliSens assay. For most samples drawn before
August 1996, the NASBA HIV-1 QT (Organon Teknika BV, Boxtel, The
Netherlands; LCL 1000 (3.0 log_{10} copies/mL) was used and occasionally
the Amplicor assay (Roche Molecular Systems, Branchburg, NJ, USA;
variable LCL around 200 (2.30 log_{10} copies/mL) was used. Measurements
were performed according to the respective manufacturers protocol.

Lymphocyte subsets

Lymphocyte immunophenotyping for peripheral CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells
and CD19\textsuperscript{+} B cells was done using two-color immunofluorescence flow
cytometry (FACS). In the successfully treated group, naive and memory T-
lymphocyte subsets were measured by using three-color flow cytometry for
the surface proteins CD45RA or CD45RO plus CD62L (L-selectin), as
previously described.\textsuperscript{[22]} T-cell subsets co-expressing CD45RA and CD62L
were regarded as truly naive T cells, whereas remaining T cells were regarded
as memory lymphocytes.\textsuperscript{[29]} Activation of CD8 cells was determined by
the percentage of CD8\textsuperscript{+}CD45RO\textsuperscript{+} cells positive for CD38.

T-cell function

The in vitro proliferative response to CD3 was determined in a lymphocyte
culture from 15 \mu L of whole blood, in the successfully treated and the
untreated groups, as previously described in detail.\textsuperscript{[30]} In brief, proliferative
responses were measured after four days of culture by means of incorporation
of \textsuperscript{3}H-thymidine, added 24 hours before harvest. Proliferative capacity was
calculated to counts per minute per 15 \mu L.

Statistical analysis

Time-dependent analyses were performed using a repeated measurement
model, Proc mixed, SAS 6.12 (SAS Systems Inc., Cary, NC), including the
respective baseline value and time since the start of treatment as covariates.
SPSS 9.0.1 (SPSS Inc., Chicago, IL) was used for the remaining statistical
analyses. Correlation coefficients were obtained by Spearman rank
correlation. Differences between groups at baseline were analyzed with
the Mann-Whitney U test for continuous variables and with the Fisher’s
exact test for categorical variables.

RESULTS

Thirteen successfully treated subjects and 13 untreated controls,
retrospectively matched with the first group for CD4\textsuperscript{+} T-cell counts and
Viral dynamics and immune reconstitution during potent antiretroviral therapy

Plasma HIV-RNA levels at entry, were included in the study. A third group consisted of eight subjects, who started with PI-containing combination therapy and experienced virological failure after several months of viral suppression. Antibody patterns in this group were expected to initially show the same pattern as in the successfully treated group, with possible differences following the return of HIV-RNA levels towards baseline. Baseline characteristics of the three groups are shown in Table 1. There were no statistically significant differences with regard to the various immune parameters between the successfully treated and the untreated groups. The treatment failure group had significantly lower CD4+ T-cell and CD19+ B-cell counts at baseline compared to the untreated controls and contained significantly more subjects with symptomatic HIV-disease (CDC class B or C).

<table>
<thead>
<tr>
<th>Group</th>
<th>Untreated Controls</th>
<th>Successfully Treated</th>
<th>Treatment Failures</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male / female)</td>
<td>13 / 0</td>
<td>12 / 1</td>
<td>7 / 1</td>
</tr>
<tr>
<td>CDC class (A / B / C)</td>
<td>11 / 2 / 0</td>
<td>7 / 3 / 3</td>
<td>2 / 1 / 5*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40 (35 - 46)</td>
<td>37 (34 - 41)</td>
<td>37 (32 - 48)</td>
</tr>
<tr>
<td>n pre-treated</td>
<td>0</td>
<td>0</td>
<td>6 (75%)*</td>
</tr>
<tr>
<td>HIV RNA (log_{10} copies/mL)</td>
<td>4.9 (4.6 - 5.5)</td>
<td>4.9 (4.7 - 5.0)</td>
<td>4.6 (4.5 - 5.3)</td>
</tr>
<tr>
<td>p24-antigen positive</td>
<td>5 (42%)</td>
<td>6 (46%)</td>
<td>5 (71%)</td>
</tr>
<tr>
<td>Anti-p24 titer (log_{10})</td>
<td>2.1 (1.4 - 2.8)</td>
<td>2.5 (1.5 - 2.8)</td>
<td>1.0 (1.0 - 2.5)</td>
</tr>
<tr>
<td>Anti-p24 below cutoff</td>
<td>5 (38%)</td>
<td>6 (46%)</td>
<td>5 (63%)</td>
</tr>
<tr>
<td>Anti-gp120 titer (log_{10})</td>
<td>4.5 (4.2 - 4.7)</td>
<td>4.4 (3.8 - 4.9)</td>
<td>4.7 (4.5 - 5.0)</td>
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<td>Total IgG (g/L)</td>
<td>16.5 (15.2 - 20.9)</td>
<td>17.6 (14.9 - 20.3)</td>
<td>20.7 (16.9 - 23.3)</td>
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<tr>
<td>CD4+ T cells (10^6 cells/L)</td>
<td>270</td>
<td>190</td>
<td>80</td>
</tr>
<tr>
<td>CD8+ T cells (10^6 cells/L)</td>
<td>(205 - 333)</td>
<td>(138 - 223)</td>
<td>(15 - 210)*</td>
</tr>
<tr>
<td>CD8+ memory T cells (10^6 cells/L)</td>
<td>740</td>
<td>940</td>
<td>640</td>
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<tr>
<td>(650 - 1230)</td>
<td>(698 - 1145)</td>
<td>(258 - 1393)</td>
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<tr>
<td>Activated (% CD38+)</td>
<td>ND</td>
<td>799</td>
<td>ND</td>
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<tr>
<td>CD8+CD44RO+ T cells</td>
<td>ND</td>
<td>25 (18 - 41)</td>
<td>ND</td>
</tr>
<tr>
<td>CD19+ B cells (10^6 cells/L)</td>
<td>215</td>
<td>150</td>
<td>55</td>
</tr>
<tr>
<td>(130 - 250)</td>
<td>(115 - 190)</td>
<td>(23 - 140)*</td>
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<tr>
<td>T-cell function (counts/min.)</td>
<td>1725</td>
<td>660</td>
<td>ND</td>
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<tr>
<td>(1083 - 2942)</td>
<td>(199 - 2020)</td>
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Table 1. Baseline characteristics of the three groups. Shown are medians and interquartile ranges (IQR). *significantly different (p < 0.05) from untreated controls with Mann-Whitney test, or Fisher’s Exact Test. ND = not done.
Baseline anti-gp120 titers, anti-p24 titers and total IgG concentrations of all three groups combined did not correlate with HIV-RNA levels, CD4⁺- or CD8⁺ T-cell- or CD19⁺ B-cell counts or T-cell function. Anti-gp120 and anti-p24 titers at baseline correlated with borderline statistical significance with the total IgG concentrations (R = 0.33, p = 0.06 and R = 0.34, p = 0.05, respectively), but not with each other. Subjects with anti-p24 titers below 1.5 at baseline were significantly more often p24-antigen-positive than subjects with anti-p24 titers that could be quantified (12/16 versus 4/16, p = 0.01, Fisher’s exact test).

**HIV-RNA and lymphocyte responses**

All subjects in the successfully treated group had a strong and persistent suppression of HIV-RNA levels to below 100 copies/mL and a substantial rise in CD4⁺ T-cell counts (Figs 1A and B). CD8⁺ T-cell counts increased during the first few weeks, but subsequently returned to baseline values (Fig. 1C). In addition, the number of circulating CD19⁺ B cells showed a rapid increase upon start of treatment (Fig. 1D). CD8⁺ memory T-cell counts increased initially, but decreased subsequently to below baseline values, while CD8⁺ naive T-cell counts slowly increased. The percentage of activated CD8⁺ memory T cells rapidly declined and the anti-CD3 T-cell function improved.125

In contrast, in the control group HIV-RNA levels remained stable at a high level while CD4⁺ and CD8⁺ T-cell and CD19⁺ B-cell counts also remained relatively stable, with a tendency for the CD4⁺ T-cell and CD19⁺ B-cell counts to decline towards the end of the study period (84 weeks). This is indicative of a normal HIV-1-induced deterioration of the immune system. Subjects in the treatment failure group, which had specifically been selected for their HIV-RNA pattern of decline and subsequent rebound, continued to have slow, persistent increases in CD4⁺ and CD8⁺ T-cell and CD19⁺ B-cell counts, despite the return of the HIV-RNA levels to baseline values (Figs 1A-D). CD8⁺ T-cell subsets were not determined in the control and treatment failure groups.

**Antibody titer changes**

In order to obtain data on the effects of antiretroviral therapy on the humoral immune system, anti-gp120 titers, anti-p24 titers and total IgG concentrations were determined on longitudinally sampled sera in the three groups. In the untreated controls, anti-gp120 titers remained stable, while anti-p24 titers tended to decline in subjects with quantifiable anti-p24 titers at baseline, and IgG concentrations tended to increase towards the end of the follow-up period (Fig. 2A-C). This pattern of stable anti-gp120 titers, slowly declining anti-p24 titers and slowly increasing IgG concentrations is as expected for individuals with reduced (median 270 cells/µL) and slowly declining CD4⁺ T-cell counts and high HIV-RNA levels (median nearly 10⁵ copies/mL).11,13,11-16

Titers and total IgG concentrations were statistically significantly different
**Viral dynamics and immune reconstitution during potent antiretroviral therapy**

Fig. 1 (A,B). HIV-RNA and lymphocyte patterns in the untreated controls, successfully treated and treatment failures during 72 to 84 weeks of follow-up. Median values and interquartile ranges are shown. A. HIV-RNA levels, with number of subjects tested and percentage with HIV-RNA levels below the lower cutoff level (LCL) of the assay used. In the successfully treated the Ultra-NucliSens assay with an LCL of 100 (2.0 log₁₀copies/mL) was used. In the controls and treatment failures HIV-RNA levels were measured with the assay available for routine patient care at that time; usually NucliSens (LCL 400 (2.6 log₁₀ copies/mL), in older samples NASBA HIV-1 QT (LCL 1000 (3.0 log₁₀ copies/mL) and occasionally Amplicor (variable LCL around 200 (2.3 log₁₀ copies/mL). B. CD⁴⁺ T-cell counts.
Fig. 1 (C,D). HIV-RNA and lymphocyte patterns in the untreated controls, successfully treated and treatment failures during 72 to 84 weeks of follow-up. C. CD8⁺ T-cell counts D. CD19⁺ B-cell counts.

In the successfully treated group, anti-gp120 titers, anti-p24 titers and total IgG concentrations all declined (Fig. 2A-F). The patterns of anti-gp120 compared to the untreated controls (p < 0.001 for each parameter), in whom anti-gp120 titers remained stable and IgG concentrations tended to increase. For anti-p24 titers, which declined in the controls as well, the difference was not statistically significant (p = 0.06). After 84 weeks of follow-up, the median IgG concentrations in the successfully treated group had declined to just below the upper limit of normal.
Viral dynamics and immune reconstitution during potent antiretroviral therapy

**A**

Number of subjects

<table>
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Week

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<th>24</th>
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<th>48</th>
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<th>72</th>
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<td>-0.4</td>
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Control --- Success --- Failures

**B**

Number of subjects

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Week

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<th>60</th>
<th>72</th>
<th>84</th>
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<td>0.4</td>
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<td>-0.4</td>
<td>-0.6</td>
<td>-0.8</td>
<td>-1.0</td>
<td>-1.3</td>
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**C**

Number of subjects

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</table>

Week

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<th>48</th>
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<th>72</th>
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<td>-0.6</td>
<td>-0.8</td>
<td>-1.0</td>
<td>-1.2</td>
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</table>

Total lgG concentration change (g/L)
Fig. 2. HIV-specific antibody titers and total IgG concentrations in the untreated controls, successfully treated and treatment failures during 72 to 84 weeks of follow-up. Median values and interquartile ranges are shown. A to C. Changes from baseline of anti-gp120 titers, anti-p24 titers in the subjects with titers above the lower cutoff level at baseline, and total IgG concentrations, respectively. D to F. Individual absolute values in the successfully treated group, with the median for the group. Anti-gp120 titers, anti-p24 titers and total IgG concentrations, respectively.
Six subjects in the successfully treated group had anti-p24 titers below the cutoff level at baseline, five of whom remained below the cutoff. The one subject with increasing titers during treatment also had the strongest CD4⁺ T-cell count increase (from 140 to 1250 cells/µL) and CD19⁺ B-cell count increase (from 100 to 530 cells/µL), one of the strongest CD8⁺ T-cell count increase (from 1010 to 1600 cells/µL), and the highest baseline HIV-RNA levels (5.56 log₁₀ copies/mL) in this group. No subject with anti-p24 titers above the cutoff level at baseline had titers declining to below the cutoff while on treatment.

Concurrent with the strong decline in HIV-RNA levels, the six subjects in the successfully treated group who were positive for p24-antigen at baseline and in whom five had anti-p24 titers below the cutoff level, became p24-antigen-negative.

In the treatment failure group, antibody titers declined at first, comparable to the pattern of the successfully treated group. With the rebound in HIV-RNA levels, anti-gp120 and anti-p24 titers and IgG concentrations approximately returned to baseline levels (Fig. 2A-C).

Of the five subjects from this group with anti-p24 titers below the cutoff level at baseline, one subject had a strong increase in anti-p24 titers. Like the subject from the success-group, this subject had the strongest CD4⁺ T-cell (from 10 to 450 cells/µL) and CD19⁺ B-cell count increase (from 80 to 260 cells/µL), one of the strongest CD8⁺ T-cell count increase (from 160 to 920 cells/µL), and one of the highest baseline HIV-RNA levels (5.42 log₁₀ copies/mL) in the treatment failure group.

**Correlations between changes in antibodies and T-cell immunity**

The relationships between a number of immunological and virological parameters were investigated by correlating the magnitude of changes of the respective parameters at the last visit of each subject. In the successfully treated group, changes in anti-gp120 titers, anti-p24 titers and total IgG concentrations did not correlate with each other, nor did they correlate with the changes in plasma HIV-RNA levels, CD4⁺ T-cell or CD19⁺ B-cell counts or anti-CD3 T-cell function.

In the subjects with anti-p24 titers above the cutoff level at baseline, changes in anti-p24 titers correlated with changes in total CD8⁺ T-cell counts (R = 0.86, p = 0.01), changes in CD8⁺ memory T-cell counts (R = 0.83, p = 0.04) and changes in percentage of activated CD8⁺ memory T-cells (R = 0.78, p = 0.04). Anti-gp120 titer changes did also correlate with changes in CD8⁺ memory T-cell counts (R = 0.73, p = 0.01), but not with changes in total CD8⁺ T-cell counts or percentage of activated CD8⁺ memory T cells. Changes in total IgG concentration did not correlate with changes in total CD8⁺ T-cell counts or CD8⁺ T-cell subsets (Table 2).

In the treatment failure group, changes in anti-gp120 titers correlated close to statistical significance with changes in total CD8⁺ T-cell counts, but changes in IgG concentrations did not (R = 0.66, p = 0.08 and R = 0.36, p = 0.38, respectively). For changes in anti-p24 titers data were
available from three subjects with anti-p24 titers above baseline only (Table 2).

<table>
<thead>
<tr>
<th>Successfully treated</th>
<th>Treatment failures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total CD8 change</strong></td>
<td><strong>Total CD8 change</strong></td>
</tr>
<tr>
<td><strong>agp120 change</strong></td>
<td><strong>ap24 change</strong></td>
</tr>
<tr>
<td>R = 0.26, p = 0.38, n = 13</td>
<td>R = 0.86, p = 0.01, n = 11</td>
</tr>
<tr>
<td>R = 0.73</td>
<td>R = 0.83</td>
</tr>
<tr>
<td>R = -0.03</td>
<td>R = 0.78</td>
</tr>
<tr>
<td>p = 0.92</td>
<td>p = 0.04</td>
</tr>
<tr>
<td>n = 13</td>
<td>n = 7</td>
</tr>
<tr>
<td><strong>IgG change</strong></td>
<td><strong>IgG change</strong></td>
</tr>
<tr>
<td>R = 0.29, p = 0.33, n = 13</td>
<td>R = 0.02, p = 0.96, n = 11</td>
</tr>
<tr>
<td>R = 0.02</td>
<td>R = 0.17</td>
</tr>
<tr>
<td>R = 0.17</td>
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</tbody>
</table>

**Table 2.** Spearman rank correlations of changes in antibodies with changes in total CD8+ T-cell counts and CD8+ T-cell subsets, in the successfully treated group and with changes in total CD8+ T-cell counts in the treatment failure group. ND = not done.

**DISCUSSION**

This study shows that in subjects treated with potent antiretroviral therapy, who had a rapid and prolonged suppression of HIV-RNA levels, both HIV-specific antibodies and total IgG slowly but steadily declined. In contrast, antibodies remained relatively stable in untreated subjects with comparable high HIV-RNA levels and decreased CD4+ T-cell counts, with a tendency of anti-p24 antibodies to decline and IgG concentration to increase during infection, both of which indicated disease progression. In subjects with a temporal suppression of HIV-RNA levels and subsequent treatment failure, antibodies followed the RNA pattern, bouncing back to baseline values.

Antibody production during HIV-infection is influenced by several factors and the suppression of viral replication has a number of effects that may influence the titers of the different antibodies, possibly in opposite directions. These are: 1) a rapid and strong decline in viral antigens following the suppression of viral replication, which would be expected to result in a decline in the stimulation of virus-specific B and T-cell immunity. 2) A strong suppression of viral replication, which would be expected to result in a decline in the general immune activation of both B and T cells, as has been shown for T cell activation. 3) An increase in T-cell immunity, both in numbers and in function, which could
stimulate T-helper cell-dependent anti-viral immunity. However, in contrast to T-helper cell-dependent antibody production to other pathogens, recovery of HIV-specific T-helper cell function is very limited. The restoration of the follicular dendritic cell (FDC) network in lymphoid tissue and an increase in B-cell- and other antigen-presenting cell (APC) function, which could potentially result in an increased virus-specific antibody production. 4) A decrease in viral antigens, which could reduce complexing of antibodies, thus possibly increasing the measured titers of circulating anti-viral antibodies. Overall, anti-p24 and anti-gp120 antibody titers and total IgG concentrations showed a pattern which was specific for each patient group. In the successfully treated subjects, all antibodies declined slowly but continuously, while in the treatment failure group a subsequent return to baseline occurred following the HIV-RNA rebound. Although all antibodies declined with successful therapy, the underlying mechanisms may not be completely identical. A decline in antigenic stimulation of the virus-specific immune system and a decline in the general immune activation are both expected to result in declining antibody titers. The first would be expected to more strongly influence the virus-specific antibodies, the second to more strongly influence the hypergammaglobulinemia. The dynamics of the decline in antibody levels were delayed compared to the decline in HIV-RNA levels. However, viral proteins, like p24, have been shown to persist longer than viral RNA on the surface of FDCs in lymphoid tissue under antiretroviral therapy. The decline in viral antigens and, in response, in antibodies might therefore not occur at a rate identical to the rapid decline in viral RNA. Anti-gp120 and anti-p24 responses might not be regulated by exactly the same factors, as is shown in the untreated group, in whom anti-gp120 titers remained stable while anti-p24 titers declined. Anti-p24 antibody production has been suggested to be T-helper cell-dependent, whereas anti-gp120 production is T-helper cell-independent and more directly responsive to the amount of viral antigen. The recovery of virus-specific T-helper cell function with the use of potent antiretroviral therapy is limited. This might explain the fact that the anti-p24 titers remained low in most subjects in the successfully treated group who had anti-p24 titers below the cutoff level at baseline. However, the two subjects with increasing anti-p24 titers in the successfully treated- and treatment failure groups showed concurrent strong rises in CD4+ T-cell and CD8+ T-cell counts, indicating that recovery of CD4+ T-helper cell function may have been involved. Complex formation of anti-p24 antibodies with p24-antigen is another factor which differentiates anti-gp120 and anti-p24 responses. The measured levels of anti-p24 antibodies are therefore thought to be lower than the actual ones. However, in the subjects who were positive for p24 antigen and who had anti-p24 levels below the cutoff level, no rebound of anti-p24 titers was observed following the clearance of viral
RNA in serum. This indicates that in these cases antibody-antigen complex formation does not play a significant role.

In an effort to relate the observations on antibody levels to other parameters of immune function and viral replication, we searched for significant correlations between these factors. As the numbers of subjects in the different groups are small, correlations between the different parameters should be treated with some caution. The groups were selected retrospectively and did not participate in a randomized trial together. Furthermore, the number of available subjects with the required HIV-RNA profile for the treatment failure group was limited. Therefore, this group was not matched for baseline CD4\(^+\) T-cell counts and HIV-RNA levels.

In the successfully treated group, significant correlations were found between the changes in anti-p24 and anti-gp120 titers and changes in CD8\(^+\) memory T-cell counts. Such a correlation was not found for total IgG concentrations. Anti-p24 titer changes also correlated with changes in total CD8\(^+\) T-cell counts and the percentage of activated CD8\(^+\) T cells in this group. In agreement with these observations, a nearly statistically significant correlation was found between changes in anti-gp120 titers, but not in changes of total IgG concentrations or changes in total CD8\(^+\) T-cell counts in the treatment failure group. These associations probably do not reflect a causal relationship, but more likely are the result of a response of both antibody production and CD8\(^+\) T cells to the decline in viral burden. This explanation is most likely if a substantial part of the declining CD8\(^+\) memory T cells were HIV-specific CTLs. HIV-specific CTLs usually are CD45RO\(^++\) and the number of circulating HIV-specific CTLs declines with the use of potent antiretroviral therapy. \[31,38,39\] Analysis of T-cell receptor (TCR) distribution has shown that the expansion of CD8\(^+\) memory T cells is oligoclonal, suggesting a virus-specific rather than a general expansion. The TCR distribution pattern slowly normalizes with antiretroviral therapy. \[40\] Furthermore, an increase of anti-p24 and anti-gp120 titers in parallel with an increase in antiviral CTLs, in subjects with increasing plasma HIV-RNA levels during treatment interruptions, has been described before.\[41\] If, on the other hand, the decline in CD8\(^+\) T-cell subsets would mostly reflect a change in general immune activation, one would expect the hypergammaglobulinemia to correlate, rather than the virus specific antibodies. In addition, like anti-p24 antibody production, CTL responses to p24 have been shown to correlate with T-helper cell function.\[42\] This may explain the stronger association of changes in antibody titers to p24 (T-cell dependent antigen) than to gp120 (T-cell independent antigen) with changes in total CD8\(^+\) T-cell counts and the percentage of activated CD8\(^+\) T cells.

In conclusion, potent antiretroviral therapy leads to a slow, continuing decline in both virus-specific antibodies and total IgG. Although different combinations of the factors mentioned above could have been affecting on the different kinds of antibodies we studied, the similarities in responses
suggest that most of the influence comes from a decline in antigenic stimulation of the virus-specific immune system and a decline in general immune activation. The first mechanism would mostly influence the virus-specific antibodies and the latter the hypergammaglobulinemia, but both are part of the normalization of the immune system with anti-retroviral therapy. The effects of anti-p24 antibody-complexing and changes in T-helper cell function on anti-p24 titer changes appear to be limited, although an improved p24-specific T-helper cell function might explain the stronger association of anti-p24 than anti-gp120 titer changes with the CD8\(^+\) T-cell subsets. Our interpretation of the relationships between changes in CD8\(^+\) memory T cells and changes in anti-p24 and anti-gp120 titers is that this reflects responses of these parameters to the decline in viral antigen load with the use of antiretroviral therapy.

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