Anatomical and cellular reservoirs for HIV-1 during potent antiretroviral therapy
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CHAPTER 6

Immuno-activation with anti-CD3 and recombinant human IL-2 in HIV-1-infected patients on potent antiretroviral therapy

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Abstract

Background A stable reservoir of latently infected, resting CD4 T cells has been demonstrated in HIV-1-infected patients despite prolonged antiretroviral treatment. This is a major barrier for the eradication of HIV by antiretroviral agents alone. Activation of these cells in the presence of antiretroviral therapy might be a strategy to increase the turnover rate of this reservoir.

Methods Three HIV-1-positive patients on potent antiretroviral therapy, in whom plasma viremia had been suppressed to below 5 copies/mL for at least 26 weeks, were treated with a combination of OKT3 (days 1–5) and recombinant human IL-2 (days 2–6).

Results The side effects were fever, headache, nausea, diarrhoea, and in one of the patients transient renal failure and seizures. The regimen resulted in profound T cell activation. In one patient plasma HIV-1 RNA transiently increased with a peak at 1500 copies/mL. In the other two patients plasma HIV-1 RNA levels remained below the detection limit, but HIV-1 RNA levels in the lymph nodes increased two- to threefold. All patients developed antibodies against OKT3.

Conclusion OKT3/IL-2 resulted in T cell activation and proliferation, and could stimulate HIV replication in patients having achieved prolonged suppression of plasma viremia. OKT3/IL-2 therapy was toxic and rapidly induced antibodies against OKT3.
**Introduction**

Recently, a small reservoir of latently infected, resting memory CD4 T cells harbouring replication competent, integrated provirus was demonstrated in patients despite prolonged antiretroviral combination therapy with suppression of plasma HIV-1 RNA levels below 50–200 copies/mL.\(^1\)\(^-\)\(^2\) The number of these cells decreased only very slowly with increasing time on therapy.\(^3\) This reservoir might be a major barrier for the eradication of HIV by antiretroviral agents alone. Treating patients for many years with antiretroviral combination therapy is undesirable given the side effects of such therapy.\(^4\)\(^-\)\(^5\) Therefore, it is necessary to develop strategies to increase the turnover rate of the reservoir of latently infected T cells. One strategy might be the activation of the resting T cells, in the continued presence of antiretroviral therapy.\(^6\)\(^-\)\(^7\) Activation of these cells may result in apoptosis of these cells,\(^8\) or in virus replication within these cells, resulting in their lysis, with antiretroviral drugs preventing new infections.\(^6\) *In vitro*, resting T cells of HIV-1 patients could be activated by CD3 monoclonal antibody or by the combination of the cytokines TNF-\(\alpha\), IL-2 and IL-6, resulting in HIV-1 replication.\(^6\)

In the present study, we investigated the concept of in-vivo stimulation with OKT3 and IL-2. OKT3 is an IgG2a murine monoclonal antibody directed against the CD3 molecule.\(^9\)\(^10\) Because IL-2 plays a critical role in OKT3-driven cell proliferation,\(^11\) the effects exerted by OKT3 treatment might be enhanced by the administration of recombinant IL-2.\(^9\)\(^-\)\(^11\)

**Materials and methods**

*Patients and treatment*

Three patients (008, 010 and 002) were studied. Antiretroviral therapy had been started 9–15 months earlier with zidovudine, lamivudine, abacavir, nevirapine, indinavir and ritonavir.\(^12\) Patient 010 stopped abacavir and nevirapine within 10 days because of hypersensitivity reactions and instead received hydroxyurea until 10 weeks before the OKT3/IL-2 treatment. When the plasma HIV-1 RNA load had been below 5 copies/mL for at least 26 weeks (Figure 1), the patients were treated with OKT3 and recombinant human IL-2. On five consecutive days 5 mg of OKT3 (Janssen-Cilag BV, Tilburg, the Netherlands) were given as a 2 h continuous infusion,\(^13\) but on the first day 2.5 mg was given for safety reasons. From days 2–6, rhIL-2 (Chiron, Amsterdam, the Netherlands) was added at a rate of 4.5 MIU twice a day subcutaneously. Antiretroviral therapy was continued. Because of the side effects experienced by the first patient (008), the IL-2 dosage was decreased to 2 MIU twice a day in patients 010 and 002. The OKT3/IL-2 cycle was to be

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repeated after 2 weeks. The study was approved by the Medical Ethics Committee of our hospital and informed consent was obtained from all three patients.

Follow-up
Lymph node biopsies were obtained by surgical excision 4–6 weeks before the OKT3 treatment and on the fourth day of the first (patient 002) or the second (patient 010) OKT3/IL-2 cycle, fixed in ParaFix™, and embedded in paraffin.

OKT3 concentrations and OKT3 antibody levels in serum were determined using enzyme-linked immunosorbent assay. To determine whether antiidiotype or anti-isotype antibodies were produced, an irrelevant mouse IgG2a antibody was used.

The quantification of HIV-1 RNA in plasma was performed using the NucliSens HIV-1 QT assay (Organon Teknika, Boxtel, the Netherlands). When RNA levels decreased to below 50 copies/mL, an initial input volume in the assay of 2 mL plasma was used combined with the ultrasensitive protocol adaptation, resulting in a lower quantification limit of 5 copies/mL.

HIV-1 RNA in tissue sections was quantitated by first performing in-situ hybridisation with 35S radiolabelled antisense HIV-1 RNA probes, with sense probes used as a control. This was followed by measurements in the phosphor storage imager. One pCi/mm² represents 555 viral genome equivalents. HIV-1 expressing mononuclear cells in the tissue sections were counted in a darkfield microscope. Positive cells were considered to have more than 20 silver grains per 200 μm².

HIV-1 DNA from at least 1x10⁶ mononuclear cells was isolated using the TRizol reagent (Gibco BRL, Life Technologies Inc., Grand Island, MD, USA). The cellular DNA was recovered, aliquoted and a mutant plasmid was added (400, 80, 16, and 3 copies). A competitive nested polymerase chain reaction was performed on the HIV-1 pol region. The DNA copy number was expressed as copy number per 10⁶ CD4 T cells present in peripheral blood mononuclear cells (PBMC).

Culture of resting CD4 T cells
HLA-DR-CD4 T cells were isolated from 50 mL freshly obtained blood. Virus was isolated from limiting-diluted cells.
Results

Clinical side-effects
The side effects consisted of spiking fever, headache, nausea, vomiting, diarrhoea and anaemia. These side effects started within several hours after administration and persisted undiminished during the entire OKT3/IL-2 cycle. Because of the side effects patients 008 and 002 declined a second cycle. Although no prolonged periods of hypotension were recorded, patient 008 developed acute renal failure caused by acute tubular necrosis after 6 days. Temporary hemodialysis was required, after which his renal function recovered completely. In the following patients the dosage of IL-2 was decreased, intravenous fluid administration was increased and dopamine 3 µg/kg/min was added as a continuous infusion. Furthermore, patient 008 developed seizures on the 19th day after the start of treatment. A magnetic resonance imaging scan showed white-matter abnormalities. Within a few weeks all neurological signs and symptoms disappeared and anti-epileptic medication was withdrawn. Finally, patient 010 developed a short period of sub-clinical hypothyroidism.

A profound lymphocytopenia persisted during the entire treatment with OKT3. Likewise, CD4 T cell counts dropped from 320–690 x 10⁶/L to below 10 x 10⁶/L as early as one hour after the start of treatment. Lymphocyte and T cell counts started to recover after day 8.

Serum levels of OKT3 and OKT3 antibodies
Serum concentrations of OKT3 at the end of the infusions were 132–225 ng/mL during the first day and 566–1148 ng/mL during days 2–5, and trough concentrations ranged from 11 to 44 ng/mL after the first dose and from 53.7 to 133 ng/mL after subsequent dosages.
None of our patients developed precipitating antibodies against OKT3. However, all patients developed both anti-idiotype and anti-isotype antibodies against OKT3, as early as 11 days after the start of treatment. On day 15, patient 010 started a second OKT3/IL-2 cycle. This time he developed only minor clinical signs and symptoms, the degree of lymphocytopenia in the peripheral blood (23%) was much less than during the first cycle (1%), and the concentration of OKT3 measured at the end of the first infusion was only 5.3 ng/mL, as opposed to 132 ng/mL during the first cycle. We concluded that the anti-OKT3 antibodies significantly reduced the effects of OKT3. Therefore, OKT3 infusions were stopped after 3 days, and the IL-2 dosage increased to 4.5 MIU twice a day.
Figure 1 Time course for changes of HIV-1 RNA in plasma (copies/mL), and changes in HIV-1 DNA copy number per 10⁶ peripheral blood CD4 T cells, after the initiation of antiretroviral therapy, which was started on day 0.

* When re-tested less than 5 copies/mL.
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Plasma HIV-1 RNA
After the start of antiretroviral therapy, plasma HIV-1 RNA levels declined within 1–9 weeks to levels below 5 copies/mL (Figure 1). Patient 008, who had a plasma HIV-1 RNA load below 5 copies/mL for 37 weeks before the start of the OKT3/IL-2 treatment, had a plasma HIV-RNA level of 110 copies/mL immediately before the first dose of OKT3. His HIV-1 RNA plasma levels peaked at 1500 copies/mL at day 5 of the OKT3/IL-2 protocol, and dropped again to below 5 copies at day 16 (Figure 1). Plasma HIV-1 RNA levels of the other two patients remained below 5 copies/mL during the entire treatment and follow-up period, up to 6 weeks after the first day of OKT3.

HIV-1 RNA in lymph nodes
During OKT3/IL-2 the number of viral RNA genome equivalents in the lymph nodes increased from 555 to 1532/mm² in patient 010 and from 683 to 1332/mm² in patient 002 (Figure 2), but no increase in the number of productively infected cells (< 1/10⁶ paracortical cells; approximately 10⁶ paracortical cells counted) was seen.

HIV-1 DNA in peripheral blood mononuclear cells
During OKT3/IL-2, HIV-1 DNA in the PBMC dropped to undetectable levels in patient 010 and 002 in parallel to the disappearance of CD4⁺ T cells from the peripheral blood. In patient 008, CD4⁺ T cells did not completely disappear from the circulation and HIV-1 DNA could be measured in all samples obtained during the OKT3/IL-2 treatment. Upon the reappearance of the CD4⁺ T cells, HIV-1 DNA returned to pre-OKT3/IL-2 levels.

Culture of resting CD4⁺ T cells
In patient 008, the number of resting CD4⁺ T lymphocytes in peripheral blood harbouring replication-competent HIV before OKT3/IL-2 treatment (10⁻¹⁹/per 10⁶ resting CD4⁺ T cells) was in the same order of magnitude as the number thereafter (13–19/10⁶). In the other two patients the frequency of these cells was one or less per 10⁶ resting CD4⁺ T cells before and after OKT3/IL-2 treatment, and it is therefore not possible to assess whether the treatment influenced the size of this HIV reservoir.
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The early development of anti-OKT3 antibodies causes difficulties in treating patients with consecutive courses. More than one T cell activating cycle is probably required to ‘flush out’ the entire latent reservoir. Therefore, additional strategies to activate these latently infected cells in vivo must be considered.

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