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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Chapter 6

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.
Immuno-activation with anti-CD3 and IL-2

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. The number of these cells decreased only very slowly with increasing time on therapy. 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. 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. Activation of these cells may result in apoptosis of these cells, or in virus replication within these cells, resulting in their lysis, with antiretroviral drugs preventing new infections. In vitro, resting T cells of HIV-1 patients could be activated by CD3 monoclonal antibody or by the combination of the cytokines TNF-α, IL-2 and IL-6, resulting in HIV-1 replication.

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. Because IL-2 plays a critical role in OKT3-driven cell proliferation, the effects exerted by OKT3 treatment might be enhanced by the administration of recombinant IL-2.

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. 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, 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
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 anti-idiotypic 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 $3 \mu g/kg/min$ 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 $320-690 \times 10^6/L$ to below $10 \times 10^6/L$ 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.

v When re-tested less than 5 copies/mL.
Immuno-activation with anti-CD3 and IL-2

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.
Chapter 6

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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