Lymphocyte subsets and viral load in patients with HIV-associated non-Hodgkin’s lymphoma treated with anti-CD20 monoclonal antibody and chemotherapy

Abstract The anti-CD20 monoclonal antibody Rituximab is a novel antitumor agent used in association with chemotherapy (CT) for the treatment of high-grade/intermediate non-Hodgkin’s lymphomas (NHL) in HIV-negative populations. This therapeutic combination is currently also being explored in HIV-positive patients with NHL (HIV-NHL). The objective of our study was to determine CD4 and CD8 T cell counts, HIV plasma viremia and proviral load in patients with CD20-positive HIV-NHL treated with Rituximab plus CT and highly active antiretroviral therapy (HAART). We studied eight patients with HIV-NHL treated by anti-CD20 and CT before, after three, and after six cycles of therapy; CD4, CD8 and CD19 lymphocyte subsets were measured by monoclonal antibodies and flow cytometry. HIV plasma viremia was determined by the b-DNA assay, and proviral load by a quantitative competitive PCR. CD4T cell counts remained stable after three cycles of therapy, while a significant reduction of this subset was present at the end of therapy. HIV plasma viremia was significantly reduced after the third cycle, but returned to pretreatment levels at the end of therapy; we also observed individual fluctuations of proviral load during therapy, this marker being increased in two out of three patients at the end of therapy. These observations suggest that Rituximab plus CT accelerated the rate of CD4 depletion and of HIV replication in the peripheral blood of HIV-NHL patients and that HAART may be able to delay these effects.

Key words HIV-NHL · Rituximab · Chemotherapy · CD4+ T Cells · HIV-Viremia

Introduction
Non-Hodgkin’s lymphomas (NHL) occurring in HIV-infected patients (HIV-NHL) are increasing in incidence in the era of highly active antiretroviral therapy (HAART) [8, 17]. The use of HAART has significantly changed the natural history of HIV infection, with increasing CD4 cell counts, decreased incidence of opportunistic infections (OI) and prolonged survival [18]. Nevertheless, the outcome of HIV-NHL remains poor [9, 23]. Although chemotherapy (CT) is associated with a sustained impairment of immunological function, it remains the preferred treatment for HIV-NHL [9, 23, 24]. The combination of CT plus HAART may be feasible, but the impact on survival needs to be evaluated [25].

Recently, significant advances have been made in the use of monoclonal antibody (mAb)-based therapies to treat HIV-negative NHL. Rituximab (IDEC-C2B8), a chimeric mAb directed against the B lymphocyte-specific antigen CD20, is a novel and promising antitumor agent. The anti-CD20 mAb alone or in combination with CT has shown good efficacy and minimal toxicity in low- as well as high-grade NHL in the general population [6, 14, 15, 16]. The combination of Rituximab plus CT is currently being explored in phase II trials within European and USA study groups in HIV-NHL patients [3, 22].

In immunocompetent patients, Rituximab exerts its functions by inducing apoptosis, by activating complement and antibody-dependent cytotoxicity, and by acting as an antiproliferative agent [13, 19]. The impact of the combined treatment with anti-CD20 mAb plus CT and HAART on HIV infection is still unknown.

The primary objective of our trial was to determine the effects of the combined therapy on T and B lymphocyte subsets in the peripheral blood, and to determine HIV burden during treatment.
Table 1 Characteristics of the patients included in the study. M male, prior ART prior anti-retroviral treatment, CDC classification according to 1993 CDC, grade according to the working formulation, Stage According to Ann Arbor classification, Rit-CT cycles number of Rituximab-chemotherapy cycles. Response: PRO progression, CR complete remission. Outcome: D dead, A alive

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Materials and methods

Patients

Eight patients with HIV-NHL were enrolled in a prospective study performed at the Aviano Cancer Center, Italy, from December 1998 to June 1999, as reported [22]. Patients' requirements for enrolment were: to have biopsy-proven anti-CD20 positive intermediate or high-grade systemic NHL, stage I-IV according to Ann Arbor classification, and to be HIV-positive. Exclusion criteria were CNS involvement, prior diagnosis of AIDS, active OI, prior CT. For the AIDS diagnosis according to WHO [4] only CDC clinical criteria were adopted [1].

Informed consent was obtained from all patients. Patients received a total of six intravenous infusions of 375 mg/m² of Rituximab and six cycles of cyclophosphamide, doxorubicin and etoposide (CDE), given every 28 days. Each CDE cycle consisted of cyclophosphamide (200 mg/m²), doxorubicin (12.5 mg/m²) and etoposide (60 mg/m²) by continuous intravenous infusion for 4 days. Rituximab was administered over 3 to 5 h, 1 day before the CDE cycle. Oral premedication with acetaminophen and diphenhydramine hydrochloride was administered 30 to 60 min before each Rituximab infusion. Therapy included central nervous system prophylaxis with methotrexate given intrathecally (12 mg) on day 1 of each cycle and HAART. Combination anti-retroviral therapy included one (five cases) or more (one case) protease inhibitors (PI) and two reverse transcriptase inhibitors (RTI).

Immunological and virological data were available at time 0 (t = 0), after the third cycle of therapy (= t middle) and at the end of therapy (cycle 5 or 6, depending on the patient; = t end).

Five patients with HIV-NHL treated by CDE alone were included for comparison of the immunological and virological data. The histopathological characteristics of these patients were similar to those of the patients treated by CDE and Rituximab. However, in these subjects, CDE was used as a second-line antineoplastic therapy without concomitant HAART in a more advanced disease stage, making the two groups slightly different from each other. Due to their clinical situation, these patients received a maximum of three CT cycles.

Lymphocyte subsets

Peripheral blood samples were obtained in EDTA and evaluated by a whole-blood lysing technique, as previously described [7]. In brief, 100 µl of blood were added to the appropriate mAb combination and incubated for 15 min; samples were then lysed and fixed by a commercial preparation (Immunoprep, Coulter, Milan). The four-color CD3/CD4/CD8/CD45 and CD3/CD19/CD56/CD45 mAb combinations (Coulter, Milan) were used. Fluorescence was then measured in an EPICS XL flow cytometer (Coulter, Milan).

Plasma viremia

Plasma viremia was determined by commercial bDNA assay (Chiron Diagnostics, Milan) with a lower detection limit of 50 copies/ml.

Prophylactic treatment

The HIV proviral copy numbers in peripheral blood mononuclear cells (PBMCs) were determined by the quantitative competitive PCR approach described elsewhere [27, 28]. In brief, PBMCs were obtained from 10 ml of peripheral blood by density gradient centrifugation on Ficoll-Hypaque (Pharmacia). For each patient, proviral DNA was extracted by standard methods [27, 28].

Two microliters of such DNA were coamplified with decreasing amounts of the pSPL-111 competitor template in a 100 µl PCR reaction containing specific primers for the reference single copy β-globin gene (primer set PCO3-PCO4) or for the HIV-1 gene gag (primer set 1-211). The construction and the use of this plasmid for the quantitation of HIV-1 nucleic acids have been already described [27, 28]. After amplification, 20 µl of each reaction mixture were resolved in an 8% nondenaturing polyacrylamide gel, stained with ethidium bromide, visualized under ultraviolet light and analyzed by densitometric scanning. The proviral DNA copies were expressed taking into account the percentage of circulating CD4 + cells between PBMCs, as described [27, 28].

Statistical analysis

Since the variables under study were not normally distributed, we used nonparametric statistical tests. The Wilcoxon rank sum test was used to analyze paired values in the same group [2].

Results

Patient characteristics

The characteristics of the eight patients included in this study are reported in Table 1.

Fig. 1 For each patient treated with anti-CD20 and chemotherapy (CT), the CD4 counts/µl of peripheral blood (scales on the left side) and the number of HIV-RNA copies/ml of plasma (scales on the right side) are shown. In patients 1, 3 and 5 the provirus copies/10⁶ peripheral blood mononuclear cells (PBMCs) are also presented (scales on the left side). The x axis shows the time when the parameters were evaluated: 0 = before therapy, middle = after the third cycle of anti-CD20 plus CT, end = at the end of therapy. Patients 1–6 received concomitant highly active antiretroviral therapy (HAART), while patients 7 and 8 did not receive any concomitant antiretroviral treatment.