Addition of granulocyte colony-stimulating factor to chemotherapy in patients with Aids-related lymphoma: effects on neutrophil Fcgamma receptor expression and soluble FcgammaRIII plasma levels

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Addition of granulocyte colony-stimulating factor to chemotherapy in patients with AIDS-related lymphoma: effects on neutrophil Fcγ receptor expression and soluble FcγRIII plasma levels

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Summary: AIDS-related neutropenia and neutrophil dysfunction can (partly) be reversed by granulocyte-colony stimulating factor (G-CSF). We studied the effect of G-CSF on neutrophil increment and levels of soluble Fcγ receptor type III in 15 patients with AIDS-related lymphoma (ARL) undergoing chemotherapy. In six of these patients we performed a detailed kinetic analysis of the membrane expression of the functionally important Fcγ-receptors type I, II and III. In all these patients G-CSF induced FcγRII positive neutrophils with a decreased expression of the FcγRIII receptor. These changes were similar to those seen both in healthy volunteers and in non-HIV-infected individuals treated with chemotherapy. Interestingly, the mean neutrophil and sFcγRIII increment were significantly lower and more patients had a nadir granulocyte count \(<0.5 \times 10^9/l\) after the first cycle than after the second cycle of chemotherapy. This may be related to a therapy-associated decrease in HIV-1 viral load.

The conclusion is that patients treated with chemotherapy for ARL have a qualitatively normal response to G-CSF.

Keywords: rh-G-CSF, FcγR expression, sFcγRIII levels, AIDS, non-Hodgkin’s lymphoma.

Although CD4-lymphocyte depletion is the hallmark of HIV-1 infection, patients with AIDS often also have neutropenia, and several studies have demonstrated evidence of neutrophil dysfunction, such as defective chemotaxis, bacterial killing and phagocytosis (Ellis et al., 1988; Murphy et al., 1988; Gabrilovich et al., 1994; Elbim et al., 1994). HIV-1 infected persons have a higher incidence of bacterial infection (e.g. pneumonia) as compared with the general population (Polsky et al., 1986; Moore et al., 1995). This may partly be iatrogenic (myelosuppressive effect of anti(retro) viral drugs), but has also been attributed to HIV-infection itself or to HIV-1-induced dysregulation of cytokines (Ellis et al., 1988; Busch et al., 1986). Myelosuppressive chemotherapy renders these patients especially sensitive to bacterial infection, causing severe morbidity and mortality (Gill et al., 1987; Sankwa et al., 1992; Kaplan et al., 1989).

Granulocyte colony stimulating factor (G-CSF) is a lineage-specific growth factor that stimulates the production, differentiation and functional activation of neutrophil granulocytes (Lieschke & Burgess, 1992). Recombinant human (r-H) G-CSF shortens the period of neutropenia induced by high-dose chemotherapeutic regimens (Frampton et al., 1994). Also in patients with AIDS, G-CSF can overcome neutropenia and neutrophil defects (Vecchiarelli et al., 1995; Laursen et al., 1995; Hermans et al., 1996).

The high affinity IgG receptor FcγRI is usually absent from mature neutrophils in healthy individuals but can be up-regulated during infection (presumably via production of gamma-interferon, IFNγ) or upon treatment with G-CSF (Gericke et al., 1995; Kerst et al., 1993a). Antibody-dependent cellular cytotoxicity is mediated by FcγRI and has been shown to be defective in patients with AIDS (Kinne & Gupta, 1989). FcγRIII (CD16), a low-affinity receptor for IgG, is expressed by neutrophils, natural killer cells and macrophages. A soluble form of FcγRIII originating mainly from release by neutrophils can be detected in plasma. The plasma concentration of sFcγRIII reflects total body mass of neutrophils, and is not influenced by shifts of neutrophils from one pool to another (Huizinga et al., 1994). sFcγRIII levels are therefore presumed to be a better measure of total body neutrophil mass and neutrophil defence than the peripheral blood neutrophil count and might be a better
predictor of the risk of infection in neutropenic patients (unpublished observation). sFcγRIII levels have been shown to be increased early in HIV-1 infection and to be severely depressed in patients with clinical AIDS (Khayat et al., 1990).

In view of the neutrophil defects found in patients with AIDS, we studied the effect of G-CSF on neutrophil increments, levels of sFcγRIII and membrane expression of FcγRI. II and III in patients with AIDS-related lymphoma undergoing chemotherapy, and related this to the rate of infection and to endogenous G-CSF levels. These data were also compared with results previously obtained in healthy volunteers (Kerst et al., 1993a).

PATIENTS AND METHODS

Patients. We studied 15 patients with AIDS-related NHL who were treated at our hospital in a phase II trial of CNOP chemotherapy in combination with G-CSF. In the first six patients detailed kinetic studies of neutrophil Fcγ receptor expression and soluble FcγRIII were performed. Clinical characteristics of the patients will be described elsewhere (unpublished observation). Briefly, they were 15 homosexual HIV-1 seropositive males with biopsy-proven, previously untreated, intermediate or high grade NHL. Two patients had bone marrow infiltration. None of the patients had symptoms or signs of an (opportunistic) infection at the start of chemotherapeutic treatment. The median CD4 cell number was 0.06 × 10^9/l (range 0.01–0.67 × 10^9/l).

Chemotherapy regimen. Chemotherapy consisted of cyclophosphamide, 750 mg/m², mitoxantrone, 10 mg/m² and vincristine, 1.4 mg/m² i.v. on day 1, and oral prednisone 50 mg/m² days 1–5. This cycle was repeated every 3 weeks.

In cases of granulocytopenia (< 1.0 × 10^9/l) and/or thrombocytopenia (< 100 × 10^9/l) chemotherapy was postponed for 1 week; in cases of persistent pancytopenia a 50% dose-adjustment for cyclophosphamide and mitoxantrone was made. G-CSF (Neupogen, Amgen, Thousand Oaks, Calif.) was self-administered on days 3–12 (300 µg subcutaneously). Antiretroviral therapy was stopped during chemotherapy, and related this to the rate of infection in neutropenic patients (unpublished observation). SFcγRIII levels have been shown to be increased early in HIV-1 infection and to be severely depressed in patients with clinical AIDS (Khayat et al., 1990).

Collection of blood samples. For the first six patients, during the first three cycles of chemotherapy venous blood samples (serum and EDTA anticoagulated blood) were obtained on day 1 (prior to start of chemotherapy), day 3 (prior to start of G-CSF), and days 7–8, 11–12, 15, 18 and 22 (prior to the next cycle of chemotherapy). For the other nine patients, blood was obtained at day 1 and prior to every cycle of chemotherapy. Total white blood cell (WBC) counts and differential counts were performed with the H1 system (Technicon Instruments, Tarrytown, N.Y.).

Fcγ receptor expression. FcγR expression was analysed by flowcytometry (FACScan, Becton Dickinson, San Jose, Calif.) essentially as described (Kerst et al., 1993a), using standard indirect immunofluorescence techniques. Monoclonal antibodies (MoAb) used were FeRgran1 (5D2) for CD16, AT10 for CD32 (CLB, Amsterdam, The Netherlands) and 32.2 for CD64 (Medarex Inc., West Lebanon). As controls, irrelevant murine antibodies of the appropriate Ig subclass were used.

Binding of the antibodies was visualized with FITC labelled polyclonal goat-anti-mouse Ig F(AB')2 (DakoPatts, Denmark). Measurements were made after live-gating on neutrophils. The mean fluorescent intensity (MFI) was used as a relative measure of receptor expression, after subtraction of background fluorescence.

Soluble FcγRIII. Plasma levels of soluble FcγRIII were determined by sandwich ELISA as described (Koene et al., 1996), using the MoAb CLB FeRgran1 as the primary antibody and a polyclonal biotinylated antibody as the secondary antibody. A calibration curve was constructed by pooling plasma of 90 healthy donors and measuring serial dilutions of this pooled plasma in the ELISA. The concentration of sFcγRIII in this pool was arbitrarily set at 100 units. G-CSF. G-CSF levels were measured with a commercial sandwich ELISA (Quantikine, R&D Systems, Minneapolis, Minn.) according to the manufacturer's instructions. The sensitivity of this assay is 10–9 pg/ml; 95% of levels in healthy volunteers are < 40 pg/ml.

Statistical analysis. Statistical analysis was performed using the Number Cruncher Statistical System (NCSS, Kaysville, Utah). A P value of < 0.05 was considered to represent statistical significance. For normally distributed values a paired Student's t-test was used and for skewed data the nonparametric Wilcoxon test was used. Baseline values of G-CSF, sFcγRIII and neutrophils were correlated with Spearman's rank-correlation coefficient.

RESULTS

Neutrophil counts

At baseline, the median WBC count was 3.5 × 10^9/l (range 0.9–12.9 × 10^9/l). Two patients had neutropenia (absolute neutrophil count < 0.4 × 10^9/l and/or thrombocytopenia < 1.0 × 10^9/l) at baseline to 8.3 × 10^9/l (range 0.6–14.8) at baseline to 8.3 × 10^9/l (range 0.6–14.8) at baseline to 8.3 × 10^9/l (range 0.6–14.8) at baseline to 8.3 × 10^9/l (range 0.6–14.8). Two patients had neutropenia (absolute neutrophil count < 0.4 × 10^9/l and/or thrombocytopenia < 1.0 × 10^9/l) at baseline to 8.3 × 10^9/l (range 0.6–14.8) at baseline to 8.3 × 10^9/l (range 0.6–14.8). Two patients had neutropenia (absolute neutrophil count < 0.4 × 10^9/l and/or thrombocytopenia < 1.0 × 10^9/l).

Of 15 patients, seven had a nadir granulocyte count < 0.5 × 10^9/l after the second cycle. Of 15 patients, seven had a nadir granulocyte count < 0.5 × 10^9/l after the second cycle.

sFcγRIII levels

Before chemotherapy, sFcγRIII levels ranged from 47 to 294 arbitrary units (mean ± SD: 129 ± 3 ± 77.6 a.u.). Baseline levels of neutrophils did not correlate with sFcγRIII levels (P > 0.1). For the six subjects studied in detail, the kinetics of sFcγRIII levels are shown in Fig 1C. Peak levels of sFcγRIII were reached 13–16 d after the start of G-CSF: 214 ± 91 a.u. (mean ± SD, P = 0.07). After the second cycle, sFcγRIII levels rose from 149 on day 22 to 328 a.u. on day 39.
Fig 1. Membrane expression of FcγRI (A) and FcγRIII (B) on neutrophils as well as soluble sFcγRIII levels (C) are shown during treatment with chemotherapy and G-CSF in patients with AIDS-related NHL. On the x-axis the time in days is shown. The arrow at days 1 and 22 indicates the start of chemotherapy; the box indicates the administration of G-CSF (days 3–12 and days 24–33). Data are shown for six individuals (mean ± SEM). Asterisks indicate statistically significant increases or decreases (P < 0.05). (○) in panels A, B and C represents the mean number of circulating neutrophils (× 10^9/l; right axis). Panel A: (●), membrane expression of FcγRI (mean fluorescent intensity (MFI; mean ± SEM; left y-axis) as measured by flow cytometry using MoAb 32. Panel B: (■), membrane expression of FcγRIII (mean fluorescent intensity (MFI; mean ± SEM; left y-axis) as measured by flow cytometry using MoAb CLB FcγRgran/1. Panel C: (▲), plasma levels of soluble FcγRIII as measured by sandwich ELISA. Data are expressed as percentage of baseline value of sFcγRIII.
Improving effector cell functions such as antibody-dependent affinity receptors for IgG is probably instrumental in (Kerst et al., 1993b). The G-CSF-induced expression of high-affinity receptors for IgG is probably instrumental in improving effector cell functions such as antibody-dependent cellular cytotoxicity (ADCC; van de Winkel & Andersen, 1991). Since patients with AIDS are at increased risk of bacterial infections, especially when treated with chemotherapy, and have been shown to have impaired ADCC, we were interested to find out whether treatment with G-CSF would induce up-regulation of FcYRI. Our results show that after G-CSF treatment virtually all circulating neutrophils are FcYRI positive. In addition, these neutrophils have decreased FcYRIII expression as compared with baseline. These changes were similar in magnitude when compared with both effects seen in healthy volunteers (Kerst et al., 1993a) and in non-HIV-infected individuals treated with chemotherapy and G-CSF (Spiekermann et al., 1994). In contrast to a previous report by Capacchini et al. (1992), we could not demonstrate expression of FcYRI on neutrophils before start of treatment. This could be due to the fact that none of our patients had signs of active infection at that time. We did in fact find FcYRI expression in one patient no longer treated with G-CSF during an infectious episode, possibly caused by increased IFNγ production.

In nine of the 15 patients the neutrophil increment was significantly higher after the second than after the first cycle of chemotherapy. Since also the increase in sFcYRIII levels was significantly higher after the second cycle, the difference in neutrophil increment is probably not merely the result of shifts from one pool to another but a reflection of a higher production of neutrophils in the bone marrow. Moreover, significantly more patients had a nadir neutrophil count <0.5·10^3/l after the first than after the second cycle (7/15 vs 1/15; P=0.02). The difference in increment or nadir could not be explained by differences in doses of chemotherapy, by presence or absence of lymphomatous bone marrow infiltration, by the use of concurrent myelosuppressive medication, or by baseline neutrophil or sFcYRIII levels. Although G-CSF levels were elevated in 10/15 patients, no correlation could be demonstrated between endogenous G-CSF levels and neutrophil increment or sFcYRIII increment.

G-CSF levels
Baseline endogenous G-CSF levels were between 16 and 421 µg/l (mean ± SD: 122 ± 136.2 µg/l), and were above 40 µg/l (the 95% confidence limit of normal values) in 10/15 patients. No correlation could be demonstrated between endogenous G-CSF levels and either pretreatment neutrophil or sFcYRIII levels (P>0.1). Also, no correlation was found between endogenous G-CSF levels and neutrophil increment or sFcYRIII increment.

Infectious episodes
In these 15 patients, four infectious episodes were seen. One patient had CMV colitis after the fifth cycle of chemotherapy, and three patients had localized bacterial infection (all three perianal abscesses). Two of the abscesses occurred after the third cycle and one after the first cycle. These patients did not have neutropenia at the time of infection. Pretreatment sFcYRIII levels were low in two of the three patients with infection (30.46 and 116 a.u.), but not statistically significantly different from levels seen in patients without infection.

Discussion
This is the first study of the effect of G-CSF on neutrophil FcYR expression in patients with AIDS. Previously, we have demonstrated in vitro that under the influence of G-CSF committed myeloid progenitor cells give rise to FcYRI-positive neutrophils, which at the same time have a strongly decreased expression of the PI-linked FcYRII receptor (Kerst et al., 1993b). The G-CSF-induced expression of high-affinity receptors for IgG is probably instrumental in improving effector cell functions such as antibody-dependent cellular cytotoxicity (ADCC; van de Winkel & Andersen, 1991). Since patients with AIDS are at increased risk of bacterial infections, especially when treated with chemotherapy, and have been shown to have impaired ADCC, we were interested to find out whether treatment with G-CSF would induce up-regulation of FcYRI. Our results show that after G-CSF treatment virtually all circulating neutrophils are FcYRI positive. In addition, these neutrophils have decreased FcYRIII expression as compared with baseline. These changes were similar in magnitude when compared with both effects seen in healthy volunteers (Kerst et al., 1993a) and in non-HIV-infected individuals treated with chemotherapy and G-CSF (Spiekermann et al., 1994). In contrast to a previous report by Capacchini et al. (1992), we could not demonstrate expression of FcYRI on neutrophils before start of treatment. This could be due to the fact that none of our patients had signs of active infection at that time. We did in fact find FcYRI expression in one patient no longer treated with G-CSF during an infectious episode, possibly caused by increased IFNγ production.

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We could not demonstrate a correlation between either neutrophil or sFcYRIII levels and endogenous G-CSF levels, as has been shown to exist for patients with aplastic anaemia (Watari et al., 1989). The fact that endogenous G-CSF levels were increased in two-thirds of the patients probably reflects decreased neutrophil production in the bone marrow, which, in view of the relatively normal sFcYRII levels, appears to be compensated. The number of infections seen in this patient group was too low to evaluate the prognostic value of sFcYRIII levels.
REFERENCES


