CD64 Surface Expression on Neutrophils and Monocytes Is Significantly Up-Regulated after Stimulation with Granulocyte Colony-Stimulating Factor during CHOP Chemotherapy for Patients with Non-Hodgkin’s Lymphoma

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Abstract

The present study was performed to examine whether the expression of CD64 Fc gamma receptor type I (FcγRI) on both neutrophils and monocytes can be modulated by multiple daily administrations of granulocyte colony-stimulating factor (G-CSF) to patients with non-Hodgkin’s lymphoma in neutropenia caused by CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy. The expression of CD64 was determined by flow cytometric analysis at the following time points: before chemotherapy, at the nadir of the neutrophil count, at the fifth day after the start of G-CSF administration, and at more than 8 days after the start of G-CSF administration. CD64 expression was enhanced in patients given G-CSF during CHOP treatment, whereas CD64 expression remained unchanged in patients not given G-CSF. CD64 expression levels on both neutrophils and monocytes were significantly up-regulated by the daily administration of G-CSF and reached peak levels at day 5 ($P = .0007$). Thereafter, expression on both cell types remained at almost the same levels as on day 5 for the rest of the treatment course, even though G-CSF therapy continued for 3 to 5 more days. Interestingly, CD64 expression on monocytes was already increased significantly ($P = .0001$) at the nadir of the neutrophil count relative to the baseline before chemotherapy and then was additionally up-regulated by day 5 after the start of G-CSF injections ($P = .019$). In antibody-dependent cellular cytotoxicity assays, we found that rituximab-mediated cell lysis was significantly enhanced at day 5 after the start of G-CSF treatment ($P = .01$). In conclusion, this study shows that multiple doses of G-CSF administered to lymphoma patients with neutropenia due to CHOP chemotherapy can enhance CD64 expression on both neutrophils and monocytes. Peak CD64 levels are reached at day 5 of G-CSF treatment, resulting in an activation of the rituximab-mediated antitumor ability of these effector cells. This finding may be useful in determining the optimal timing of administration for an antibody such as rituximab in a chemotherapeutic strategy designed to exert a maximal effect against tumor cells. Int J Hematol. 2004;79:55-62.

Key words: CD64; G-CSF; Neutrophil; Monocyte; ADCC

1. Introduction

Rituximab (IDEC-C2B8), a chimeric immunoglobulin G1 (IgG1) kappa monoclonal antibody with mouse variable and human constant regions that recognizes the CD20 antigen[1], is used increasingly in the treatment of patients with CD20\(^+\) non-Hodgkin’s lymphoma (NHL), particularly low-grade NHL.

The CD20 antigen is a 35-kd nonglycosylated hydrophobic phosphoprotein that is expressed on the surface of more than 95% of normal and malignant B-cells [2]. The CD20 antigen is present from the pre-B-cell stage onward and is absent from the surfaces of plasma cells. Several studies suggest that CD20 acts as a calcium channel and plays a role in B-cell activation, proliferation, and differentiation [3]. Importantly, CD20 is not expressed on other hematologic cells or on non-lymphoid cells, does not modulate in response to antibody
binding, and is not shed from the cell surface [4]; thus, CD20 provides an ideal target for monoclonal antibody therapy.

Several antitumor mechanisms have been proposed for the depletion of B-cells by rituximab, including activation of intracellular signaling and apoptosis, complement-dependent cytotoxicity, and antibody-dependent cellular cytotoxicity (ADCC) [1,5-8], and these effects have been demonstrated in experiments with cultured malignant B-cell lines. Recently, several studies have been performed to define the contributions of these different mechanisms of rituximab action to normal and malignant B-cells [9-11]. These studies have found that in vitro B-cell depletion with rituximab is observed in the presence of mononuclear cells, natural killer (NK) cells, and monocytes/macrophages and in the absence of complement and significant direct apoptosis, suggesting that a cell-mediated mechanism (that is, ADCC) plays a predominant role in the action of rituximab. Although NK cells and monocytes/macrophages are the major natural mediators of ADCC, neutrophils can also function as effector cells after exposure to some cytokines, such as interferon γ or granulocyte colony-stimulating factor (G-CSF), whereby the cytotoxicity of neutrophils in ADCC can be greatly enhanced [12-16].

In the peripheral blood of healthy people, neutrophils constitutively express CD16 (Fc gamma receptor type III, FcγRIII) and CD32 (FcγRI) [17]. CD32 is thought to be the main cytotoxic FcγR of unstimulated neutrophils [17,18]. On the other hand, CD64 (FcγRI) is expressed constitutively under unstimulated physiological conditions on monocytes/macrophages and NK cells but not on neutrophils. The expression of CD64 on neutrophils, however, can be induced in vivo by G-CSF, and CD64 is the only FcγR that can bind monomeric IgG with high affinity [17,19]. CD64 has been reported to be the main cytotoxic FcγR molecule involved in ADCC with G-CSF–primed neutrophils [14-17,20]. Therefore, the administration of G-CSF may enhance ADCC through an increase in CD64 expression on neutrophils and facilitate the clinical antitumor efficacy of rituximab. Rituximab has significant activity as a single agent in the treatment of relapsed [21-24] or previously untreated [25,26] low-grade NHL. Moreover, recent clinical study has shown that rituximab combined with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy appears to be more effective [27-29].

So far, no detailed data are available on the kinetics of CD64 expression on neutrophils, let alone on monocytes, derived from lymphoma patients with neutropenia who have received G-CSF, whereas there are some data on the kinetics in healthy donors who have received G-CSF injections [17] and in cancer patients who have received G-CSF for the collection of peripheral blood progenitors in a hematologically steady state [30].

Thus, we investigated whether CD64 expression on neutrophils and monocytes can be up-regulated by multiple daily administrations of G-CSF to NHL patients with neutropenia during CHOP treatment. We found that the changes in CD64 expression on both cell types can be modulated by G-CSF in a certain pattern during the treatment course and that rituximab-mediated cell lysis is correspondingly enhanced by G-CSF. This study appears to provide a therapeutic strategy for using rituximab under the optimal conditions of a maximal antitumor effect.

2. Materials and Methods

2.1. Patients

After informed consent was given, blood samples were obtained from patients with newly diagnosed NHL who were undergoing a CHOP chemotherapy regimen. Twelve patients ranging in age from 43 to 84 years and with a mean age of 65 years were enrolled. Eight patients were female, and 4 were male. Histologically, 9 patients had a diagnosis of diffuse, large cell NHL, and 3 patients had follicular center cell disease.

2.2. Administration of G-CSF

G-CSF (nartograstim, 50 μg/m² per day; Kyowa Hakko Kogyo, Tokyo, Japan) was administered subcutaneously in 8 patients. G-CSF was given for 5 days to 5 patients, for 8 days to 2 patients, and for 10 days to 1 patient, with the length of treatment depending on the recovery of neutrophils. Venous blood was obtained before chemotherapy, just before starting G-CSF at a white blood cell (WBC) count of 2000/μL (pre–G-CSF), on the fifth day of daily G-CSF injections (G-CSF5), and after the last administration when G-CSF was used for 8 or more days (G-CSF >8). Because the kinetics of neutrophil recovery during G-CSF treatment vary, the following criteria were defined for analysis: (1) starting G-CSF at a WBC count of 2000/μL, (2) G-CSF treatment for at least 5 days, and (3) continued use if the doctors in charge decided on the basis of clinical indications that more G-CSF was needed. G-CSF was not administered in 4 patients. In these cases, blood samples were obtained before chemotherapy, at approximately the nadir of the WBC count (nadir), and just before the next chemotherapy (postnadir).

2.3. Monoclonal Antibodies

Fluorescein isothiocyanate (FITC)-conjugated anti-CD16b (clone 1D3, mouse IgM), FITC-conjugated mouse IgM, and R-phycocerythrin (R-PE)-conjugated mouse IgG1 were from Beckman Coulter (Fullerton, CA, USA). R-PE-conjugated anti-CD64 (clone 10.1, mouse IgG1) was from Caltag Laboratories (Burlingame, CA, USA). The chimeric anti-CD20 antibody rituximab was from Roche Pharmaceuticals (Basel, Switzerland).

2.4. Immunofluorescence Analysis by Flow Cytometry

The surface expression of the CD64 molecule on neutrophils and monocytes was determined by indirect immunofluorescence using flow cytometry and monoclonal antibodies. Whole blood was used to avoid any in vitro manipulation that would activate these cells. Aliquots of heparinized blood (100 μL) were washed twice with phosphate-buffered saline (PBS) and incubated with pooled normal rabbit serum (Cedarlane Laboratories, Hornby, Ontario, Canada) for 5