Biologic Response to Anti-CD16 Monoclonal Antibody Therapy in a Human Immunodeficiency Virus-Related Immune Thrombocytopenic Purpura Patient


A patient with refractory human immunodeficiency virus (HIV)-related immune thrombocytopenic purpura (ITP) was treated with 3G8 (anti-CD16) monoclonal antibody on days 1, 3, and 8 (25, 25, and 50 mg were administered intravenously, respectively). Side effects were those expected after the administration of a xenogenic protein, but a severe bone pain occurred from the second injection. At the time of the initiation of the treatment the platelet count was 20,000/mm² and the absolute CD4 number was 100/mm². We obtained a long-term correction of thrombocytopenia and, to a lesser extent, there was a stabilization of CD4 lymphocytes for 18 months. We observed a significant stimulation of natural killer (NK) function and an elevation in the serum level of tumor necrosis factor α, interferon γ, and granulocyte-macrophage colony-stimulating factor. This suggests that in HIV-related ITP the removal of platelets is mediated by low-affinity Fcγ receptors (CD16). The stimulation of NK function and elevation in CD4⁺ lymphocytes may be related to the production of cytokines by activated human NK cells through the interaction of their CD16-bearing receptor with the 3G8 monoclonal antibody. This observation warrants confirmation and further clinical trials.

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Yvelines, France), soluble receptor to IL-2 (sIL-2R) (Immunotech, Marseille, France), tumor necrosis factor α (TNFα), interferon γ (IFNγ), and IL-1α (Endogen, Clinisciences, Paris, France). Soluble CD16 was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) test previously described by Khayat et al. Human anti-mouse antibodies (HAMA) (Immunomedics, Biotechnic, Rungis, France) were also tested from day 1 to day 48. Pharmacokinetic of 3G8 in serum was measured using a classical ELISA test for the detection of mouse IgG in body fluids. NK activity was assessed on days 0, 1, 2, 3, 7, 8, 18, and 138. Peripheral blood mononuclear cell (PBMC) activity was tested against K562 cells labeled with 51 Cr 100 pCi for 1 hour. Spontaneous chromium release by K562 never exceeded 10% of the maximum release.

ADCC activity has been measured according to the method described by Perussia et al. with Epstein-Barr virus (EBV)-transformed B cells from a normal individual serving as target cells. A monoclonal-specific antibody for monomorphic class I antigens (B 9-12-1) (a generous gift from E. Gomard, Inserm U 152, Cochin Hospital, Paris) was used to mediate antibody-dependent cellular cytotoxicity (ADCC). Effector cells were the patient’s PBMCs. Controls were made evaluating lysis caused by effectors alone in the absence of MoAb (C1), or caused by MoAb alone in the absence of effectors (C2).

Specific ADCC is calculated as the lysis of target cells caused by the presence of MoAb and effectors, ie:

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\% \text{ ADCC Activity} = \frac{\exp - C2}{\max - C2} \times 100
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Drug administration and side effects. On June 20 (day 1), June 22 (day 3), and June 27 (day 8) the patient received 25 mg, 25 mg, and 50 mg, respectively of MoAb 3G8 infused intravenously (IV) over 1 (first and second injection) or 2 hours (third injection) diluted in human serum albumin.

Chills, fever, and nausea were observed after each injection requiring the administration of antihistaminic drug (dexchlorpheniramine: 10 mg/d); they resolved within 2 hours. From the second injection, intense bone pain occurred requiring the prescription of analgesic drugs (paracetamol: 3 g/d). These pains increased after the third injection requiring morphine treatment and lasted for 4 days.

RESULTS

Clinical status remained unchanged after the whole treatment cycle except for the side effects described above.

Effects on platelet counts. The platelet count went down with each injection and then rebounded to a higher level. However, the double-dose injection was followed by an increase in the platelet number up to 60,000 from day 18 remaining above 95,000 for 18 months (Fig 1).

Effects on WBCs. After each injection WBCs showed a transient (3 hours) but intense decrease. At day 114, leukocyte count was 10,000/mm³. Myelemia concurrently appeared with circulating metamyelocytes, with myelocytes representing up to 5,000 cells/mm³ (Fig 2).

Effects on T-lymphocyte subsets. CD4 count per milliliter transiently decreased from 170 to less than 100 after each injection, reincreased up to 425 at day 18 and then progressively decreased (160 at 18 months; ie, approximately the pretreatment level). One of the most interesting figures is that after treatment we observed a long-lasting CD4 stability (Fig 3) leading to a permanent break in the decreasing slope of CD4 count observed in the pre-3G8 treatment period. CD8 count per cubic millimeter showed the same type of curve so that the CD4:CD8 ratio remained constant during therapy.
p24 Antigen in serum. p24 antigen level in serum did not change during the follow-up (Fig 4).

Serum cytokines and growth factors. We observed after each 3G8 infusion a release of TNFα, IFNγ, and GM-CSF (Fig 4). There was a suggestion of a lesser response to the second and third 3G8 infusions for each of the cytokines indicated as compared with the initial infusion. There seems to be a tendency for IL-2 to increase in parallel with a small decrease in the sIL-2R but, if specific, these changes were minimal (Fig 5). IL-1α remained unchanged.

Soluble human FcγRIII (CD16) could not be detected in this patient at any time after injections. HAMA were undetectable. 3G8 pharmakokinetic showed a peak followed by a decrease after each injection then entirely disappeared from serum from day 9.

NK activity. NK function was depressed before the initiation of the treatment, which is often observed in HIV-infected patients. Surprisingly, NK function increased a few days after the last injection and continued to be elevated for greater than 4 months (Fig 6).

ADCC activity. The ADCC activity was decreased in the 24 hours after the infusion with the same kinetic at each treatment (Fig 6).

DISCUSSION

HIV-infected patients with thrombocytopenia may often present early in the course of their HIV infection such that the thrombocytopenia may occur years before any opportunistic infection. They can be considered to have “isolated” thrombocytopenia because, in general, WBC counts are nor-
mal and only very rarely there is any evidence of anemia, as was the case for our patient before receiving the MoAb therapy. Clinical platelet-vascular function appears to be rather normal as seen in classical ITP, but serious, life-threatening hemorrhages have occurred, in general at platelet counts less than 20,000/µL. The relative roles of antiplatelet antibody (including immune complexes) and direct viral infection of the marrow in causing the thrombocytopenia remain to be clarified.

Overall, the optimal treatment strategy for those HIV-ITP patients requiring treatment remains uncertain. Steroids and splenectomy may have a role but the effects of treatment on the underlying HIV disease are more important than those on the platelet count in view of the low incidence of central nervous system hemorrhage. For this reason, AZT is often the initial treatment of choice with refractory patients receiving a lot of concomitant therapies.

It appears clear that treatments reacting with Fc receptors may be efficacious in increasing the platelet count in even refractory patients. The examples of this are IVIg and anti-D. Clarkson et al reported the successful treatment of a woman with a highly resistant ITP using 3G8 MoAb (anti-FcγRIII or CD16) infusion.

We report here the clinical, biological, and immunological results of an anti-CD16 MoAb (3G8) treatment of ITP in an HIV-infected patient. During the treatment, an increase of the platelet count was observed suggesting that the removal of platelets was, at least partly, mediated by low affinity Fcγ receptors (CD16) in this HIV-related ITP. Surprisingly, platelet counts continued to remain elevated for several months while free 3G8 MoAb had disappeared from serum with no detectable level since the ninth day after the beginning of the treatment.

Interestingly, we observed a modification of the CD4 number. Before the treatment, as shown in Fig 3, the decrease of CD4 count was significantly related to time (t = 3.896; P < .1). But after the 3G8 therapy, a relative stabilization for 18 months was observed. This fact is relatively unusual with other therapies, ie, corticosteroids, danazol, or splenectomy with which T-helper count normally show only transient in-

Concerning the secretion of cytokines among all TNFα, INFγ, and GM-CSF, several factors may have contributed to the activation of secreting cells leading to a stimulation of cytokine production or release: HIV may itself initiate the process, a phenomenon recorded previously by in vitro experiments. Human NK cells have been shown to release soluble factors as TNF. As reported by Cuturi et al using a 10-day culture preparation of NK cells, it has been shown that NK cells can be induced to transcribe TNF and IFNγ genes on specific stimulation with rIL-2 in combination with ligands for the NK-cell receptor for IgG-Fc, (FcR), CD 16 antigen, or on nonspecific stimulation with calcium ionophore. These data have been recently confirmed by Robertson and Ritz showing that NK cells can secrete soluble factors with either stimulatory or inhibitory activity and that the activation of NK cells is required for either activity.

In our patient, we observed an important stimulation of NK function and an increase in cytokine levels, suggesting that, at least in this particular case, these results could be related to the production of cytokines by activated human NK cells through the interaction of their CD16 bearing receptor with the 3G8 MoAb, as previously shown in vitro. We also observed the release of GM-CSF, IFNγ, and perhaps IL-2 following each injection of the MoAb. According to Cuturi et al the interaction of CD16 with their specific ligands as MoAbs stimulate GM-CSF production of NK cells, which, in return, activates the secretion of a factor-enhancing myeloid precursor colony formation explaining the concurrent and important myelemia observed including erythroblasts, metamyelocytes, myelocytes, and promyelocytes. As
reported by Robertson and Ritz, the activation of NK cells by their contact with early myeloid progenitor cells would seem to be an adequate stimulus. Whether this myeloid cell production is in relation with the severe pain observed immediately after the second and third anti-CD16 injection remains unclear.

Regarding the soluble CD16 titer, a factor that we previously showed to be related to the prognosis of HIV-related disease, no significant level could be detected in the patient’s serum at any time suggesting that there is probably no interaction at this point between the injection of an anti-CD16 MoAb and the release of the molecule itself.

The role of low-affinity Fcγ receptors (CD16-CD32) in HIV infection has been recently reevaluated. Several investigators have shown their possible involvement in the penetration of HIV in the monocyte macrophage reservoir. The key role of CD16 in the activation of NK cells and the control of a cytokine cascade initiated by the secretion of lymphokines and growth factors by CD16-mediated NK cell activation may contribute to its involvement in the control of the growth of lymphoid and myeloid lineages that are frequently affected in HIV patients. Its release, as soluble particles in serum, has been shown to be related to the prognosis of HIV infection, and to have a significant relationship with all the most important biologic factors of HIV infection (CD4, p24). The results reported here suggest that HIV-related thrombopenic purpura seems to be related to the sequestration of the Ig-coated platelet. However, the data presented here are rather limited and immunotherapy with mouse anti-CD16 antibody should be used only under research conditions as part of a controlled trial and not as part of the therapeutic options for HIV-related ITP.

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