Treatment with Megestrol Acetate Improves Human Immunodeficiency Virus-Associated Immune Thrombocytopenia

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Splenomegaly in vivo and in vitro macrophages Fc receptors participate in the pathophysiology of immune cytopenias, and in such disorders, the beneficial effects of glucocorticoids are in part mediated by decreased expression of macrophage Fcγ receptors. In the animal model, progesterones, like glucocorticoids, inhibit expression of these receptors. Megestrol acetate (MA) is a progestogen frequently used for treating human immunodeficiency virus (HIV)-associated anorexia-cachexia. Twenty-eight patients with HIV-associated thrombocytopenia with shortened platelet survival and increased platelet-associated immunoglobulin G (IgG) who were being treated with MA for anorexia-cachexia were prospectively studied for a 6-month period to assess the potential role of progesterone in the treatment of immune thrombocytopenia. Treatment with MA for nonconsecutive periods of 2 months and 1 month significantly increased platelet count and platelet survival without significant alteration of platelet-associated immunoglobulin levels. Of the 28 patients studied, 22 presented a complete response, 19 presented a complete response 1 month after finishing the MA treatment regimen, and 12 remained in complete response for a further month. Expression of Fcγ receptors (FcγRI and FcγRII) by peripheral blood monocytes and the in vitro recognition of IgG-sensitized cells by monocytes were significantly decreased by the MA treatment. Decreased expression and functioning of these receptors significantly correlated with platelet counts and survival times, but no relationship was found with platelet-associated immunoglobulin, circulating immune complexes, body mass index, plasma HIV load, or CD4 lymphocyte levels. These results suggest that treatment with progesterones, like MA, may be an alternative therapy for immune cytopenias, with few side effects.

Preliminary data have suggested that treatment with megestrol acetate (MA) enhances the platelet count of malnourished patients with human immunodeficiency virus (HIV)-associated thrombocytopenia (21), most of whom present immunoglobulin G (IgG) antiplatelet antibodies (13, 27). Receptors for the Fc fragment of IgG (FcγRs) on macrophages play an important role in host defense against infection (10, 19), particularly in the pathophysiology of immune cytopenias (3, 5, 7, 8, 22, 23). Hence, regulation of the expression of these splenic receptors is an important target in the immunotherapeutic treatment of those disorders.

Glucocorticoid treatment is the standard therapy for immune cytopenias such as immune thrombocytopenic purpura and immune hemolytic anemia (1, 8), but its usefulness is limited by significant side effects. Glucocorticoids inhibit the expression of splenic macrophage Fcγ receptors and increase cell survival (6, 8, 22, 23). In an animal model (the guinea pig), progesterones have been shown to decrease the clearance of IgG-sensitized cells (11, 24) through their effect on the expression of these receptors. However, this effect of progesterone has not been reported before in humans.

MA is a progestogen already approved for the treatment of HIV-associated anorexia-cachexia (14, 25, 26) but not yet for thrombocytopenia. We have performed a prospective study of 28 patients presenting HIV-associated thrombocytopenia, with shortened platelet survival and elevated platelet-associated IgG, who were being treated with MA for anorexia-cachexia. The objective was to assess the role of MA in the specific treatment of HIV-associated thrombocytopenia by monitoring the platelet count and platelet survival and the surface expression and functioning of peripheral blood monocyte FcγRI and FcγRII.

MATERIALS AND METHODS

Patients. We prospectively studied patients with HIV-associated thrombocytopenia treated in the outpatient clinic of our hospital between January 1992 and December 1995. Data on 28 of these patients who were taking MA for anorexia-cachexia (4 females and 24 males; age, 29 ± 12 years) and who fulfilled the inclusion criteria and completed the 6-month follow-up period were analyzed (Table 1).

The patients eligible for this study were between 18 and 60 years old, with at least three platelet counts of less than 50,000/ml during the previous 6 months, shortened platelet survival, and elevated platelet-associated IgG levels. We excluded patients with renal disease (plasma creatinine ≥2 mg/dl), liver disease (prothrombin or prothrombin time below 80% of the levels of controls, serum albumin <3.5 mg/dl, or bilirubin ≥3 mg/dl), cirrhosis, ultrasonographic signs of portal hypertension, active infection, sepsis, neoplasia, and autoimmune disorders. Patients with an AIDS-defining event or those receiving immunosuppressive treatment during follow-up were also excluded. Patients gave informed consent before enrollment. The study was approved by the Ethic and Clinical Trials Committee of our university hospital.

The characteristics of the patients are given in Table 1. None of the patients were excluded because they developed an AIDS-defining event. Two patients were excluded because they failed to comply with MA treatment during the first month of treatment. These two patients were not considered for analysis because they generated no more data than the parameters before commencement of the treatment.

Seventeen patients acquired HIV infection by intravenous drug abuse (60.71%), 6 patients by heterosexual transmission (21.43%), and 5 patients by homosexual or bisexual practices (17.86%). Six patients were being treated with...
two nuclease reverse transcriptase analogs (four with zidovudine [AZT] plus zalcitabine [ddC] and two with AZT plus didanosine [ddI]), and 22 patients were being treated with AZT monotherapy when this study commenced. No patients had received protease inhibitors, and all of them had detectable virus loads (200 HIV RNA copies/ml) in plasma on enrollment or during follow-up.

**Treatment and response.** Oral treatment with MA, 320 mg twice per day for the first month, was increased to 320 mg three times per day during the second month if a complete response was not obtained at the end of the first month. MA was not administered during the third month after enrollment. After the fourth month, patients were again treated with MA at the same dose as that given during the second month. The MA was provided to patients on their weekly visits to the clinic, at which time the attending nurse applied a questionnaire to confirm that the patient had complied with the treatment regimen (Table 1).

An increase in the platelet count of ≥80,000/ml over the baseline value was taken as a complete response, an increase in the platelet count of ≥40,000/ml but ≤79,999/ml as a partial response, and an increase in the platelet count of ≤38,999/ml as no response.

**Study protocol.** Complete blood count, the Westergern sedimentation rate, urinalysis, and renal and liver function tests were done on enrollment and monthly thereafter. Serologic tests for hepatitis B and C virus, syphilis, antinuclear antibodies, and bone marrow aspiration were performed on enrollment.

The number of CD4 and CD8 lymphocytes and the plasma HIV RNA load were determined on enrollment and after 3 and 6 months. CD4 and CD8 cells were analyzed by flow cytometry, and the plasma HIV RNA load was determined using a commercial PCR technique (AmpliSens, Roche Diagnostics, Madrid, Spain) in samples frozen at −70°C.

Platelet survival, platelet-associated immunoglobulin (IgG and IgM), and circulating immune complexes were measured both on enrollment and at the end of the second, fourth, and sixth months. Survival of autologous platelets was calculated immune complexes were measured both on enrollment and at the end of the study (without Ca²⁺ or Mg²⁺) containing 0.5 mM EDTA. Cells presented the appearance of monocytes after Wright-Giemsa staining (Diff-Quik, Dade Diagnostics, Madrid, Spain); more than 95% stained positive with nonspecific esterase, more than 90% were able to phagocytose latex particles and to readhere to plastic, and more than 95% were viable as assayed by trypsin blue dye exclusion (9, 17, 18).

**Flow cytometry.** Monoclonal antibodies (MAbs) directed against FcγRI (MAb32.2), FcγRII (MAbHIV.3), and FcγRIII (MAb3G8) were used in indirect immunofluorescence binding studies to assess protein expression of these receptors on monocytes. Experiments were performed as previously described (9, 17, 18). Cells were incubated with antibodies for 30 min at 4°C and washed twice with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin and 0.02% sodium azide. Bound antibodies were labeled by incubation with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse Ig antibody (Tago, Inc., Burlingame, Calif.) for 30 min at 4°C. Cells were washed twice, fixed with 4% paraformaldehyde, and analyzed by flow cytometry. Fluorescence was measured by a FACScan cytometer with Consort 32 software (Becton-Dickinson, Madrid, Spain). For all samples, 10,000 events were recorded on a logarithmic fluorescence scale, and the actual mean fluorescence intensity (MFI) for each sample was determined. In order to correct for autofluorescence, the MFI of a nonreactive murine IgG1 antibody (P3×63) was subtracted from the MFI of the anti-FcγR-stained cells. Change in mean fluorescence intensity was calculated as ∆MFI (change in MFI) = MFI of anti-FcγR MA-treated cells − MFI of P3×63 MA-treated cells = MFI of anti-FcγR control cells − MFI of P3×63 control cells.

**Preparation of IgG-sensitized RBCs.** Antibody-sensitized sheep erythrocytes were prepared as previously described (9, 17, 18). In brief, 1 × 10⁹ sheep RBCs in 1.0 ml of 0.01-mol/liter EDTA buffer were sensitized by adding mouse monoclonal antibody Sp2/0/HL subclass IgG2b (Serotec Ltd., Bicester, Oxon, England), in 0.1 ml at 37°C for 1 h. We used the optimal antibody dilutions found in previous studies, 1:20 and 1:80 (9, 17, 18). The IgG-sensitized sheep RBCs were washed twice and resuspended in Hank’s balanced salt solution to a final concentration of 10⁹ cells/ml.

**Monocyte recognition of sheep IgG-sensitized RBCs.** In vitro recognition of sheep IgG-sensitized red blood cells RBCs by peripheral blood monocytes and the surface expression of peripheral blood monocyte Fcy receptors, FcγRI and FcγRII, were determined on inclusion and at the end of the first, second, fourth, and sixth months after enrollment.

**Preparation of monocytes.** Human monocytes were isolated in suspension as previously described (9, 17, 18). Briefly, mononuclear cells were isolated from heparinized blood by density gradient centrifugation. Between 40 × 10⁶ and 40 × 10⁶ mononuclear cells in RPMI 1640 medium containing 25 mg of glutamine, 100 U of penicillin, 100 mg of streptomycin, and 10% heat-inactivated fetal calf serum (FCS) per ml were incubated in 125-cm² tissue culture flasks (Corning, Madrid, Spain) pretreated with heat-inactivated FCS (M.A. Bioproducts, Madrid, Spain) and were then incubated for 1 h at room temperature and washed five times with RPMI 1640 containing 10% FCS to remove nonadherent cells. Adherent cells were harvested by striking the flasks against a soft surface to dislodge the cells and were then rinsed with Hank’s balanced salt solution (without Ca²⁺ or Mg²⁺) containing 0.5 mM EDTA. Cells presented the appearance of monocytes after Wright-Giemsa staining (Diff-Quik, Dade Diagnostics, Madrid, Spain); more than 95% stained positive with nonspecific esterase, more

**RESULTS**

Treatment with MA under the regimen described enhanced the platelet count in all 28 patients. Platelet count increased from 22,280 ± 2,110 per ml (mean ± standard error of the mean [SEM]) before treatment (to 232,472 ± 3,122 per ml after 2 months of MA treatment (P < 0.001) (Fig. 1). One month after MA withdrawal (i.e., in months 3 and 5), the mean platelet counts (171,830 ± 2,742 per ml and 247,378 ± 3,014 per ml, respectively)

### Table 1. Patient characteristics

| Time of treatment | MA dose (mg/kg/day) | Response at end of mo (no. of patients) | Body mass index (kg/m²) | CD4 count (cells/µl) | HIV RNA load in plasma (RNA copies/ml) |
|-------------------|---------------------|----------------------------------------|--------------------------|----------------------|-------------------------------------|
|                   |                     | Complete | Partial | No response |                     |                                 |
| Before            |                     | 640      | 15      | 5           | 19 ± 3               | 252 ± 23                         | 24,947 ± 1,201                   |
| Mo 1              | 826 ± 51            | 20       | 5       | 3           | 19 ± 2               | 242 ± 21                         | 27,645 ± 2,471                   |
| Mo 2              | 826 ± 51            | 22       | 3       | 3           | 21 ± 5               | 22 ± 3                           | 267 ± 19                         | 27,273 ± 2,211                   |
| Mo 3              | None                | 15       | 8       | 3           | None                | None                             | None                             |
| Mo 4              | None                | 12       | 8       | 7           | None                | None                             | None                             |
| Mo 5              | None                | 19       | 6       | 3           | None                | None                             | None                             |
| Mo 6              | None                | 12       | 8       | 8           | None                | None                             | None                             |

*Twenty-eight patients (4 female, 24 male; mean age ± SEM, 29 ± 7 years) with HIV-associated thrombocytopenia, with shortened platelet survival and elevated platelet-associated IgG, treated with MA for anorexia-cachexia, participated in the study. The characteristics of patients on enrollment (before treatment), the dose of MA and the evolution of HIV infection during follow-up after are shown. Results are expressed as means ± SEM. HIV-pVL = HIV-RNA load in plasma.
respectively) were significantly higher than pretreatment values ($P < 0.001$). Two months after MA withdrawal (i.e., in month 6), the mean platelet count ($41,170 \pm 2,436$ per ml) did not differ significantly from baseline values ($P = $ not significant). Twenty two patients (78.57%) presented a complete response with MA treatment (i.e., in month 4). One month after terminating MA treatment (i.e., in month 5), 19 patients (67.86%) presented a complete response, 6 patients a partial response, and 3 patients no response, while a month later (i.e., in month 6) 12 patients (42.86%) were still in complete response.

Platelet survival (mean $\pm$ SEM) was significantly shortened on enrollment ($T_{1/4} = 118.50 \pm 28$ min) ($P < 0.001$). Two months of MA treatment increased the platelet survival in all patients; 17 of the 28 patients (60.07%) attained normal platelet survival. Mean platelet survival increased significantly to $T_{1/4} = 350.10 \pm 43$ min after 2 months of MA treatment (i.e., by month 2) ($P < 0.001$). Two months after MA withdrawal (i.e., in month 6), mean platelet survival ($156.90 \pm 87$ min) did not differ significantly from baseline values (Fig. 1). Platelet-associated immunoglobulins, change in platelet-associated IgG, and change in platelet-associated IgM were not significantly altered by MA treatment at any stage of the study. Results are expressed as mean $\pm$ SEM. $*, P < 0.001$.

We assessed the surface expression of both Fc$\gamma$ receptors expressed by peripheral blood monocytes, Fc$\gamma$RI and Fc$\gamma$RII by using flow cytometry (Table 2). Results are expressed as percent inhibition of the mean fluorescence intensity below pretreatment values (mean $\pm$ SEM). The expression of peripheral blood monocyte Fc$\gamma$RI decreased significantly 1 and 2 months after MA treatment, by $41.13\% \pm 3.71\%$ and $51.80\% \pm 4.73\%$, respectively ($P < 0.001$). The expression of peripheral blood monocyte Fc$\gamma$RII decreased significantly 1 and 2 months after MA treatment by $36.83\% \pm 4.21\%$ and $45.53\% \pm 4.47\%$, respectively ($P < 0.001$). Two months after MA withdrawal, the expression of peripheral blood monocyte Fc$\gamma$ receptors Fc$\gamma$RI and Fc$\gamma$RII did not differ significantly from baseline (Table 2).

The in vitro recognition of IgG2b-sensitized RBCs by peripheral blood monocytes at two ionic strengths (0.15 and 0.07) was determined to assess the functioning of Fc$\gamma$RI ($\Delta \mu = 0.15$) and Fc$\gamma$RII ($\Delta \mu = 0.07$) (9, 18). Results are expressed as
The data obtained in this short-term study suggest that treatment with MA significantly enhances platelet count and survival; it decreases the surface expression of macrophage Fcγ receptors, FcγRI and FcγRII, and does not alter the platelet-associated immunoglobulin. Treatment with MA for 1 month produced a complete response in the majority (78.57%) of the patients studied, while 2 months after MA withdrawal, 42.86% of patients were still in complete response.

Platelet-associated IgG or IgM and circulating immune complexes were not altered by MA treatment. The increased platelet count and survival and decreased expression of peripheral blood monocyte Fcγ receptors, FcγRI and FcγRII, observed do not seem to be due to weight gain or to progression of HIV infection, since those effects of MA were not correlated with the body mass index or with the number of CD4 cells and HIV RNA plasma viral load, respectively. No significant changes were observed in the body mass index, number of CD4 cells, or plasma HIV load during follow-up.

Enhanced platelet production by MA may in part explain the increased platelet count. Nevertheless, our findings of enhanced platelet survival and decreased expression of macrophage Fcγ receptors, with no alteration of platelet-associated immunoglobulin following MA treatment, cannot be explained by an improved bone marrow production of platelets. Therefore, the most consistent mechanism for the observed MA treatment effect is a decreased macrophage Fcγ receptor-dependent phagocytosis, resulting in longer platelet survival and platelet count.

It has recently been observed that the beneficial effects of treatment with intravenous immunoglobulin for immune cytopenias depends upon induction of the surface expression of macrophage FcγRIIB (21). Our finding of decreased monocyte FcγRII expression is not in contradiction with that observation, since our experimental design does not differentiate between the expression of receptor isotypes FcγRIIA and FcγRIIB. Thus, while the surface expression of the monocyte receptor FcγRIIB may indeed be enhanced, the surface expression of the other receptor isotype FcγRIIA may have been decreased. Another pathophysiologic limitation of our study is the lack of data on the macrophage FcγRIII that is also involved in the pathophysiology of immune cytopenias (2, 3, 5–7, 12). We have not performed invasive studies to harvest macrophages (peritoneal, pulmonary, or splenic). Nevertheless, we did determine the surface expression of FcγRIII by peripheral blood monocytes in some patients, but its expression was either very low or absent.

Our results indicate that MA treatment improves platelet count and platelet survival in patients with HIV-associated thrombocytopenia, with shortened platelet survival and with elevated platelet-associated immunoglobulin. Therefore, progestergones such as megestrol acetate may be used in the treat-
ment of immune cytopenias, with few short-term side effects. The effect of MA compared with glucocorticoids and the risk-benefit ratio of glucocorticoids in combination with MA for the treatment of immune cytopenias await confirmation by appropriate clinical trials.

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