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Granulocyte-Colony Stimulating Factor Treatment of Lupus Autoimmune Disease in MRL-lpr/lpr Mice

Flora Zavala,* Annie Masson,* Karine Hadaya,* Sophie Ezine,† Elke Schneider,‡ Olivier Babin,* and Jean-François Bach*

G-CSF not only functions as an endogenous hematopoietic growth factor for neutrophils, but also displays pro-Th2 and anti-inflammatory properties that could be of therapeutic benefit in autoimmune settings. We evaluated the effect of treatment with G-CSF in a murine model of spontaneous systemic lupus erythematosus, a disease in which G-CSF is already administered to patients to alleviate neutropenia, a common complication. Chronic treatment of lupus-prone MRL-lpr/lpr mice with low doses (10 μg/kg) of recombinant human G-CSF, despite the induction of a shift toward the Th2 phenotype of the autoimmune response, increased glomerular deposition of Igs and accelerated lupus disease. Conversely, high-dose (200 μg/kg) treatment with G-CSF induced substantial protection, prolonging survival by >2 mo. In the animals treated with these high doses of G-CSF, neither the Th1/Th2 profile nor the serum levels of TNF-α and IL-10 were modified. Despite the presence of immune complexes in their kidney glomeruli, no inflammation ensued, and serum IL-12 and soluble TNF receptors remained at pre-disease levels. This uncoupling of immune complex deposition and kidney damage resulted from a local down-modulation of FcγRIII (CD16) expression within the glomeruli by G-CSF. Our results demonstrate a beneficial effect of high doses of G-CSF in the prevention of lupus nephritis that may hold promise for future clinical applications, provided caution is taken in dose adjustment. The Journal of Immunology, 1999, 163: 5125–5132.

Granulocyte CSF was first described and cloned in 1986 (1) and has been used since with high efficiency and inoquity as a key molecule for the mobilization of early myeloid progenitors belonging chiefly to the granulocyte lineage. In clinical trials, it has been shown to improve circulating neutrophil counts after irradiation or chemotherapy, as well as after infection (2).

G-CSF treatment likewise modifies the monocyte/macrophage lineage, both quantitatively and functionally, as assessed, respectively, by increased cell counts in the blood and a shift to an anti-inflammatory compartment (3) that conferred protection in murine models of experimental endotoxemia (4).

The T cell compartment is also affected because G-CSF-mobilized blood cells display a significant reduction in T cell responses (5). Alloreactive responses are decreased by treatment with G-CSF as assessed by the reduced acute rejection of liver transplants (6) and the lower incidence of graft-versus-host disease (GVHD) in recipients of G-CSF-mobilized progenitor cells (7).

Other studies in mice have confirmed that treatment with G-CSF confers a significant protection against acute GVHD (8) that correlates with a down-regulation of Th1-derived cytokines and an increased production of Th2-derived cytokines. These T cell effects of G-CSF are most probably mediated indirectly, because receptors for G-CSF have not been detected on this cell type (1).

In autoimmune diseases, and particularly in systemic lupus erythematosus, polarized T cell phenotypes (Th1 vs Th2) often display a complex relationship to the pathogenesis. Systemic lupus erythematosus is a humoral autoimmune syndrome, characterized by the serological appearance of antinuclear autoantibodies. Glomerular immune complex deposition may lead to renal failure and mortality. MRL-lpr/lpr mice spontaneously develop a severe form of lupus-like disease. Recent studies (9) have demonstrated that disease acceleration correlated with increased IgG2a/IgG1 and IgG3/IgG1 isotype ratios of the autoantibodies as well as increased production of IFN-γ over IL-4 by spleen and lymph node CD4 T cells. Several reports have demonstrated that neutralization of Th1 cytokines was beneficial in lupus treatment. Indeed, MRL-lpr/lpr mice deficient for the IFN-γ gene (10, 11) or the IFN-γ receptor gene (12) were protected from disease, as were (NZB × NZW)F1 mice treated with anti-IFN-γ Abs (13) or IFN-γ-soluble receptors (14). Similarly, MRL-lpr/lpr mice treated with anti-IL-12 Abs showed enhanced survival (15). Moreover, (NZW × C57BL/6.Jaa)F1 mice transgenic for IL-4 (16) exhibited an antigenic response shifted toward the Th2 phenotype and did not develop the disease. However, Th2-derived cytokines may also play a deleterious role, because MRL-lpr/lpr mice deficient for the IL-4 gene were protected from disease (10), and anti-IL-4 Ab prevented the onset of lupus nephritis in (NZB × NZW)F1 mice (17).

In addition to this complex interplay of Th1/Th2 cytokines at the initial phase of the disease, an inflammatory reaction develops at a more advanced stage, and IL-12 (18), TNF-α, and IL-1 (19) mRNA expression within the renal cortex has been reported in MRL-lpr/lpr mice suffering from nephritis.

This line of evidence, along with the fact that G-CSF is already administered to lupus patients to alleviate neutropenia (20), prompted us to investigate whether the reported pro-Th2 and anti-inflammatory properties of G-CSF might be of therapeutic benefit
in the treatment of lupus disease. Therefore, we evaluated the effect of different dose regimens of G-CSF on the development of the lupus syndrome in MRL-lpr/lpr mice.

Materials and Methods

Mice

Six-week-old female MRL-lpr/lpr mice were purchased from Harlan Olac (Oxon, U.K.) and maintained in our animal facilities under specific pathogen-free conditions. Animals were randomly distributed into the different treatment groups (n = 12 per group).

Treatment of MRL-lpr/lpr mice with recombinant human G-CSF

Recombinant human G-CSF (Amgen, Thousand Oaks, CA) was injected s.c. every 6 wk for five consecutive days, starting at 9 wk of age. Mice at this age were no longer on amlodipine. They had detectable anti-nucleosome but no anti-dsDNA Abs, typical of the onset of the autoantibody response in young lupus mice (21). Two doses of G-CSF were used: 10 μg/kg and 200 μg/kg, reported to protect C57BL/6 mice from GVHD (8) and eliciting stem cell mobilization (22), respectively. The control group received the carrier solution (5% dextrose in sterile H2O).

A second protocol consisted in a single late treatment with 200 μg/kg of G-CSF for five consecutive days at 13 wk of age, when mice presented high levels of circulating anti-nucleosome Abs with occasional low levels of anti-dsDNA Ab and emerging albuminuria.

Assessment of renal disease

Renal disease was evaluated by the development of albuminuria and histological modifications of the kidney. Albuminuria was measured colorimetrically using commercially available sticks (Albyn-Test, Boehringer Mannheim, Mannheim, Germany).

Albumin concentrations over 1 mg/ml were considered positive. Kidney histology was performed on mice that were killed at 20 wk of age. The kidneys were removed, fixed in 3.6% paraformaldehyde in PBS, and in- cluded in paraffin. Sections of 5 μm were stained with hematoxylin-eosin and analyzed for glomerulonephritis. Evaluation of the Ig deposits in the glomeruli was performed on kidneys frozen in OCT (Tissue Tek-Miles, Elkart, IN). Fresh cryostat sections of 5 μm were fixed in acetone for 5 min, air-dried, and saturated with 10% goat serum (Vector, Burlingame, CA) in PBS for 30 min at room temperature. After three washes, they were incubated for 30 min at room temperature with FITC-conjugated rat anti-mouse IgG3 (or anti-IgG2a) Ab (Southern Biotechnology Associates, Birmingham, AL), diluted 1/100 in PBS, supplemented with 1% goat serum. Stained sections were observed with a conventional fluorescence microscope.

Immunohistochemical detection of Fcy receptors on kidney sections

Acetone-fixed cryostat sections (5 μm) were washed twice and then incubated with 2 μg of 2.4G2 mAb (produced in our laboratory from hybridoma) for 60 min. After three washes, biotinylated anti-rat Ab (Zymed Laboratories, San Francisco, CA) was added for 15 min, sections were washed three times, and HRP-coupled streptavidin (Zymed, San Francisco, CA) for 60 min. After three washes, the supernatants were removed and stored at −80°C until cytokine assays.

The following pairs of mAbs (prepared in our laboratory from hybridoma or purchased from Pharmingen (Becton Dickinson, Le Pont de Clai, France)) were used for the ELISA measurements: AN 18 (2 μg/ml) and R46A2 (0.5 μg/ml) for IFN-γ, 11B11 (2 μg/ml) and BV6D6 (0.25 μg/ml) for IL-4, JES2.A5 (5 μg/ml) and JES5-16E3 (0.5 μg/ml) for IL-10, for capture and detection, respectively. Bound secondary Abs were detected with peroxidase-conjugated streptavidin from Amersham (Amer- sham, Buckingham, U.K.) for IL-4 and IL-10 and from Vector Laboratories (Burlingame, CA) for IFN-γ, with o-phenylene-diamine dichloride (Sigma) as substrate. ODs were measured at 490 nm with a reference filter at 630 nm using a Dynatech microplate reader (Dynex Technologies, Issy les Moulineaux, France).

TNF-α was measured with a commercial ELISA from Genzyme.

FACS analysis of spleen and bone marrow cells

The cell-surface phenotype of splenocytes and bone marrow cells was analyzed by flow cytometry. All cells were incubated in PBS supplemented with 2% FCS and 0.02% sodium azide. One million cells per sample were incubated in 20 μl for 15 min at room temperature under constant shaking. The following mAbs were used: anti-CD4 (clone GK1.5), anti-CD8 (clone 53-6.7), anti-αβ TCR (clone H57-597), anti-B220 (clone RA3-6B2), anti-CD3 (clone 145-2C11), and anti-Gr1 (clone Mac1 (clone M170), and anti-GR-1 (clone RA3-8CS)). They were pro- duced in our laboratory from hybridomas or purchased from Pharmingen and used coupled to biotin and revealed by streptavidin-tricolor (Caltag, South San Francisco, CA), PE, or FITC.

For general phenotypic analysis, 10,000 events were acquired using the FACScan flow cytometer (Becton Dickinson, San Jose, CA). Analysis of the acquired data was performed using Lysis II software (Becton Dickinson).

In vitro colony-forming assay

Total CFU-C were quantified in “Complete Methylcellulose Medium with Recombinant Cytokines and Erythropoietin” (MethoCult GF M3434, Stem Cell Technologies, Vancouver, Canada). Cells were plated in a final vol- ume of 1 ml at concentrations ranging from 5–50 × 10³ cells per culture dish (Falcon 1008, Becton Dickinson, Lincoln Park, NJ). Colonies were scored on day 7–8.

Statistical analysis

Differences in serological parameters between the different treatment groups were evaluated with Student’s t test. The occurrence of mortality and albuminuria in the different treatment groups were plotted using the Kaplan-Meier method, i.e., nonparametric cumulated survival plots. The statistical comparison between the different curves was performed using the Mantel-Cox log rank test, which provided the corresponding χ² values. Values of p < 0.05 were considered significant.
Results

High-dose G-CSF efficiently retards the development of albuminuria and mortality in lupus-prone MRL-1pr/lpr mice, whereas treatment with low-dose G-CSF accelerates disease

In control MRL-1pr/lpr mice, the onset of albuminuria occurred at the age of 13 wk. Fifty percent of the animals were positive at 19 wk and 100% at 25 wk (Fig. 1A). The mortality rate reached 50% in 23-wk-old mice (Fig. 1B).

Chronic administration of 200 μg/kg of G-CSF significantly delayed the onset of the disease, with 50% albuminuric mice at 23 wk of age, i.e., 1 mo later than in the excipient group. This dose regimen notably prolonged survival, as 50% mortality was not even reached at 32 wk of age, when the experiment was stopped. It is noteworthy that a single, late injection of G-CSF at 200 μg/kg given to 13-wk-old mice caused an initial delay in the progression of albuminuria, which attained the 50% level only at the age of 22 wk. However, the treated group caught up with excipient controls thereafter (data not shown).

In contrast, repeated administration of low-dose G-CSF at 10 μg/kg per injection markedly accelerated the onset of albuminuria, which was already detected at the age of 15 wk in one mouse of two, i.e., 1 mo earlier than in the excipient group. Mice died rapidly in this group (80–90% at 20 wk of age).

Renal histology and glomerular Ab deposits in high vs low-dose G-CSF-treated MRL-lpr/lpr mice

Kidneys from 20-wk-old mice representative for each treatment group were collected and sectioned. For light microscopy, the histologic slides were routinely processed and stained with hematoxylin-eosin by standard procedures (Fig. 2, a–c). In the control group (Fig. 2a), the lesions, essentially localized in the cortex, revealed a moderate endoproliferative glomerulonephritis with initial crescent formation and deposits along the capillary walls, “wire loops.” Almost intact glomeruli were observed in kidneys from mice treated with 200 μg/kg of G-CSF (Fig. 2b), the group that was significantly protected from disease. By contrast, mice injected with G-CSF at 10 μg/kg (Fig. 2c) had a severe renal disease, consisting of a diffuse proliferative and crescentic glomerulonephritis with widespread “wire loops” and fibrinoid necrosis, interstitial and perivascular inflammatory cells, and moderate tubulo-interstitial abnormalities. Neither vasculitis nor thrombotic microangiopathy were detected in any of the three groups.

Indirect immunofluorescence analysis performed with mAbs directed against two IgG subclasses, IgG2a and IgG3, revealed that labeling with anti-IgG3 (shown in Fig. 2, d–f) was slightly weaker in the kidney of mice chronically injected with 200 μg/kg of G-CSF (Fig. 2e) than in control mice (Fig. 2d). Conversely, IgG2a deposits were present at similar levels in both treated and control mice (not shown). In mice treated with 10 μg/kg of G-CSF, IgG3 deposits (Fig. 2f) were much increased relative to the control group, as were IgG2a (not shown).

Inflammatory cytokine levels are reduced by treatment with high-dose G-CSF

Inflammation is a hallmark of disease progression in MRL-1pr/lpr mice. TNF soluble receptors (TNF-sR55 and TNF-sR75) represent stable and sensitive markers of an ongoing inflammatory reaction, and their levels are increased in the circulation of relapsing lupus patients (24, 25). In addition, IL-12 has been detected in the circulation of MRL-1pr/lpr mice but not in MRL +/+ mice, which develop only mild and late nephritis, or in nonautoimmune BALB/c mice (15).

As can be seen in Fig. 3A, the two soluble TNF receptors were increased in the serum of control MRL-1pr/lpr mice between 14 and 20 wk of age but did not significantly change with age (particularly TNF-sR55) in mice subjected to the high-dose G-CSF regimen. At 20 wk of age (Fig. 3B), serum IL-12 was significantly decreased in MRL-1pr/lpr mice that had been treated chronically with G-CSF at 200 μg/kg.

There was no significant difference in serum levels of soluble TNF receptors or of IL-12 between mice injected with low-dose G-CSF and controls.

Serum levels of IL-10 (6.4 ± 4.3 pg/ml vs 9.3 ± 1.7 pg/ml and 6.6 ± 2.8 pg/ml, for the excipient vs G-CSF 10 μg/kg and 200 μg/kg groups, respectively) at 20 wk of age, were not significantly different between the three groups of mice. Any possible increase in TNF-α levels measured at the same age had no relationship to disease outcome (10.45 ± 3.9 pg/ml vs 25.4 ± 13 pg/ml and 27.2 ± 16 pg/ml, for the excipient vs G-CSF 10 μg/kg and 200 μg/kg groups, respectively).

G-CSF-treatment modulates the glomerular expression of FcγR in MRL-lpr/lpr mice

Knowing that G-CSF modulates FcγR expression on neutrophils and macrophages (26) and that FcγRIII (CD16) is expressed by mesangial cells and mediates the inflammatory response to im-
mune complexes (27), we examined the glomerular expression of CD16 in kidneys of mice from the different treatment groups. Immunohistochemical staining of glomeruli (Fig. 2g) from 20-wk-old control mice with the 2.4G2 Ab revealed FcγRII+RIII) receptors expression on infiltrating inflammatory cells as well as mesangial cells. Interestingly, the well-preserved, noninflamed glomeruli

FIGURE 2. Effect of G-CSF on glomerulonephritis development and Fc receptor expression in MRL-lpr/lpr mice. Representative kidney sections were obtained from 20-wk-old mice. Top, Hematoxylin-eosin staining of an untreated mouse (a) showing an endocapillary glomerulonephritis with initial deposits along the capillary walls; mice treated with G-CSF 200 μg/kg (b) had almost intact glomeruli, whereas mice injected with G-CSF 10 μg/kg (c) displayed accelerated endoproliferative and crescentic glomerulonephritis with enlarged glomeruli, heavy “wire loops” deposits, and fibrinoid necrosis (×40). Middle, Immunofluorescence showing IgG3 deposits was slightly reduced in high-dose G-CSF treated mice (e) relative to excipient (d), whereas abundant deposits in all glomeruli were present in the low-dose G-CSF-treated mice (f) (×25). Bottom, Immunostaining for FcγRII+RIII) with the 2.4G2 Ab was almost undetectable in the 200 μg/kg G-CSF-treated mice (h), whereas the low-dose G-CSF-treated mice (i) displayed an intense staining (×40). j, Semiquantitative evaluation of the effect of G-CSF treatment on 2.4G2 labeling intensity in kidney glomeruli in mice. Mice (three per group) were sacrificed at the age of 20 wk, and evaluation of the percentage of glomeruli with graded 2.4G2 labeling intensity was performed on at least 30 glomeruli per kidney section, in a blinded fashion, using an arbitrarily defined semiquantitative score. Open bars represent values of mice treated with excipient (Exc), dark hatched bars represent values of mice treated with 10 μg/kg G-CSF (G10), and bars with gray points represent values of mice treated with 200 μg/kg G-CSF (G200). *, p = 0.014; **, p = 0.003; ***, p = 0.001, as assessed by Student’s t test.
from mice treated with G-CSF at 200 μg/kg (Fig. 2h) were practically negative for 2.4G2 staining, contrasting with the increased staining exhibited by mice treated with low-dose G-CSF (Fig. 2i).

This was confirmed using a semiquantitative score to evaluate in a blinded fashion the intensity of labeling of at least 30 glomeruli per kidney section for each of three mice per group (Fig. 2j).

**Effect of G-CSF on autoantibody production and isotype balance**

The humoral autoimmune response in treated mice was evaluated both quantitatively, by measuring circulating autoantibody levels, and qualitatively, by determining their isotype profile.

As shown in Fig. 4, excepting a lower mean level of circulating anti-nucleosome IgG2a isotype (p < 0.05, relative to controls), no significant changes in isotype ratio (calculated as the mean of individual ratios) were detectable in the high-dose G-CSF group when compared with excipient controls, whether the anti-nucleosome (Fig. 4A) or the anti-dsDNA (Fig. 4B) response was considered. Total circulating anti-nucleosome IgG of all four isotypes were reduced by 50% as compared with the control group, whereas those directed against dsDNA did not significantly differ from controls.

Treatment with 10 μg/kg of G-CSF induced a 5- and 10-fold drop in the concentration of anti-nucleosome IgG2a and IgG3 Abs, respectively (Fig. 4A). Concomitantly, a 10-fold increase in IgG1 levels occurred relative to the control group, resulting in a dramatic reduction of IgG2a/IgG1 and IgG3/IgG1 ratios. These changes were detectable at 17 wk of age, i.e., 8 wk after the first treatment period, and remained stable afterward (not shown). The sum of all four isotypes of circulating anti-nucleosome IgG Abs was reduced by ~30%. Interestingly, this marked switch in the isotype profile was confined to the anti-nucleosome Ab response, as no such changes could be detected in the anti-dsDNA response (Fig. 4B) at 20 wk of age and/or later. However, total anti-dsDNA activity comprising all four isotypes was 2-fold increased in mice treated with 10 μg/kg of G-CSF.

None of the groups of mice exhibited significant differences in circulating levels of total IgG of each of the four isotypes (not shown).

**Effect of G-CSF treatment on cellularity and on the cytokine profile in MRL-lpr/lpr mice**

At 20 wk of age, i.e., 4 wk after the last series of G-CSF injections, when differences in the disease incidence were maximal between the three groups of mice, cellular analysis in spleen and bone marrow was performed at the same time as cytokine assays.

![FIGURE 3.](http://www.jimmunol.org/)

**FIGURE 3.** Serum levels of soluble TNF receptors and IL-12 in G-CSF-treated MRL-lpr/lpr mice. Sera collected at 14 and/or 20 wk of age were evaluated for TNF-sR55, TNF-sR75, and IL-12 contents using specific ELISA. Data represent the mean ± SEM of soluble receptor or cytokine serum levels in six mice per group. High-dose G-CSF treatment lowered the levels of TNF-sR55 (**, p = 0.026), TNF-sR75 (NS), and IL-12 (***, p = 0.003) in 20-wk-old mice. For bar symbols, refer to Fig. 2.

![FIGURE 4.](http://www.jimmunol.org/)

**FIGURE 4.** Autoantibody activities in G-CSF-treated MRL-lpr/lpr mice. Every 6 wk, starting at 9 wk of age, MRL-lpr/lpr mice were injected on five consecutive days with G-CSF at 200 μg/kg, 10 μg/kg, or excipient. Sera were collected every 3 wk and assayed by ELISA for isotypes of anti-nucleosome (A) and anti-ds-DNA (B) IgG isotypes. Data are means ± SEM from sera collected from 12 mice per group at 14 and 17 wk of age for anti-nucleosome autoantibodies and at 17 and 20 wk of age for anti-ds-DNA activity. Isotype ratios are means ± SEM of 12 individual IgG2a/IgG1 and IgG3/IgG1 ratios in each group. ***, p < 0.01; **, p < 0.05. For bar symbols, refer to Fig. 2.)
Changes in total spleen cellularity are depicted in Table I. Double negative (DN) T cells, typical of the lpr phenotype, were reduced in both G-CSF treated groups. Total spleen cell numbers were reduced (−45%) in mice treated with low-dose G-CSF, but were unchanged in the group treated with high-dose G-CSF, in which Mac-1⁺ cells had considerably expanded (+70%). No significant changes were observed in the granulocyte population recognized by the GR-1 Ab. In the bone marrow, both cellularity and incidence of phenotypically defined cell subsets were similar to those of excipient controls. The frequency of spleen and bone marrow progenitors forming colonies in methylcellulose remained also unchanged at this particular time point, 4 wk after cessation of treatment (data not shown).

When CD4 spleen cells were compared for their cytokine production in response to anti-CD3 Ab (Fig. 5), no modification of the cytokine profile in response to anti-CD3 was seen when G-CSF was administered at 200 μg/kg per injection. However, the low-dose group displayed a significant decrease in IFN-γ production and a tendency, although not statistically significant, to generate more IL-4. TNF-α production was also reduced but IL-10 remained unaffected.

Discussion

We have investigated the effect of G-CSF treatment on the development of lupus autoimmune disease because it has been reported that G-CSF may have pro-Th2 and antiinflammatory properties in certain models (3, 8, 26).

In the present study, we demonstrate that chronic treatment of lupus-prone MRL-lpr/lpr mice with G-CSF at 200 μg/kg exerted a significant protection from nephritis. The TNF-α/IL-10 balance in the circulation, which has been reported to be a determinant for the disease progression in (NZB × NZW)F₁ mice (28), was not affected by administration of G-CSF to MRL-lpr/lpr mice. Instead, the reduced serum levels of IL-12 in 20-wk-old mice and the fact that soluble TNF receptors did not increase with age, but remained at predisease levels highly suggest that antiinflammatory effects of G-CSF were responsible for the protection from nephritis observed in the high-dose treatment. Moreover, the absence of changes in the Th1/Th2 balance in the autoreactive response of the mice injected with these high doses of G-CSF indicates that the antiinflammatory effect was exerted locally, at the glomerular level. At 20 wk of age, kidney histology revealed almost intact glomeruli in treated mice, despite the presence of immune complexes. This observation is reminiscent of the uncoupling of immune complex deposits and kidney damage observed in (NZB × NZW)F₁ mice deficient for the γ-chain of the Fc receptor (29). Interestingly, these γ⁻/⁻ animals, even with significant proteinuria, did not progress to renal failure (29), a feature shared with our group of high-dose G-CSF-treated mice (Fig. 1). The binding of immune complexes to FcγRIII (CD16) expressed by mesangial cells triggers an inflammatory reaction (production of IL-6, TNF-α, and IL-12 in the renal cortex) (18, 27, 30) and contributes to glomerular injury that leads to nephritis. The absence of staining by 2.4G2 Ab (which recognizes FcγRII and FcγRIII) in these kidneys suggests that the protective effect of G-CSF may involve a decrease in FcγRIII (CD16) expression on mesangial cells, similar to the effect seen on neutrophils and macrophages (26), where G-CSF induces the shedding of membrane CD16 in its soluble form but has no effect on FcγRII (CD32). Interestingly, soluble CD16 has been shown to be beneficial in the treatment of murine lupus nephritis (31). In addition, a reduced mesangial proliferation was also observed, which may further contribute to the long-term reduction in 2.4G2 expression induced by high-dose G-CSF treatment.

The paradoxical effect of the low-dose treatment with G-CSF represents a particularly interesting aspect of our study. Indeed, treatment with G-CSF at 10 μg/kg has been shown to protect from GVHD in C57BL/6 mice by inducing a shift toward the Th2 phenotype (8), a model from which, unfortunately, no information is available at high doses. In keeping with this observation, we found that this low dose of growth factor induced a decrease in IFN-γ production, a relative increase in IL-4 secretion, and a corresponding shift in the IgG2a/IgG1 isotype balance of the anti-nucleosome response in lupus-prone MRL-lpr/lpr mice. Nevertheless, in Th2-oriented MRL-lpr/lpr mice, the disease progress was markedly accelerated. This result could be explained by the participation of both Th1- and Th2-derived cytokines in the disease process, a notion that is emerging from an increasing body of evidence obtained in murine lupus models (9–11, 14, 17). The present results provide further support to the above-cited evidence that a shift

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<td>Mac-1⁺</td>
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* Absolute cell numbers are expressed as mean ± SEM from three mice per group.

**Figure 5.** Effect of G-CSF treatment on anti-CD3 induced cytokine production. CD4⁺ cells were purified (≥95%) from 20-wk-old MRL-lpr/lpr mice of the three groups (excipient, 10, and 200 μg/kg of G-CSF) and stimulated for 24 h with coated anti-CD3 (10 μg/ml) in 96-well microplates (2.10⁶ cells/well). Supernatants were collected and stored at −80°C until use. Cytokine assays were performed by ELISA as described in Materials and Methods. Results are expressed as means ± SEM from three mice per group. (**, p < 0.05). For bar symbols, refer to Fig. 2j.
toward Th2 is not necessarily protective in autoimmunity and suggest that autoimmunity should not be reduced to a mere imbalance between Th1 and Th2 cytokines. In particular, it should be taken into account that the evolution of the renal disease is subject to a balance between production and glomerular trapping and/or deposition of specific isotopes of the autoantibodies. Indeed, immunofluorescence studies of kidney sections demonstrated that the aggravation of lupus disease in the low-dose group of G-CSF-treated mice corresponded to enhanced Ig deposits, particularly of the nephritogenic IgG3 and IgG2a isotopes, that might be explained by an increased glomerular expression of FcγRII induced by G-CSF (26).

Interestingly, IgG isotype changes concerned only the autoimmune response and specifically the anti-nucleosome activity, whereas the anti-dsDNA activity remained unchanged. The production of anti-nucleosome Abs that recognize quaternary epitopes of the Ag (21, 32) precedes that of anti-dsDNA in MRL-lpr/lpr mice. Nucleosome-specific T cells are able to accelerate disease progression in (NZB × SWR)F1 mice (33), suggesting that the nucleosome particle, but not its individual components, is the primary T cell Ag in lupus. Therefore, the Th1/Th2 balance of T cell-derived cytokines may preferentially influence the isotype profile of anti-nucleosome Ab production. In addition, studies in lupus patients have recently demonstrated that increased Ab levels of specific isotype during disease relapses were observed only among anti-nucleosome, but not among anti-dsDNA or anti-dsDNA, Abs (Z. Amoura, unpublished observations).

The effect of G-CSF administration on spleen and bone marrow cellularity and phenotype was analyzed in 20-wk-old mice. This time point was chosen because the differences in disease progression between control and treated mice were the most striking. The treatment with G-CSF had been interrupted 4 wk before, which might explain why the numbers of myeloid progenitors and GR1+ granulocytes was not modified. This observation is in agreement with the previously reported transient effect of G-CSF on neutrophil and progenitor cell mobilization (22, 26, 34, 35). The increase in the Mac-1+ population, which is known to be already expanded in MRL-lpr/lpr mice (36), indicates that G-CSF has nevertheless exerted some hemopoietic effect. Interestingly, as previously noted for pluripotent stem cells in other autoimmune strains (37), the number of progenitors forming colonies in methylcellulose was remarkably high in spleens of MRL-lpr/lpr mice (97, 123.8 ± 2.131.0; mean ± SEM from six mice), as compared with age-matched MRL-Mp+/- controls (26,074.3 ± 7.177.6; mean ± SEM from six mice; p < 0.05), possibly hampering their further expansion in response to G-CSF.

Both treatment regimens significantly reduced TCRβ+ CD4+ CD8+ DN cell numbers. It has been documented that the accumulation of DN T cells in spleen and lymph nodes is clearly dissociated from the autoimmune renal disease (38–40). The opposite disease outcome promoted by the high- and low-dose treatment with G-CSF, in spite of a similar reduction in this DN population, confirms that DN cells play no pathogenic role in lupus-prone MRL-lpr/lpr mice. However, it remains to be established whether this apparent decrease of DN cells is effectively due to a reduction or a redistribution to other sites such as liver or peritoneal cavity.

Our data provide the first evidence for a therapeutic impact of hemopoietic growth factors on autoimmune diseases. The protection against lupus nephritis by antiinflammatory doses of G-CSF may hold promise for a clinical evaluation of this factor in lupus patients. Our finding that a 5-day treatment with G-CSF at 200 μg/kg, initiated in 13-wk-old mice suffering already from a beginning proteinuria, still delayed the disease onset, suggests that chronic administration of G-CSF might be beneficial for lupus patients suffering from nephritis, i.e., even at a relatively advanced stage of the disease.

In addition to their fundamental interest, our results are of clinical relevance because G-CSF is already administered to lupus patients to relieve neutropenia, which is a common complication of the lupus syndrome. This is well illustrated by the reports showing that G-CSF efficiently restores normal neutrophil counts in lupus patients. Yet, in the light of our data, the dose of G-CSF injected has to be chosen with caution. Indeed, vasculitis and aggravation of neurolupus were observed in one and two of nine patients treated with G-CSF, respectively (20). Finally, recent clinical trials using G-CSF-mobilized autologous stem cell transplantation in patients with autoimmune diseases, including lupus patients (41), have provided interesting results. Therefore, it is even more important to elucidate the dose-dependent effect of G-CSF on the progression of lupus disease.

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References


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