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CIPROFLOXACIN IMMUNOMODULATION OF EXPERIMENTAL ANTIPHOSPHOLIPID SYNDROME ASSOCIATED WITH ELEVATION OF INTERLEUKIN-3 AND GRANULOCYTE–MACROPHAGE COLONY-STIMULATING FACTOR EXPRESSION

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Objective. **To evaluate the immunomodulatory potential of ciprofloxacin in mice with experimental antiphospholipid syndrome (APS).**

Methods. **Ciprofloxacin or ceftazidime (control antibiotic) was given to mice with experimentally induced APS. The titers of autoantibodies, levels of cytokines, and number of cytokine-producing cells were determined by enzyme-linked immunosorbent assay. Myeloid progenitor cells were determined by granulocyte–macrophage colony-forming unit, and interleukin-3 (IL-3) messenger RNA (mRNA) was tested by Northern analysis.**

Results. **A decrease in the incidence of pregnancy loss and an improvement in the clinical manifestations of APS were noted in the mice treated with ciprofloxacin, compared with the mice given ceftazidime. The effect of ciprofloxacin was found to be associated with increased serum levels of IL-3 and with increased IL-3 mRNA transcription in the splenocytes. Expression of granulocyte–macrophage colony-stimulating factor (GM-CSF) was documented by elevated titers in the sera and elevated numbers of colony-forming cells in the bone marrow.**

Conclusion. **Ciprofloxacin prevents the manifestations of experimental APS. This effect may be associated with increased IL-3 levels and GM-CSF expression.**

The antiphospholipid syndrome (APS) is characterized by the presence of anticardiolipin antibodies (aCL) or/and lupus anticoagulant associated with thromboembolic phenomena, thrombocytopenia, and recurrent fetal loss (1–8). The syndrome may be primary or secondary to systemic lupus erythematosus or associated with other autoimmune disorders, acute myocardial infarction, stroke, or infection (9–15).

In previous studies, we (16–19) and others (20–23) have been able to induce the partial or complete expression of experimental APS in naive mice, following passive transfer of human and mouse monoclonal and polyclonal aCL antibodies to the tail vein of BALB/c and ICR mice, or following active immunization with these aCL. The availability of this experimental model has enabled us to evaluate various therapeutic modalities at different stages of disease induction and development (24–32).

Ciprofloxacin is a potent broad-spectrum antibacterial agent of the quinolone family, currently in wide clinical use. Recent studies have shown that ciprofloxacin may have inhibitory or stimulatory effects on human and murine immune systems. It was found to enhance the production of interleukin-1 (IL-1) by murine macrophages (33) and to increase IL-2 production by phytohemagglutinin-stimulated human lymphocytes (34–36). Furthermore, increased synthesis of interferon-γ (IFNγ), granulocyte–macrophage colony-stimulating factor (GM-CSF), and IL-3 in the presence of ciprofloxacin has been reported (37,38). Ciprofloxacin was found to interfere with a regulatory pathway common to several cytokines, resulting in up-regulation of

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messenger RNA (mRNA) for IL-1, IL-2 and its receptors, IFNγ, GM-CSF, and IL-3 (39). Ciprofloxacin enhanced the repopulation of murine hematopoietic organs in sublethally irradiated mice, possibly by stimulating IL-3 and GM-CSF production (38,40). However, the production of human tumor necrosis factor α (TNFα), IL-1α, lymphotoxin tumor necrosis factor β (TNFβ), and GM-CSF was inhibited at higher concentrations of the drug (41). Some authors suggested that adding ciprofloxacin to cultures of mitogen-stimulated lymphocytes exerts inhibitory effects on cell cycle progression and immunoglobulin secretion (42).

Previously, we have shown decreased levels of IL-3 production in women with APS and in mice with experimental APS (26,43). The fetal wastage in these mice was prevented by prior administration of recombinant IL-3 (28) or by treatment with aspirin, which as we have shown, accelerates IL-3 production in APS mice (29). It is well established that IL-3 and GM-CSF favorably affect various stages of pregnancy (44–46). Both cytokines were shown to participate in at least 2 crucial stages responsible for embryo development: trophoblast invasion and trophoblast expansion (47–50). Moreover, IL-3 was found to affect the regulation of placental growth and to increase platelet number because of a de novo production of megakaryocytes (44,51).

In the current study, we assessed the immunomodulatory potential of ciprofloxacin and ceftazidime (as a control antibiotic) in mice with experimentally induced APS.

MATERIALS AND METHODS

Mice. Female BALB/c mice ages 10–12 weeks were purchased from Tel-Aviv University animal house. BALB/c females were checked for estrus, caged overnight with BALB/c males, and examined for vaginal plug next morning. The presence of the plug was considered to be day 1 of pregnancy in all the studied groups.

Induction of experimental APS and treatment design. H-3 is a human monoclonal IgM aCL (17), generated following fusion of peripheral blood lymphocytes from a healthy subject immunized with diphtheria and tetanus with the GM4672 lymphoblastoid cell line.

BALB/c mice were immunized intradermally in the hind footpads with 10 μg of H-3 in Freund's complete adjuvant. Three weeks later, a booster injection of the immunoglobulin (10 μg) in phosphate buffered saline (PBS) was given at the same site, as previously described by us (17,18). The mice were bled every month, and the antibody titers were determined in the sera by the modified aCL enzyme-linked immunosorbent assay (ELISA) (10). The titers of anti-β_2-glycoprotein I (anti-β_2-GPI) antibodies were tested with the use of CL-coated plates, incubated with 10 μg/ml of β_2-GPI, and subsequently with the addition of sera in different dilutions (10).

We evaluated the mice for experimental APS parameters (activated partial thromboplastin time [APTT], thrombocytopenia, increased fetal loss) as we have detailed previously (16–19,24–32). The percentage of fetal loss was calculated according to a method previously described (52).

Two sets of experiments were performed. First, mice with experimental APS at 1 month after the booster injection, were injected intraperitoneally with ciprofloxacin or ceftazidime 30 mg/kg/dose or with PBS given 3 times daily for 5 consecutive days, starting on day 1 of pregnancy (when the vaginal plug was detected).The second set of experiments was performed over 10 consecutive days, starting 5 days before mating. The following parameters were studied in the treated groups: titers of aCL in the sera, APTT, platelet counts, percentage of fetal resorptions, number of megakaryocytes in the bone marrow, levels of IL-3 and GM-CSF produced by the splenocytes, number of cytokine-producing cells (IL-3, GM-CSF, IL-2, and IL-4), IL-3 mRNA, and the number of myeloid progenitors (colony-forming unit–culture [CFU-C]).

Preparation of spleen conditioned medium (SCM). Spleen cells from individual mice from each group were prepared as a single-cell suspension at a concentration of 1×10^7 cells/ml in tissue culture medium, with or without the addition of 10% fetal calf serum (FCS) or 0.5% normal mouse serum. The cells were cocultured in a 24-well plate (Nunclon, Dalta, Denmark) at 5×10^6 cells/well/500 μl, with or without concanavalin A (Con A; 2 μg/ml). Plates were incubated for 24–48 hours at 37°C and an atmosphere of 5% CO_2. Cell-free supernatants were collected, centrifuged at 600*g* for 15 minutes, filtered (0.22 μm), and stored at −70°C until tested.

Cytokine-secreting enzyme-linked immunospot (ELISPOT) assay. The cytokine-secreting cells were determined by a cytokine-secreting ELISPOT assay as previously described (53): Briefly, ELISA plates (Nunc) were coated with a capture monoclonal antibody (anti–IL-3, anti–GM-CSF, anti–IL-2, anti–IL-4; PharMingen, San Diego, CA), 2 μg/ml, diluted in coating buffer, and incubated overnight at 4°C. After blocking with 25% FCS for 2 hours at 37°C, splenocytes (1×10^7 cells/well) diluted in complete medium were added, and the cells were incubated overnight at 37°C in 7% CO_2. The presence of a specific cytokine was probed with biotinylated anticytokine-detecting monoclonal antibody (2 μg/ml; PharMingen) for 2 hours at 37°C. Streptavidin–alkaline phosphatase (PharMingen) was added according to the manufacturer's instructions for 30 minutes at 37°C. Substrate BCIP (Sigma, St. Louis, MO) mixed with 3% agarose was added. Incubation at 37°C for 8 hours resulted in blue spots. Between each step, extensive washings in PBS–0.05% Tween were performed.

Cytokine assays. The levels of IL-3 and GM-CSF in the SCM were detected by ELISA kits (PharMingen).

RNA isolation and Northern blot. Total mRNA was prepared from spleens by use of Tri-Reagent solution (Tri-Reagent MRC, Cincinnati, OH). RNA (0.5 μg) was examined on ethidium bromide–stained agarose gels to verify that every sample was of a comparable concentration and purity. The RNA was then ethanol precipitated, dried, resuspended in 15× saline–sodium citrate, 7.5% formaldehyde, and incubated at 60°C for 15 minutes. The RNA (10 μg) was loaded

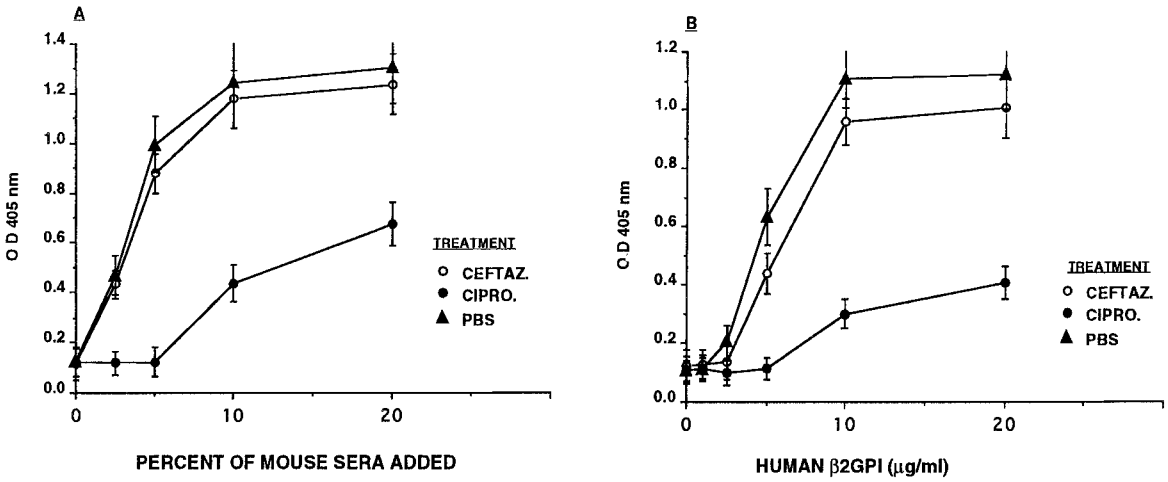

Figure 1. Binding of IgG from mice with experimental antiphospholipid syndrome (APS) to cardiolipin in the presence of mouse sera and human β_2-glycoprotein I (β2GPI). For determination of anti-β_2-GPI antibodies, the modified anticardiolipin antibody (aCL) enzyme-linked immunosorbent assay (ELISA) was employed. IgG was eluted from the sera of mice with experimental APS by use of a protein G column, and was then biotinylated. The labeled mouse IgG (10 μg/ml) was tested with the modified aCL ELISA using different concentrations of **A,** mouse sera and **B,** human β_2-GPI. Binding was probed using streptavidin–alkaline phosphatase and an appropriate substrate. Values are the mean ± SD optical density (O D). Ceftaz. = ceftazidime; cipro. = ciprofloxacin; PBS = phosphate buffered saline.

onto formaldehyde–agarose gels and blotted to nylon filters (Hybond-N+; Amersham, Little Chalfont, UK). Filters were hybridized according to standard protocols and exposed to x-ray films for 1–5 days at −70°C by using intensifying screen. Autoradiographs were quantified by scanning laser densitometry.

DNA probe. The IL-3 probe was a 550-basepair *Eco* RI fragment. The DNA fragment was labeled with α^{32}P-dCTP (specific activity 3,000 Ci/mmole; PB 10205; Amersham) by random priming (Amersham). Free nucleotides were separated on a spin column (Costar, Cambridge, MA) containing Sephadex G-50 (Pharmacia, Piscataway, NJ). The specific activity of the probe was never $<10^8$ counts per minute per microgram of DNA.

Assay for myeloid progenitors (CFU-C) in bone marrow. Bone marrow (BM) cells were flushed from femurs and tibias of the mice with Dulbecco's modified Eagle's medium into sterile polystyrene tubes, and spleens were dispersed into single-cell suspensions. The cells were plated at a concentration of 7.5×10^5/ml and 1.5×10^5/ml of splenocytes and BM cells, respectively, in a medium containing 0.3% agar, 15% FCS, and Con A. Colonies were counted on day 7. The counted number of CFU-C was multiplied by the number of spleen or marrow cells (flushed from the organ) to obtain the total CFU-C in each hematopoietic organ. Results are expressed as the mean ± SD of CFU-C of 2 experiments.

Statistical analysis. Student's *t*-test was used to evaluate differences between the binding properties of the various groups studied. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effect of ciprofloxacin treatment on antibody production and other parameters of experimental APS. H-3 was found to bind negatively charged phospholipids in the standard aCL ELISA and in the modified aCL ELISA (β_2-GPI added to CL-coated plates). H-3 does not bind CL in the absence of β_2-GPI, but binds β_2-GPI (from human and murine origins) in the absence of CL. Immunization with H-3 results in the production of mouse antibodies with binding specificities similar to H-3 (reactive with β_2-GPI/CL and with β_2-GPI–coated plates but not with CL alone [Figure 1]) and with an experimental APS manifested by fetal wastage, prolongation of APTT, and thrombocytopenia (17,27).

BALB/c mice immunized with H-3 developed high titers of "self" anti-β_2-GPI antibodies. No anti-DNA antibodies were detected in the sera of the immunized mice (employed as an irrelevant antigen). Anti-β_2-GPI antibodies (detected using the modified aCL ELISA) were significantly decreased in the ciprofloxacin-treated mice (as compared with ceftazidime- and PBS-treated mice) when the sera were assayed at dilutions of 1:400 to 1:3,200 (Figure 2). These findings were evident in both treatment regimens (the agent given 5 days before mating for 5 days; the agent given on

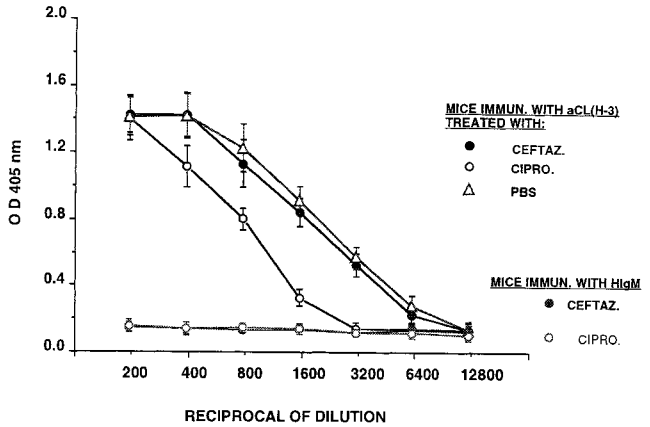

Figure 2. Binding of sera from mice with experimental APS to β_2-GPI/CL–coated plates. Sera from mice with experimental APS obtained at the end of the experiment (treated 5 days prior to mating for 10 consecutive days) were assessed at different dilutions (1:200–1:12,800) for binding to β_2-GPI/CL–coated plates using the modified aCL ELISA. Values are the mean ± SD (10 mice per group). See Figure 1 for abbreviations.

day 1 of pregnancy for 5 days). When total IgG levels were assayed in the mice from all study groups by use of a capture ELISA (goat anti-mouse IgG employed for coating), no differences were evident.

An effect on the APS parameters (prolonged APTT, thrombocytopenia, and fetal loss) was observed in the immunized mice that had been exposed to ciprofloxacin (Table 1). The mice treated with ciprofloxacin for 5 days had a mean ± SD APTT of 45 ± 3 seconds, compared with 62 ± 3 seconds in mice that received ceftazidime ($P < 0.002$) and 67 ± 4 seconds in PBS-treated mice. The APTT was 37 ± 2 seconds in the non-APS mice. Platelet counts in the immunized mice were restored (mean ± SD 985 ± 101 cells/mm^3 × 10^3) ($P < 0.05$) upon treatment with ciprofloxacin, an effect that was not evident after treatment with ceftazidime (559 ± 89 cells/mm^3 × 10^3); in non-APS mice, a value of 998 ± 102 cells/mm^3 × 10^3 was noted.

The percentage of fetal resorptions (equivalent to pregnancy loss in humans) decreased in the ciprofloxacin-treated mice, from 52 ± 5% (mean ± SD) to 17 ± 3% compared with the resorption rate in the mice treated with ceftazidime ($P < 0.001$). A 51 ± 4% resorption rate was found in the mice that received PBS (5 ± 2% in the non-APS group).

A decline in APTT and thrombocytosis and a reduction in the percentage of pregnancy loss was also observed when treatment of the immunized mice started 5 days before mating and continued for 10 days, as shown in Table 1.

Effect of ciprofloxacin on megakaryocyte numbers in mice with experimental APS. The percentage of megakaryocytes in the BM cells of the ciprofloxacin-treated mice was increased 4-fold compared with the

Table 1. Effect of ciprofloxacin on clinical parameters of experimental antiphospholipid syndrome*

Parameter	Immunization with anti-β_2-GPI (H-3)			Immunization with HIgM		Nonimmunized	
	Cipro.	Ceftaz.	PBS	Cipro.	Ceftaz.	Cipro.	Ceftaz.
Started on day 1 of pregnancy, for 5 days							
No. of mice	15	11	9	11	9	10	8
Anti-β_2-GPI	1:400†	1:2,000	1:2,500	1:200	1:200	1:200	1:200
APTT (seconds)	45 ± 3‡	62 ± 3	67 ± 4	30 ± 2	33 ± 1	35 ± 3	37 ± 2
Platelets (/mm^3 × 10^3)	985 ± 101§	559 ± 89	603 ± 111	997 ± 101	1,182 ± 261	1,065 ± 241	998 ± 102
% resorptions	17 ± 3¶	52 ± 5	51 ± 4	8 ± 2	7 ± 1	9 ± 2	5 ± 2
Started 5 days before mating, for 10 days							
No. of mice	18	16	14	17	18	16	17
Anti-β_2-GPI	1:450#	1:2,000	1:2,500	1:200	1:200	1:200	1:200
APTT (seconds)	42 ± 3**	59 ± 5	62 ± 4	34 ± 2	33 ± 4	32 ± 2	30 ± 3
Platelets (/mm^3 × 10^3)	879 ± 124§	602 ± 97	598 ± 103	1,128 ± 231	1,142 ± 219	997 ± 89	1,063 ± 245
% resorptions	18 ± 4¶	49 ± 4	53 ± 3	7 ± 1	8 ± 2	6 ± 1	8 ± 3

* Anti-β_2-GPI = anti-β_2-glycoprotein 1; HIgM = human IgM; Cipro. = ciprofloxacin; Ceftaz. = ceftazidime; PBS = phosphate buffered saline; APTT = activated partial thromboplastin time.
† $P < 0.006$.
‡ $P < 0.02$.
§ $P < 0.05$.
¶ $P < 0.001$.
# $P < 0.004$.
** $P < 0.04$.

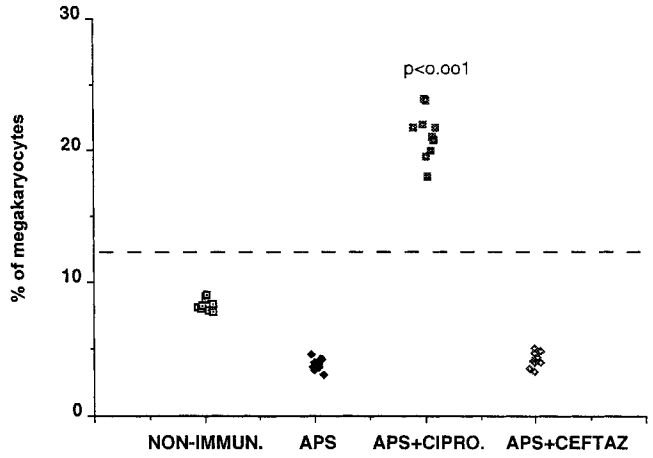

Figure 3. Megakaryocyte count in the bone marrow (BM) of mice with experimental APS treated with ciprofloxacin, ceftazidime, or PBS. Megakaryocytes were counted on day 17 of pregnancy (2 weeks after the end of a 5-day treatment). Values are the percentage of megakaryocytes from the total count of BM cells (10 mice per group). Non-immun. = nonimmunized. See Figure 1 for other definitions.

percentage of BM megakaryocytes in the ceftazidime- or PBS-treated APS mice ($P < 0.001$) (Figure 3).

Effect of ciprofloxacin on IL-3 and GM-CSF production in mice with experimental APS. To study the mechanism by which ciprofloxacin improved the APS parameters in our study, we analyzed the expression of IL-3 and GM-CSF in the mice following exposure to ciprofloxacin. Therefore, IL-3 and GM-CSF expression were measured in the following experiments in splenocytes incubated with Con A for 20 hours; IL-3 mRNA levels were also measured.

Culture supernatants of the splenic population derived from the APS mice exposed to ciprofloxacin showed at least a 10-fold increase in the level of IL-3 in the absence of Con A and an 8.6-fold increase in the presence of Con A, in comparison with supernatants from the ceftazidime- or PBS-treated mice (Figure 4A). By 2 weeks after the end of treatment, the levels of IL-3 were decreased by half when the splenocytes were derived from APS mice treated with ciprofloxacin (Figure 4B). The level of GM-CSF was elevated 2-fold in the absence and presence of Con A in the ciprofloxacin-treated APS mice, in comparison to ceftazidime- or PBS-treated mice (Figure 4B).

The number of IL-3–and GM-CSF–secreting cells in the splenocytes of ciprofloxacin-treated APS mice increased 9.7-fold and 7.4-fold ($P < 0.005$), respectively, compared with the ceftazidime- or PBS-treated mice (Figure 5). No change in the number of IL-2–and IL-4–secreting spleen cells was noted in the ciprofloxacin-treated APS mice.

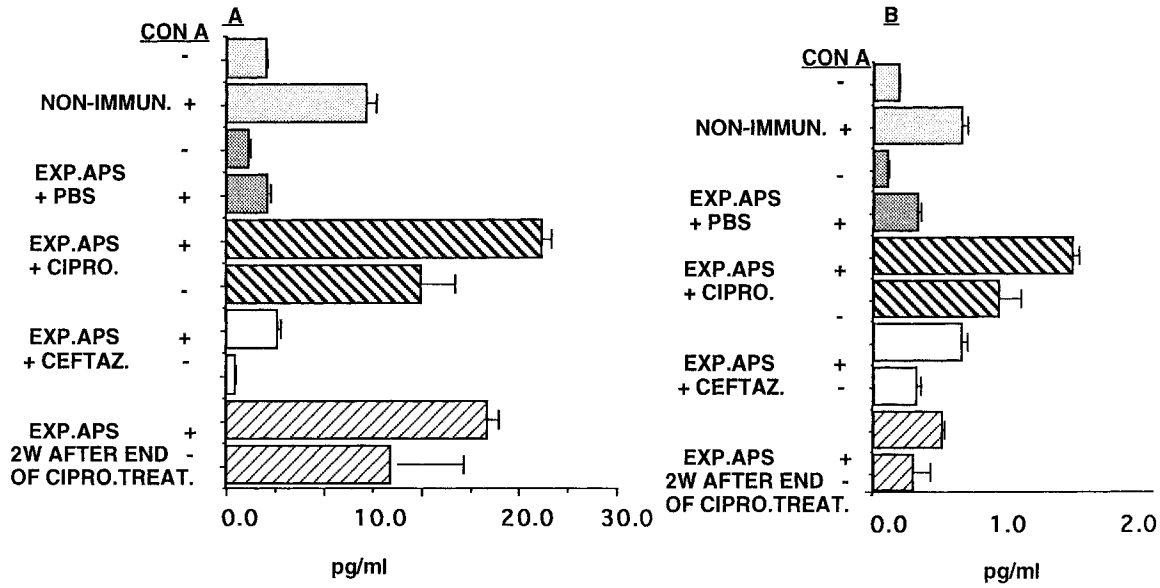

Figure 4. Concentrations of **A,** interleukin-3 and **B,** granulocyte–macrophage colony-stimulating factor in splenocyte culture fluid from mice with experimental (exp.) APS treated with ciprofloxacin, with or without concanavalin A (Con A). Splenocytes were obtained at the end of treatment (treat.) and 2 weeks later (2W) from APS mice treated for 10 days with ciprofloxacin, ceftazidime, or PBS. Values are the mean and SD pg/ml (5–8 mice per group). See Figure 1 for other definitions.

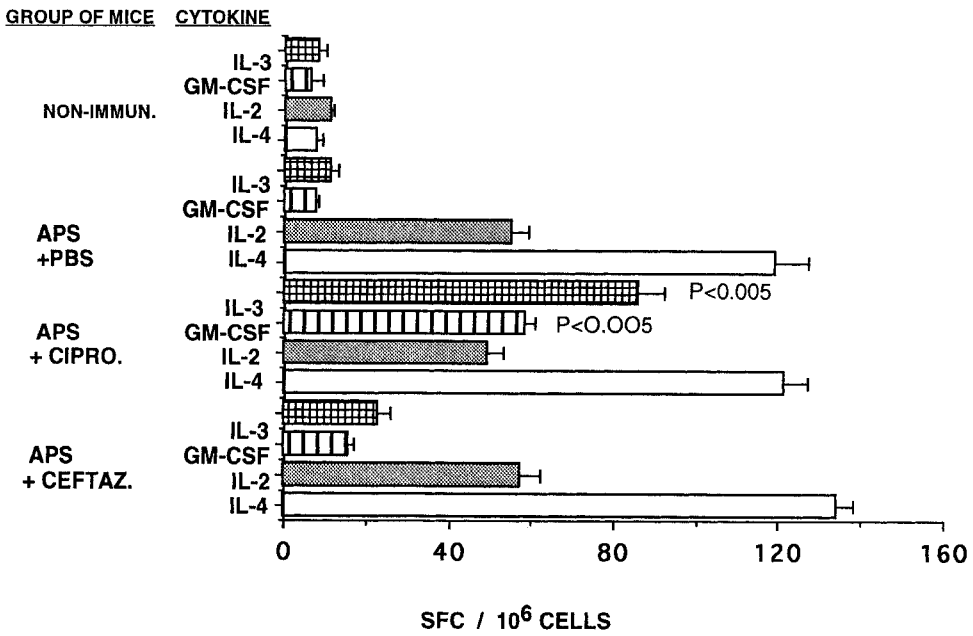

Figure 5. Effect of ciprofloxacin on interleukin-3 (IL-3)–and granulocyte–macrophage colony-stimulating factor (GM-CSF)–secreting spleen cells from APS mice. The numbers of IL-3, GM-CSF, IL-2, and IL-4 cytokine-secreting splenocytes were studied in APS mice treated for 10 days with ciprofloxacin, ceftazidime, and PBS. Values are the mean and SD spot-forming cells (SFC) per 10^6 cells (8–10 mice per group). Non-immun. = nonimmunized. See Figure 1 for other definitions.

A 4-fold increase in the IL-3 mRNA transcription level was detected in the ciprofloxacin-treated APS mice compared with the ceftazidime- and PBS-treated groups ($P < 0.05$) (Figure 6).

Effect of ciprofloxacin on myeloid progenitors in the BM cells and spleens of mice with experimental APS. We compared the number of CFU-C from BM cells and spleens of APS mice treated with ciprofloxacin

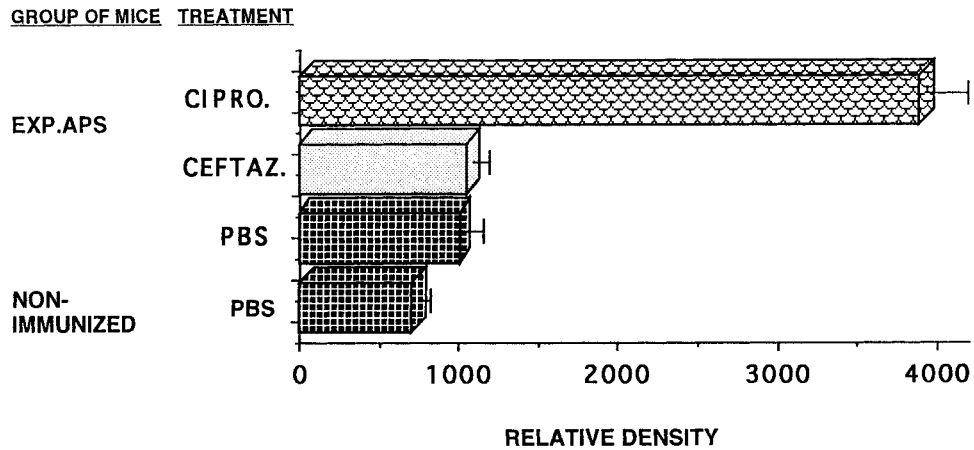

Figure 6. Effect of ciprofloxacin on levels of interleukin-3 (IL-3) messenger RNA (mRNA) in the spleens of mice with experimental (exp.) APS, as determined by Northern blot analysis. Autoradiographs were quantified by scanning laser densitometry. Values are the mean and SD (5 mice per group). See Figure 1 for other definitions.

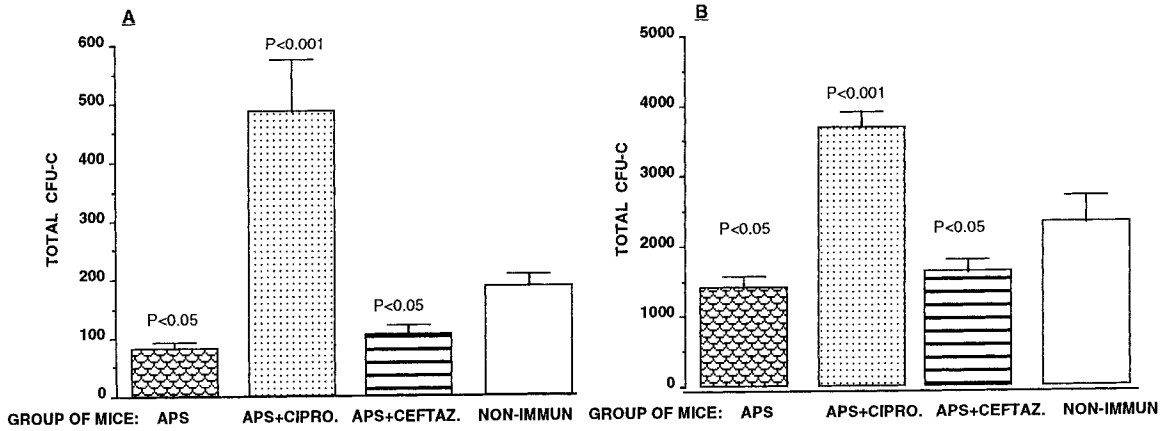

Figure 7. Effect of ciprofloxacin treatment on total myeloid progenitors in mice with experimental APS. Comparison of myeloid colony formation (colony-forming unit–culture [CFU-C]) in **A,** bone marrow and **B,** spleen cells obtained from APS mice treated for 10 days with ciprofloxacin, ceftazidime, and PBS. Values are the mean and SD (8–10 mice per group). Non-immun = nonimmunized. See Figure 1 for other definitions.

30 mg/kg/day with those of APS mice treated with ceftazidime or PBS. Figures 7A and B show that there were 4.2-fold and 2-fold increases in the number of CFU-C in the BM cells and spleens, respectively, following exposure of the APS mice to ciprofloxacin, as compared with ceftazidime or PBS. Threefold or 1.4-fold increases in the number of CFU-C in the BM cells and spleens of the ciprofloxacin-treated mice were detected when compared with the nonimmunized mice.

DISCUSSION

The rationale for the mode of therapy used in the present study was based on our previous reports showing a low production of IL-3 and GM-CSF in humans with APS and in mice with experimental APS (26,43). Since IL-3 plays an active role both in modulating placental growth and in increasing megakaryocyte numbers, it is conceivable that its down-regulation in APS could explain the clinical features of the syndrome (i.e., thrombocytopenia and fetal loss). Accordingly, exogenous administration of recombinant IL-3, or a potentiator of IL-3 production such as low-dose aspirin, in mice with APS prevented fetal loss, improved platelet numbers, and restored APTT values to normal (28,29). Ciprofloxacin was found to accelerate IL-3 and GM-CSF expression in vivo and in vitro in different mouse models and in humans (37–40). Therefore, the goal of our study was to determine whether in vivo exposure of mice with experimental APS to ciprofloxacin could lead to improvement in the clinical parameters of APS based on its ability to enhance IL-3 and GM-CSF production.

In the current study, we have shown that clinically relevant concentrations of ciprofloxacin given to BALB/c mice with experimental APS, before mating and during implantation time, or, alternatively, only during early pregnancy, ameliorated the manifestations of the disease. This was evidenced by decreased titers of anti–β_2-GPI/CL antibodies, shortened APTT, increased platelet counts (and even thrombocytosis), elevated megakaryocyte counts in the BM, as well as a lower percentage of fetal loss. Ceftazidime, which has a similar clinical spectrum as ciprofloxacin, had no effect on the parameters of the disease in the APS mice.

Ciprofloxacin caused an up-regulation of IL-3 and GM-CSF expression in the treated mice with experimental APS. This observation was based on: (a) elevation of IL-3 and GM-CSF production by splenocytes that increased when the splenocytes were exposed in vitro to a mitogen (Con A); (b) increase in the number of total myeloid progenitors (CFU-C) in the BM and spleen; (c) rise in the number of IL-3–and GM-CSF–producing cells in the splenocytes, without change in the number of IL-2 and IL-4 cytokine-producing cells which, in this stage of the disease, are already elevated; and (d) increased IL-3 mRNA expression in the spleens of the ciprofloxacin-treated mice. There was no change in IL-3 and GM-CSF expression in the spleens of the mice that received the antibiotic ceftazidime.

The results described in this study, confirm previous observations demonstrating that IL-3 and GM-CSF favorably affect the various stages of pregnancy (47–50). Moreover, IL-3 was found to affect the regula-

tion of placental growth and functional maturation, as well as to increase the numbers of platelets due to de novo production of megakaryocytes (44,51).

Recently, the safety of ciprofloxacin use during pregnancy was studied (54). Thirty-eight women who received ciprofloxacin were followed up for perinatal complications, birth weight, birth defects, and developmental milestones, with particular emphasis on the musculoskeletal system. These data were compared with those in control subjects matched for both maternal age and indication for antibacterial therapy. No differences in achievement of the developmental milestones or in the development of the musculoskeletal system were detected between the 2 groups. A recent report details the use of ciprofloxacin in 130 pregnant women who were mostly in their first trimester; no congenital abnormalities were noted in the children (55).

We found that the titers of anti-β_2-GPI antibodies in the sera of the APS mice treated with ciprofloxacin were decreased in the current study. Our data, together with those from other reports (42,56,57), suggest that modification of the immune responses by ciprofloxacin is a complex phenomenon that may be influenced by factors such as the route of administration, the duration of treatment, the timing of administration, and the dosage. Previous in vitro and in vivo studies showed different effects of ciprofloxacin on immunoglobulin generation. Adding ciprofloxacin to in vitro cultures of mitogen-stimulated lymphocytes exerted inhibitory effects on cell cycle progression and immunoglobulin secretion (56). Intraperitoneal administration of ciprofloxacin to mice for 3 consecutive days and then immunization with sheep erythrocytes showed a significant suppression of hemolytic IgG-forming cells (42). Potentiation of both the direct and the indirect plaque-forming cell response in mice treated subcutaneously was observed by other investigators (57). In our study the reduction of autoantibody production upon exposure of APS mice to ciprofloxacin was indeed correlated with improvement in the clinical markers of APS in the mice, a point which strengthens the causal role of the antibodies in the pathogenesis of the experimental syndrome.

Taken together, these data indicate that ciprofloxacin improves manifestations of experimental APS and results in thrombocytosis, reduction of the APTT, and prevention of fetal loss. This effect may be mediated via immunomodulation of IL-3 and GM-CSF expression. If the favorable effects of ciprofloxacin demonstrated in this study could be reproduced in other experimental models, it could be considered a possible alternative treatment after careful clinical evaluation in patients with APS.

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