Interleukin 10 Inhibits Growth and Granulocyte/Macrophage Colony–stimulating Factor Production in Chronic Myelomonocytic Leukemia Cells

By Klaus Geissler,* Leopold Öhler,* Manuela Födinger,‡ Irene Virgolini,§ Maria Leimer,§ Eva Kabrna,* Marie-ta Kollars,* Sonja Skoupy,‡ Barbara Bohle,‡ Michael Rogy,‖ and Klaus Lechner*

*From the *Division of Hematology–Internal Medicine I, the ‡Department of Laboratory Medicine, the §Department of Nuclear Medicine, and the ‖Department of General Surgery, University of Vienna, A-1090 Vienna, Austria*

Summary

Autonomous release of hematopoietic growth factors may play a crucial role in the pathogenesis of certain hematological malignancies. Because of its cytokine synthesis–inhibiting action, interleukin 10 (IL-10) could be a potentially useful molecule to affect leukemic cell growth in such disorders. Chronic myelomonocytic leukemia (CMML) cells spontaneously form myeloid colonies (colony-forming units–granulocyte/macrophage) in methylcellulose, suggesting an autocrine growth factor–mediated mechanism. We studied the effect of recombinant human IL-10 (rhIL-10) on the in vitro growth of mononuclear cells obtained from peripheral blood or bone marrow of patients with CMML. IL-10 specifically binding to leukemic cells had a profound and dose-dependent inhibitory effect on autonomous in vitro growth of CMML cells. IL-10 significantly inhibited the spontaneous growth of myeloid colonies in methylcellulose in 10/11 patients, and autonomous CMML cell growth in suspension in 5/5 patients tested. Spontaneous colony growth from CMML cells was also markedly reduced by addition of anti–granulocyte/macrophage colony–stimulating factor (GM-CSF) antibodies, but not by addition of antibodies against G-CSF, IL-3, or IL-6. IL-10-induced suppression of CMML cell growth was reversed by the addition of exogenous GM-CSF and correlated with a substantial decrease in GM-CSF production by leukemic cells, both at the mRNA and protein levels. Our data indicate that IL-10 profoundly inhibits the autonomous growth of CMML cells in vitro most likely through suppression of endogenous GM-CSF release. This observation suggests therapeutic evaluation of rhIL-10 in patients with CMML.

Chronic myelomonocytic leukemia (CMML)[1] is a hematopoietic malignancy of the elderly that is characterized by leukocytosis with monocytes and granulocytic cells in all stages of development, marked dysmyelopoiesis, a chronic course, and unresponsiveness to aggressive chemotherapy (1–3). CMML differs from chronic myeloid leukemia in several ways, including cytologic criteria, lack of Philadelphia chromosome, and as we have originally shown, spontaneous colony formation by CMML cells in semisolid medium (4). The growth of hematopoietic colonies without addition of exogenous growth factors raises the possibility that CMML cells secrete their own growth factors that might subsequently lead to the proliferation of leukemic cells through an autocrine growth factor–mediated mechanism in vitro and possibly also in vivo (5). Later work by others confirmed our original observation and suggested GM-CSF and IL-6 as candidate growth factors for CMML cells (6).

IL-10 is a 35-kD protein, originally identified by virtue of its ability to inhibit cytokine synthesis in T helper 1 clones (7, 8). It is primarily produced by mononuclear cells (MNC; 9) and possesses a wide range of activities on a number of cell types, including B cells (10), T cells (11), NK cells (12), mast cells (13), neutrophils (14), eosinophils (15), and monocytes (16). The main feature of this cytokine is a suppressive effect on cytokine expression. Thus, IL-10 profoundly suppresses the induced production of IL-1α, IL-1β, IL-6, IL-8, TNF-α, and GM-CSF by human monocytes (16) and mouse peritoneal macrophages (17).

Because of its cytokine synthesis–inhibiting action, IL-10 could be a potentially useful molecule in hematological malignancies in which autostimulatory mechanisms may

[1] *Abbreviations used in this paper:* BM, bone marrow; CMML, chronic myelomonocytic leukemia; MNC, mononuclear cell; PB, peripheral blood; RT, reverse transcription.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/96/10/1377/08 $2.00
Volume 184 October 1996 1377–1384

Table 1. *Clinical and Laboratory Data in Patients with CMML*

Patient	Age/sex	Duration	PB findings						Karyotype	CT
			WBC	Hb	Plt	Mono	LDH	Lysozyme		
		mo	*× 10⁹/liter*	*g/dl*	*× 10⁹/liter*		*U/liter*	*μg/ml*		
JL	55/F	15	160	9.3	125	17	658	49	45XX,−7	+
PM	70/F	22	181	8.4	118	26	503	126	46XX,t2/11	+
RL	66/F	60	20	14.2	387	30	287	27	46XX	−
MR	68/M	1	55.8	9.9	130	23	1280	62	45XO	−
ML	59/F	8	8.5	9.9	72	23	138	49	46XX	+
HL	68/M	105	19.9	9.7	87	15	440	34	NA	+
DH	88/F	5	7	10.0	101	27	520	114	NA	−
MA	79/F	36	14.3	8.5	130	30	165	146	46XX	−
WM	81/F	6	9.8	8.6	8	29	94	51	46XX	−
FJ	83/M	1	39.8	9.9	13	55	373	106	NA	−
KR	80/M	180	22.4	14.7	345	12	246	32	NA	−

CT, chemotherapy; Hb, hemoglobin; Mono, monocytes; NA, not available; Plt, platelets; WBC, white blood cells.

play a crucial role in pathogenesis. Here, we investigated the effect of IL-10 on the autonomous in vitro growth of MNC from patients with CMML. We found that IL-10 profoundly inhibits the spontaneous proliferation of CMML cells in methylcellulose and in suspension, most likely through suppression of spontaneous release of GM-CSF.

Materials and Methods

Patients. All 11 patients met the diagnostic criteria for CMML according to the FAB group, i.e., dyspoietic features of myelodysplastic syndromes associated with a blood monocytosis $>10^9$/l, an increase in bone marrow (BM) monocyte precursors, and a blast cell percentage <5% in the peripheral blood (PB) and <30% in BM. Clinical and laboratory data of these patients are shown in Table 1. In four patients, cytoreductive chemotherapy was given before or at the time of the study.

Preparation of Cells. After informed consent, PB was collected into sterile tubes containing 4 ml EDTA. BM samples were obtained by aspiration into sterile tubes containing heparin with no preservative. PB MNC and BM MNC were harvested after a Ficoll-Hypaque gradient centrifugation (400 g, 40 min, 1.007 g/ml).

Reagents. Recombinant human IL-10 (rhIL-10; specific activity = 1–2 × 10^6 U/mg) was kindly provided by Schering-Plough Corp. (Kenilworth, NJ). A neutralizing anti–IL-10 antibody was obtained from R&D Systems Europe Ltd. (UK), antibodies directed against G-CSF, GM-CSF, IL-3, IL-6, and rhIL-6 were purchased from Genzyme Corp. (Cambridge, MA), rhGM-CSF and rhIL-3 were kindly provided by Sandoz (Basel, Switzerland), and rhG-CSF was purchased from British Biotechnology (Oxan, UK). Cytokines were added at the onset of the culture. The neutralizing antibody against IL-10 was preincubated with IL-10 for 2 h at room temperature. Neutralizing antibodies against G-CSF, GM-CSF, IL-3, and IL-6 were used as recommended by the manufacturer.

CFU-GM Assay. PB MNC or BM MNC were cultured in 0.9% methylcellulose, 30% FCS (INLIFE, Wiener Neudorf, Austria), and IMDM (Gibco, Paisley, Scotland) with or without the addition of cytokines or anticytokine antibodies. Cultures were plated in triplicate at 10–100 × 10^3 MNC/ml. After a culture period of 14 d (37°C, 5% CO_2, full humidity) cultures were examined under an inverted microscope. Aggregates with at least 40 cells were counted as CFU-GM.

Suspension Cultures. PB MNC from CMML patients were cultured at 5 × 10^5 per milliliter for 7 d in IMDM supplemented with 30% FCS with or without IL-10. Cell numbers were determined using a hemocytometer. For morphological examination, cytospins were stained by May-Grünwald-Giemsa.

Radiolabeling of rIL-10. IL-10 was labeled with ^{125}I using standard techniques (18). Briefly, 250 μg IL-10 (nmol), 2 mCi ^{125}I-NaI, and 5 μg lactoperoxidase were added into a vial containing a magnetic stirrer. The reaction mixture was slowly stirred at pH 7 (0.01 M balance phosphate buffer) for 10 min and thereafter subjected to a preparative gradient HPLC (RP C18 column, MeCN gradient). The column eluent was passed through a scintillation radioactivity detector and a UV (280 nm) detector in series. The system was calibrated with unlabeled IL-10 and allowed collection of pure radioiodinated IL-10, separated from unlabeled IL-10, as well as from reagents and inorganic iodine species. ^{125}I-IL-10 was isolated in a 40% yield at a specific activity of 2,000 Ci/mmol. The ^{125}I-IL-10 product fraction was stabilized by addition of 2% human serum albumin and analyzed by analytic HPLC (same system as the preparative one), paper electrophoresis (zone electrophoresis on 3MM paper [Whatman Chemical Co., Clifton, NJ], 0.1 M barbital buffer, pH 8.6, field 300 V for 10 min), and TCA precipitation (10% final TCA). Radiochemical purity was >97% and remained stable during >24 h.

Binding Assay. In saturation studies, intact cells (5 × 10^5 cells per tube) were incubated in the assay buffer containing 50 mmol/liter Tris-HCl (pH 7.4) and 5 mmol/liter $MgCl_2$ with increasing concentrations of ^{125}I-IL-10 (0.01–25 nmol/liter) in the absence (total binding) and the presence of unlabeled IL-10 (1 μmol/liter, nonspecific binding) for 45 min at 4°C. Displacement studies

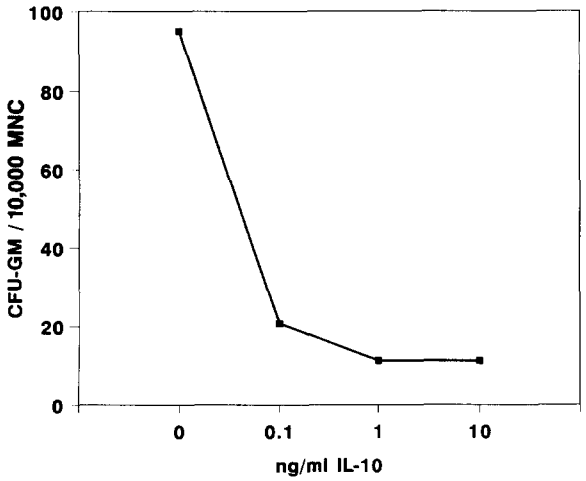

Figure 1. Dose-dependent inhibitory effect of IL-10 on spontaneous CFU-GM growth of CMML cells in patient JL. PB MNC were cultured in methylcellulose with medium alone or with increasing concentrations of IL-10. Colony growth was assessed after 14 d. Results represent the mean values from triplicates.

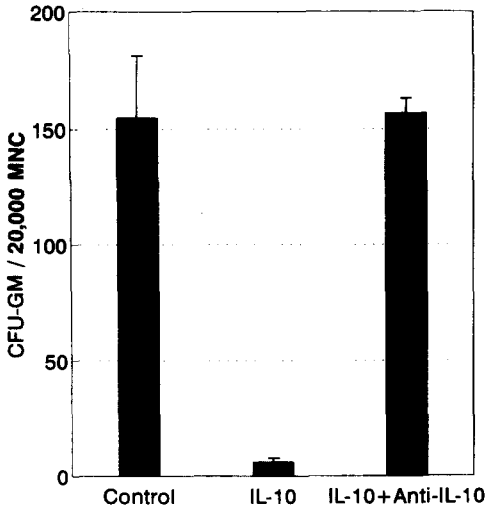

Figure 2. Specificity of IL-10–induced inhibition of spontaneous CFU-GM growth of CMML cells in patient WM. PB MNC were cultured in methylcellulose with medium alone, with 5 ng/ml IL-10, or with IL-10 plus a neutralizing antibody against IL-10. Colony growth was assessed after 14 d. Results represent the mean values ± SD from triplicates.

were performed with 5 nmol/liter ^{125}I-IL-10 (total binding) and increasing concentrations of unlabeled IL-10 (0.01–1 μmol/liter, nonspecific binding). After incubation, the reaction mixture was diluted 1:10 with assay buffer (4°C) and centrifuged (5,000 *g*, 10 min, 4°C) to separate the membrane-bound from free ligand. The resulting pellet was washed twice in buffer and counted in a gamma counter for 1 min. Binding data were calculated according to Scatchard (19).

Semiquantitative Reverse Transcriptase PCR (RT-PCR) Analysis of GM-CSF Transcripts. 10^7 PB MNC were cultured in suspension with or without IL-10 (10 ng/ml) for 24 h. After incubation, cells were washed twice in diethylpyrocarbonate-treated water, and 10^7 cell aliquots were lysed by the addition of 1.6 ml RNAzol B (Biotecx, Houston, TX). Total RNA was extracted as described (20). The integrity of RNA was controlled by electrophoresis through formaldehyde agarose gels. High quality RNA was quantitated by measuring absorbance at 260 nm, and 1 μg of total RNA was subjected to cDNA synthesis as previously described (21).

For semiquantitative analysis of GM-CSF mRNA, an RT-PCR technique that allows measurement of relative transcript levels was applied (22, 23). The oligonucleotide primer sequences for the amplification of GM-CSF were: 5′-CTGCTGCTGAG-ATGAATGAAACAG-3′ and 5′-TGGACTGGCTCCCAGCAG-TCAAAG-3′, which bracketed a GM-CSF fragment of 286 bp (24). PCR amplification of ABL transcripts was used as a reference to assess variations of total RNA or cDNA between samples. The primer sequences for amplification of ABL were as follows: 5′-CAGCGGCCAGTAGCATCTGACTTTG-3′ and 5′-CCA-TTTTTGGTTTGGGCATCACACCATTCC-3′, resulting in the production of a PCR fragment of 228 bp (21). The linear ranges of PCR amplifications of GM-CSF and ABL were established for each patient as a function of cycle number and cDNA concentrations, as described (22, 23). Reaction conditions included 3 μl cDNA, 20 pmol of each primer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 2.5 U Ampli Taq DNA polymerase (Perkin-Elmer Cetus Corp., Norwalk, CT), and [^{32}P]dCTP (150,000 cpm) in a 50-μl reaction volume. The thermol cycling conditions were denaturation at 94°C (1 min), annealing at 60°C (1 min), and extension at 72°C (2 min), preceded by an initial denaturation step at 94°C for 5 min and followed by a terminal extension of 10 min at 72°C. The number of PCR cycles for amplification of GM-CSF or ABL transcripts was 32 cycles for each patient. Reaction products were subjected to 6% polyacrylamide gels (Novex, San Diego, CA), and dried gels were exposed to XAR-5 films (Eastman Kodak, Rochester, NY) at −70°C for 12 h.

For quantification of the PCR products incorporated, [^{32}P]dCTP was measured on autoradiograms using an imaging densitometer (model 670; Bio Rad Laboratories, Hercules, CA) and the system's volume integration program (Bio Rad Gel DOC 1000 system, Molecular Analyst/PC software). Potential differences of total cellular RNA/cDNA in PCR analyses were corrected by dividing GM-CSF values by the ABL value (mean value of six PCR analyses). The relative level of GM-CSF transcripts was measured in all patient samples in three PCR analyses in duplicate using freshly synthesized cDNA.

GM-CSF Assay. PB MNC from CMML patients were cultured at 5×10^5 per milliliter in IMDM supplemented with 30% FCS with or without IL-10, and the supernatants were collected at day 2, 5, and 7, respectively. GM-CSF contents in supernatants were measured by an immunoenzymetric assay (EASIA; Medgenix Diagnostics, Fleurus, Belgium). The detection limit for GM-CSF was 3 pg/ml.

Statistical Analysis. Data were expressed as mean values ± SD. The Student's *t*-test was used to compare differences with regard to CFU-GM growth and cell growth in suspension. The paired *t* test was used to test for significant differences between GM-CSF transcript levels in CMML cells treated with or without IL-10. $P < 0.05$ was considered statistically significant.

Results and Discussion

Inhibitory Effect of IL-10 on Spontaneous Growth of CMML Cells in Methylcellulose and Suspension. As has been found previously by us (4) and by others (6), MNC obtained from PB or BM of CMML patients form a high number of mye-

Table 2. *Inhibitory Effect of IL-10 on Spontaneous CFU-GM Growth in Methylcellulose in CMML Patients*

Patient	Source	MNC/dish $\times 10^3$	GFU-GM ± SD/dish		Percent of inhibition	*P*
			Control	+IL-10		
JL	PB	10	130.0 ± 12.5	22.3 ± 11.5	83	<0.001
PM	PB	15	124.3 ± 7.5	28.0 ± 14.9	77	<0.001
RL	PB	100	23.7 ± 2.9	9.3 ± 4.2	61	<0.01
MR	BM	50	77.7 ± 1.5	1.0 ± 1.0	99	<0.001
ML	PB	34	75.0 ± 1.7	7.0 ± 2.0	91	<0.001
HL	PB	50	19.3 ± 4.2	8.3 ± 2.3	57	<0.05
DH	PB	50	54.0 ± 12.0	6.0 ± 2.0	89	<0.005
MA	PB	50	9.7 ± 3.5	6.7 ± 1.5	31	>0.05
WM*	PB	20	154.7 ± 23.5	6.0 ± 1.0	96	<0.001
FJ	PB	25	45.3 ± 6.1	13.0 ± 6.9	71	<0.005
KR	PB	16	130.0 ± 1.4	3.0 ± 2.7	98	<0.001

PB MNC or BM MNC from CMML patients were cultured as described in Materials and Methods with or without 10 ng/ml IL-10 (* in patient WM, 5 ng/ml IL-10 was used). Colony growth was assessed after 14 d. Results represent mean values ± SD from triplicates.

loid colonies in methylcellulose in the absence of exogenous growth factors. Treatment of CMML cell cultures with IL-10 resulted in a profound and dose-dependent inhibition of autonomous CFU-GM growth (Fig. 1). IL-10 at a concentration of 0.1 ng/ml already markedly suppressed colony growth, and maximal inhibition was observed at concentrations of 1 and 10 ng/ml, respectively. The effect of 10 ng/ml IL-10 on leukemic cell growth was investigated in PB MNC or BM MNC from 11 CMML patients (Table 2). Because of the great variation of autonomous CFU-GM growth between individuals, cell cultures from different patients had to be plated with different cell numbers to allow reliable scoring of colony growth. As shown in Table 2, 10 ng/ml IL-10 significantly inhibited autonomous formation of myeloid colonies in 10/11 patients tested. In all patients, there was a mean inhibition by 78% ranging from 31 to 99%. The effect was specific, since a neutralizing anti–IL-10 antibody was able to prevent IL-10–induced suppression of CFU-GM growth (Fig. 2).

In five patients with CMML, suspension cultures were performed using PB MNC. As shown in Fig. 3, cell numbers increased in the absence of exogenous growth factors after 7 d in all five patients tested. IL-10 significantly decreased CMML cell growth in three patients and completely prevented cell growth in two patients. By examining cell morphology on May-Grünwald-Giemsa–stained cytospins (Fig. 4), it was found that IL-10 cultures, as compared to medium alone, contained more mature cells (both in terms of relative and absolute cell numbers), suggesting that IL-10 had not only growth-inhibiting, but also some differentiation-inducing activity.

Specific Binding of Radiolabeled IL-10 to CMML Cells. Since in CMML patients with marked leukocytosis PB MNC preparations contained only small amounts of normal cells as compared to malignant cells, it was unlikely that the inhibitory effects of IL-10 on cell growth were mediated by normal lymphocytes and/or monocytes. To further prove the direct action of IL-10 on the leukemic clone, it was important to examine wether CMML cells expressed receptors for IL-10. Cell preparations from three patients (FJ, JL, and PM) containing ≥90% malignant cells, as determined by morphological examination, were used for binding studies with ^{125}I-IL-10. A typical binding isotherm is shown in Fig. 5. In all three patients, significant binding of ^{125}I-IL-10

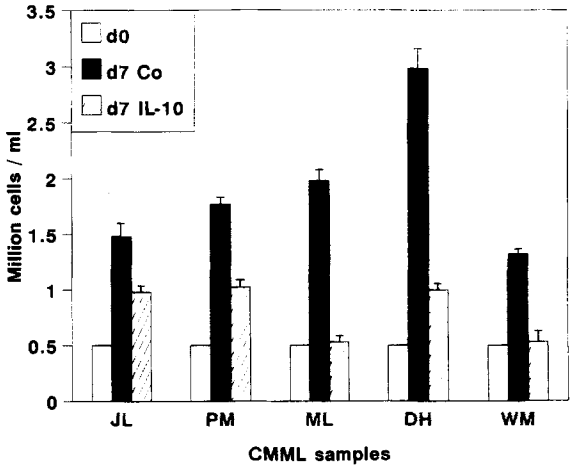

Figure 3. Inhibitory effect of IL-10 on spontaneous proliferation of CMML cells in suspension in five patients. PB MNC were cultured with medium alone or with 10 ng/ml IL-10. Cell numbers were determined after 7 d. Results represent mean values ± SD from triplicates.

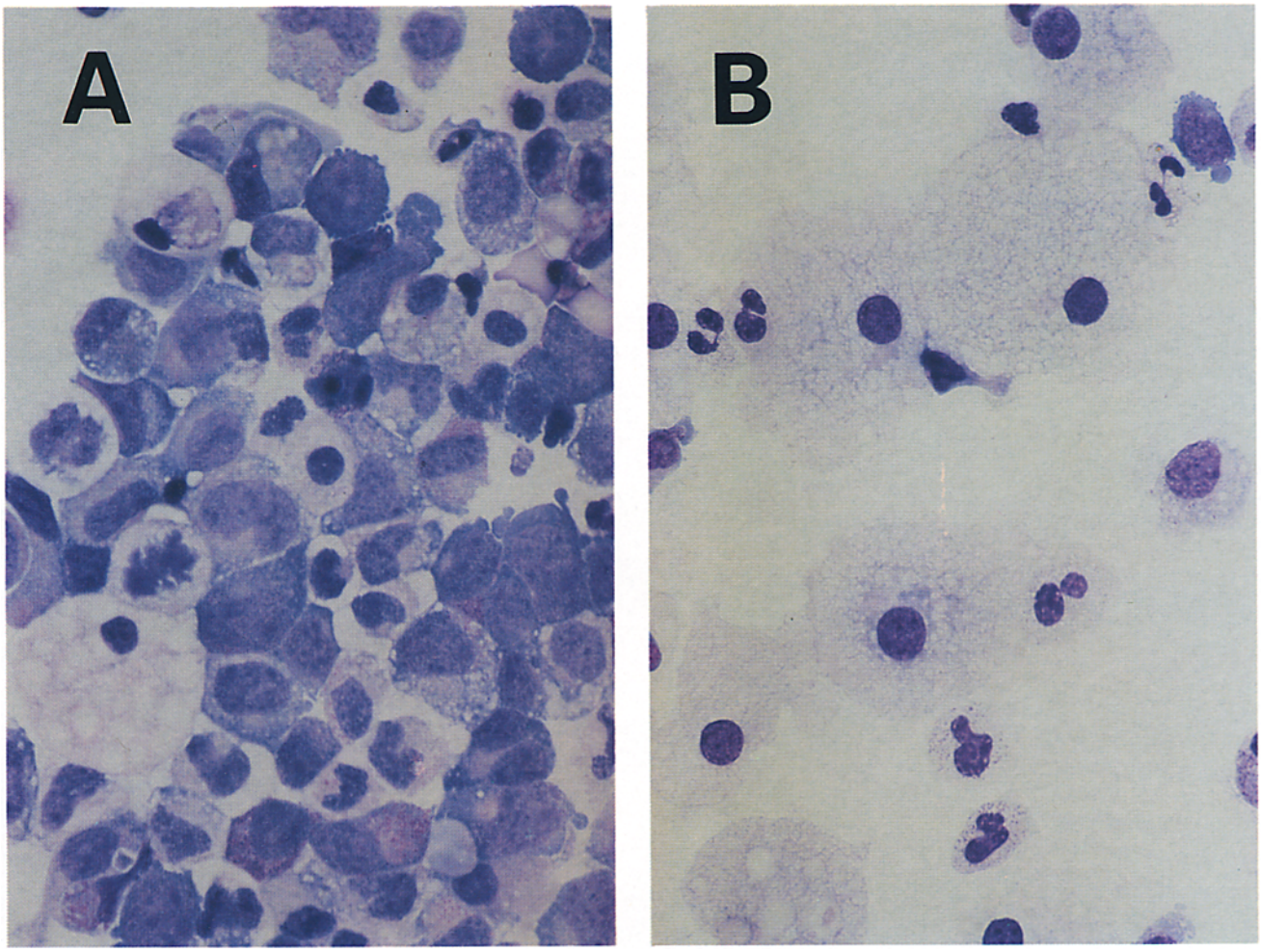

Figure 4. May-Grünwald-Giemsa staining of CMML cells cultured for 7 d with medium alone (*A*) or with 10 ng/ml IL-10 (*B*) in patient PM.

to intact cells was found. The linear Scatchard transformation suggested a single class of specific binding sites. CMML cells of these patients expressed 5.6, 5.0, and 5.2 × 10^4 IL-10–binding sites per cell with K_d of 1.8, 1.2, and 1.2 nmol/liter, respectively. The corresponding inhibitory constants (IC_{50}) were 2.1, 1.8, and 2.4 nmol/l.

Inhibitory Effect of Anti–GM-CSF Antibody on Autonomous CFU-GM Growth from CMML Cells. To elucidate the possible mechanism of the inhibitory action of IL-10, we first tried to identify the factor responsible for autonomous colony growth by adding neutralizing antibodies against GM-CSF, G-CSF, IL-3, and IL-6 to cell cultures from three pa-

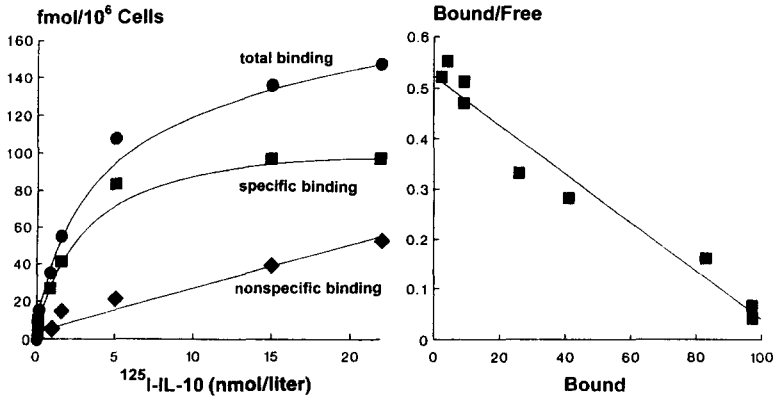

Figure 5. Binding of ^{125}I-IL-10 to CMML cells in patient FJ. The left panel shows the saturation curve and the right panel shows the Scatchard plot. Specific binding was determined as total binding − nonspecific binding. In this patient, the binding capacity amounted to 93 fmol/10^6 cells (i.e., 56,000 sites per cell) with a corresponding K_d of 1.8 nmol/liter.

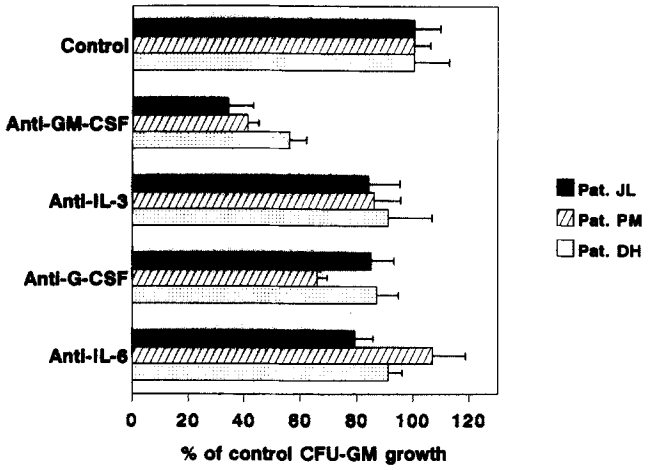

Figure 6. Effect of anti cytokine antibodies on spontaneous CFU-GM growth of CMML cells in three patients. PB MNC were cultured with medium alone or with antibodies against GM-CSF, G-CSF, IL-3, or IL-6, respectively. Colony growth was assessed after 14 d. Results represent mean values ± SD from triplicates. ■, patient JL; ▨, patient PM; ▩, patient DH.

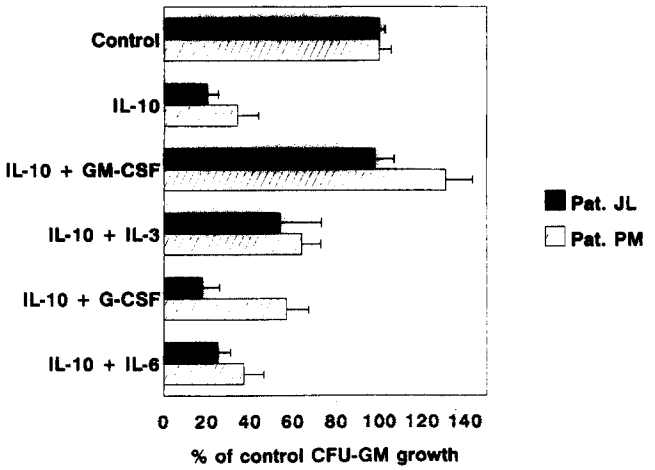

Figure 7. Effect of exogenous growth factors on IL-10–induced suppression of CFU-GM growth from CMML cells. PB MNC were cultured in methylcellulose with 10 ng/ml IL-10 in the presence or absence of exogenous GM-CSF (100 U/ml), G-CSF (100 U/ml), IL-3 (10 U/ml), and IL-6 (10 ng/ml), respectively. Colony growth was assessed after 14 d. Results represent mean values ± SD from triplicates. ■, Patient JL; ▨, patient PM.

tients with excessive colony growth. A previous study has suggested GM-CSF and IL-6 as candidate growth factors in CMML, since both cytokines were detectable in conditioned media from CMML cells, and antibodies against GM-CSF and IL-6 could partially inhibit spontaneous colony formation by CMML cells (6). In our patients, only antibody against GM-CSF, however, reproducibly inhibited autonomous CFU-GM growth, suggesting GM-CSF as a major autocrine growth factor (Fig. 6).

Effect of Exogenous Growth Factors on IL-10 Induced Suppression of CFU-GM Growth from CMML Cells. The antiproliferative action of anti–GM-CSF antibody in CMML cells and the fact that IL-10 has been shown to inhibit cytokine synthesis, including GM-CSF, in human monocytes

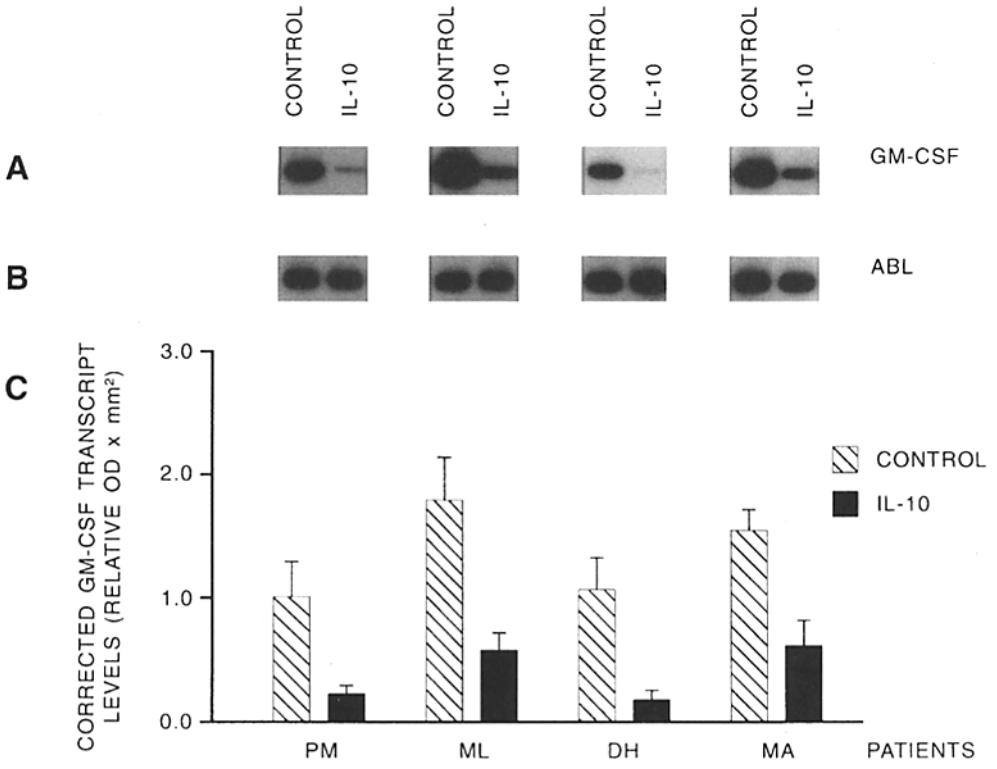

Figure 8. Semiquantitative RT-PCR analysis of GM-CSF transcript levels. (*A*) Autoradiograms showing incorporated radioactivity of amplification products obtained from PB MNC cultured in suspension with or without IL-10 for 24 h. (*B*) Autoradiograms showing ABL transcripts that served as a reference to correct for potential variations of RNA or cDNA samples. (*C*) Corrected GM-CSF transcript levels in cultured CMML cells. Each patient sample was analyzed in three radioactive PCR analyses in duplicate using freshly synthesized cDNA. The quantity of ^{32}P incorporated into the PCR product was determined by densitometric scanning of the autoradiograms. Results were corrected by dividing GM-CSF values by the mean values obtained from six ABL transcripts of that cDNA. The results are shown as mean values ± SD.

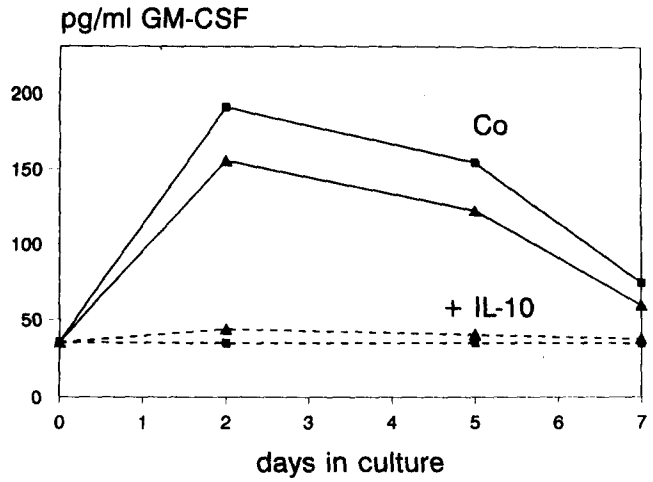

Figure 9. GM-CSF levels in supernatants of CMML cell cultures with medium alone or with 10 ng/ml IL-10 in patient JL (▲) and patient PM (■).

(16), led us to hypothesize that inhibition of CMML growth by IL-10 was secondary to IL-10–induced suppression of endogenous GM-CSF release. If this was the case, one would expect that exogenous addition of GM-CSF could largely reverse growth inhibition by IL-10. In contrast, restoration of colony growth by exogenous growth factors would not be observed if IL-10 had a direct cytotoxic effect on CMML cells. In fact, exogenous GM-CSF was able to completely overcome IL-10–induced suppression in two patients tested (Fig. 7). Not surprisingly, G-CSF, IL-3, or IL-6 were ineffective or only moderately effective in correcting IL-10–induced growth inhibition.

Inhibitory Effect of IL-10 on GM-CSF Messenger RNA Expression in CMML Cells. Analyses of the effect of IL-10 on GM-CSF expression in CMML cells at the mRNA level were performed in four patients. GM-CSF transcripts, as measured by the semiquantitative RT-PCR technique (22, 23), were already present in freshly isolated CMML cells and further increased in these cells after 24 h of suspension culture with medium alone (not shown). In comparison, ABL transcripts, which served as a control, remained unchanged during culture. In the presence of IL-10 (10 ng/ml), GM-CSF transcript levels after 24 h were markedly decreased, as compared to cells kept in suspension without IL-10 (Fig. 8). Fig. 8 *C* shows corrected GM-CSF mRNA levels in the four patients. Comparison of corrected GM-CSF transcript levels of CMML cells cultured with and without IL-10 revealed a mean decrease of 79% in patient PM ($P < 0.0005$), 68% in patient ML ($P < 0.0001$), 83% in patient DH ($P < 0.005$), and 60% in patient MA ($P < 0.005$), respectively, by IL-10.

Inhibitory Effect of IL-10 on GM-CSF Production by CMML Cells. To confirm the inhibitory effect of IL-10 on GM-CSF production in CMML cells at the protein level, supernatants from CMML cell suspension cultures obtained at different time points were analyzed for GM-CSF by an immunoenzymetric assay (EASIA) in two patients. As shown in Fig. 9, CMML cells cultured in medium alone secreted GM-CSF spontaneously, with levels peaking at day 2 and gradually declining thereafter. In contrast, GM-CSF secretion by CMML cells was almost completely abrogated in the presence of IL-10 (10 ng/ml) at all time points studied.

In summary, we demonstrate here a hitherto unknown profound inhibitory effect of IL-10 on the in vitro growth of CMML cells. IL-10 not only inhibited growth of CFU-GM in semisolid medium, but also clearly inhibited the growth of CMML cells in suspension. IL-10 was remarkebly effective in the majority of patients tested. The demonstration of specific binding of IL-10 to CMML cells suggests a direct effect of IL-10 on the leukemic cell population. Although the exact mechanism of the inhibitory action of IL-10 on CMML cells remains to be shown, our data provide strong evidence that a modulation of endogenous GM-CSF release in CMML cells by IL-10 plays a major role. Therefore, growth inhibition by IL-10 may represent a novel strategy to interrupt autostimulatory loops in hematological malignancy. Administration of IL-10 as a therapeutic option should be harmless to normal hematopoietic cells, since we have not observed any inhibitory effect of IL-10 on normal $CD34^+$ cells (data not shown).

Regardless of the mechanism involved the profound inhibitory effect of IL-10 on CMML cell growth in vitro has clear clinical implication. Currently, patients with CMML appear to have a poor prognosis with conventional cytotoxic drugs (3). Recently, IL-10 has been administered in healthy volunteers and serum levels of the cytokine that are effective in vitro were achieved at tolerable administered doses (25). Thus, biologic chemotherapy of CMML with IL-10 is feasible and our findings provide experimental support for clinical trials of IL-10 in patients with CMML.

Address correspondence to Dr. Klaus Geissler, Division of Hematology–Internal Medicine I, Room 6.I9.29, University of Vienna, Währinger Gürtel 18-10, A-1090 Vienna, Austria.

Received for publication 23 January 1996 and in revised form 3 July 1996.

References

1. Geary, C.G., D. Catovsky, E. Wiltshow, G.R. Milner, M.C. Scholes, S. Van Noorden, L.D. Wadworth, S. Muldal, J.E. MacIver, and D.A.G. Galton. 1975. Chronic myelomonocytic leukemia. *Br. J. Haematol.* 30:289–302.

2. Bennett, J.M., D. Catovsky, M.T. Daniel, G. Flandrin, D.A.G. Galton, H.R. Gralnick, and C. Sultan. The French-American-British (FAB) Co-operative Group (1982) proposals for the classification of the myelodysplastic syndromes. *Br.*

J. Haematol. 51:189–199.

3. Solal-Celigny, P., B. Desaint, A. Herrera, C. Chastang, M. Amar, M. Vroclans, N. Brousse, F. Mancilla, M. Renoux, J.F. Bernard, and P. Boivin. 1984. Chronic myelomonocytic leukemia according to FAB classification: analysis of 35 cases. *Blood.* 63:634–638.
4. Geissler, K., W. Hinterberger, P. Bettelheim, O. Haas, and K. Lechner. 1988. Colony growth characteristics in chronic myelomonocytic leukemia. *Leuk. Res.* 12:373–377.
5. Schrader, J.W. 1993. Re-Examining the autocrine loop in acute myeloid leukaemia. *Adv. Cancer Ther.* 4:5–16.
6. Everson, M.P., C.B. Brown, and M.B. Lilly. 1989. Interleukin-6 and granulocyte-macrophage colony-stimulating factor are candidate growth factors for chronic myelomonocytic leukemia cells. *Blood.* 74:1472–1476.
7. Fiorentino, D.F., M.W. Bond, and T.R. Mosmann. 1989. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170:2081–2095.
8. Veiira, P., R. de Waal-Malefyt, M.-W. Dang, K.E. Johnson, R. Kastelein, D.F. Fiorentino, J.E. de Vries, M.-G. Roncarolo, T.R. Mosmann, and K.W. Moore. 1991. Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. *Proc. Natl. Acad. Sci. USA.* 88:1172–1176.
9. Spits, H., and R. de Waal Malefyt. 1992. Functional characterization of IL-10. *Int. Arch. Allergy. Immunol.* 99:8–15.
10. Rousset, F., E. Garcia, T. Defrance, C. Péronne, N. Vezzio, D.H. Hsu, R. Kastelstein, K.W. Moore, and J. Banchereau. 1992. Human and viral IL-10 are potent growth and differentiation factors for activated human B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 89:1890–1893.
11. Ralph, P., I. Nakoinz, A. Sampson-Johannes, S. Fong, D. Lowe, H.Y. Min, and L. Lin. 1992. IL-10, T lymphocyte inhibitor of human blood cell production of IL-1 and tumor necrosis factor. *J. Immunol.* 148:808–814.
12. Hsu, D.-H., K.W. Moore, and H. Spits. 1992. Differential effects of interleukin-4 and -10 on interleukin-2-induced interferon-gamma synthesis and lymphokine-activated killer activity. *Int. Immunol.* 4:563–569.
13. Thompson-Snipes, L.A., V. Dhar, M.W. Bond, T.R. Mosmann, K.W. Moore, and D.M. Rennick. 1991. Interleukin 10: a novel stimulatory factor for mast cells and their progenitors. *J. Exp. Med.* 173:507–510.
14. Cassatella, M.A., L. Meda, S. Bonora, M. Ceska, and G. Constantin. 1993. Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1β mediating the production of IL-8 triggered by lipopolysaccharide. *J. Exp. Med.* 178:2207–2211.
15. Takanaski, S., R. Nonaka, Z. Xing, P. O'Byrne, J. Dolovich, and M. Jordana. 1994. Interleukin 10 inhibits lipopolysaccharide-induced survival and cytokine production by human peripheral blood eosinophils. *J. Exp. Med.* 180:711–715.
16. De Waal Malefyt, R., J. Abrams, B. Bennett, C.G. Figdor, and J.E. de Vries. 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL- 10 produced by monocytes. *J. Exp. Med.* 174:1209–1220.
17. Fiorentino, D.F., A. Zlotnik, T.R. Mosmann, M. Howard, and A. O'Garra. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147:3815–3822.
18. Virgolini, I., M. Raderer, A. Kurtaran, P. Angelberger, S. Banyai, Q. Yang, S. Li, M. Banyai, J. Pidlich, B. Niederle, W. Scheithauer, and P. Valent. 1994. Vasoactive intestinal peptide-receptor imaging for the localization of intestinal adenocarcinomas and endocrine tumors. *N. Engl. J. Med.* 331: 1116–1121.
19. Scatchard, G. 1949. The attractions of proteins for small molecules and ions. *Ann. NY Acad. Sci.* 51:660–672.
20. Chomczynski, P., and N. Sacchi. 1987. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.
21. Maurer, J., J.W. Janssen, E. Thiel, J. van Denderen, W.D. Ludwig, Ü. Aydemir, B. Heinze, C. Fonatsch, J. Harbott, H. Riehm, D. Hoelzer, and C.R. Bartram. 1991. Detection of chimeric BCR-ABL genes in acute lymphoblastic leukaemia by the polymerase chain reaction. *Lancet.* 337:1055–1058.
22. Russell, M.E., D.H. Adamd, L.R. Wyner, Y. Yamashita, N.J. Halnon, and M.J. Karnovsky. 1993. Early and persistent induction of monocyte chemoattractant protein in rat cardiac allografts. *Proc. Natl. Acad. Sci. USA.* 90:6086–6090.
23. Khoury, S.J., L. Gallon, W. Chen, K. Betres, M.E. Russell, W.W. Hancock, C.B. Carpenter, M.H. Sayegh, and H.L. Weiner. 1995. Mechanism of acquired thymic tolerance in experimental autoimmune encephalomyelitis: thymic dendritic-enriched cells induce specific peripheral T cell unresponsiveness in vivo. *J. Exp. Med.* 182:357–366.
24. Miyatake, S., T. Otsuka, T. Yokota, F. Lee, and K. Arai. 1985. Structure of the chromosomal gene for granulocyte/macrophage colony stimulating factor: comparison of the mouse and human genes. *EMBO (Eur. Mol. Biol. Organ.) J.* 4: 2561–2568.
25. Chernoff, A.E., E.V. Granowitz, L. Shapiro, E. Vannier, G. Lonnemann, J.B. Angel, J.S. Kennedy, A.R. Rabson, S.M. Wolff, and C.A. Dinarello. 1995. A randomized, controlled trial of IL-10 in humans. Inhibition of inflammatory cytokine production and immune responses. *J. Immunol.* 154:5492–5499.