

Reciprocal regulation of cultured human mast cell cytokine production by IL-4 and IFN- γ

Hiroshi Tachimoto, MD, PhD,^a Motohiro Ebisawa, MD, PhD,^{a,b} Tomohide Hasegawa,^a Tomoko Kashiwabara, PhD,^c Chisei Ra, MD, PhD,^d Bruce S. Bochner, MD,^e Katsushi Miura, MD,^e and Hirohisa Saito, MD, PhD^a *Tokyo and Kanagawa, Japan, and Baltimore, Md*

Background: T_H1 and T_H2 cytokines are thought to regulate allergic inflammation.

Objective: Two key regulatory cytokines, IL-4 and IFN- γ , were examined for their effects on cytokine production by cultured human mast cells (CHMCs).

Methods: CHMCs were obtained by culturing cord blood-derived CD34⁺ cells in the presence of stem cell factor and IL-6 for 14 to 16 weeks. CHMCs were passively sensitized with human myeloma IgE and supplemented with or without IL-4 or IFN- γ . After the sensitization, CHMCs were stimulated with anti-Fc ϵ RI α mAb. Concentrations of secreted cytokines were measured by using ELISA, and cytokine messenger RNA was analyzed by using quantitative competitive RT-PCR.

Results: IL-4 profoundly enhanced Fc ϵ RI-mediated production of macrophage inflammatory protein (MIP) 1 α , IL-8, and GM-CSF. For example, the enhancement by IL-4 (10 ng/mL) of the production of MIP-1 α , IL-8, and GM-CSF was 25-, 7-, and 90-fold, respectively, after 6 hours. IL-4 also enhanced levels of Fc ϵ RI-induced cytokine messenger RNA but to a lesser degree. In contrast, IFN- γ inhibited Fc ϵ RI-induced production of MIP-1 α , IL-8, and GM-CSF. For example, the inhibition by IFN- γ (10 ng/mL) of Fc ϵ RI-mediated production of MIP-1 α , IL-8, and GM-CSF was 80%, 75%, and 95%, respectively. IFN- γ also suppressed Fc ϵ RI-induced messenger RNA expression of these cytokines. Neither IL-4 nor IFN- γ affected the kinetics of cytokine production by CHMCs.

Conclusion: These data suggest that IL-4 and IFN- γ may influence allergic reactions by modulating human mast cell cytokine production. (*J Allergy Clin Immunol* 2000;106:141-9.)

Key words: Human, mast cell, cellular activation, cytokines, chemokines, IgE, allergy

Abbreviations used

CHMC:	Cultured human mast cell
CRA-1:	Anti-human Fc ϵ RI α -chain mAb
dNTP:	Deoxyribonucleoside triphosphate
MIP-1 α :	Macrophage inflammatory protein 1 α
SCF:	Stem cell factor

Mast cells are important effector cells in IgE-mediated allergic reactions. It has been reported that in addition to the release of chemical mediators (histamine, leukotrienes, and prostaglandins), mast cells are capable of producing several cytokines after Fc ϵ RI cross-linking.¹ In human systems expression of IL-4, IL-5, IL-6, IL-8, TNF- α , and IL-13 has been reported in lung, skin, or intestinal tissue mast cells,²⁻⁹ although there is much controversy about the expression of IL-4 in mast cells.^{6,10,11} These mast cell-derived cytokines may contribute to the late-phase allergic reaction and to chronic allergic diseases through the recruitment of inflammatory cells, such as lymphocytes, eosinophils, and basophils, into the site of allergic inflammation. However, little is known about the regulation of mast cell cytokine production because of difficulties in purification of human mast cells, small numbers, and the lack of appropriate cell lines.

Recently, we established a culture system to derive human mast cells from CD34⁺ cells in cord blood in the presence of stem cell factor (SCF) and IL-6.¹¹⁻¹³ These cells have functional Fc ϵ RI α , and most contain tryptase and chymase.¹¹ These cultured human mast cells (CHMCs) released histamine after stimulation with calcium ionophore A23187 or cross-linking of Fc ϵ RI but not with compound 48/80 or substance P,¹⁴ indicating that CHMCs have characteristics typical of mature mast cells from lung tissue. CHMCs are capable of producing cytokines, such as GM-CSF, IL-5, macrophage inflammatory protein (MIP)-1 α , IL-8, and TNF- α , but not IL-4, within hours of cross-linking of Fc ϵ RI.^{11,13} The availability of CHMCs has made it possible to study the regulation of mast cell cytokine production by means of Fc ϵ RI cross-linking.

From ^athe Department of Allergy, National Children's Medical Research Center, Tokyo; ^bthe Department of Pediatrics, National Sagami Hospital, Kanagawa; ^cGenox Research, Inc, Tokyo; ^dthe Department of Immunology, Juntendo University School of Medicine, Tokyo; and ^ethe Johns Hopkins Asthma and Allergy Center, Baltimore.

Received for publication Jan 10, 2000; revised Mar 2, 2000; accepted for publication Mar 3, 2000.

Reprint requests: Motohiro Ebisawa, MD, PhD, Department of Allergy, National Children's Medical Research Center 3-35-31, Taishido, Setagaya-ku, Tokyo, 154-8509, Japan.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/107043

doi:10.1067/mai.2000.107043

We hypothesized that FcεRI-mediated cytokine production by CHMCs could be regulated by cytokines, such as IL-4 and IFN-γ. In the present study we examined the effect of IL-4 or IFN-γ on FcεRI-mediated mast cell cytokine production by using CHMCs.

METHODS

Human mast cell culture

CHMCs were generated as previously reported.^{11,12} In brief, heparin-treated umbilical cord blood that was obtained (under an informed consent approved by the hospital's ethical committee) was diluted and layered over lymphocyte separation medium (Organon Teknica Corp) at room temperature. Cord blood mononuclear cells were purified by density gradient centrifugation methods followed by immunomagnetic positive selection with mAb against CD34 (Miltenyi Biotec Inc). The purified CD34⁺ cells were cultured in IBL Media I containing 10 μg/mL insulin, 10 μg/mL transferrin, 5×10^{-5} mol/L 2-mercaptoethanol, 25 mmol/L HEPES, and 2.6 ng/mL NaSeO₃ (IBL Men-eki Seibutu Kenkyujo) supplemented with 5% FCS (Cansera), 100 ng/mL SCF, and 50 ng/mL IL-6 (both generously provided by Kirin Brewery Co, Ltd; mast cell culture medium) for 14 to 16 weeks. The purity of mast cells was determined by staining with May-Grünwald and Giemsa reagents. The purity of mast cells reached 99% to 100% by 14 to 16 weeks of culture.

Cell treatment and IgE sensitization

CHMCs (0.5 to 1×10^5 /mL) were suspended in mast cell culture medium. The cells were incubated with 5 μg/mL human myeloma IgE (a generous gift from Dr Kimishige Ishizaka, La Jolla, Calif) at 37°C for 2 days. During the incubation with IgE, CHMCs were treated with or without recombinant human IL-4 or IFN-γ (R&D Systems) at the concentrations indicated. To examine the kinetics of IL-4- or IFN-γ-mediated events, CHMCs were incubated with or without 10 ng/mL IL-4 or IFN-γ for the last 15 minutes and 1, 2, 4, or 8 days of the culture before rinsing away free IgE. In some experiments cells were cultured with IgE in the presence or absence of IFN-γ for 2 days and the effect of IFN-γ on FcεRI expression and histamine release to anti-human FcεRIα-chain mAb (CRA-1) was determined as previously described.^{10,11} After the incubation, cell viability was assessed by means of trypan blue dye exclusion. IL-4 and IFN-γ did not affect CHMC viability after 8 days (control vs 10 ng/mL IL-4: $97.1\% \pm 1.0\%$ vs $96.8\% \pm 1.0\%$, $n = 4$; control vs 10 ng/mL IFN-γ: $95.4\% \pm 2.0\%$ vs $94.7\% \pm 2.0\%$, $n = 5$).

Assays for cytokine production by CHMCs

After incubation with IgE and treatment with IL-4 or IFN-γ, cells were washed twice and resuspended in mast cell culture medium at 2 to 5×10^5 cells/mL with or without various concentrations of IL-4 and IFN-γ (0.1–10 ng/mL). CHMCs were challenged with an optimal concentration (1 μg/mL) of CRA-1 at 37°C for 6 hours. In the kinetic experiments CHMCs were resuspended at 2 to 5×10^5 cells/mL in mast cell culture medium supplemented with or without 10 ng/mL IL-4 or IFN-γ and then stimulated with CRA-1 at 37°C for 1, 3, 6, and 18 hours. In the other kinetics experiments with pretreatment with IL-4 or IFN-γ, CHMCs were resuspended in mast cell culture medium with or without 10 ng/mL IL-4 or IFN-γ and then stimulated with CRA-1 at 37°C for 6 hours. After incubation with CRA-1, the supernatants were collected and stored at –20°C. GM-CSF, IL-8, and MIP-1α protein levels were measured by using ELISA (BOTRAK cytokine ELISA system, Amersham International, Amersham Life Science Inc). The detection limits were as follows: MIP-1α of 6 pg/mL and IL-8 and GM-CSF of 2 pg/mL.

Analysis of cytokine messenger RNA expression by quantitative competitive RT-PCR

CHMCs (5×10^5 cells/sample) were preincubated with IgE (5 μg/mL) for 2 days with or without IL-4 (10 ng/mL) or IFN-γ (10 ng/mL) in mast cell culture medium. After washing twice, CHMCs were incubated with 1 μg/mL CRA-1 for 2 hours before harvesting. Total cellular RNA was prepared with RNeasy Mini Kits (Qiagen) according to the manufacturer's instructions. Total cellular RNA was reverse-transcribed to complementary (c)DNA by using first-strand cDNA synthesis kits (Pharmacia Biotech). The RNA extract was denatured at 65°C for 10 minutes and mixed with RT solution containing a reverse transcriptase, deoxyribonucleoside triphosphate (dNTP), Not I-d(18), and dithiothreitol solution. The mixture was incubated at 37°C for 60 minutes and then at 65°C for 5 minutes to inactivate reverse transcriptase.

Levels of cDNA were quantified by means of a competitive PCR method.^{15,16} PCR mimics were constructed by using the PCR MIMIC construction kit (Clontech). Cytokine mimics were prepared for GM-CSF, IL-8, and MIP-1α by using PCR for 16 or 21 cycles. To construct the PCR mimics, two rounds of PCR amplification were performed. In the first PCR reaction two composite primers were used. Each composite primer had the target cytokine gene primer sequence attached to a short nucleotide stretch of sequence designed to hybridize to opposite strands of a fragment of v-erbB gene DNA. The first PCR was cycled at 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 90 seconds. The sequences of composite primers were as follows: GM-CSF, 5'-CTG AAC CTG AGT AGA GAC ACT GCG CAA GTG AAA TCT CCT CCG-3' and 5'-GGG GAT GAC AAG CAG AAA GTC TCT GTC AAT GCA GTT TGT AG-3'; IL-8, 5'-TGT GTG AAG GTG CAG TTT TGC CCG CAA GTG AAA TCT CCT CCG-3' and 5'-CCC TCT TCA AAA ACT TCT CCA CGG GAC AAG ATA CTC ATC TGC-3'; MIP-1α, 5'-TCT GCA CCA TGG CTC TCT GCG CAA GTG AAA TCT CCT CCG-3' and 5'-CAC TCC TCA CTG GGG TCA GGG ACA AGA TAC TCA TCT GC-3'; β-actin, 5'-GTA CGT TGC TAT CCA GGC TGC GCA AGT GAA ATC TCC TCC G-3' and 5'-CTT AAT GTC ACG CAC GAT TTC CTC TGT CAA TGC AGC AGT TTG TAG-3'. The first PCR reaction products were diluted and amplified in a second round of PCR for 18 cycles with cytokine-specific primers to ensure that all PCR mimics had the complete gene-specific primer sequences. The sequences of cytokine specific primers were as follows: GM-CSF, 5'-CTG AAC CTG AGT AGA GAC ACT G-3' and 5'-GGG GAT GAC AAG CAG AAA GTC-3'; IL-8, 5'-TGT GTG AAG GTG CAG TTT TGC C-3' and 5'-CCC TCT TCA AAA ACT TCT CCA C-3'; MIP-1α, 5'-TCT GCA CCA TGG CTC TCT G-3' and 5'-CAC TCC TCA CTG GGG TCA-3'; β-actin, 5'-GTA CGT TGC TAT CCA GGC TG-3' and 5'-CTT AAT GTC ACG CAC GAT TTC C-3'. After the second PCR amplification, the PCR mimics were purified by passage through CHROMA SPIN+TE-100 Columns (Clontech). The yield of PCR mimic was calculated and diluted to 100 attomoles/mL. For determination of cytokine messenger (m)RNA levels, 10^{-3} to 10^2 attmoles of PCR mimics were added to PCR amplification reactions containing a constant amount of the experimental cDNA sample. To perform competitive PCR, aliquots (2 mL of cDNA) of sample and PCR mimics were added to buffers containing 0.8 mL of dNTP mixture (final concentration, 0.5 mol/L; GeneAmp dNTPs; Perkin-Elmer), 0.5 mL Taq polymerase (2.5 U/20 mL reaction; AmpliTaq DNA Polymerase), 2 mL of paired primers, and 2 mL of 10× PCR buffer (1.5 mmol/L MgCl₂, Perkin-Elmer). The PCR cycled according to the following protocol in a Perkin-Elmer DNA thermal cycler: denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 min-

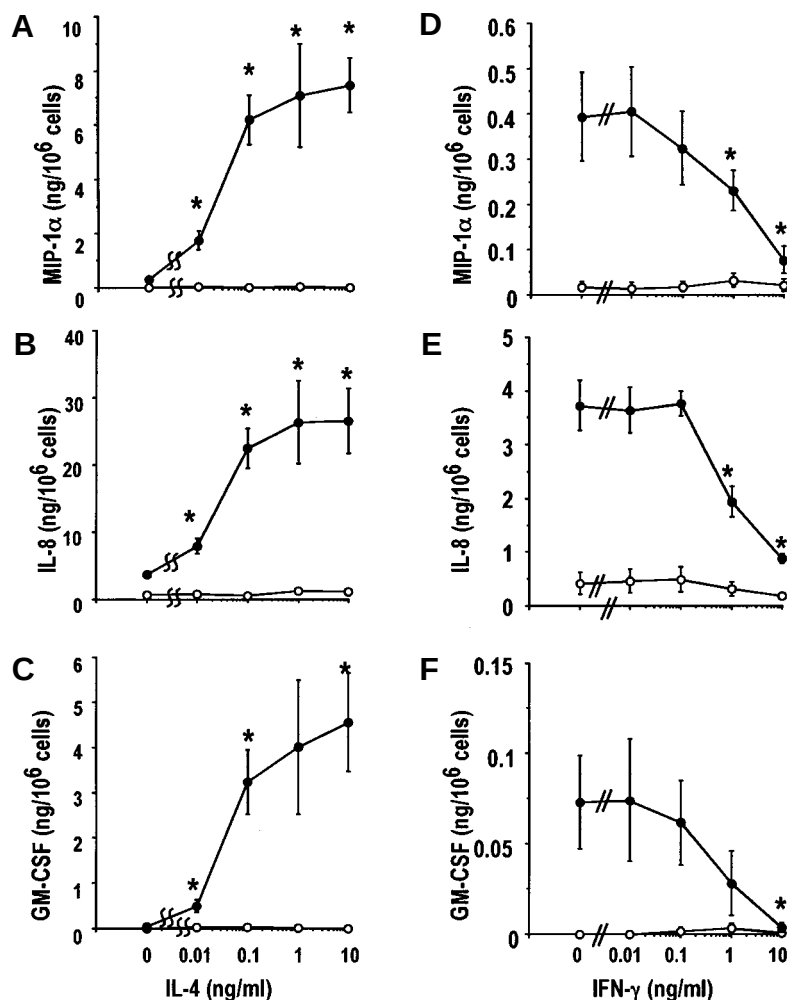


FIG 1. Effect of IL-4 or IFN- γ on Fc ϵ RI-mediated cytokine production by CHMCs. CHMCs were cultured with 0 to 10 ng/mL IL-4 (A-C) or IFN- γ (D-F). Cells were incubated with (filled circles) or without (open circles) 1 μ g/mL CRA-1 at 37°C for 6 hours. The concentrations of MIP-1 α (A and D), IL-8 (B and E), and GM-CSF (C and F) in the supernatants were measured by using ELISA. Values represent mean $\pm$ SEM of 4 separate experiments. * P < .05 compared with the control condition without IL-4 or IFN- γ .

utes. β -Actin and cytokines were cycled 32 times before a 4-minute incubation at 72°C for a final extension. PCR products were visualized on a 1.5% agarose gel in a buffer containing 0.5 mg/mL ethidium bromide.

Statistics

The data were analyzed by using the paired Student t test. Data are displayed as means $\pm$ SEM. The level of significance was set at a P value of less than .05 for all tests.

RESULTS

Effect of IL-4 or IFN- γ on cytokine production by CHMCs

CHMCs were incubated with IgE in the presence or absence of IL-4 for 2 days as concentration indicated. The sensitized CHMCs were washed and resuspended in mast

cell culture medium containing 0 to 10 ng/mL IL-4 and 1 μ g/mL CRA-1 for 6 hours. Supernatants were harvested and analyzed for various cytokines by using ELISA. IL-4 did not affect spontaneous MIP-1 α production by CHMCs. Fc ϵ RI-mediated MIP-1 α production by CHMCs was 0.3 ± 0.1 ng/10⁶ cells in the absence of IL-4 (Fig 1, A). MIP-1 α production by CHMCs was strongly enhanced up to 7.5 ± 1.0 ng/10⁶ cells in the presence of 10 ng/mL IL-4 (25-fold increase). Although the lowest concentration of IL-4 to significantly enhance MIP-1 α production was 0.01 ng/mL, MIP-1 α production was remarkably enhanced by IL-4 above concentrations of 0.1 ng/mL. The effects of IL-4 on the production of IL-8 and those of GM-CSF by CHMCs were also examined (Fig 1, B and C). Fc ϵ RI-mediated IL-8 or GM-CSF production by CHMCs was 3.7 ± 0.5 ng/10⁶ cells or 0.05 ± 0.03 ng/10⁶ cells in the

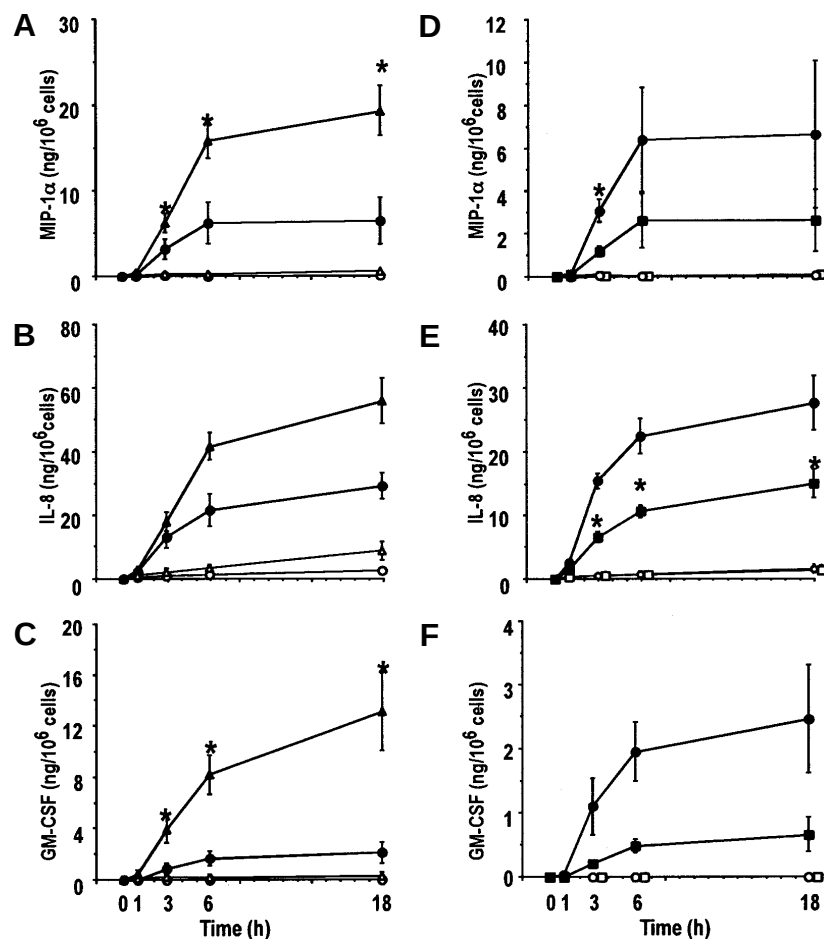


FIG 2. Effect of IL-4 or IFN- γ on the kinetics of Fc ϵ RI-mediated cytokine production by CHMCs. CHMCs were cultured with 5 μ g/mL IgE in the presence (IL-4, triangles; IFN- γ , squares) or absence (circles) of 10 ng/mL IL-4 (A-C) or IFN- γ (D-F). Then cells were incubated with (filled symbols) or without (open symbols) 1 μ g/mL CRA-1 at 37°C for 1, 3, 6, and 18 hours; supernatants were harvested; and concentrations of MIP-1 α (A and D), IL-8 (B and E), and GM-CSF (C and F) were determined. Values represent mean $\pm$ SEM of 4 separate experiments. * P < .05 compared with the control condition (without IL-4 or IFN- γ) at each time point.

absence of IL-4 (10 ng/mL), respectively. IL-4 enhanced IL-8 or GM-CSF production to 26.5 ± 4.8 ng/10⁶ cells (7-fold increase) or 4.6 ± 1.1 ng/10⁶ cells (90-fold increase), respectively. IL-4 did not affect spontaneous production of these 3 cytokines, as shown in Fig 1.

We next examined the effect of IFN- γ , a T_H1 cytokine, on mast cell cytokine production (Fig 1, D-F). CHMCs were first sensitized with IgE in the presence of up to 10 ng/mL IFN- γ for 2 days, washed, and resuspended in mast cell culture medium with IFN- γ at the concentrations indicated. Cells were then incubated with or without CRA-1 for 6 hours, and supernatants were assayed for various cytokines. Spontaneous production of MIP-1 α , IL-8, and GM-CSF was not affected by exposure of cells to IFN- γ for 2 days. However, IFN- γ treatment, in contrast to the IL-4 effects, inhibited the production of these cytokines. Fc ϵ RI-mediated MIP-1 α production by CHMCs without IFN- γ was 394 ± 98 pg/10⁶ cells,

whereas production was 77 ± 30 pg/10⁶ cells (80% inhibition) after pretreatment with IFN- γ . IL-8 production by CHMCs was also suppressed by IFN- γ , from 3.7 ± 0.5 ng/10⁶ cells to 0.9 ± 0.09 ng/10⁶ cells (75% inhibition), as was GM-CSF production, from 72.8 ± 25.7 pg/10⁶ cells to 3.8 ± 2.5 pg/10⁶ cells (95% inhibition). We also examined the effect of IFN- γ on Fc ϵ RI expression induced by IgE. In the absence of IgE, Fc ϵ RI expression was minimal on CHMCs (net mean fluorescence intensity, 1.8 ± 1.0 ; $n = 4$), which is consistent with previous observations.¹⁷⁻¹⁹ IFN- γ inhibited IgE-induced Fc ϵ RI expression by 32% (from a net mean fluorescence intensity of 11.8 ± 2.6 to 8.0 ± 2.5 , $n = 4$). Despite the reduction of Fc ϵ RI levels, histamine release was enhanced by 74% (from $12.1\% \pm 1.4\%$ to $21.1\% \pm 3.4\%$ histamine release, $n = 4$). These data demonstrate different effects of IFN- γ on histamine release compared with effects on Fc ϵ RI expression and cytokine production.

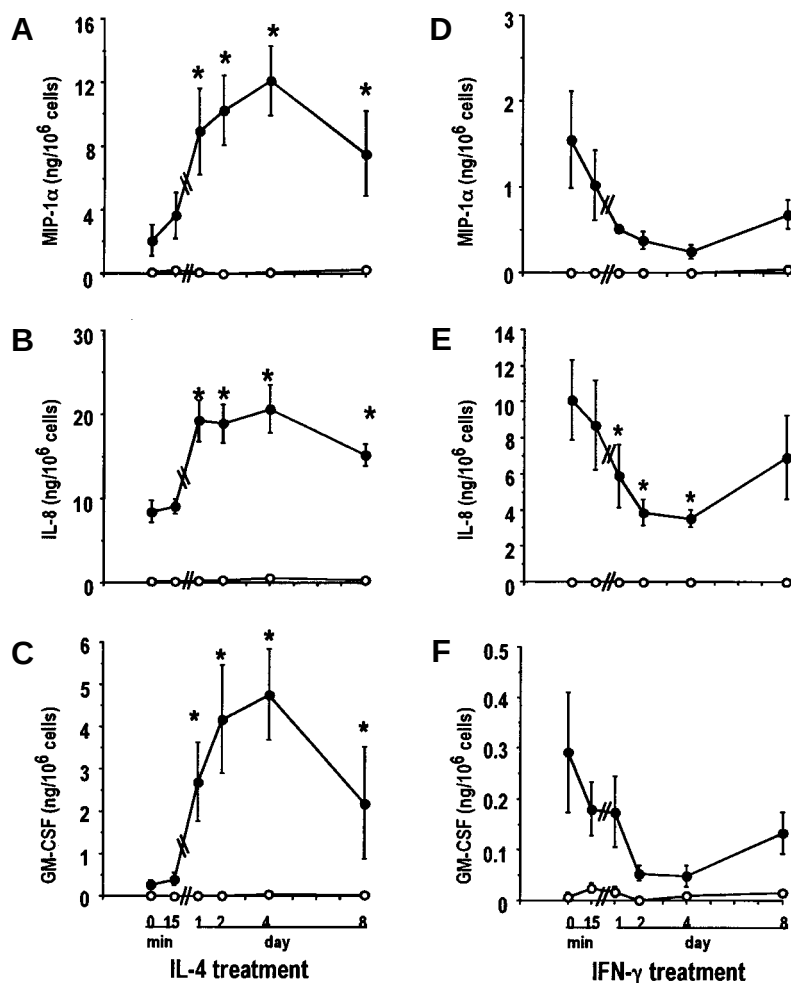


FIG 3. Kinetics of IL-4-induced enhancement of FcεRI-mediated cytokine production by CHMCs. CHMCs were cultured with or without IL-4 (10 ng/mL, **A-C**) or IFN-γ (10 ng/mL, **D-F**) in the presence of SCF (100 ng/mL) and IL-6 (50 ng/mL) for 15 minutes and 1, 2, 4 or 8 days and human myeloma IgE (5 μg/mL) for 2 days. Then cells were incubated with (filled circles) or without (open circles) CRA-1 (1 μg/mL) at 37°C for 6 hours, supernatants were harvested, and concentrations of MIP-1α (**A** and **D**), IL-8 (**B** and **E**), and GM-CSF (**C** and **F**) were measured. Values represent mean ± SEM of 4 separate experiments. **P* < .05 compared with the control condition at day 0 with or without IL-4 or IFN-γ.

Effect of IL-4 and IFN-γ on the kinetics of cytokine production by CHMCs

In these experiments the kinetics of cytokine production by CHMCs was first examined in the presence of IL-4 (10 ng/mL). MIP-1α, IL-8, and GM-CSF production in the absence of IL-4 reached a plateau at 6 hours after the CRA-1 stimulation. Pretreatment of CHMCs with IL-4 greatly enhanced FcεRI-mediated MIP-1α, IL-8, and GM-CSF production at each time point (Fig 2, A-C). However, IL-4 did not affect spontaneous production of MIP-1α and GM-CSF after stimulation. Spontaneous IL-8 production by CHMCs after 18 hours of pretreatment with IL-4 increased slightly, but this did not reach statistical significance. To examine the kinetics of IL-4-induced enhance-

ment of FcεRI-mediated cytokine production by CHMCs, cells were exposed to IL-4 during the last 15 minutes and 1, 2, 4, or 8 days of the culture. Then CHMCs were washed and stimulated with CRA-1 for another 6 hours. Enhancement by IL-4 was seen as early as 1 day after pretreatment with IL-4 and reached a maximum at day 4 (Fig 3, A-C). Although pretreatment of CHMCs with IL-4 for 8 days slightly decreased FcεRI-mediated cytokine production by CHMCs compared with maximum cytokine production at day 4, the enhancement of FcεRI-mediated cytokine production by IL-4 was still significant.

Next the kinetics of cytokine production by CHMCs was tested in the presence of IFN-γ (10 ng/mL). IFN-γ did not spontaneously affect these cytokine productions by CHMCs. Pretreatment of CHMCs with IFN-γ sup-

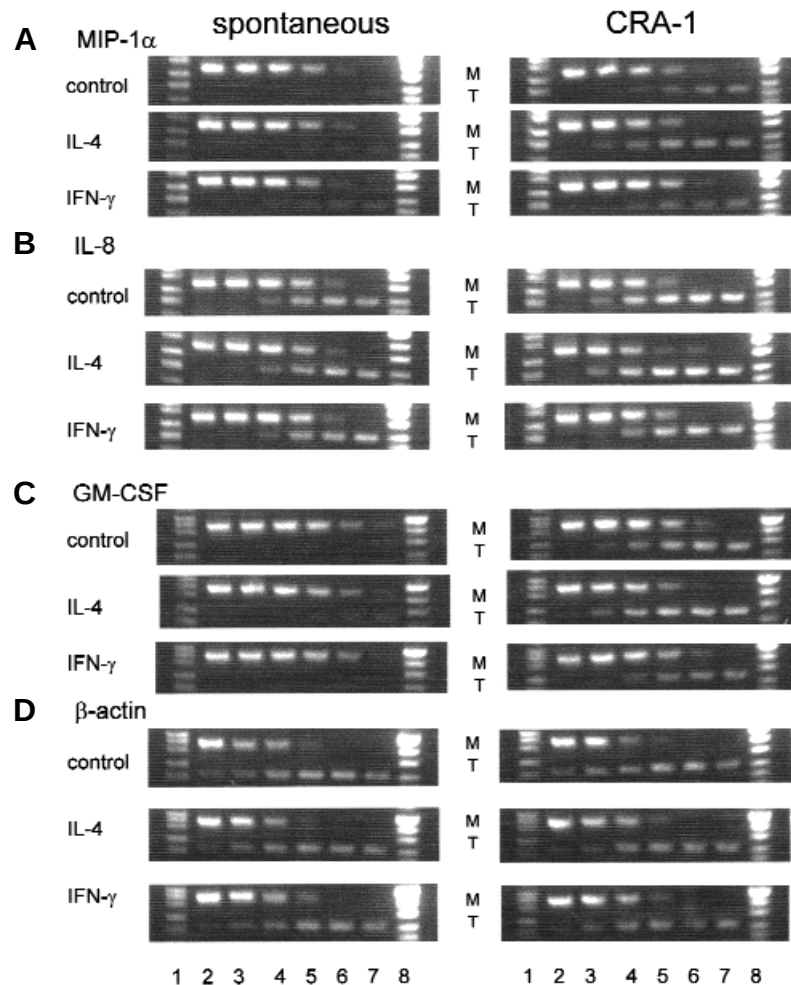


FIG 4. Analysis of the effects of IL-4 and IFN- γ on expression of cytokine mRNA by using competitive RT-PCR. CHMCs were cultured with IL-4 (10 ng/mL) or IFN- γ (10 ng/mL) in the presence of SCF (100 ng/mL), IL-6 (50 ng/mL), and human myeloma IgE (5 μ g/mL) for 2 days and then washed and challenged with 1 μ g/mL CRA-1 at 37°C for 2 hours. cDNA levels for MIP-1 α (**A**), IL-8 (**B**), GM-CSF (**C**), and β -actin (**D**) are shown. M, Mimic cDNA; T, target cDNA. Lanes 1 and 8 indicate molecular weight markers. Lanes 2 to 7 show progressive 10-fold increments of mimic DNA spiked into the PCR tube with a constant amount of target cDNA.

TABLE I. Effect of IL-4 or IFN- γ on Fc ϵ RI-mediated cytokine mRNA expression by cultured human mast cells

Experiment No.	Condition	β -Actin mRNA (attomol/10 ⁶ cells)	MIP-1 α / β -actin	IL-8/ β -actin	GM-CSF/ β -actin
1	Control	1069.9	0.035	0.708	0.127
	IL-4	1060.9	0.154	1.804	0.839
	IFN- γ	986.0	0.011	0.342	0.035
2	Control	389.8	0.061	1.900	0.216
	IL-4	306.0	0.422	3.035	0.632
	IFN- γ	329.2	0.016	0.748	0.071

Image data shown in Fig 4 were converted to the digital values by using computer software (FMBIO Analysis version 6.0; Hitachi Software Engineering Co, Ltd, Tokyo, Japan).

pressed Fc ϵ RI-mediated MIP-1 α , IL-8, and GM-CSF production at each time point tested (Fig 2, D-F). Fc ϵ RI-mediated IL-8 production by CHMCs at 3, 6, and 18 hours after stimulation was significantly inhibited by IFN- γ . IFN- γ also showed inhibitory effects on Fc ϵ RI-

mediated GM-CSF and MIP-1 α production, although it did not reach statistical significance. To further examine the kinetics of IFN- γ -mediated suppression of Fc ϵ RI-mediated cytokine production by CHMCs, cells were exposed to IFN- γ during the last 15 minutes and 1, 2, 4,

or 8 days of the culture, washed, and then stimulated with CRA-1 for another 6 hours. The inhibition by IFN- γ was very consistent from experiment to experiment. Maximum inhibition of MIP-1 α , IL-8, and GM-CSF by IFN- γ was greater than 70% and occurred at days 2 and 4. However, these effects did not reach statistical significance, except for IL-8, because of the wide variation among individual batches of CHMCs in their ability to produce cytokines in response to Fc ϵ RI cross-linking on day 0. Finally, the observed IFN- γ -mediated suppression of cytokine production appeared to be reversible and be consistently less at day 8 (Fig 3, D-F).

The effects of IL-4 and IFN- γ on the expression of cytokine mRNA levels

In beginning to examine the mechanism of IL-4-induced upregulation and IFN- γ -induced downregulation of cytokine production by CHMCs, we performed semiquantitative analysis of the expression of cytokine mRNA in the presence or absence of IL-4 or IFN- γ by using competitive PCR (Fig 4 and Table I). Fc ϵ RI-mediated expression of MIP-1 α , IL-8, and GM-CSF mRNA was enhanced by IL-4. In contrast, Fc ϵ RI-mediated expression of MIP-1 α , IL-8, and GM-CSF mRNA was suppressed by IFN- γ . Levels of GM-CSF, MIP-1 α , and IL-8 mRNA without CRA-1 mAb stimulation were unaffected by IL-4 or IFN- γ . Along with effects on cytokine protein production shown in the previous figures, these data demonstrate effects on both mRNA and protein levels induced by IL-4 and IFN- γ in CHMCs.

DISCUSSION

In this study we showed that IL-4 and IFN- γ have opposite effects on human mast cell cytokine production induced by means of Fc ϵ RI cross-linking. For example, not only does IL-4 upregulate Fc ϵ RI-dependent production of IL-3, IL-5, and IL-13 in CHMCs or human intestinal mast cells,^{9,20,21} but it also enhances production of MIP-1 α , IL-8, and GM-CSF (Figs 1-3). This occurs at the level of both mRNA and protein synthesis in addition to enhancement of expression of Fc ϵ RI and Fc ϵ RI-dependent histamine release as previously reported.^{18,22} The kinetics of Fc ϵ RI-mediated IL-13 production reached a plateau at 3 days after cross-linking of Fc ϵ RI²⁰ and differed from other cytokines (Fig 2), suggesting that regulation of MIP-1 α , IL-8, and GM-CSF production may differ from that of IL-13. The enhancement of Fc ϵ RI-mediated cytokine production from CHMCs by IL-4 required pretreatment for at least 1 day and was maximal at 4 days. In contrast to IL-4, IFN- γ inhibited cytokine production. This inhibition occurred at the level of both mRNA and protein synthesis in CHMCs. This is the first report that IFN- γ inhibits Fc ϵ RI-mediated mRNA and protein synthesis of cytokines in human mast cells. This does not appear to be due to CHMC apoptosis because CHMC viability in the presence or absence of IFN- γ supplementation was not different (see "Methods" section). In fact, IFN- γ itself supports CHMC survival,

even in the absence of SCF and IL-6.²³ Also, if the inhibition was due to apoptosis, all cytokines should have been inhibited equally. Neither IL-4 nor IFN- γ had any effect on spontaneous MIP-1 α , IL-8, and GM-CSF production and mRNA expression in CHMCs.

Cytokine production by CHMCs was detected 3 hours after Fc ϵ RI cross-linking and reached a plateau by 6 hours.¹³ Neither IL-4 nor IFN- γ had any effect on the kinetics of Fc ϵ RI-mediated mast cell cytokine production. It thus appears that IL-4 and IFN- γ do not affect posttranscriptional events in CHMCs for these cytokines. Although it is clear that IL-4 priming dramatically enhanced Fc ϵ RI-mediated cytokine production, the mechanism responsible for this is not yet known. We did observe that 2 days' culture with IL-4, even in the absence of IgE, profoundly enhanced Fc ϵ RI-mediated mast cell cytokine production (data not shown). Because IgE and IL-4 can enhance Fc ϵ RI expression,^{17,18,22,24} it is possible that a modest enhancement of Fc ϵ RI contributed to increased mast cell cytokine production induced by anti-Fc ϵ RI mAb. However, given the dramatic potentiation by IL-4 on Fc ϵ RI-mediated mast cell cytokine production, it seems more likely that some other mechanism besides simply increasing Fc ϵ RI expression is primarily responsible for this effect. On the other hand, we consistently observed that IFN- γ inhibited Fc ϵ RI-mediated cytokine production. IFN- γ only moderately inhibited IgE-induced Fc ϵ RI expression (32% reduction), but it enhanced Fc ϵ RI-mediated histamine release (74% increase), which is similar to findings from previous reports.²³ These data suggest that the downregulation of Fc ϵ RI-mediated cytokine production by IFN- γ occurs by mechanisms other than decreasing the expression of Fc ϵ RI on CHMCs and that the effects of IFN- γ on Fc ϵ RI-mediated histamine release and cytokine production were regulated differently. The precise mechanisms for this effect by IFN- γ on CHMCs are not clear and will require further studies.

The potency of the inhibition of production of MIP-1 α , IL-8, and GM-CSF by IFN- γ was different between Figs 1 and 2 at 6 hours. The reason for these different responses to IFN- γ might in part be due to a wide variation among the individual batches of CHMCs as previously reported.²² However, we consistently observed that IFN- γ inhibited Fc ϵ RI-mediated cytokine production by CHMCs. These data demonstrate that IFN- γ can act as an inhibitor of mast cell function.

The enhancement by IL-4 of the production of MIP-1 α , IL-8, and GM-CSF was 25-, 7-, and 90-fold, respectively, after 6 hours. The enhancement by IL-4 of the expression of mRNA for MIP-1 α , IL-8, and GM-CSF was 4.4- to 6.9-, 1.5- to 2.5-, and 2.9- to 6.6-fold, respectively, after 2 hours. It seems that the changes of protein for these cytokines were more impressive than that of expression of mRNA. Although the precise reason for the discrepancy is not known, there are several possible explanations for this. First, time points studied were different between protein and mRNA measurement. We may not have measured peak mRNA. Second,

some of the changes of expression may involve effects on transcription, whereas some of the changes may involve mRNA stability or mRNA translation. Third, the quantitative accuracy of the ELISA may be greater than that of competitive PCR. To clarify this point would require molecular studies that would be difficult to perform with these cells and are well beyond the scope of this report.

IL-4 profoundly enhanced FcεRI-mediated MIP-1α, IL-8, and GM-CSF production. Equally dramatic was the inhibitory effect of IFN-γ on cytokine production by CHMCs. Thus our data showed that CHMCs could be functionally driven into two directions after incubation with IL-4, a T_{H2} cytokine, or IFN-γ, a T_{H1} cytokine. Which effect would predominate in CHMCs cultured with both IL-4 and IFN-γ is not known. While we were preparing this article, Lorentz et al²¹ reported that semi-purified intestinal human mast cells produced IL-6 at 50 to 100 pg/10⁶ cells with or without IgE-dependent stimulation and that the production of IL-6 was blocked by preincubation with IL-4, whereas IL-4 enhanced the production of IL-3, IL-5, and IL-13. We presented here the effects of IL-4 only on the production of cytokines that can be produced at 1 ng/10⁶ cells by IgE-dependent stimuli. We wanted to exclude the possibility that contaminated macrophages might contribute to the net cytokine production and that IL-4 may block it through the effect on macrophages.^{25,26}

The clinical significance of our observations remains to be determined, but the potential implications of our findings are extremely important. Although the roles of IL-8 and MIP-1α in allergic inflammation are probably less significant than those of some of the other mast cell-derived cytokines, such as IL-13, IL-5, and GM-CSF, the analysis of the production by CHMCs of these cytokines is important to clarify the regulation of mast cell cytokine production. We previously reported that the supernatant derived from CHMCs after FcεRI cross-linking is of sufficient potency to support eosinophil survival in vitro.¹² Recently, it has been reported that IFN-γ therapy was effective for atopic dermatitis.²⁷ Although most mast cells in the skin are MC_{TC} mast cells, the inhibition of mast cell cytokine production might be among the mechanisms by which IFN-γ therapy is effective. Taken together, these observations and our findings may point to a potential positive or negative regulatory mechanism in allergic disorders in which elevations of IL-4 or IFN-γ result in enhanced or decreased mast cell cytokine production, respectively. Thus IL-4 and IFN-γ may alter allergic reactions not only by balancing the function of lymphocytes in their traditional roles as T_{H2} and T_{H1} cytokines but also by modulating human mast cell cytokine production.

We thank Dr Kiyoshi Kawashima, Dr Shigenobu Shoda, and the staff of the Department of Obstetrics, Gyoda Chuo Hospital, for their continuous support by generously providing the umbilical cord blood. We also thank Dr Akira Akasawa, Dr Toshio Katsunuma, Dr Hidetoshi Kawahara, Dr Ichiro Nomura, and Mr Hisashi Tomita for their technical assistance and advice.

REFERENCES

- Galli SJ. New concepts about the mast cell. *N Engl J Med* 1993;328:257-65.
- Moller A, Lippert U, Lessmann D, Kolde G, Hamann K, Welker P, et al. Human mast cells produce IL-8. *J Immunol* 1993;151:3261-6.
- Pawankar RU, Okuda M, Hasegawa S, Suzuki K, Yssel H, Okubo K, et al. Interleukin-13 expression in the nasal mucosa of perennial allergic rhinitis. *Am J Respir Crit Care Med* 1995;152:2059-67.
- Steffen M, Abboud M, Potter GK, Yung YP, Moore MA. Presence of tumour necrosis factor or a related factor in human basophil/mast cells. *Immunology* 1989;66:445-50.
- Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, et al. Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992;176:1381-6.
- Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993;151:3853-65.
- Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994;10:471-80.
- Bradding P, Okayama Y, Howarth PH, Church MK, Holgate ST. Heterogeneity of human mast cells based on cytokine content. *J Immunol* 1995;155:297-307.
- Bischoff SC, Sellge G, Lorentz A, Sebald W, Raab R, Manns MP. IL-4 enhances proliferation and mediator release in mature human mast cells. *Proc Natl Acad Sci USA* 1999;96:8080-5.
- Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the FcεRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. *J Clin Invest* 1997;99:1492-9.
- Saito H, Ebisawa M, Tachimoto H, Shichijo M, Fukagawa K, Matsumoto K, et al. Selective growth of human mast cells induced by Steel factor, IL-6, and prostaglandin E2 from cord blood mononuclear cells. *J Immunol* 1996;157:343-50.
- Tachimoto H, Ebisawa M, Iikura Y, Kimata M, Mori K, Sakaguchi N, et al. Activation human mast cells release factors supporting eosinophil survival in vitro. *Int Arch Allergy Immunol* 1997;113:293-4.
- Ebisawa M, Shichijo M, Miura K, Tachimoto H, Katsunuma T, Onda T, et al. Human mast cell produce TNF-α, MIP-1α and IL-5 following activation through FcεRI. *J Allergy Clin Immunol* 1995;95:219.
- Igarashi Y, Kurosawa M, Ishikawa O, Miyachi Y, Saito H, Ebisawa M, et al. Characteristics of histamine release from cultured human mast cells. *Clin Exp Allergy* 1996;26:597-602.
- Siebert PD, Larrick JW. PCR MIMICS: competitive DNA fragments for use as internal standards in quantitative PCR. *Biotechniques* 1993;14:244-9.
- Siebert PD, Larrick JW. Competitive PCR. *Nature* 1992;359:557-8.
- Yamaguchi M, Lantz CS, Oettgen HC, Katona IM, Fleming T, Miyajima I, et al. IgE enhances mouse mast cell FcRI expression in vitro and in vivo: evidence for a novel amplification mechanism in IgE-dependent reactions. *J Exp Med* 1997;185:663-72.
- Toru H, Ra C, Nonoyama S, Suzuki K, Yata J, Nakahata T. Induction of the high-affinity IgE receptor (FcεRI) on human mast cells by IL-4. *Int Immunol* 1996;8:1367-73.
- Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA. T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells in vitro. *J Exp Med* 1999;190:267-80.
- Toru H, Pawankar R, Ra C, Yata J, Nakahata T. Human mast cells produce IL-13 by high-affinity IgE receptor cross-linking: enhanced IL-13 production by IL-4-primed human mast cells. *J Allergy Clin Immunol* 1998;102:491-502.
- Lorentz A, Schwengberg S, Sellge G, Manns MP, Bischoff SC. Human intestinal mast cells are capable of producing different cytokine profiles: role of IgE receptor cross-linking and IL-4. *J Immunol* 2000;164:43-8.
- Yamaguchi M, Sayama K, Yano K, Lantz CS, Noben-Trauth N, Ra C, et al. IgE enhances FcεRI expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell FcεRI expression and mediator release. *J Immunol* 1999;162:5455-65.

23. Yanagida M, Fukamachi H, Takei M, Hagiwara T, Uzumaki H, Tokiwa T, et al. Interferon- γ promotes the survival and Fc ϵ RI-mediated histamine release in cultured human mast cells. *Immunology* 1996;89:547-52.
24. Yano K, Yamaguchi M, de Mora F, Lantz CS, Butterfield JH, Costa JJ, et al. Production of macrophage inflammatory protein-1 α by human mast cells: increased anti-IgE-dependent secretion after IgE-dependent enhancement of mast cell IgE-binding ability. *Lab Invest* 1997;77:185-93.
25. Kluth DC, Rees AJ. Inhibiting inflammatory cytokines. *Semin Nephrol* 1996;16:576-82.
26. Bonder CS, Finlay-Jones JJ, Hart PH. Interleukin-4 regulation of human monocyte and macrophage interleukin-10 and interleukin-12 production. Role of a functional interleukin-2 receptor γ -chain. *Immunology* 1999;96:529-36.
27. Sur S, Wild JS, Choudhury BK, Sur N, Alam R, Klinman DM. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J Immunol* 1999;162:6284-93.

Bound volumes available to subscribers

Bound volumes of The Journal of Allergy and Clinical Immunology are available to subscribers (only) for the 2000 issues from the Publisher, at a cost of \$107.00 for domestic, and \$128.00 for international subscribers for Vol. 105 (January-June) and Vol. 106 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby, Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887; phone (800) 654-2452 or (407) 345-4000.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.