Brief Definitive Report

Interleukin 8 (IL-8) Selectively Inhibits Immunoglobulin E Production Induced by IL-4 in Human B Cells

By H. Kimata, A. Yoshida, C. Ishioka, I. Lindley,* and H. Mikawa

*From the Department of Pediatrics, Kyoto University Hospital, Kyoto 606, Japan; and the *Sandoz Research Institute, A-1235 Vienna, Austria*

Summary

The effect of interleukin 8 (IL-8) on IL-4–induced immunoglobulin E (IgE) production was studied. IL-4 induced IgE and IgG4 production by tonsillar mononuclear cells (MNC) without affecting IgM, IgG1, IgA, IgG2, or IgG3 production. IL-8 inhibited IL-4–induced IgE and IgG4 production, whereas it had no effect on IgM, IgG1, IgA, IgG2, and IgG3 production. The inhibitory effect by IL-8 was specific, since it was blocked by anti–IL-8 mAb, but not by control IgG1. Although interferon γ (IFN-γ) also inhibited IgE and IgG4 production by MNC stimulated with IL-4, the inhibitory effect of IL-8 was not mediated by IFN-γ, since the IL-8–induced inhibition could not be blocked by anti–IFN-γ mAb. Furthermore, anti–IL-8 mAb had no effect on IFN-γ–induced inhibition. Moreover, addition of IL-5 or IL-6 did not reverse IL-8–induced inhibition of IgE production. In contrast to these observations with MNC, IL-4 failed to induce IgE and IgG4 production by purified B cells. However, combined treatment of purified B cells with IL-4 and anti-CD40 antibody resulted in IgE but not IgG4 production. IL-8 inhibited this IgE production without affecting IgM, IgG1, IgG2, IgG3, IgG4, or IgA production, whereas IFN-γ, IFN-α, or prostaglandin E_2 (PGE_2) failed to do so. These results indicate that IL-8 antagonizes IL-4–induced IgE production by directly affecting B cells through a specific mechanism that is different from IFN-γ, IFN-α, or PGE_2.

IgE production has been shown to be regulated by many factors in various in vitro systems. In mononuclear cells (MNC), IL-4 induces IgE production, which can be inhibited by IFN-α, IFN-γ, or prostaglandin E_2 (PGE_2) (1), and enhanced by IL-5 or IL-6 (2, 3). IL-4 has also been shown to inhibit IFN-γ synthesis or release of PGE_2 (4, 5), indicating mutual interaction of these factors. In contrast, in purified B cells, IL-4 alone cannot induce IgE production. However, stimulation of purified B cells with IL-4 and anti-CD40 ab induces IgE production, which is IFN-α– and IFN-γ–independent (6–8). We have previously reported that soluble CD23 enhances IgE production without affecting cell growth in the human IgE-producing cell line AF-10, which is a subclone of U266 (9). We have also found that IgE production is regulated by erythropoietin or disodium cromoglycate in an IFN-α– and IFN-γ–independent, but in a T cell- and monocyte-dependent fashion (10, 11).

IL-8 was initially characterized and cloned as a neutrophil chemotactic and activating agent (12). However, it has subsequently been shown that IL-8 is also chemotactic for T cells, induces histamine release from IL-3–primed basophils, and inhibits neutrophil adhesion to endothelium (13–15). A report that IL-4 inhibits IL-8 production from monocytes (16) prompted us to study the effect of IL-8 on IL-4–induced IgE production to see whether there was mutual interaction. We demonstrate that IL-8 inhibits IgE production in MNC and in purified B cells in an IFN-α– and IFN-γ-independent fashion.

Materials and Methods

Reagents. The following recombinant human cytokines were kindly provided by the companies as described previously (10, 11): IL-4 (Ono Pharmaceutical Company, Osaka, Japan); IL-5 (Suntory Research Center, Osaka); IL-6 (kind gift from Drs. T. Hirano and T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka); and IFN-α and IFN-γ (Takeda Chemical Industries, Osaka). Human rIL-8 which has the same neutrophil-activating potency as the natural product isolated from LPS-stimulated MNC and immunosorbent purified mouse IgG1 monoclonal anti–IL-8 ab (4G9/A5/A7) were obtained from Sandoz Research Institute (Vienna, Austria) (12, 14). Chemotactic assay for fresh human neutrophils showed that activity was detected at 0.06–1.7 μg/ml, with maximal activity at 0.2 μg/ml. Mouse IgM anti-CD40 antibody (MA6), mouse IgG3 anti-human IFN-γ ab and control mouse IgG1 were purchased from Cosmo Bio Co. (Tokyo, Japan). PGE_2 was purchased from Nakalai Chemicals (Kyoto, Japan).

Cell Cultures. Tonsillar MNC from nonatopic donors were cultured (2×10^5/0.2 ml/well) in 96-well U-bottomed microtiter

plates (Costar Corp., Cambridge, MA) for 14 d in RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD) containing 10% FCS (Irvine Scientific, Santa Ana, CA), 2 mM L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin, and stimulated with IL-4 (800 U/ml) and various factors as described in Results. Highly purified B cells were obtained by SRBC rosetting, followed by L-leucine methyl ester incubation as described previously (11). Purified B cell fractions contained, <1% $CD3^+$ T cells, <1% $CD11^+$ monocytes, <1% $CD16^+$ NK cells, and >98% $CD20^+$ B cells. Purified B cells were cultured (10^5 cells/0.2 ml/well) with various factors for 14 d. Control cultures for the evaluation of preformed Ig were carried out in the presence of cycloheximide (100 μg/ml). The amount of IgE, IgG subclasses, IgM, and IgA in the supernatants were determined by ELISA (9, 11).

Results

The effect of IL-8 on IL-4–induced IgE production was studied in MNC. As shown in Fig. 1 *A*, IL-8 inhibited IgE production in a dose-dependent fashion. IL-8 also inhibited IL-4–induced IgG4 production. In contrast, IL-8 had no effect on IgM, IgG1, IgA, IgG2, and IgG3 production (Fig. 1 *B*). Ig production by MNC in the absence of IL-4 were as follows: IgE <0.2 ng/ml; IgG1 705 ± 128 ng/ml; IgG2 217 ± 59 ng/ml; IgG3 108 ± 25 ng/ml; IgG4 3.2 ± 0.5 ng/ml; IgM 910 ± 129 ng/ml; and IgA 528 ± 91 ng/ml (mean ± one SD from two experiments). IL-8 did not affect production of these Igs in the absence of IL-4.

We next studied the effect of delayed addition of IL-8 on IL-4–induced IgE and IgG4 production. As shown in Fig. 2, IL-8 inhibited IgE and IgG4 production only when added at the initiation of the 14-d culture. After 1 d of culture, IL-8 had no effect on IgE and IgG4 production. These results suggested that IL-8 interfered with IL-4 stimulation at the early stage, and that the inhibition was not due to simple cytotoxic effects.

Specificity of the IL-8 effect is documented in Table 1. Inhibition of IgE and IgG4 production by IL-8 was blocked by anti–IL-8 mAb, but not by control IgG1, although anti–IL-8 did not affect IgE and IgG4 production in the absence of IL-8. IFN-γ also inhibited IgE and IgG4 production in IL-4–stimulated cultures, and anti–IFN-γ mAb completely blocked the inhibition by IFN-γ. However, inhibition by IL-8 was not mediated by IFN-γ, since the IL-8 effect was not blocked by anti–IFN-γ mAb, and conversely, the IFN-γ effect was not blocked by anti–IL-8 mAb. Moreover, addition of IL-5 or IL-6 did not reverse IL-8–induced inhibition of IgE production (Table 1).

We also studied the effect of IL-8 on IgE production by purified B cells. It has been reported that stimulation of IL-4 and anti-CD40 ab induces IgE production by purified B cells in the absence of T cells (6–8). Thus, highly purified B cells were stimulated with IL-4 and anti-CD40 ab, and IL-8 and/or other factors were added. As shown in Table 2, IL-8 had no effect on IgE, IgM, IgA, and IgG4 production by B cells without stimuli. Stimulation of IL-4 and anti-CD40 induced IgE but not IgM, IgA, or IgG4 production. IgG1, IgG2, or IgG3 production was also not induced (data not shown). Addition of IFN-α, IFN-γ, or PGE_2, which inhibits IL-4–induced IgE production by MNC (1), did not inhibit IgE

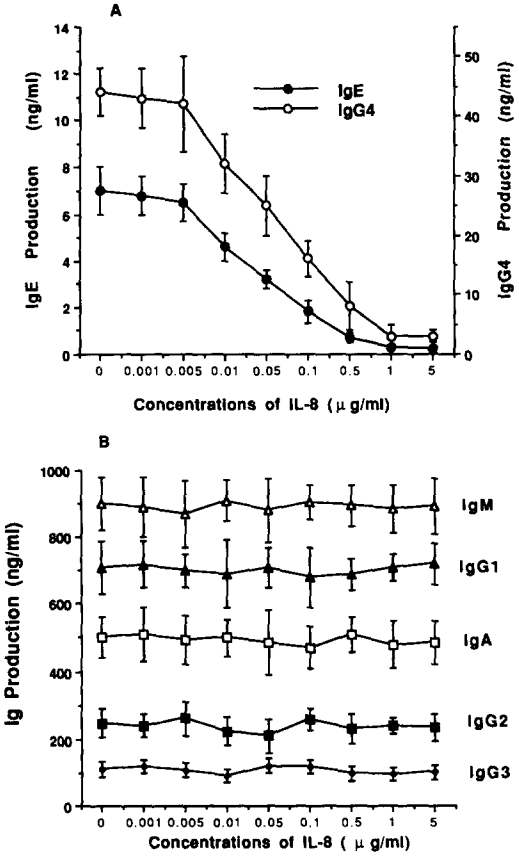

Figure 1. Effect of IL-8 on Ig production by MNC stimulated with IL-4. MNC were stimulated with IL-4 (800 U/ml), and various concentrations of IL-8 were added. After 14 d of culture, IgE and IgG4 (*A*) and IgM, IgG1, IgA, IgG2 and IgG3 (*B*) were determined. Values are means ± one SD of triplicate cultures from two experiments.

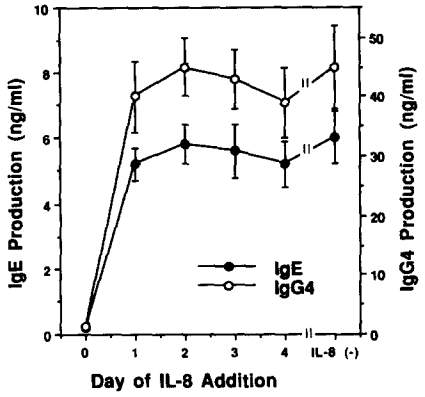

Figure 2. Kinetics of the addition of IL-8 to IL-4–stimulated MNC. MNC were stimulated with IL-4 (800 U/ml), and IL-8 (1 μg/ml) was added at the initiation (day 0), or after 1–4 days of culture. As controls, MNC were cultured with IL-4 (800 U/ml) in the absence of IL-8; IL-8 (−). After 14 d of culture, IgE and IgG4 production were determined. Values are means ± one SD of triplicate cultures of IgE (●) and IgG4 (○).

Table 1. *Specificity of the IL-8 Effect on IgE and IgG4 Production by MNC*

Factors	Ig production					
	Expt. 1		Expt. 2		Expt. 3	
	IgE	IgG4	IgE	IgG4	IgE	IgG4
	ng/ml					
Medium	5.2	27.7	2.2	31.0	1.9	14.8
IL-8	<0.2	3.0	0.6	2.1	<0.2	<0.6
Anti-IL-8 mAb	6.2	31.2	3.0	40.4	2.7	17.2
IL-8 + anti-IL-8 mAb	4.9	25.2	2.0	25.8	1.6	12.0
IL-8 + control IgG1	<0.2	3.6	0.7	3.4	<0.2	0.5
IL-8 + anti-IFN-γ mAb	<0.2	4.2	0.9	3.5	<0.2	0.9
IL-8 + IL-5	<0.2	4.1	0.9	3.4	<0.2	0.8
IL-8 + IL-6	<0.2	4.5	1.0	3.2	<0.2	0.9
IFN-γ	<0.2	2.6	0.3	1.0	<0.2	<0.6
Anti-IFN-γ mAb	7.2	37.6	3.1	43.0	2.0	20.8
IFN-γ + anti-IFN-γ mAb	5.0	30.1	1.9	30.0	1.6	12.9
IFN-γ + anti-IL-8 mAb	<0.2	2.0	0.7	1.4	<0.2	0.6

Tonsillar MNC were stimulated with IL-4 (800 U/ml), and cultured with indicated factors. IL-8 was used at 1 μg/ml, anti-IL-8 mAb at 10 μg/ml, control IgG1 at 10 μg/ml, anti-IFN-γ at 10 μg/ml, IL-5 at 100 ng/ml, IL-6 at 100 U/ml, and IFN at 1,000 U/ml. Values are means of triplicate cultures. SD were <15%.

Table 2. *Effect of IL-8 on IgE and IgG4 Production by Purified B Cells Stimulated with IL-4 and Anti-CD40 Antibody*

Factors	Ig production					
	Expt. 1		Expt. 2		Expt. 3	
	IgE	IgM	IgE	IgA	IgE	IgG4
	ng/ml					
Medium	<0.2	38.1	<0.2	5.0	<0.2	0.8
IL-8	<0.2	37.0	<0.2	4.4	<0.2	0.7
IL-4 + anti-CD40	3.1	40.1	8.7	6.4	2.9	0.6
IL-4 + anti-CD40 + IFN-γ	3.3	32.3	8.5	5.2	2.5	0.7
IL-4 + anti-CD40 + IFN-α	3.0	36.4	9.1	5.5	3.1	0.7
IL-4 + anti-CD40 + PGE_2	2.8	40.9	8.2	5.9	3.0	0.8
IL-4 + anti-CD40 + IL-8	<0.2	41.2	0.5	5.6	0.7	0.7
IL-4 + anti-CD40 + IL-8 + anti-IL-8 mAb	3.2	36.6	9.2	5.9	3.0	0.8
IL-4 + anti-CD40 + IL-8 + control IgG1	<0.2	41.2	0.6	5.6	0.8	0.7
IL-4 + anti-CD40 + IL-8 + IL-5	<0.2	42.2	0.8	6.1	0.8	0.8
IL-4 + anti-CD40 + IL-8 + IL-6	<0.2	40.6	0.7	5.5	0.7	0.8

Purified B cells were cultured with indicated factors. IL-8 was used at 1 μg/ml, IL-4 at 800 U/ml, anti-CD 40 antibody at 0.1 μg/ml, IFN-γ at 1,000 U/ml, IFN-α at 1,000 U/ml, PGE_2 at 10^{-6} M, anti-IL-8 mAb at 10 μg/ml, control mouse IgG1 at 10 μg/ml, IL-5 at 100 ng/ml, and IL-6 at 100 U/ml. Values are means of triplicate cultures. SD were <15%.

production by purified B cells. In contrast, addition of IL-8 inhibited IgE production without affecting IgM, IgA, or IgG4 production (Table 2). IL-8 also had no effect on IgG1, IgG2, or IgG3 production (data not shown). IL-8–induced inhibition of IgE production was blocked by anti–IL-8 mAb, but not by control IgG1. Addition of IL-5 (up to 100 ng/ml) or IL-6 (up to 100 U/ml) did not reverse the IL-8–induced inhibition of IgE production.

Discussion

We have demonstrated that IL-8 selectively inhibits IgE and IgG4 production in MNC stimulated with IL-4. Inhibition by IL-8 was specific, since it could be blocked by anti–IL-8 mAb but not by control mouse IgG1. Kinetic experiments showed that IL-8 had to be added at the initiation of the culture. Delayed addition of IL-8 after 1 d of culture had no effect. These results indicate that IL-8 inhibits the early activation step during IL-4 stimulation, and that the inhibition is not due to cytotoxicity. Although IFN-γ also inhibited IgE and IgG4 production in those cultures, the IL-8 effect was not mediated by IFN-γ, since IL-8–mediated inhibition was not blocked by anti–IFN-γ mAb, and conversely, the IFN-γ effect was not blocked by anti–IL-8 mAb. Moreover, IL-5 or IL-6, which enhanced IL-4–induced IgE production, did not reverse IL-8–induced inhibition of IgE production.

IL-8 also selectively inhibited IgE production by highly purified B cells stimulated with IL-4 and anti-CD40 antibody. In purified B cells, IL-8–induced inhibition of IgE production was specific and direct, since inhibition was not mediated through IFN-γ, IFN-α, or PGE_2, which failed to inhibit IgE production. This is not surprising since they all inhibit IL-4–induced IgE production by MNC via interactions of T cells and B cells (1). Moreover, the IL-8 effect was blocked by anti–IL-8 mAb but not by control mouse IgG1, and was not reversed by IL-5 or IL-6. Taken together, these results indicate that IL-8 could inhibit IL-4–induced IgE production in both T cell–dependent and –independent systems, and the inhibition was not dependent on IFN-γ, IFN-α, IL-5, or IL-6.

Monocyte-derived IL-8 has been characterized and cloned as a neutrophil chemotaxin and activator (12). In addition to these activities, IL-8 is chemotactic for T cells (13), and induces histamine release by IL-3–primed basophils (14). IL-8 mRNA can be induced in endothelial cells, fibroblasts, epithelial cells, hepatoma cells, neutrophils, and T cells (17, 18). IL-8 receptors were detected on neutrophils, monocytes, lymphocytes, T cell and monoblast cell lines (19, 20). To our knowledge, this is the first report of a direct effect on B cells, resulting in inhibition of IgE production. Preliminary work in our laboratory showed that IL-8 also inhibits proliferation in human B cell line stimulated with IL-4 (Kimata et al., manuscript in preparation).

The exact mechanism of the IL-8–induced inhibition of IgE production is currently under investigation. This system will be very useful to dissect the inhibition of IgE regulation. The in vivo role of IL-8 in IgE production remains to be elucidated. It has been reported that IL-5, IL-6, or IFN-γ, which play an important role in IgE regulation in vitro, do not affect IgE regulation in vivo in atopic patients or in patients with hyper-IgE syndrome (21–23). It might be interesting to assess IL-8 levels in those patients. We are currently studying the effect of IL-8 on IgE production by B cells from atopic patients.

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Address correspondence to H. Kimata, Department of Pediatrics, Kyoto University, 54, Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606, Japan.

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