

RANTES and Macrophage Inflammatory Protein 1 α Selectively Enhance Immunoglobulin (IgE) and IgG4 Production by Human B Cells

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Summary

We studied the effects of various chemokines including neutrophil-activating peptide 2 (NAP-2), β -thromboglobulin (β -TG), platelet factor 4 (PF-4), melanoma growth stimulating activity (GRO), γ interferon-induced protein (IP-10), regulated on activation, normal T expressed and secreted (RANTES), macrophage inflammatory protein 1 α (MIP-1 α), MIP-1 β , and monocyte chemotactic protein 1 (MCP-1) on Immunoglobulin (IgE) and IgG4 production by human B cells. None of these chemokines with or without interleukin (IL-4), anti-CD40 or -CD58 monoclonal antibody (mAb), induced IgE and IgG4 production by B cells from nonatopic donors. However, RANTES and MIP-1 α selectively enhanced IgE and IgG4 production induced by IL-4 plus anti-CD40 or -CD58 mAb without affecting production of IgM, IgG1, IgG2, IgG3, IgA1, or IgA2, whereas other chemokines failed to do so. Enhancement of IgE and IgG4 production by RANTES and MIP-1 α was specifically blocked by anti-RANTES mAb and anti-MIP-1 α antibody (Ab), respectively, whereas anti-IL-5 mAb, anti-IL-6 mAb, anti-IL-10 Ab, anti-IL-13 Ab, and anti-tumor necrosis factor- α mAb failed to do so. Purified surface IgE positive (sIgE⁺) and sIgG4⁺ B cells generated either in vitro or in vivo spontaneously produced IgE and IgG4, respectively, whereas sIgE⁻ and sIgG4⁻ B cells failed to do so. RANTES and MIP-1 α enhanced spontaneous IgE and IgG4 production in sIgE⁺ and sIgG4⁺ B cells, respectively, whereas neither RANTES nor MIP-1 α did so in sIgE⁻ or sIgG4⁻ B cells. Purified sIgE⁺ and sIgG4⁺, but not sIgE⁻ or sIgG4⁻ B cells, generated in vitro and in vivo expressed receptors for RANTES and MIP-1 α , whereas they failed to express receptors for other chemokines. These findings indicate that RANTES and MIP-1 α enhance IgE and IgG4 production by directly stimulating sIgE⁺ and sIgG4⁺ B cells.

Human IgE and IgG4 production is regulated by various cytokines and factors. IL-4 and IL-13 induced IgE and IgG4 production in mononuclear cells or in B cells stimulated with anti-CD40 mAb by isotype switching (1–3). IL-5, -6, -9, -10, and TNF- α enhance IL-4- and IL-13-induced IgE and IgG4 production (1–6), whereas IFN- α , IFN- γ , TGF- β , and IL-12 inhibit their production depending on the condition of culture (1, 4, 6, 7). Moreover, IL-4 plus anti-CD58 mAb also induced IgE production by purified B cells, and this production was IFN- γ and IL-6 independent (8). We have also reported that some neuropeptides selectively modulated IL-4 induced and spontaneous IgE and IgG4 production, which was not mediated by these cytokines (9, 10).

The chemokines consists of α subfamily members including IL-8, melanoma growth-stimulating activity (GRO), neutrophil-activating peptide 2 (NAP-2), β -thromboglobulin (β -TG), γ interferon-induced protein (IP-10), and platelet factor 4 (PF-4), and β subfamily members including regulated on activation, normal T expressed and secreted (RANTES), macrophage inflammatory protein 1 α (MIP-1 α), MIP-1 β , and monocyte chemotactic protein 1 (MCP-1) (11). Recently, it has been reported that these chemokines act on various cell types, including neutrophils, eosinophils, basophils, monocytes, myeloid progenitors, and T and B cells (11–15). In addition, we have found that IL-8 selectively inhibited IgE and IgG4 production induced by IL-4 (16). Here, we demonstrate that RANTES and MIP-1 α

selectively enhance IgE and IgG4 production, whereas other chemokines fail to do so.

Materials and Methods

Reagents. The following recombinant human cytokines and Abs were kindly provided by companies noted previously (2, 4): IL-4 and rabbit anti-IL-4 Ab (Ono Pharmaceutical Company, Osaka, Japan), and IL-2 and IFN- α (Takeda Chemical Industries, Osaka, Japan). Recombinant human IL-13 was purchased from Pepro Tech Inc. (Rocky Hill, NJ) (2). Recombinant human IL-8 and mouse IgG1 anti-IL-8 mAb were obtained from Sandoz Research Institute (Vienna, Austria) (16). Recombinant human IL-10, -6, -12, TGF- β , RANTES, GRO, MIP-1 α , MIP-1 β , and MCP-1, and mouse IgG1 anti-IL-5, anti-IL-6, anti-TNF- α mAb, anti-RANTES mAb, and goat anti-IL-10 and anti-MIP-1 α Ab were purchased from R&D Systems, Inc. (Minneapolis, MN). Recombinant human NAP-2, PF-4, highly purified native human β -TG, IP-10, mouse IgM anti-CD40 mAb (BL-C4), mouse IgG2a anti-CD58 mAb (BRIC5), and rabbit anti-IL-13 Ab were purchased from Cosmo Bio Co. (Tokyo, Japan) (2, 4, 16). The culture medium was DME, supplemented with Ham's Nutrient (DME/F-12) (Sigma Chemical Co., St. Louis, MO), 0.5% BSA, and 50 μ g/ml transferrin (2).

Cell Cultures. Tonsillar mononuclear cells were obtained from nonatopic donors (serum IgE level <50 U/ml) and atopic patients (serum IgE level 1,578–12,259 IU/ml). Highly purified B cells were separated by SRBC rosetting, followed by L-leucine methyl ester incubation as described previously (2). Purified B cell fractions contained >98% CD20⁺ B cells. Purified B cells were depleted of surface IgE positive (sIgE⁺) and sIgG4⁺ B cells by panning. The percentage of sIgE⁺ and sIgG4⁺ B cells was <0.1%. The sIgE⁻, sIgG4⁻ B cells were cultured (2×10^5 /0.2 ml/well) in U-bottomed microtiter plates (Costar Corp., Cambridge, MA) for 14 d in the presence or absence of various factors with or without Abs as described in Results. All the Abs to cytokines were used at 10 μ g/ml, because anti-IL-5 mAb, anti-IL-6 mAb, anti-IL-10 Ab, anti-IL-13 Ab, and anti-TNF- α mAb (all at 10 μ g/ml) completely neutralized induction of IgE and IgG4 production by IL-5 (100 ng/ml), IL-6 (100 ng/ml), IL-10 (100 ng/ml), IL-13 (500 ng/ml), and TNF- α (50 ng/ml), respectively (2, 4, 6). In some experiments, purified sIgE⁻, sIgG4⁻ B cells were cultured with IL-4 (1,000 U/ml) plus anti-CD40 mAb (0.1 μ g/ml) or IL-4 plus anti-CD58 mAb (0.1 μ g/ml) for 5–7 d, and then sIgE⁺, sIgE⁻, sIgG4⁺, and sIgG4⁻ B cells were purified by panning (2, 4). Alternatively, sIgE⁺, sIgE⁻, sIgG4⁺, and sIgG4⁻ B cells were purified from tonsillar B cells of atopic patients by panning. Purified sIgE⁺ and sIgG4⁺ B cell fractions contained >98% sIgE⁺ B cells and >98% sIgG4⁺ B cells, respectively (2, 4). Purified sIgE⁺ and sIgG4⁺ B cells were cultured (2×10^4 /0.2 ml/well) for 14 d as described in Results. The amounts of IgE, IgG subclasses, IgM, and IgA subclasses in the supernatants were determined by ELISA (2, 4). Results were expressed as the means \pm 1 SD of triplicate cultures from one experiment, representative of four or five.

In some experiments, sIg⁺ and sIg⁻ B cells were tested for the binding of chemokines by immunofluorescence using biotinylated chemokines, as previously reported (6, 17). The mean fluorescence intensity (MFI) value of biotinylated ligand-specific binding, determined after subtraction of the nonspecific binding in the presence of a 100-fold excess of unlabeled ligand, was expressed as Δ MFI (6, 17). Binding (Δ MFI, $n = 4$) of RANTES, MIP-1 α , IP-10, MIP-1 β , and MCP-1 in purified human mono-

cytes was 76 ± 14 , 68 ± 20 , 58 ± 21 , 72 ± 17 , and 61 ± 18 , respectively, whereas binding (Δ MFI, $n = 4$) of NAP-2, β -TG, PF-4, and GRO in purified human neutrophils was 92 ± 31 , 89 ± 27 , 80 ± 16 , and 79 ± 22 , respectively.

Results and Discussion

Preliminary experiments showed that none of the chemokines (1 pM–1 μ M) with or without IL-4, anti-CD40 mAb, or anti-CD58 mAb induced IgE (<0.3 ng/ml), IgG4 (<0.3 ng/ml), or other Ig (data not shown) production by purified B cells. However, as shown in Fig. 1, A and B, of the various chemokines tested, RANTES and MIP-1 α enhanced IgE and IgG4 production induced by IL-4 plus anti-CD40 mAb in a dose-dependent fashion, but failed to enhance IgM, IgG1, IgG2, IgG3, IgA1, and IgA2 production (Fig. 1, C and D). In contrast, none of the other chemokines, including NAP-2, β -TG, PF-4, GRO, IP-10, MIP-1 β , and MCP-1 had any effect on the production of IgE, IgG4 (Fig. 1, A and B) or other Igs (data not shown) at any concentrations tested. Similarly, RANTES and MIP-

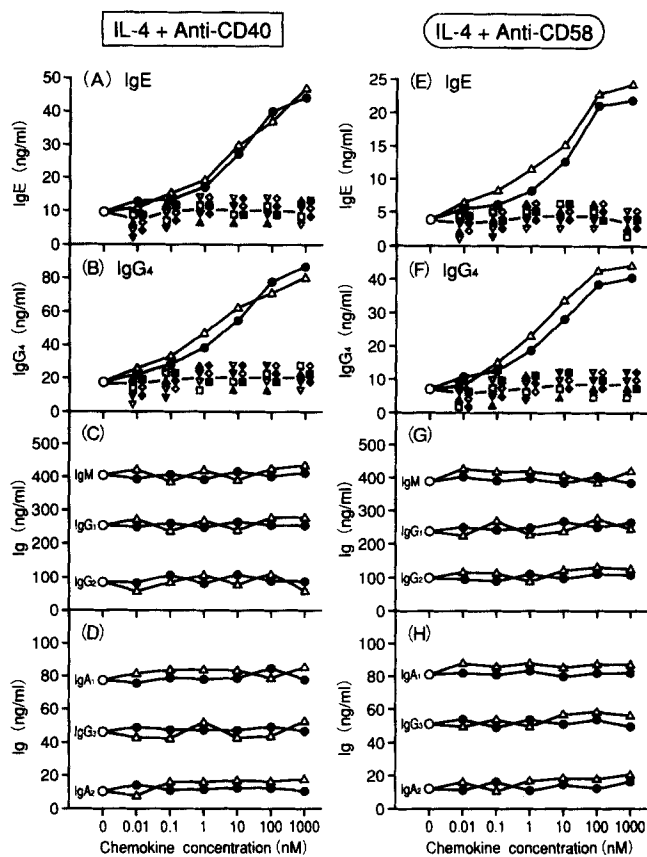


Figure 1. Effects of chemokines on Ig production. Nonatopic donors' sIgE⁻, sIgG4⁻ B cells were cultured with IL-4 (1,000 U/ml) plus anti-CD40 mAb (0.1 μ g/ml) (A–D), or IL-4 plus anti-CD58 mAb (0.1 μ g/ml) (E–H). Medium (○) or various concentrations of RANTES (●), MIP-1 α (△), GRO (▲), NAP-2 (▽), β -TG (▼), PF-4 (□), IP-10 (■), MIP-1 β (◇), or MCP-1 (◆) were added, and production of IgE (A and E), IgG4 (B and F), IgM, IgG1, and IgG2 (C and G), or IgG3, IgA1, and IgA2 (D and H) was determined.

1 α enhanced IgE and IgG4 production induced by IL-4 plus anti-CD58 mAb, but did not enhance the production of other Igs (Fig. 1, E-H). None of NAP-2, β -TG, PF-4, GRO, IP-10, MIP-1 β , or MCP-1 had any effect on the production of IgE, IgG4 (Fig. 1, E and F), or other Igs (data not shown). In five experiments performed, the ranges of enhancement of IgE and IgG4 production by RANTES (100 nM) and MIP-1 α (100 nM) were 3.3–5.1-fold and 2.9–5.2-fold, respectively, in cultures stimulated with IL-4 plus anti-CD40 mAb, and 2.7–5.2-fold and 2.5–4.9-fold, respectively, in cultures stimulated with IL-4 plus anti-CD58 mAb.

We and others have previously reported that IL-4-prestimulated B cells produce IgE spontaneously in vitro after switching to sIgE⁺ B cells (18, 19). Therefore, in this study, sIgE⁻, sIgG4⁻ B cells were prestimulated with IL-4 plus anti-CD40 mAb or IL-4 plus anti-CD58 mAb, and sIgE⁺, sIgG4⁺, sIgE⁻, and sIgG4⁻ B cells were purified. We also studied the effects of RANTES and MIP-1 α on in vivo-generated sIgE⁺ and sIgG4⁺ B cells obtained from atopic patients. As shown in Fig. 2, A-C, RANTES and MIP-1 α enhanced IgE production in sIgE⁺ B cells induced by IL-4 plus anti-CD40 mAb, IL-4 plus anti-CD58 mAb, and in vivo, but failed to induce IgE production in sIgE⁻ B cells. Enhancement of IgE production by RANTES was specific, since it was blocked by anti-RANTES mAb but not by

anti-MIP-1 α Ab or control mouse IgG1 (data not shown). Conversely, enhancement of IgE production by MIP-1 α was blocked by anti-MIP-1 α Ab, but not by anti-RANTES mAb or control goat IgG (data not shown). Identical results were obtained for IgG4 production (Fig. 2, D-F).

These findings indicate that RANTES and MIP-1 α directly stimulated sIgE⁺ and sIgG4⁺ B cells generated in vitro and in vivo, but had no effect on sIgE⁻ and sIgG4⁻ B cells. Kinetic experiments showed that enhancement of IgE and IgG4 production could be detected on day 4 (Fig. 3). That enhancement was caused by stimulation of Ig production and not by proliferation of sIgE⁺ and sIgG4⁺ B cells, since RANTES and MIP-1 α had no effect on cell number on any day tested (Fig. 3).

We and others have reported that IL-6, IL-10, and TNF- α each enhances IgE and IgG4 production induced by IL-4 plus anti-CD40 mAb (4–6). As shown in Fig. 4 A, addition of anti-IL-6 mAb and anti-IL-10 Ab each inhibited IgE and IgG4 production in sIgE⁺ and sIgG4⁺ B cells, respectively, induced by IL-4 plus anti-CD40 mAb, whereas anti-TNF- α mAb, IFN- α , and IFN- γ failed to do so. In contrast, anti-IL-10 Ab and anti-TNF- α mAb each inhibited IgE and IgG4 production in those cells induced by IL-4 plus anti-CD58 mAb, whereas anti-IL-6 mAb, IFN- α , and IFN- γ each failed to do so (Fig. 4 B). On the other hand, anti-IL-6 mAb, anti-IL-10 Ab, and anti-anti-TNF- α mAb

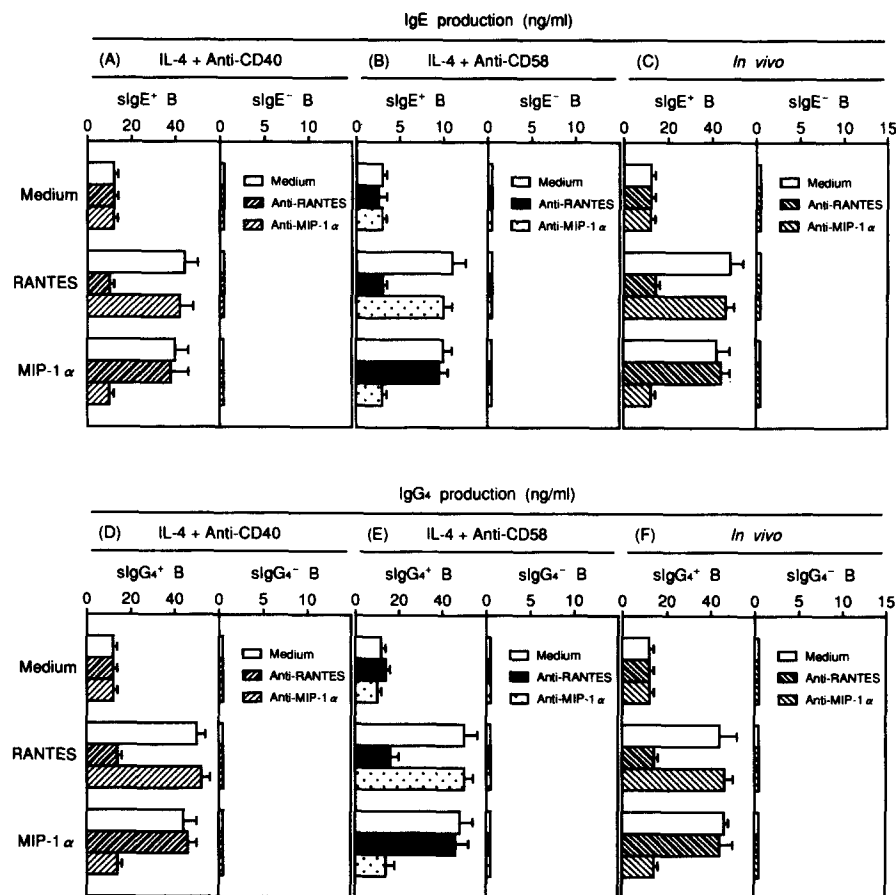


Figure 2. Effects of RANTES or MIP-1 α on IgE and IgG4 production in sIgE⁺ or sIgE⁻ B cells. sIgE⁺ or sIgE⁻ B cells (A-C), and sIgG4⁺ or sIgG4⁻ B cells (D-F) induced by IL-4 plus anti-CD40 mAb (A and D), IL-4 plus anti-CD58 mAb (B and E), or in vivo (C and F) were cultured with RANTES (100 nM) or MIP-1 α (100 nM) in the presence or absence of Abs (10 μ g/ml), and production of IgE (A-C) and IgG4 (D-F) was determined.

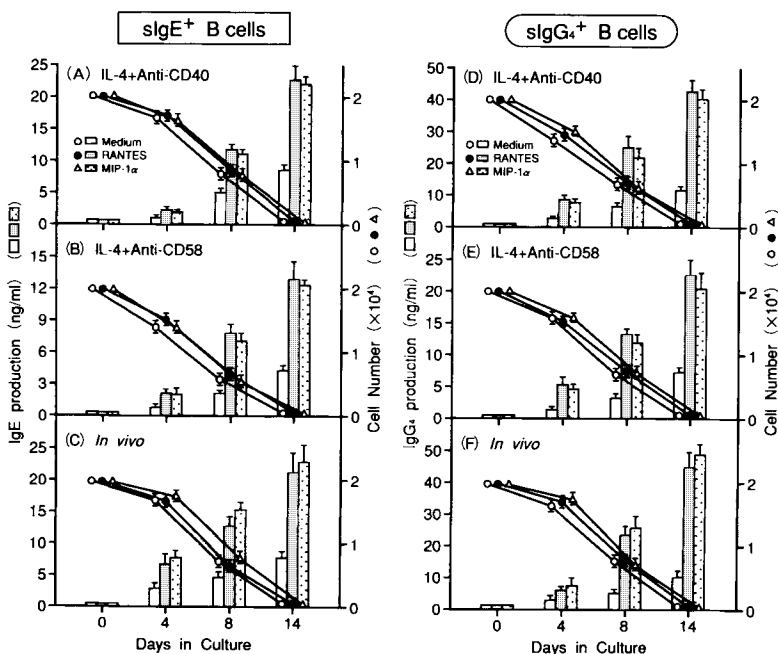


Figure 3. Kinetic study of the effects of RANTES and MIP-1 α on sIgE⁺ and sIgG4⁺ B cells. Purified sIgE⁺ B cells (A–C) or sIgG4⁺ B cells (D–F) induced by IL-4 plus anti-CD40 mAb (A and D), IL-4 plus anti-CD58 mAb (B and E) or in vivo (C and F) were cultured with medium, RANTES (100 nM), or MIP-1 α (100 nM), and Ig production and cell number were determined on days 0–14.

each inhibited IgE and IgG4 production in *in vivo*-generated sIgE⁺ and sIgG4⁺ B cells, although neither IFN- α nor IFN- γ inhibited those responses (Fig. 4 C). However, none of these Abs inhibited enhancement induced by RANTES or MIP-1 α (Fig. 4, A–C). None of IL-1 β , -2, -3, -7, or -9 at 300 U/ml, and none of IL-5, -11, -12, or -13 at 500 ng/ml affected IgE and IgG4 production by sIgE⁺ and sIgG4⁺ B cells, respectively, in these culture systems. Moreover, Abs to these cytokines also were without effects (<20% enhancement or inhibition). The effective neutralizing effects of endogenously produced cytokines with neutralizing Abs may be due to the FCS-free medium used in our culture system, since FCS induced TNF- α production

endogenously (20). We also measured IL-10 production by *in vivo*-generated sIgE⁺ and sIgG4⁺ B cells. After 3 d of culture, IL-10 production (picograms per milliliter) by sIgE⁺ and sIgG4⁺ B cells cultured with medium, RANTES (100 nM) and MIP-1 α (100 nM) was 322 \pm 41 and 410 \pm 62, 338 \pm 57 and 425 \pm 31, and 309 \pm 48 and 402 \pm 55, respectively (n = 4). Similarly, neither RANTES nor MIP-1 α induced mRNA for IL-10 by PCR (<20% enhancement of control by densitometry). Similar findings were observed in sIgE⁺ and sIgG4⁺ B cells induced by IL-4 plus anti-CD40 mAb, or by IL-4 plus anti-CD58 mAb (our manuscript in preparation).

Taken together, these findings indicate that the require-

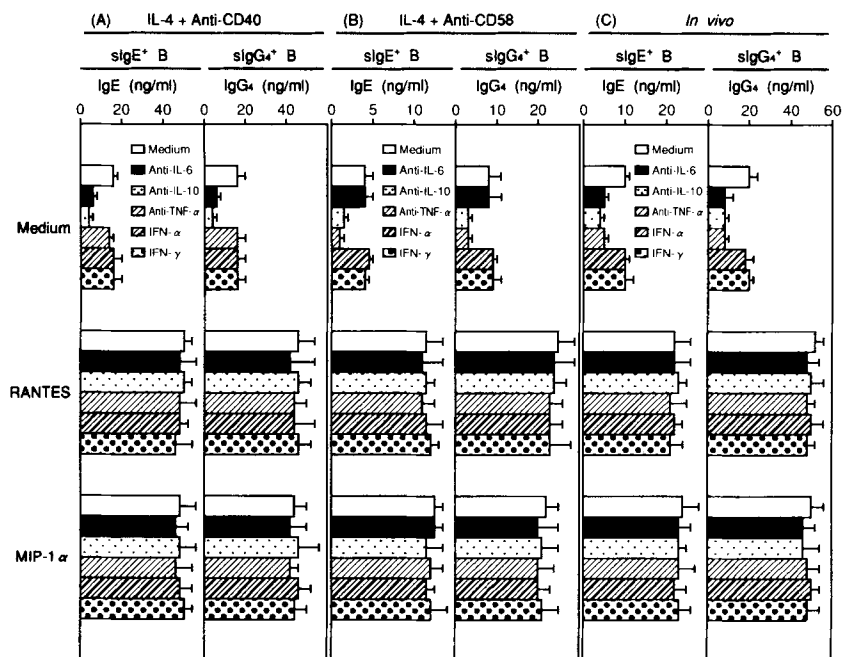


Figure 4. Effects of various factors on enhancement of IgE and IgG4 production by RANTES and MIP-1 α . Purified sIgE⁺ B cells and sIgG4⁺ B cells induced by IL-4 plus anti-CD40 mAb (A), IL-4 plus anti-CD58 mAb (B), or in vivo (C) were cultured with medium, RANTES (100 nM) or MIP-1 α (100 nM), and various factors were added. All the Abs were used at 10 μ g/ml, and IFN- α and IFN- γ at 1,000 U/ml. After 14 d of culture, IgE and IgG4 production were determined.

ment for endogenous cytokines for IgE and IgG4 production in sIgE⁺ and sIgG4⁺ B cells differed depending on the inductive stimuli. IL-6 and IL-10, but not TNF- α , were required by sIgE⁺ and sIgG4⁺ B cells induced by IL-4 plus anti-CD40 mAb, whereas IL-10 and TNF- α , but not IL-6, were required by sIgE⁺ and sIgG4⁺ B cells induced by IL-4 plus anti-CD58 mAb. In contrast, IL-6, IL-10, and TNF- α were all required by sIgE⁺ and sIgG4⁺ B cells generated in vivo. These findings were consistent with previous findings that anti-TNF- α mAb failed to block IgE and IgG4 production induced by IL-4 plus anti-CD40 mAb (5), and that IL-6 did not affect IgE production induced by IL-4 plus anti-CD58 mAb (8).

It is possible that the selective stimulation of sIgE⁺ and sIgG4⁺, but not sIgE⁻ and sIgG4⁻ B cells, by RANTES and MIP-1 α may be due to the presence or absence of receptors on these cells. Therefore, expression of receptors for RANTES, MIP-1 α , and other chemokines was studied by binding assay. Binding (Δ MFI) of RANTES in sIgE⁺ and sIgG4⁺ B cells generated by IL-4 plus anti-CD40 mAb, IL-4 plus anti-CD58 mAb, and in vivo, was 39 ± 11 and 43 ± 16 , 42 ± 10 and 34 ± 7 , and 48 ± 9 and 46 ± 10 , respectively ($n = 4$). On the other hand, binding (Δ MFI) of MIP-1 α in sIgE⁺ and sIgG4⁺ B cells generated by IL-4 plus anti-CD40 mAb, IL-4 plus anti-CD58 mAb, and in vivo, was 37 ± 12 and 42 ± 7 , 33 ± 6 and 35 ± 7 , and 49 ± 8 and 43 ± 8 , respectively ($n = 4$). In contrast, none of the sIgE⁻ and sIgG4⁻ B cells generated in vitro or in vivo expressed receptors for RANTES or MIP-1 α ($<3 \Delta$ MFI) ($n = 4$). Moreover, none of sIgE⁺, sIgG4⁺, sIgE⁻ or sIgG4⁻ B cells expressed receptors for NAP-2, β -TG, PF-4, GRO, IP-10, MIP-1 β , or MCP-1 ($<3 \Delta$ MFI) ($n = 4$).

In conclusion, of various chemokines tested, RANTES and MIP-1 α selectively enhanced IgE and IgG4 production by directly stimulating sIgE⁺ and sIgG4⁺ B cells generated in vitro and in vivo. The differences between the effects of RANTES and MIP-1 β and those of other chemokines were due to selective expression of receptors for RANTES and MIP-1 α on sIgE⁺ and sIgG4⁺ B cells. In contrast, re-

ceptors for other chemokines including NAP-2, β -TG, PF-4, GRO, IP-10, MIP-1 β , and MCP-1 were not found on sIgE⁺ and sIgG4⁺ B cells. This is not surprising. It has been reported that receptors for RANTES and MIP-1 α , but not for MIP-1 β , were found in subpopulations of B cells (21, 22). We have previously reported that IL-8 selectively inhibited IgE and IgG4 production (16). Moreover, it is well established that chemokines do differ in their effects depending upon target cells or experimental conditions. For example, MIP-1 α inhibited colony formation of myeloid progenitor cells stimulated with steel factor, whereas MIP-1 β , GRO, and NAP-2 failed to do so (13). RANTES and MIP-1 α induced migration of eosinophils, while MIP-1 β or MCP-1 did not (23).

The in vivo effects of RANTES and MIP-1 α on IgE and IgG4 production remain to be elucidated. However, it has been reported that alveolar macrophages from patients with asthma produced RANTES and MIP-1 α , and that IgE and IgG4 concentrations in bronchoalveolar lavage fluid (BALF) were elevated in such patients (2, 24–26). Consistent with this, we found that RANTES and MIP-1 α concentrations in BALF were higher in asthmatic patients (58 ± 13 pg/ml RANTES and 48 ± 9 pg/ml MIP-1 α , $n = 4$) with elevated concentrations of BALF IgE ($2,785 \pm 648$ pg/ml) and IgG4 (738 ± 149 ng/ml) than in asthmatic patients (24 ± 5 pg/ml RANTES and 19 ± 4 pg/ml MIP-1 α , $n = 4$) without elevated concentrations of BALF IgE (312 ± 172 pg/ml) or IgG4 (129 ± 42 ng/ml).

We have previously reported that chemokine IL-8 selectively inhibits IgE and IgG4 production, whereas it also inhibits the growth of B cells, and that mRNA for IL-8RI and IL-8RII is found in B cells (16, 27, 28). Conversely, the number of B cells was found to be greatly increased in mice that lacked IL-8R homologue (15). Taken together, these findings indicate that chemokines may play roles in Ig production by and growth of B cells. Detailed molecular analysis of the effects of RANTES and MIP-1 α on B cells is currently in progress.

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