

## **RANTES and Macrophage Inflammatory Protein 1 $\alpha$ 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),  $\beta$ -thromboglobulin ( $\beta$ -TG), platelet factor 4 (PF-4), melanoma growth stimulating activity (GRO),  $\gamma$  interferon-induced protein (IP-10), regulated on activation, normal T expressed and secreted (RANTES), macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ), MIP-1 $\beta$ , 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 $\alpha$  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 $\alpha$  was specifically blocked by anti-RANTES mAb and anti-MIP-1 $\alpha$  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- $\alpha$  mAb failed to do so. Purified surface IgE positive (sIgE<sup>+</sup>) and sIgG4<sup>+</sup> B cells generated either in vitro or in vivo spontaneously produced IgE and IgG4, respectively, whereas sIgE<sup>-</sup> and sIgG4<sup>-</sup> B cells failed to do so. RANTES and MIP-1 $\alpha$  enhanced spontaneous IgE and IgG4 production in sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells, respectively, whereas neither RANTES nor MIP-1 $\alpha$  did so in sIgE<sup>-</sup> or sIgG4<sup>-</sup> B cells. Purified sIgE<sup>+</sup> and sIgG4<sup>+</sup>, but not sIgE<sup>-</sup> or sIgG4<sup>-</sup> B cells, generated in vitro and in vivo expressed receptors for RANTES and MIP-1 $\alpha$ , whereas they failed to express receptors for other chemokines. These findings indicate that RANTES and MIP-1 $\alpha$  enhance IgE and IgG4 production by directly stimulating sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells.

**H**uman 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- $\alpha$  enhance IL-4- and IL-13-induced IgE and IgG4 production (1–6), whereas IFN- $\alpha$ , IFN- $\gamma$ , TGF- $\beta$ , 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- $\gamma$  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  $\alpha$  subfamily members including IL-8, melanoma growth-stimulating activity (GRO), neutrophil-activating peptide 2 (NAP-2),  $\beta$ -thromboglobulin ( $\beta$ -TG),  $\gamma$  interferon-induced protein (IP-10), and platelet factor 4 (PF-4), and  $\beta$  subfamily members including regulated on activation, normal T expressed and secreted (RANTES), macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ ), MIP-1 $\beta$ , 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 $\alpha$

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- $\alpha$  (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- $\beta$ , RANTES, GRO, MIP-1 $\alpha$ , MIP-1 $\beta$ , and MCP-1, and mouse IgG1 anti-IL-5, anti-IL-6, anti-TNF- $\alpha$  mAb, anti-RANTES mAb, and goat anti-IL-10 and anti-MIP-1 $\alpha$  Ab were purchased from R&D Systems, Inc. (Minneapolis, MN). Recombinant human NAP-2, PF-4, highly purified native human  $\beta$ -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  $\mu$ g/ml transferrin (2).

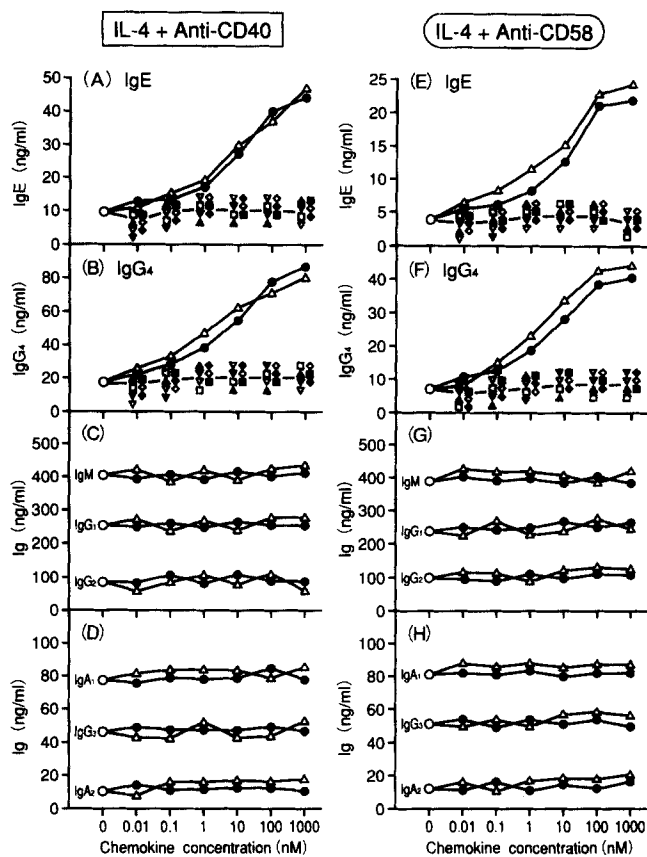
**Cell Cultures.** Tonsillar mononuclear cells were obtained from nonatopic donors (serum IgE level <50 U/ml) and atopic patients (serum IgE level 1,578–12,259 IU/ml). Highly purified B cells were separated by SRBC rosetting, followed by L-leucine methyl ester incubation as described previously (2). Purified B cell fractions contained >98% CD20<sup>+</sup> B cells. Purified B cells were depleted of surface IgE positive (sIgE<sup>+</sup>) and sIgG4<sup>+</sup> B cells by panning. The percentage of sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells was <0.1%. The sIgE<sup>-</sup>, sIgG4<sup>-</sup> B cells were cultured ( $2 \times 10^5$ /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  $\mu$ g/ml, because anti-IL-5 mAb, anti-IL-6 mAb, anti-IL-10 Ab, anti-IL-13 Ab, and anti-TNF- $\alpha$  mAb (all at 10  $\mu$ 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- $\alpha$  (50 ng/ml), respectively (2, 4, 6). In some experiments, purified sIgE<sup>-</sup>, sIgG4<sup>-</sup> B cells were cultured with IL-4 (1,000 U/ml) plus anti-CD40 mAb (0.1  $\mu$ g/ml) or IL-4 plus anti-CD58 mAb (0.1  $\mu$ g/ml) for 5–7 d, and then sIgE<sup>+</sup>, sIgE<sup>-</sup>, sIgG4<sup>+</sup>, and sIgG4<sup>-</sup> B cells were purified by panning (2, 4). Alternatively, sIgE<sup>+</sup>, sIgE<sup>-</sup>, sIgG4<sup>+</sup>, and sIgG4<sup>-</sup> B cells were purified from tonsillar B cells of atopic patients by panning. Purified sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cell fractions contained >98% sIgE<sup>+</sup> B cells and >98% sIgG4<sup>+</sup> B cells, respectively (2, 4). Purified sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells were cultured ( $2 \times 10^4$ /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<sup>+</sup> and sIg<sup>-</sup> 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  $\Delta$ MFI (6, 17). Binding ( $\Delta$ MFI,  $n = 4$ ) of RANTES, MIP-1 $\alpha$ , IP-10, MIP-1 $\beta$ , and MCP-1 in purified human mono-

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

## Results and Discussion

Preliminary experiments showed that none of the chemokines (1 pM–1  $\mu$ 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 $\alpha$  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,  $\beta$ -TG, PF-4, GRO, IP-10, MIP-1 $\beta$ , 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<sup>-</sup>, sIgG4<sup>-</sup> B cells were cultured with IL-4 (1,000 U/ml) plus anti-CD40 mAb (0.1  $\mu$ g/ml) (A–D), or IL-4 plus anti-CD58 mAb (0.1  $\mu$ g/ml) (E–H). Medium (○) or various concentrations of RANTES (●), MIP-1 $\alpha$  (△), GRO (▲), NAP-2 (▽),  $\beta$ -TG (▼), PF-4 (□), IP-10 (■), MIP-1 $\beta$  (◇), 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.

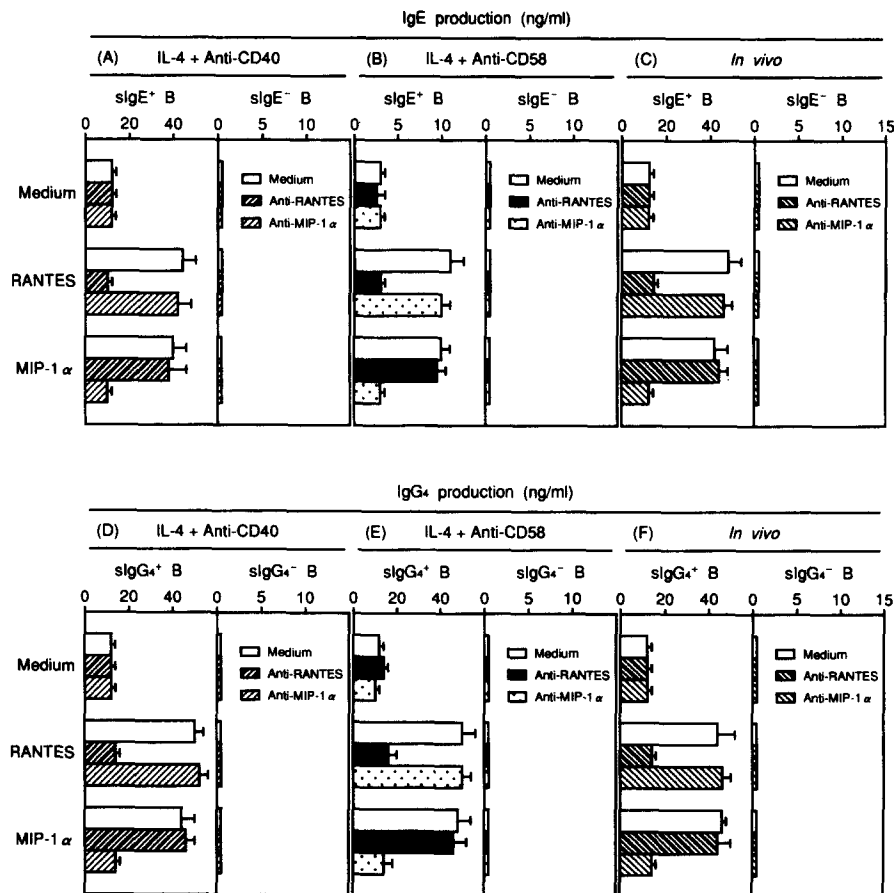
1 $\alpha$  enhanced IgE and IgG4 production induced by IL-4 plus anti-CD58 mAb, but did not enhance the production of other Igs (Fig. 1, E-H). None of NAP-2,  $\beta$ -TG, PF-4, GRO, IP-10, MIP-1 $\beta$ , or MCP-1 had any effect on the production of IgE, IgG4 (Fig. 1, E and F), or other Igs (data not shown). In five experiments performed, the ranges of enhancement of IgE and IgG4 production by RANTES (100 nM) and MIP-1 $\alpha$  (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<sup>+</sup> B cells (18, 19). Therefore, in this study, sIgE<sup>-</sup>, sIgG4<sup>-</sup> B cells were prestimulated with IL-4 plus anti-CD40 mAb or IL-4 plus anti-CD58 mAb, and sIgE<sup>+</sup>, sIgG4<sup>+</sup>, sIgE<sup>-</sup>, and sIgG4<sup>-</sup> B cells were purified. We also studied the effects of RANTES and MIP-1 $\alpha$  on in vivo-generated sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells obtained from atopic patients. As shown in Fig. 2, A-C, RANTES and MIP-1 $\alpha$  enhanced IgE production in sIgE<sup>+</sup> 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<sup>-</sup> B cells. Enhancement of IgE production by RANTES was specific, since it was blocked by anti-RANTES mAb but not by

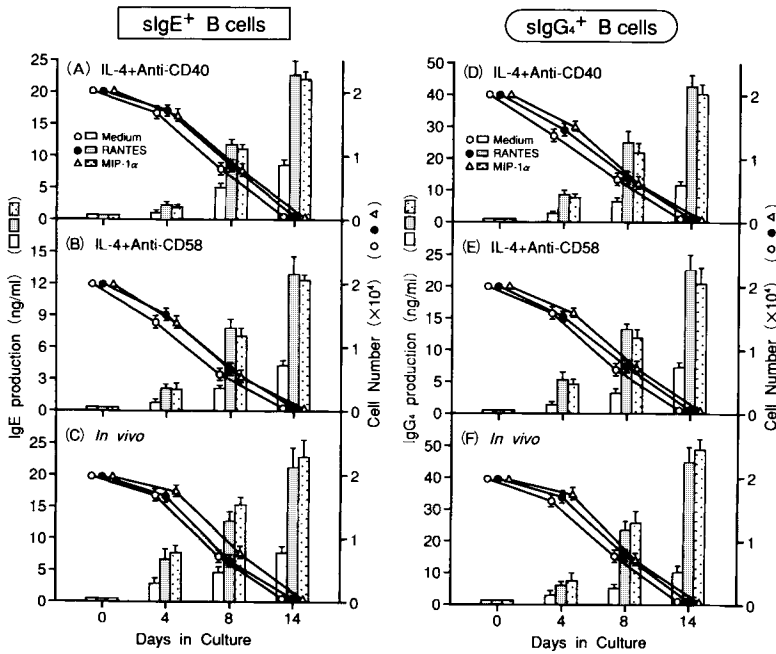
anti-MIP-1 $\alpha$  Ab or control mouse IgG1 (data not shown). Conversely, enhancement of IgE production by MIP-1 $\alpha$  was blocked by anti-MIP-1 $\alpha$  Ab, but not by anti-RANTES mAb or control goat IgG (data not shown). Identical results were obtained for IgG4 production (Fig. 2, D-F).

These findings indicate that RANTES and MIP-1 $\alpha$  directly stimulated sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells generated in vitro and in vivo, but had no effect on sIgE<sup>-</sup> and sIgG4<sup>-</sup> 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<sup>+</sup> and sIgG4<sup>+</sup> B cells, since RANTES and MIP-1 $\alpha$  had no effect on cell number on any day tested (Fig. 3).

We and others have reported that IL-6, IL-10, and TNF- $\alpha$  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<sup>+</sup> and sIgG4<sup>+</sup> B cells, respectively, induced by IL-4 plus anti-CD40 mAb, whereas anti-TNF- $\alpha$  mAb, IFN- $\alpha$ , and IFN- $\gamma$  failed to do so. In contrast, anti-IL-10 Ab and anti-TNF- $\alpha$  mAb each inhibited IgE and IgG4 production in those cells induced by IL-4 plus anti-CD58 mAb, whereas anti-IL-6 mAb, IFN- $\alpha$ , and IFN- $\gamma$  each failed to do so (Fig. 4 B). On the other hand, anti-IL-6 mAb, anti-IL-10 Ab, and anti-anti-TNF- $\alpha$  mAb



**Figure 2.** Effects of RANTES or MIP-1 $\alpha$  on IgE and IgG4 production in sIgE<sup>+</sup> or sIgE<sup>-</sup> B cells. sIgE<sup>+</sup> or sIgE<sup>-</sup> B cells (A-C), and sIgG4<sup>+</sup> or sIgG4<sup>-</sup> 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 $\alpha$  (100 nM) in the presence or absence of Abs (10  $\mu$ g/ml), and production of IgE (A-C) and IgG4 (D-F) was determined.

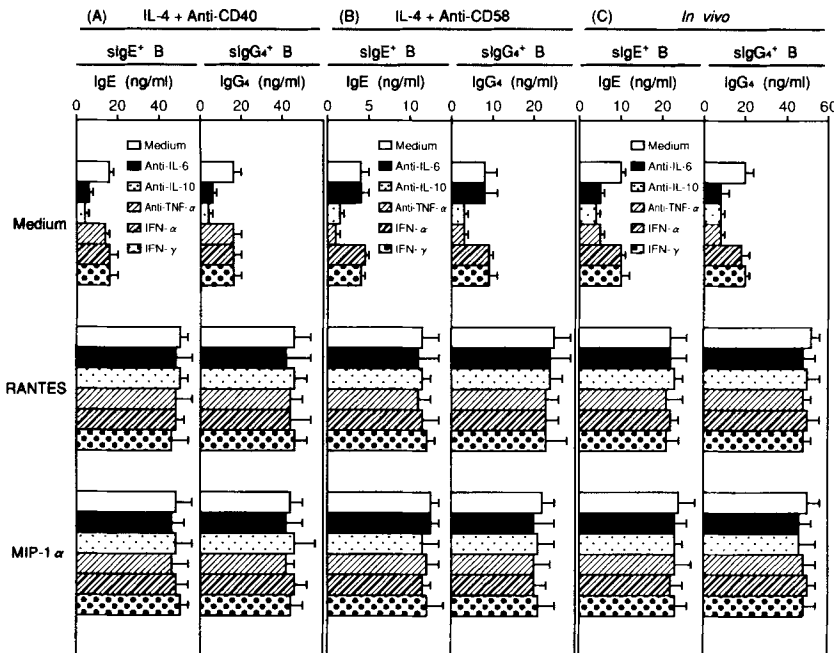


**Figure 3.** Kinetic study of the effects of RANTES and MIP-1 $\alpha$  on sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells. Purified sIgE<sup>+</sup> B cells (A–C) or sIgG4<sup>+</sup> B cells (D–F) induced by IL-4 plus anti-CD40 mAb (A and D), IL-4 plus anti-CD58 mAb (B and E) or *in vivo* (C and F) were cultured with medium, RANTES (100 nM), or MIP-1 $\alpha$  (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<sup>+</sup> and sIgG4<sup>+</sup> B cells, although neither IFN- $\alpha$  nor IFN- $\gamma$  inhibited those responses (Fig. 4 C). However, none of these Abs inhibited enhancement induced by RANTES or MIP-1 $\alpha$  (Fig. 4, A–C). None of IL-1 $\beta$ , -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<sup>+</sup> and sIgG4<sup>+</sup> 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- $\alpha$  production

endogenously (20). We also measured IL-10 production by *in vivo*-generated sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells. After 3 d of culture, IL-10 production (picograms per milliliter) by sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells cultured with medium, RANTES (100 nM) and MIP-1 $\alpha$  (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 $\alpha$  induced mRNA for IL-10 by PCR (<20% enhancement of control by densitometry). Similar findings were observed in sIgE<sup>+</sup> and sIgG4<sup>+</sup> 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 $\alpha$ . Purified sIgE<sup>+</sup> B cells and sIgG4<sup>+</sup> 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 $\alpha$  (100 nM), and various factors were added. All the Abs were used at 10  $\mu$ g/ml, and IFN- $\alpha$  and IFN- $\gamma$  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<sup>+</sup> and sIgG4<sup>+</sup> B cells differed depending on the inductive stimuli. IL-6 and IL-10, but not TNF- $\alpha$ , were required by sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells induced by IL-4 plus anti-CD40 mAb, whereas IL-10 and TNF- $\alpha$ , but not IL-6, were required by sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells induced by IL-4 plus anti-CD58 mAb. In contrast, IL-6, IL-10, and TNF- $\alpha$  were all required by sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells generated in vivo. These findings were consistent with previous findings that anti-TNF- $\alpha$  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<sup>+</sup> and sIgG4<sup>+</sup>, but not sIgE<sup>-</sup> and sIgG4<sup>-</sup> B cells, by RANTES and MIP-1 $\alpha$  may be due to the presence or absence of receptors on these cells. Therefore, expression of receptors for RANTES, MIP-1 $\alpha$ , and other chemokines was studied by binding assay. Binding ( $\Delta$ MFI) of RANTES in sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells generated by IL-4 plus anti-CD40 mAb, IL-4 plus anti-CD58 mAb, and in vivo, was  $39 \pm 11$  and  $43 \pm 16$ ,  $42 \pm 10$  and  $34 \pm 7$ , and  $48 \pm 9$  and  $46 \pm 10$ , respectively ( $n = 4$ ). On the other hand, binding ( $\Delta$ MFI) of MIP-1 $\alpha$  in sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells generated by IL-4 plus anti-CD40 mAb, IL-4 plus anti-CD58 mAb, and in vivo, was  $37 \pm 12$  and  $42 \pm 7$ ,  $33 \pm 6$  and  $35 \pm 7$ , and  $49 \pm 8$  and  $43 \pm 8$ , respectively ( $n = 4$ ). In contrast, none of the sIgE<sup>-</sup> and sIgG4<sup>-</sup> B cells generated in vitro or in vivo expressed receptors for RANTES or MIP-1 $\alpha$  ( $<3 \Delta$ MFI) ( $n = 4$ ). Moreover, none of sIgE<sup>+</sup>, sIgG4<sup>+</sup>, sIgE<sup>-</sup> or sIgG4<sup>-</sup> B cells expressed receptors for NAP-2,  $\beta$ -TG, PF-4, GRO, IP-10, MIP-1 $\beta$ , or MCP-1 ( $<3 \Delta$ MFI) ( $n = 4$ ).

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

ceptors for other chemokines including NAP-2,  $\beta$ -TG, PF-4, GRO, IP-10, MIP-1 $\beta$ , and MCP-1 were not found on sIgE<sup>+</sup> and sIgG4<sup>+</sup> B cells. This is not surprising. It has been reported that receptors for RANTES and MIP-1 $\alpha$ , but not for MIP-1 $\beta$ , 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 $\alpha$  inhibited colony formation of myeloid progenitor cells stimulated with steel factor, whereas MIP-1 $\beta$ , GRO, and NAP-2 failed to do so (13). RANTES and MIP-1 $\alpha$  induced migration of eosinophils, while MIP-1 $\beta$  or MCP-1 did not (23).

The in vivo effects of RANTES and MIP-1 $\alpha$  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 $\alpha$ , 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 $\alpha$  concentrations in BALF were higher in asthmatic patients ( $58 \pm 13$  pg/ml RANTES and  $48 \pm 9$  pg/ml MIP-1 $\alpha$ ,  $n = 4$ ) with elevated concentrations of BALF IgE ( $2,785 \pm 648$  pg/ml) and IgG4 ( $738 \pm 149$  ng/ml) than in asthmatic patients ( $24 \pm 5$  pg/ml RANTES and  $19 \pm 4$  pg/ml MIP-1 $\alpha$ ,  $n = 4$ ) without elevated concentrations of BALF IgE ( $312 \pm 172$  pg/ml) or IgG4 ( $129 \pm 42$  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 $\alpha$  on B cells is currently in progress.

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