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IgE Versus IgG4 Production Can Be Differentially Regulated by IL-10

Pascale Jeannin,[1] Sybille Lecoanet, Yves Delneste, Jean-François Gauchat, and Jean-Yves Bonnefoy

Allergen-specific IgE plays a key role in the physiopathology of allergic disorders. This IgE response is usually accompanied by a production of IgG4. Indirect evidence suggests that IgG4 may not be a sensitizing Ab but, in contrast, could be protective. As such, it may be of potential therapeutic interest to selectively modulate IgE vs IgG4 production. To date, IgE and IgG4 switching seems to be controlled by common mechanisms. We report here that IL-10 has a differential effect on IgE vs IgG4 production by PBMC. IL-10 decreases ϵ transcript expression and IgE production induced by IL-4 when added during the first 3 days of in vitro culture, suggesting that IL-10 decreases IL-4-induced IgE switching. In contrast, if added later on B cells that are already IgE switched, IL-10 potentiates IgE production. Interestingly, whatever the time of addition, IL-10 augments IL-4-induced $\gamma4$ transcript expression and IgG4 production, with a maximal effect when added during the first 3 days. As IL-10 is not a switch factor for IgG4, it is likely that IL-10 enhances IgG4 production by potentiating IL-4-induced IgG4 switching. However, IL-10 may also act by enhancing the growth and/or differentiation of cells that are already IgG4 committed. Finally, CD40 ligation reverses the early down-regulating effect of IL-10 on IgE production. These results are the first evidence of a molecule that differentially regulates IgE vs IgG4 production, thereby suggesting the existence of a pathway(s) selectively controlling their production. *The Journal of Immunology,* **1998, 160: 3555–3561.**

I nduction of IgE switching in vitro requires two primary signals. The first one, given by IL-4 or IL-13, induces the expression of the sterile ϵ transcript (1–3). The second one, provided by the triggering of CD40 with anti-CD40 mAb, recombinant CD40 ligand (CD40L),[2] or membrane CD40L expressed on different cell types including activated T cells, induces the expression of the mature ϵ transcript encoding for IgE (4–6). The production of IgE is controlled by additional secondary signals provided by soluble mediators or by direct T:B cell contact. The cytokines IL-2, IL-5, IL-6, IL-9, and TNF-α (7–11); some chemokines (12, 13); histamine (14); and the ligation of the B cell accessory molecules CD21, CD54, CD58 (15–17), CD44, and CD86 (18) enhance IgE production. Conversely, the soluble factors PGE-2, IFN-γ, IFN-α, IL-12, and TGFβ down-regulate this production (10, 19, 20). To date, all the signals recognized to be involved in IgE switching also control IgG4, suggesting that IgE and IgG4 synthesis are regulated by common mechanisms (6, 12–14, 21).

In contrast to healthy subjects, allergic patients develop specific IgE directed against sensitizing allergens that play a key role in the physiopathology of allergic diseases (22). In parallel to the IgE response, allergic patients usually produce high levels of total and allergen-specific IgG4 Ab (23–25). However, a potential role for IgG4 Ab in allergic diseases has not yet been clearly established. In allergic patients, the finding of seric IgG4 Ab directed against allergens to which the patients were not sensitive suggests that they are not sensitizing Ab (26). On the contrary, the existence of a positive correlation between a successful *Hymenoptera* venom desensitization and the high levels of specific IgG4 Ab generated suggests that IgG4 may be protective (25, 27, 28). Therefore, it may be of potential therapeutic interest to be able to modulate selectively the production of IgE vs IgG4.

IL-10 is a lymphokine produced by numerous cell types including activated T cells, mast cells, and macrophages. By blocking cytokine production and APC functions of monocytes and/or macrophages, IL-10 plays a major role in suppressing immune and inflammatory responses (reviewed in Ref. 29). IL-10 also acts on human B cells activated with an anti-CD40 mAb by enhancing: 1) switching to IgA, IgG1, and IgG3 isotypes (30–32); 2) short-term proliferation (33, 34); and 3) differentiation into Ig-secreting plasma cells (33, 35). Interestingly, IL-10 decreases IgE production by IL-4-stimulated PBMC (36). The aim of this work was to evaluate the effect of IL-10 on the production of the other isotypes by IL-4-stimulated PBMC. Results showed that IL-10 has opposite effects on IgE vs IgG4 synthesis according to the nature of the stimulus and the kinetic of addition of IL-10. These data suggest the existence of a pathway selectively involved in the control of IgE and IgG4 production.

Materials and Methods

Subjects

Five patients sensitive to the house dust mite *Dermatophagoides pteronyssinus* were selected on the basis of the following criteria: history of allergic symptoms (asthma and/or perennial rhinitis), positive cutaneous prick tests toward house dust mite allergens (diameter >9 mm), and specific anti-*D. pteronyssinus* IgE. The healthy subjects ($n = 7$) had no history of allergic disorders.

Ig assays

PBMC were isolated by centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Human tonsillar B cells were purified as described (21).

Surface IgD$^+$ B cells were purified by cell sorting using a FACS Vantage (Becton Dickinson, Erembodegem, Belgium) after labeling with a FITC-labeled anti-human IgD mAb (Dako, Glostrup, Denmark). Purity was routinely >98%. Cells ($2 \times 10^5/200$ μl/well) were cultured in enriched Iscove's medium in 96-well plates (Nunc, Roskilde, Denmark) and stimulated, in quintuplicate, with 200 U/ml of IL-4 (Geneva Biomedical Research Institute (GBRI), Geneva, Switzerland), 0.1 μg/ml of anti-CD40 mAb (Serotec, Oxford, U.K.), or combinations of IL-4 plus anti-CD40 mAb in the presence or absence of 0.4 to 50 ng/ml of IL-10 (R&D Systems, Abingdon, U.K.). In some experiments, anti-CD40 mAb was replaced by 5 μg/ml of rCD40L (GBRI). PBMC from allergic patients were stimulated as described above in the presence or absence of 1 IR/ml (biologic units used by the Laboratoire des Stallergènes, Fresnes, France) of *D. pteronyssinus* crude extract (Laboratoire des Stallergènes). In some sets of experiments, IL-10 was added at different time points following stimulation of PBMC with IL-4. In other sets of experiments, PBMC were stimulated with IL-4 in the presence of IL-1α, IL-2, IL-3, IL-5 (all from GBRI), IL-8, IL-9, IL-11, IFN-γ (all from R&D Systems), IL-6, TNF-α (both from AMS Biotechnology, Lugano, Switzerland), or TGFβ (Sigma, St. Louis, MO). Supernatants were collected at day 12 to quantify Ig.

Ig quantification

The total Ig were quantified by ELISA. IgE and IgG4 ELISA were performed as described (21). IgA1, IgA2, IgG1, IgG2, and IgG3 were quantified as follows: 96-well plates (Nunc) were coated overnight at 4°C with 0.1 μg/well of anti-IgA1 mAb (Sigma), 0.2 μg/well of anti-IgA2 mAb (Nordic Immunological Laboratories, Tilburg, The Netherlands), 1 μg/well of anti-IgG1 mAb (clone NL16) (Oxoid Unipath, Hampshire, U.K.), 0.2 μg/well of anti-IgG2 mAb (clone HP6002), or 0.2 μg/well of anti-IgG3 mAb (clone HP6050) (both from Calbiochem, La Jolla, CA) in 0.1 M carbonate-bicarbonate buffer, pH 9.6, and then saturated with 1% (w/v) BSA in PBS. Cell culture supernatants, diluted in PBS/BSA containing 0.1% (v/v) Tween-20, were incubated for 4 h at room temperature. After washing, peroxidase-conjugated anti-human α- or γ-chain immune sera (Sigma) were incubated at room temperature for 3 h, for quantification of IgA and IgG, respectively. After washing, bound Abs were detected using o-phenylenediamine substrate, and the colorimetric reaction was stopped with 2 M H$_2$SO$_4$. OD values were determined at 492 nm. The standard curves were done using purified human Ig (Sigma). The sensitivity of the ELISA was higher than 5 ng/ml. The specificity was verified using purified IgA1-2, IgG1-4 (Sigma), and IgE (The Binding Site, Birmingham, U.K.). Results are expressed in ng/ml, or as a percentage of increase or decrease of Ig production defined as $(A - O \div O) \times 100$ or $(O - A \div O) \times 100$, where A and O are the concentrations of Ig produced in the presence or absence of IL-10, respectively.

Proliferation assays

PBMC or B cells ($2 \times 10^5/200$ μl/well) were stimulated in triplicate in 96-well plates with IL-4 and/or anti-CD40 mAb in the presence of 50 ng/ml of IL-10. After 48 h of incubation, cells were pulsed with 0.25 μCi/well of [^{3}H]TdR (Amersham International, Amersham, U.K.) for 6 h. Radioactive incorporation was measured by standard liquid scintillation counting. Results are given as proliferation index (PI) calculated as follows: $A \div O$ where A and O were the cpm values obtained with cells stimulated or not with IL-10, respectively.

Analysis of IgE mRNA transcription by Northern blotting

PBMC were either unstimulated or stimulated for 10 days with 200 U/ml of IL-4, 50 ng/ml of IL-10, or IL-4 plus IL-10. In this last condition, IL-10 was added together with IL-4 (at day 0) or 4 days later. Total RNA extraction and hybridizations were performed with cRNA probes complementary to Cϵ and β-actin mRNA (2). IgE protein was quantified in the supernatants by ELISA.

Analysis of mature $\gamma4$ mRNA expression by PCR

The single-strand cDNA was synthesized using 5 μg of total RNA, prepared as described above, by reverse transcription using an oligo(dT) primer (Pharmacia). cDNA corresponding to 0.5 μg of total RNA were used for the amplification reactions. Each PCR tube contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl$_2$, 0.01% gelatin, 2 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT), 0.2 mM concentrations of each deoxynucleotide (Pharmacia), and 10 μM of each primer. Sequences of the oligonucleotides for the $\gamma4$ mature transcripts are as follows: 5'J$_H$ consensus 5'-ACC$^C/_A$TGGTCACCGTCTCCTCA-3' and 3' C$\gamma4$ 5'-GGGACCATATTTGGACTC-3' (37, 38). Control consisted of amplifying β-actin transcripts using the following primers: 5'-CGATTTC

CCGCTCGGCCGTGGTGGTGAAGC-3' and 5'-GGCGACGAGGCCCA GAGCAAGAGAGGCATC-3'. The amplification consisted of 1 cycle for 4 min at 94°C followed by 35 cycles for 30 s at 94°C, 60°C for 1 min, and 72°C for 1 min. The final extension cycle was for 5 min at 72°C. PCR products were size separated on a 1% agarose gel and visualized by ethidium bromide staining.

Statistical analysis

Statistical significance was determined using Student's t test.

Results

Differential effect of IL-10 on IgE and IgG4 production by IL-4- or IL-13-stimulated PBMC from healthy subjects

Stimulation of PBMC from healthy subjects with IL-4 (Fig. 1*A*) or IL-13 (data not shown) induces IgE and IgG4 production (1, 3, 5). In agreement with others (36), IL-10 when added together with IL-4 (at day 0) decreased IL-4-induced germ line and productive ϵ transcript expression and IgE production (decrease of 82 $\pm$ 11%, mean $\pm$ SD, $n = 6$) (Fig. 1*A*, *top*, and 2*A*). Surprisingly, IL-10 potentiated IL-4-induced productive $\gamma4$ transcript expression and IgG4 production (increase of 902 $\pm$ 136% at day 0) (Fig. 1*A*, *bottom*, and 2*B*). These effects of IL-10 on IgE and IgG4 production were dose dependent, in the range of 1 to 50 ng/ml (Fig. 1*B*). IL-10 also modulated IL-13-induced IgE and IgG4 production (decrease of 72 $\pm$ 15% and increase of 1035 $\pm$ 322%, respectively, mean $\pm$ SD, $n = 3$) (data not shown). Among the different cytokines tested, IL-10 was the only one that had an opposite effect on IgE vs IgG4 synthesis by IL-4-stimulated PBMC (Fig. 3). IL-10 also enhanced IgA1-2 and IgG1-3 production by IL-4- or IL-13-stimulated PBMC (increase of 157 $\pm$ 34%, 228 $\pm$ 15%, 380 $\pm$ 36%, 272 $\pm$ 96%, and 214 $\pm$ 75%, respectively, mean $\pm$ SD, $n = 3$) (Fig. 1*C*).

Depending on the subject, resting PBMC produced undetectable levels of IgE and low or undetectable levels of IgG4 (12 $\pm$ 9 ng/ml, mean $\pm$ SD, $n = 6$). Addition of IL-10 did not induce IgE production but enhanced IgG4 production (125 $\pm$ 35 ng/ml) (Fig. 1*A*). In parallel to these effects on Ig production, IL-10 did not significantly modulate the proliferation of PBMC either unstimulated or stimulated with IL-4 (PI < 2) (data not shown).

Thus, IL-10 has a dual effect on IgE vs IgG4 production since it prevents IgE but potentiates IgG4 synthesis by IL-4- or IL-13-stimulated PBMC.

IL-10 potentiates IL-4 plus anti-CD40 mAb-induced IgE and IgG4 production by PBMC from healthy subjects

IgE synthesis by B cells requires a signaling through IL-4 and/or IL-13 receptors and CD40 (4, 5). As such, the stimulation of PBMC with both IL-4 plus anti-CD40 mAb results in a superinduction of IgE and IgG4 production. In this condition of stimulation, IL-10 increased both IgE and IgG4 production (increase of 715 $\pm$ 144% and 878 $\pm$ 122%, respectively with 50 ng/ml IL-10, mean $\pm$ SD, $n = 6$) (Fig. 4, *A* and *B*). IL-10 also enhanced IL-4 plus rCD40L-induced IgE and IgG4 production by PBMC (increase of 624 $\pm$ 127% and 792 $\pm$ 125%, respectively, mean $\pm$ SD, $n = 3$). This effect was dose dependent in the range of 1 to 50 ng/ml of IL-10 (data not shown). IL-10 also increased IgA1-2 and IgG1-3 production (increase of 611 $\pm$ 221%, 914 $\pm$ 215%, 545 $\pm$ 156%, 879 $\pm$ 211%, and 541 $\pm$ 108%, respectively, mean $\pm$ SD, $n = 3$) (Fig. 4*C*).

PBMC stimulated with anti-CD40 mAb or rCD40L alone did not produce IgE and produced undetectable or low levels of IgG4 (15 $\pm$ 8 ng/ml and 12 $\pm$ 5 ng/ml, respectively, mean $\pm$ SD, $n = 6$). Addition of IL-10 did not induce IgE production but enhanced IgG4 production (315 $\pm$ 98 ng/ml and 285 $\pm$ 66 ng/ml, respectively). In parallel, IL-10 did not modulate the proliferation of
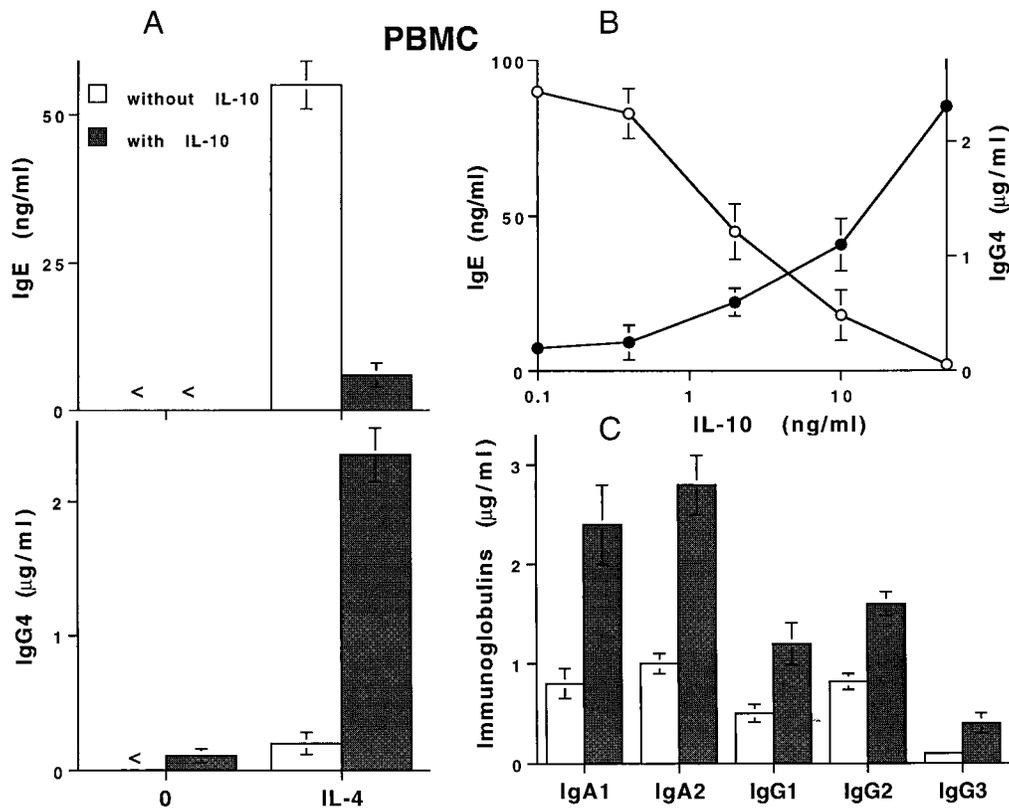
FIGURE 1. Effect of IL-10 on Ig production by IL-4-stimulated PBMC. *A*, IL-10 decreases IgE and increases IgG4 production. PBMC from healthy subjects were stimulated or not with IL-4 in the absence (□) or presence (■) of 50 ng/ml of IL-10. IgE (*top*) and IgG4 (*bottom*) were quantified in 12-day supernatants. *B*, IL-10 affects IgE and IgG4 production in a dose-dependent manner. PBMC were stimulated with IL-4 in the absence or presence of 0.1 to 50 ng/ml of IL-10. IgE ○ and IgG4 (●) were quantified in 12-day supernatants. *C*, IL-10 increases IgA1, IgA2, and IgG1-3 production. PBMC were stimulated by IL-4 without (□) or with (■) 50 ng/ml of IL-10. IgA1, IgA2, and IgG1-3 were quantified in 12-day supernatants. *A*, *B*, and *C*, Results are expressed in ng/ml or in μg/ml (mean ± SD of quintuplicate values) and are representative of one of six (*A*) or three (*B* and *C*) experiments.

PBMC induced by an anti-CD40 mAb in the absence or presence of IL-4 (PI < 2) (data not shown). Thus, IL-10 potentiates both IgE and IgG4 production by PBMC stimulated through the IL-4 receptor and CD40.

Differential effect of IL-10 on IgE and IgG4 production by PBMC from allergic patients stimulated by the sensitizing allergen

We have evaluated the clinical relevance of the above results on PBMC from *D. pteronyssinus*-sensitive patients. Resting and *D. pteronyssinus*-stimulated PBMC from allergic patients produced undetectable or low levels of IgE (4.5 ± 2 and 6 ± 3 ng/ml, respectively, mean ± SD, $n = 5$) and IgG4 (12 ± 3 and 15 ± 4 ng/ml, respectively) (Fig. 5, *A* and *B*). Addition of IL-10 significantly enhanced both IgE (22 ± 6 and 24 ± 5 ng/ml, respectively) and IgG4 production (389 ± 96 and 332 ± 72 ng/ml, respectively) ($p < 0.01$) (Fig. 5, *A* and *B*).

For each patient, the production of IgE and IgG4 induced by IL-4 plus allergen was higher than those induced by IL-4 alone (Fig. 5, *A* and *B*). In both these conditions of stimulation, IL-10 decreased IgE (decrease of 85 ± 16% and 80 ± 15%, respectively, mean ± SD, $n = 5$) but enhanced IgG4 production (increase of 951 ± 201% and 742 ± 105%, respectively) (Fig. 5, *A* and *B*). In contrast, IL-10 enhanced both IgE and IgG4 production induced by IL-4 plus anti-CD40 mAb (increase of 789 ± 120% and 620 ± 93%, respectively, $n = 5$) (Fig. 5*C*) or by IL-4 plus rCD40L (data not shown). Similar effects of IL-10 were observed using cells from patients sensitive to grass pollen (data not shown). In parallel,
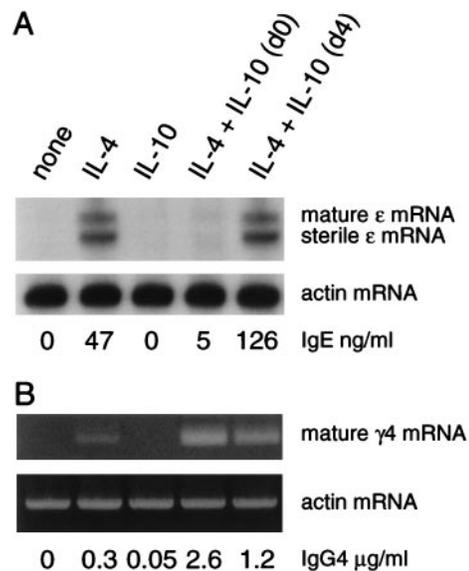

FIGURE 2. Effect of IL-10 on ε and γ4 transcript expression by IL-4-stimulated PBMC. PBMC were either unstimulated or stimulated with IL-4, IL-10, or IL-4 plus IL-10; in this last condition, IL-10 was added together with IL-4 (day 0) or 4 days later. RNA were collected at day 10. *A*, The expression of germline and productive ε transcripts (*top*) and of β-actin (*bottom*), used as control, was evaluated by Northern blotting. *B*, The expression of productive γ4 and β-actin transcripts was evaluated by reverse transcriptase-PCR. IgE (*A*) and IgG4 (*B*) were quantified in the 10-day supernatants.
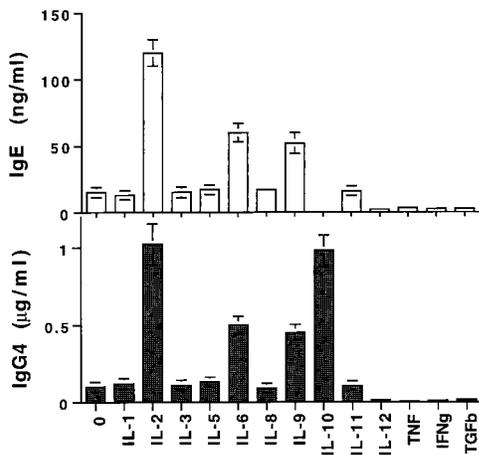
FIGURE 3. Effect of different cytokines on IgE and IgG4 production by IL-4-stimulated PBMC. PBMC were stimulated with IL-4 in the absence or presence of 50 ng/ml of different cytokines. IgE (*top*) and IgG4 (*bottom*) were quantified in 12-day supernatants. Results are expressed in ng/ml or μg/ml (mean ± SD of quintuplicate values) and are representative of one out three separate experiments.

and whatever the conditions of stimulation, IL-10 also enhanced IgA1-2 and IgG1-3 production (data not shown).

These data indicate that in the absence of CD40 ligation, IL-10 decreases IgE and increases IgG4 production by PBMC from allergic patients specifically stimulated in the presence of IL-4.

Time-dependent effect of IL-10 on IL-4-induced IgE synthesis by PBMC

To investigate the mechanism underlying the dual effect of IL-10 on IgE synthesis, IL-10 was added at different time points to IL-4-stimulated PBMC. When added during the first 2 days following IL-4 stimulation, IL-10 prevents IgE production with a maximal effect when added at day 0 (decrease of 82 ± 12% and 35 ± 8% at days 0 and 2, respectively, $n = 4$) (Fig. 6*A*). When added later, IL-10 potentiates IgE production with a maximum occurring at days 4 or 6, according to the subject (maximal increase of 302 ± 36%) (Fig. 6*A*). Furthermore, IL-4-induced ε transcript expression was decreased when IL-10 was added at day 0, whereas it was weakly increased when added at day 4 (Fig. 2*A*). Thus, according

to the time of addition, IL-10 presents an opposite effect on IgE production by IL-4-stimulated PBMC. In contrast, whatever the time of addition, IL-10 increased IgG4 production with a maximal effect when added at day 0 (increase of 895 ± 136% and 372 ± 57% when added at days 0 and 4, respectively) (Fig. 6*B*). In parallel, IL-4-induced productive γ4 transcript expression was enhanced to a higher extent when IL-10 was added at day 0 rather than at day 4 (Fig. 2*B*).

Therefore, the inhibitory effect of IL-10 on IgE synthesis by IL-4-stimulated PBMC occurs only during the first 2 days after stimulation.

IL-10 is not a switch factor for IgG4

On the basis of the above results, it was tempting to speculate that IL-10 may differentially affect IgE and IgG4 switching. As such, we have first evaluated the effect of IL-10 on IgE and IgG4 production by purified tonsillar B cells, in the absence of signals provided by T cells (Table I). B cells either unstimulated or stimulated with IL-4 or anti-CD40 mAb did not produce IgE or IgG4. In these conditions of stimulation, IL-10 induced a weak production of IgG4 (<200 ng/ml) but no IgE. However, IL-10 potentiated IL-4 plus anti-CD40 mAb-induced IgE and IgG4 production (increase of 703 ± 105% and 948 ± 203%, respectively, mean ± SD, $n = 4$). In parallel, in agreement with previous data, IL-10 enhanced anti-CD40 mAb and anti-CD40 mAb plus IL-4-induced B cell proliferation (SI = 2.2 ± 0.4 and 4.5 ± 0.6, respectively, mean ± SD, $n = 4$) (Table I) (31–33). Thus, we have determined the effect of IL-10 on IgE and IgG4 production by naive surface IgD$^+$ B cells. IL-10 did not induce IgE or IgG4 production by non-isotype-committed B cells that were either unstimulated, or stimulated with IL-4, anti-CD40 mAb (Table I), or rCD40L alone (data not shown). However, IL-10 potentiated IgE and IgG4 production induced by IL-4 plus anti-CD40 mAb (Table I).

These results suggest that IL-10 is not a switch factor for IgE and IgG4 since it is unable to replace IL-4 or CD40 triggering in inducing IgE and IgG4 synthesis.

Discussion

The molecules involved in the induction (IL-4, IL-13, and CD40L) and regulation (see Fig. 3) of IgE production also control IgG4 synthesis (5, 12–14, 21). We report here that IL-10 inhibits IgE and increases IgG4 synthesis by IL-4-stimulated PBMC. Thus, IL-10
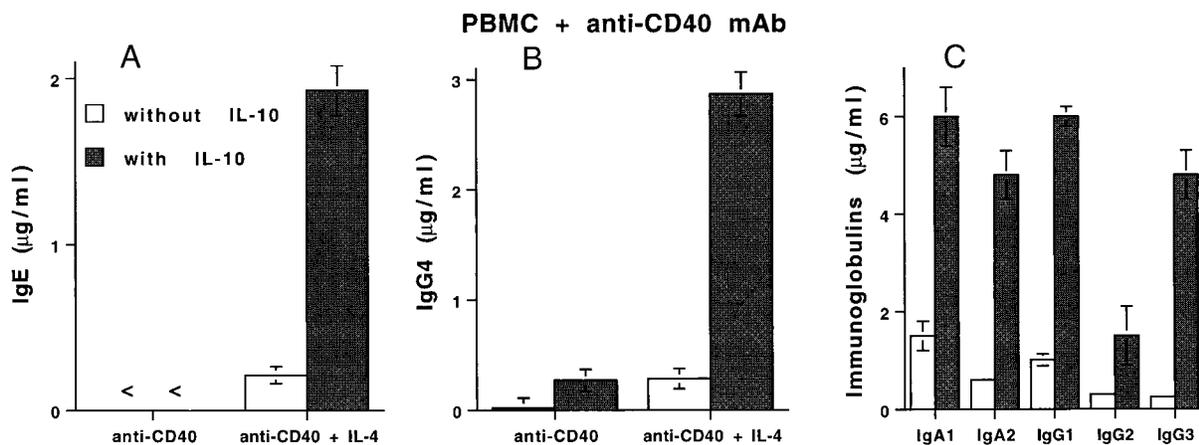

FIGURE 4. Effect of IL-10 on IgE and IgG4 production by IL-4 plus anti-CD40 mAb-stimulated PBMC. PBMC from healthy subjects were stimulated with an anti-CD40 mAb without or with IL-4 in the absence (□) or presence (■) of 50 ng/ml of IL-10. IgE (*A*), IgG4 (*B*), IgA1, IgA2, and IgG1-3 (*C*) were quantified in 12-day supernatants. Results are expressed in μg/ml (mean ± SD of quintuplicate values) and are representative of one of six (*A* and *B*) or three (*C*) experiments.

FIGURE 5. Effect of IL-10 on IgE and IgG4 production by allergen-stimulated PBMC from a *D. pteronyssinus*-sensitive patient. PBMC from a *D. pteronyssinus*-sensitive patient either unstimulated (*A*), stimulated with *D. pteronyssinus* (*B*), or with an anti-CD40 mAb (*C*) were or were not incubated with IL-4 in the absence (□) or presence (■) of 50 ng/ml of IL-10. IgE and IgG4 were quantified in 12-day supernatants. Results are expressed in μg/ml (mean ± SD of quintuplicate values) and are representative of one of five experiments.
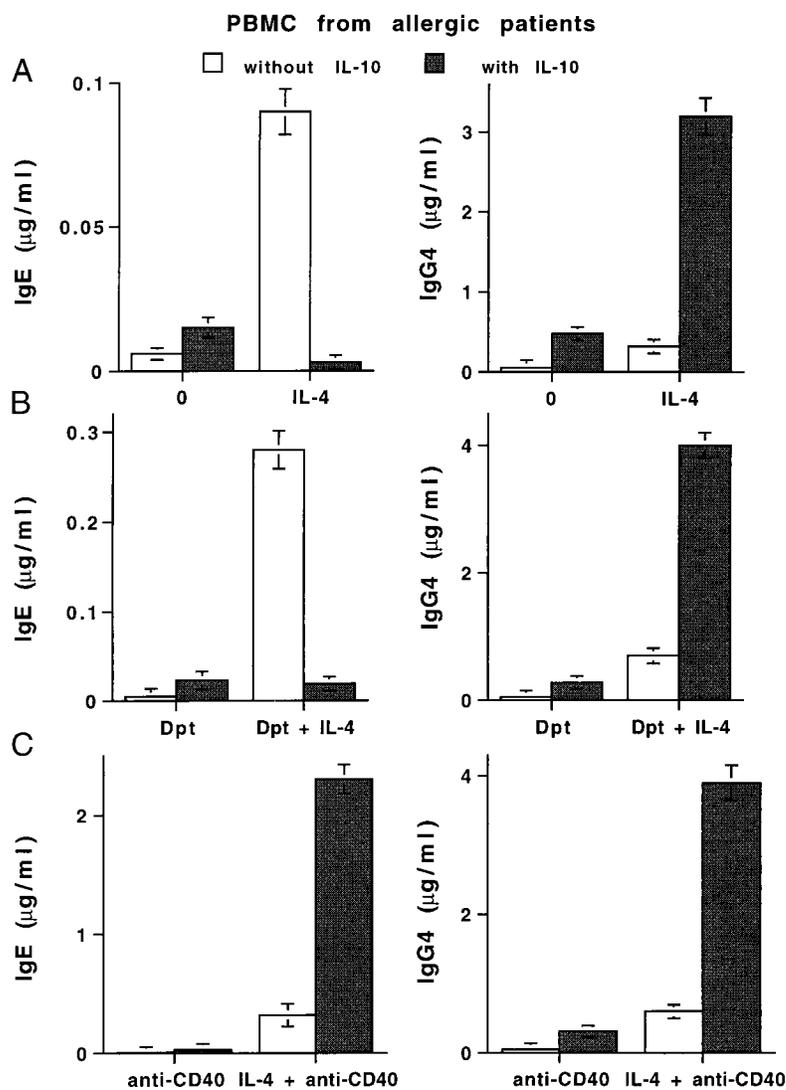
is, to our knowledge, the only molecule that differentially affects IgE vs IgG4 production, thereby suggesting the existence of a pathway(s) selectively involved in the control of their production.

In agreement with others (36), we have observed that IL-10 decreases ε transcript expression and IgE production by IL-4- or IL-13-stimulated PBMC. This effect was extended to PBMC from allergic patients specifically stimulated with the sensitizing allergen. More precisely, we found that IL-10 inhibited ε transcript expression and IgE synthesis only when added during the first 2 days after addition of IL-4, at the time when the switch process takes place. These results suggest that IL-10 inhibits IL-4-induced IgE switching. Previous data showing that IL-10 decreases the frequency of IgE-secreting cells in IL-4-stimulated PBMC from allergic patients reinforces this observation (39).

On the contrary, IL-10 potentiated γ4 transcript expression and IgG4 production by IL-4-stimulated PBMC. This effect could result from a positive effect of IL-10 on IgG4 switching and/or on the growth and differentiation of IgG4-secreting B cells. IL-10 is a switch factor for IgA, IgG1, and IgG3 (30–32). Moreover, we have observed that IL-10 enhanced IgG4 production by PBMC even in the absence of IL-4. In view of these effects, it was tempting to speculate that IL-10 may induce IgG4 switching. However, in agreement with others (31), we found that IL-10 was unable to replace IL-4 or CD40 triggering in inducing IgG4 switching in

noncommitted IgD+ B cells, thereby confirming that IL-10 is not a switch factor for IgG4 (31). Nevertheless, IL-10 has been shown to increase the growth of CD40-activated B cells and to be a potent plasma cell differentiation factor (33–35). In accordance with these properties, we have observed that IL-10 acted on PBMC by enhancing the production of 1) IgE by B cells that were already IgE switched, 2) IgG4 in the absence of IL-4, and 3) IgA and IgG1-3 in the absence (data not shown) or presence of IL-4. In addition, we report that IL-10 up-regulates γ4 transcript expression with a maximal effect when added together with IL-4. Thus, IL-10 may increase IgG4 synthesis both by enhancing the growth and/or differentiation of the IL-4-induced IgG4-producing cells and by potentiating IL-4-induced IgG4 switching.

In summary, when added together with IL-4, IL-10 decreases IgE synthesis by IL-4-stimulated PBMC. This decrease seems to result from a dual effect: an inhibition of IgE switching and a potentiation of the proliferation and/or differentiation of IgE-secreting cells. In parallel, IL-10 potentiates IL-4-induced IgG4 by PBMC. As IL-10 is not a switch factor for IgG4, this effect seems to result from the combined effects of the two lymphokines: 1) IL-4 induces IgG4-switching and 2) IL-10 potentiates IL-4-induced IgG4 switching and/or favors the growth and differentiation of IgG4-producing cells.
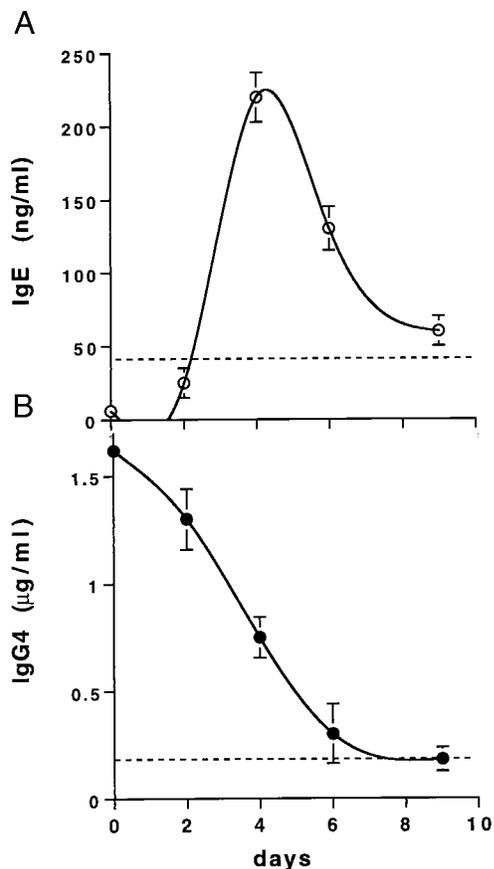
FIGURE 6. Kinetics for the addition of IL-10 on IL-4-stimulated PBMC. PBMC from a healthy subject were stimulated at day 0 with IL-4, and IL-10 (50 ng/ml) was added at different time points. IgE (*A*) and IgG4 (*B*) were quantified in day-12 supernatants. The levels of IgE and IgG4 obtained in the absence of IL-10 are represented as horizontal hatched bars. Results are expressed in ng/ml or μg/ml (mean $\pm$ SD of quintuplicate values) and are representative of one of four experiments.

It has been suggested that IL-10 inhibited IgE synthesis of PBMC by acting on monocytes (36). However, we have observed that IL-10-decreased IgE production required the presence of T cells and could occur in the absence of monocytes (data not shown). This result suggests that IL-10 interferes with signal(s) provided by T cells and involved in the control of IgE synthesis. It is unlikely that IL-10 decreases IgE synthesis by modulating IL-4 production, since the experiments were performed with optimal concentrations of IL-4. Interestingly, CD40 triggering bypasses the down-regulating effect of IL-10 on IgE production, suggesting that IL-10 may prevent IgE production by decreasing CD40L expression on T cells. However, CD40-CD40L interaction also participates to the regulation of IgG4 production (5) and different concentrations of anti-CD40 mAb or rCD40L modulate in parallel, and in a dose-dependent manner, IgE and IgG4 production (our unpublished observations). Moreover, we found that IL-10 does not affect CD40L expression by IL-4-stimulated PBMC (data not shown). As such, a modulation of this pathway cannot explain the differential effect of IL-10. Thus, these data suggest the existence of a T cell-derived signal(s), different from CD40L and selectively involved in the control of IgE synthesis. In line with this hypothesis, it has been reported that T cells from hyper-IgM patients, which express a nonfunctional CD40L, were still able to provide help for IgE synthesis (40). IL-10 may also interfere with costimulatory signals involved in IgE synthesis. IL-10 has been shown to decrease IL-4-induced CD23 expression on monocytes and IL-6 production by T cells and monocytes (41, 42). Nevertheless, a potential modulation of CD23 expression and IL-6 production is not sufficient to explain the selective effect of IL-10 on IgE vs IgG4 synthesis. Indeed, IgG4 production also requires CD23-CD21 interaction (21) and is also potentiated by IL-6 (Table I). Thus, until now, the mechanism(s) responsible for the selective effect of IL-10 on IgE vs IgG4 production by IL-4-stimulated PBMC remained undetermined. Finally, an important finding is that CD40 triggering bypasses the inhibitory activity of IL-10. This observation underlines that using anti-CD40 mAb or rCD40L in vitro may bypass signals provided by different cell types and involved in the tight regulation of IgE synthesis.

While IgE and IgG4 synthesis in vitro require identical signals, in vivo observations suggested that these isotypes can be produced independently. Allergic patients have IgG4 Ab directed against allergens to which they are not sensitive (i.e., in the absence of a specific IgE Ab) (26). Moreover, in response to an immunization with keyhole limpet hemocyanin, atopics develop a specific IgG4 response but no IgE (23). Both of these observations suggest that IgG4 Ab, in contrast to IgE, are not sensitizing Ab. Moreover, the development of a potent and specific IgG4 Ab response during hymenoptera venom desensitization has been associated with a positive outcome (25, 27, 28). Consequently, in IgE-mediated diseases such as allergic disorders, it could be of clinical interest to prevent IgE production (43) without affecting IgG4 response. Due to its ability to inhibit IgE and to potentiate IgG4 production by allergen-stimulated PBMC from allergic individuals, IL-10 would appear to be a potential candidate. However, different data suggest

Table I. *Effect of IL-10 on IgE and IgG4 production by purified tonsillar B cells*[a]

| Stimuli | IL-10 | Unseparated B Cells | | | IgD+ B Cells | |
		IgE (ng/ml)	IgG4 (ng/ml)	Proliferation (cpm $\times 10^{-3}$)	IgE (ng/ml)	IgG4 (ng/ml)
None	−	<	<	1.5 ± 0.2	<	<
	+	<	58 ± 6	1.4 ± 0.3	<	<
IL-4	−	<	<	1.6 ± 0.2	<	<
	+	<	110 ± 10	1.5 ± 0.3	<	<
Anti-CD40	−	<	<	4.2 ± 0.6	<	<
	+	<	418 ± 95	9.5 ± 0.8	<	<
IL-4 + Anti-CD40	−	98 ± 18	331 ± 59	8.1 ± 0.9	20 ± 5	50 ± 11
	+	648 ± 150	1579 ± 150	36.5 ± 6	120 ± 20	600 ± 120

[a] Unseparated and naive IgD+ tonsillar B cells were either unstimulated or stimulated with IL-4, anti-CD40 mAb, or IL-4 plus anti-CD40 mAb in the absence or presence of 50 ng/ml IL-10. IgE and IgG4 were quantified in day 12 supernatants. The proliferation of unseparated B cells was measured at day 3. Results are expressed in cpm $\times 10^{-3}$. Data are means $\pm$ SD of quintuplicate values and are representative of one of four experiments.

the contrary: 1) IgE-producing B cells already exist in allergic individuals and IL-10 may increase their proliferation and/or differentiation and consequently favor IgE synthesis, and 2) in response to an allergenic stimulation, IL-10 could be produced later than IL-4 and then may favor the differentiation of IgE-producing B cells generated by IL-4 (44).

Altogether, our results show that, in addition to common pathways such as signaling through IL-4 or IL-13 receptors and CD40, IgE and IgG4 switching can be induced through different mechanisms, as evidenced by the ability of IL-10 to inhibit IgE and potentiate IgG4 synthesis by IL-4-stimulated PBMC.

Acknowledgments

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