

Effects of interleukin-4 and interferon- γ on the secretion of IgG4 from human peripheral blood mononuclear cells

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Whereas IgE antibodies are linked with allergy, IgG4 antibodies may reflect the state of immunity and protection against a particular antigen. It has been shown that interleukin (IL)-4 is required for induction of IgE synthesis. In order to elucidate the role of IL-4 in the production of IgG4 and to compare IgG4 and IgE regulatory processes, we quantified these immunoglobulin isotypes after *in vitro* culture of peripheral blood mononuclear cells (PBMC) in the presence of IL-4. The production of IgG4 was increased by IL-4 under the same conditions which are optimal for IgE production but not among PBMC from all donors, depending on the magnitude of spontaneous IgG4 secretion: IL-4 was effective only when the spontaneous secretion of IgG4 was <7% of the total IgG secretion; it had no effect when spontaneous IgG4 production was >7% of total IgG. The IL-4-induced IgE response was consistently obtained when IgG4 was <7% of total IgG but was markedly diminished or absent when IgG4 was >7% of total IgG. If *Staphylococcus aureus* strain Cowan 1 (SAC) was present during the 48-h preincubation step, spontaneous IgG4 production was increased, but the stimulatory effect of this mitogen on immunoglobulin production, including IgG4, was markedly blocked by the addition of IL-4. In contrast, IL-4-induced IgE synthesis was strongly blocked by the presence of SAC. Finally, secretion of IgG4 (spontaneous and IL-4-induced) was suppressed among cells from most donors by interferon- γ (IFN- γ). These results suggest that IL-4 has opposite effects on *in vitro* IgG4 production and that the *in vitro* synthesis of both IgG4 and IgE appears to be regulated similarly by IL-4 and IFN- γ , whereas additional signals promote the production of one isotype in preference to the other. It is possible that activated B cells respond to IL-4 less well than do nonactivated cells.

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Allergen-specific IgG4 antibodies have been found in the serum of allergic and nonallergic patients. In allergic diseases, IgG4 has been believed to be either an anaphylactic antibody or a blocking antibody, but its exact role is still under scrutiny. However, it appears that, in some instances, IgG4 and IgE are cosynthesized, suggesting a common regulatory mechanism (1, 19, 20, 33).

The secretion of human IgE *in vitro* is modulated by a number of cytokines (8, 9, 24, 25, 27, 30). Interleukin (IL)-4 appears to be a crucial factor for promoting the induction or enhancement of IgE secretion by mononuclear cells (MNC) (9, 18, 24, 26, 27, 34). This promoting effect of IL-4 on IgE synthesis is suppressed by interferon- γ (IFN- γ) (23, 24, 27).

These two cytokines regulate the IgE synthesis by inhibiting the activity of each other (5, 22, 31). In mice, it has been demonstrated that in addition to IgE, IL-4 increased and IFN- γ suppressed the production of the IgG1 subclass (28, 32). Interestingly, it was suggested that murine IgG1 is immunologically similar to human IgG4 (21), although there are no similarities at the genetic and biochemical levels (3). The roles of IL-4 and IFN- γ in the regulation of IgG4 synthesis are still unclear. Several recent reports have showed an increased IgG4 synthesis in the presence of IL-4 (12, 13, 15, 21), while another has failed to find it (11). One of these reports (15) also presented evidence that IFN- γ inhibits IL-4-induced of IgG4 synthesis.

In order to clarify the effects of IL-4 and IFN- γ on IgG4 synthesis, we studied the synthesis of IgE and IgG4 in the same experimental conditions that are optimal for IgE induction by normal PBMC. We also investigated whether particular experimental conditions can promote the synthesis of one isotype at the expense of the other as well as the heterogeneity of the synthesis of both immunoglobulin isotypes according to donors.

Material and methods

Subjects

Blood samples or leukopheresis preparations from healthy donors were obtained from Dr B. Villiger (Thurgauisch-Schaffahausische Höhenklinik, Davos, Switzerland) and Dr M. Fopp (Blutspendezentrum, Kantonsspital, Saint-Gallen, Switzerland), respectively.

Fourteen subjects were included in this study. None of the subjects had any family history of allergy or any clinical symptoms of allergy or atopy.

Separation and culturing of PBMC

Peripheral blood mononuclear cells (PBMC) were isolated as previously described (5). In brief, anticoagulated blood was diluted with phosphate-buffered saline (PBS) and layered over Ficoll (Seromed, Berlin, Germany), and the PBMC were collected following centrifugation. The PBMC were resuspended in Yssel's culture medium (35) in the absence of saturated and unsaturated fatty acids, supplemented with 5% fetal calf serum (FCS), and incubated at 37°C in a humidified atmosphere containing 5% CO₂.

In order to determine the optimal concentration of IL-4 to be used as well as the optimal time of incubation, we initially preincubated PBMC at 5×10^6 cells/ml in culture flasks (Costar, Cambridge, MA, USA) for 48 h in 200 U/ml of purified recombinant IL-4 (10^7 U/ml, donated by Dr J. E. De Vries, DNAX Research Institute, Palo Alto, CA, USA). After the preincubation step, nonadherent cells were collected, washed three times with PBS containing 1% FCS, resuspended in culture medium, and plated at 1×10^6 cells/ml in 24-well cluster plates (Costar), 1 ml/well, with or without various amounts of IL-4 (0, 0.01, 0.1, 1.0, 10, 100, or 500 U/ml) added in triplicate. The day of washing and reculturing the cells was designated day 0. Culture supernatants (CSN) of each treatment were harvested at 2-day intervals, and were assayed for IgG, IgG4, IgA, and IgM content by ELISA specific for each isotype (see below).

For immunoglobulin (Ig) production, PBMC were similarly preincubated at 5×10^6 cells/ml in the pres-

ence of 200 U/ml of IL-4; in certain experiments, PBMC were preincubated in the presence of 0.01% *Staphylococcus aureus*, strain Cowan 1 (SAC) (Pansorbin Cells, CalBiochem, La Jolla, CA, USA) for 48 h. After washing, the nonadherent cells were resuspended in culture medium and cultured with or without additional IL-4 or recombinant IFN- γ (0.7×10^6 IU/ml, donated by Dr C. Heusser, Ciba Geigy, Basel, Switzerland). After an additional 10 days of culture, CSN were harvested, and levels of Ig were determined by specific ELISA (see below).

Measurement of secreted immunoglobulins

All ELISA were performed with 96-well, flat-bottomed microtiter plates (Nunc-Immuno Plate Maxisorp, Roskilde, Denmark). Unless otherwise stated, ELISA plates were washed three times between each step with PBS pH 7.4, supplemented with 0.02% Tween 20 and 0.02% sodium azide; dilutions of samples and standards were made in PBS pH 7.4, supplemented with 0.05% Tween 20, 0.2% sodium azide, and 1% casein hydrolysate (Oxoid, Hampshire, UK).

For IgG4 determination, wells were coated with 100 μ l of 0.5 μ g/ml affinity-purified goat antimouse IgG (Cappel, West Chester, PA, USA) in sodium carbonate buffer pH 9.6 for 18 h at 4°C. After washing, antihuman IgG4 monoclonal antibody (mAb) MCA 271 (clone HP 6025, as described in a World Health Organization (WHO) document, Serotec, Oxford, UK) was added to the plates at a concentration of 0.25 μ g/ml and incubated for 6 h at room temperature. The mAb MCA 271 had been previously purified by Sepharose-protein G chromatography (Pharmacia, Uppsala, Sweden). Next, CSN were appropriately diluted and added to the wells in duplicate. Dilutions of standard serum were incorporated in parallel to the wells as a reference. Initially, this assay was calibrated by the WHO reference serum 67/97, which has an IgG4 concentration of 0.5 mg/ml. Then, a pool of sera from healthy donors randomly chosen in Davos was determined in this assay to have an IgG4 concentration of 0.4 mg/ml; dilutions of this pooled serum gave a parallel curve to the WHO reference serum. Thus, this pooled serum was subsequently used as the standard reference for measurement of IgG4 content in the CSN. After 18 h at room temperature, a mixture of alkaline phosphatase-labeled goat antihuman κ -L chain (1:3000, Tago, Burlingame, CA, USA) and goat antihuman λ -L chain (1:4000, Tago) was added to detect bound IgG4. After 2 h at room temperature, the plates were washed four times with 0.9% NaCl + 0.02% Tween 20, and IgG4 binding was visualized by incubation with 1.5 mg/ml 4-nitrophenylphosphate disodium salt, hexahydrate (Sigma)

in 1 M diethanolamine-HCl buffer, pH 9.8, for 30 min at 37°C. Absorbance was measured by a VMAX kinetic microplate reader (Molecular Devices, Menlo Park, CA, USA) at 405 nm. The limit of sensitivity of the assay was 0.3 ng/ml. We used IgG1, IgG2, and IgG3 myeloma sera in order to meet the specificity of the assay. It was found that a marginal cross-reaction could be observed with IgG1 and IgG2 subclasses, but more than 300 ng/ml of these isotypes was required to produce an OD similar to that obtained by 1 ng/ml of IgG4 proteins. There was also a slight cross-reaction with IgG3 since 40 ng/ml of IgG3 gave an OD similar to 1 ng/ml of IgG4. However, since IgG3 and IgG4 are produced in similar amounts in cell culture supernatants from normal persons (2, 10), it is unlikely that IgG3 would cause a significant error in IgG4 quantification.

IgA and IgM ELISA was modified from previously described assays (24). Goat anti-IgA (1:10000; Pasteur Institute, Paris, France) or rabbit anti-IgM (1:1000; Behring, Marburg, Germany) was used as coating antibody. Appropriate dilutions of the CSN and standard sera (Behring) were incubated for 18 h at room temperature. Later steps were as described above for IgG4. The sensitivities of the assays were 2 ng/ml and 4 ng/ml for IgA and IgM, respectively.

For IgG determination, the plates were coated for 18 h at room temperature with 1 µg/ml goat antihuman IgG (Tago). Free protein binding sites were blocked with 1% casein hydrolysate in PBS pH 7.4 containing 5% Tween 20. Samples and standards (purified standard human IgG, CalBiochem) were diluted in the blocking buffer and incubated for 18 h at room temperature. Bound IgG was detected with goat antihuman IgG antibodies conjugated to alkaline phosphatase (1:4000; Tago) and incubated for 1 h at 37°C. ELISA development and reading were as described in the IgG4 ELISA. The sensitivity of the assay was 0.6 ng/ml.

For IgE determination (5), ELISA plates were coated with rabbit anti-IgE (Dakopatts, Glostrup, Denmark) diluted 1:2000 in pH 9.6 sodium carbonate buffer. The plates were incubated 18 h at 4°C and then washed with PBS containing 0.05% Tween 20. This wash was used between all steps of the assay. Next, the wells were blocked with 2% BSA in PBS containing 0.05% Tween 20 for 1 h at room temperature. Dilutions of CSN and standard IgE serum (Behring) were added in parallel in PBS containing 0.05% Tween 20 and 0.2% BSA. After 18 h at room temperature, the murine antihuman IgE mAb I-27 (4) (donated by Dr J. E. De Vries, DNAX Research Institute) was added and incubated for 6 h at room temperature. After washing, the plates were incubated for 4 h at room temperature with peroxidase conjugated goat antimouse Ig (1:2500; Tago). Finally, 1 mg/ml of [2,2'-azinobis(3-ethylbenz-

thiazoline-6-sulfonic-acid)] (Sigma), in 0.1 M sodium citrate-0.1 M sodium phosphate buffer, pH 4.5, containing 0.003% H₂O₂, was added as enzyme substrate. The OD was measured at 405 nm after 30 min at room temperature. The limit of sensitivity of the assay was 0.15 ng/ml.

Design of the study

Several experiments were performed. First, we analyzed the spontaneous release of IgG4 and IgE. Then we studied their production in order to investigate the role of IL-4 on IgG4 production and compare its effects on the IgG4 and IgE productions. The optimal dose and the optimal time of incubation of IL-4 were studied. A third experiment series investigated the inhibitory effect of IFN-γ. Finally, we tested the regulation of IgG4 production by using a polyclonal B-cell activator (SAC) which increases the production of IgG in the presence or absence of IL-4 to determine whether this cytokine modulates IgG4 production.

Statistical analysis was performed by nonparametric tests. The Wilcoxon two-sample test for paired, nonparametric data was used to analyze the results. The results were considered to be significantly different when the calculated significance was less than or equal to 0.05.

Results

Spontaneous IgE and IgG4 production

There was no spontaneous secretion of IgE. The spontaneous production of IgG4 was variable among the donors, ranging from ≤0.3 to 32.3 ng/ml (mean ± SD: 9.4 ± 10.1 ng/ml). IgG4 represented 0.5–22.1% of total IgG production (mean ± SD: 6.9 ± 6.3%).

Enhancement of the spontaneous IgE and IgG4 production by IL-4

The optimal dose of IL-4 which enhanced immunoglobulin production by PBMC ranged between 100 and 500 U/ml for both IgG4 and IgE (not shown). No further increase in IgE and IgG4 levels was observed for higher amounts of IL-4 added. Thus, all results reported below refer to the optimal dose of 100 U/ml. The optimal incubation time is presented in Fig. 1. It is shown that the production of both IgE and IgG4 required 10–12 days. Thus, all results reported below refer to the optimal incubation time of 10 days.

IL-4 added at day 0 induced the IgE production in 11/14 experiments (range: ≤0.15 ng/ml to 18.8 ng/ml; $P < 0.05$, Wilcoxon W test). IL-4 added at day

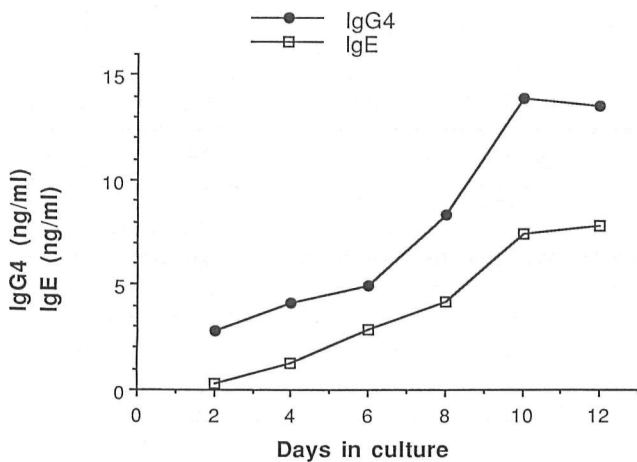


Fig. 1. Kinetics of IL-4-induced IgG4 and IgE synthesis. PBMC were preincubated in 200 U/ml IL-4 for 48 h, harvested, washed, and reincubated in presence of 100 U/ml IL-4 added in triplicate at day 0; CSN were harvested at 2-day intervals, and levels of accumulated IgG4 (●) and IgE (□) determined. Results are reported as mean $\pm$ SD for triplicate determinations.

0 increased the IgG4 production, but this effect was dependent upon the level of its spontaneous release expressed as the percentage of IgG secreted as IgG4

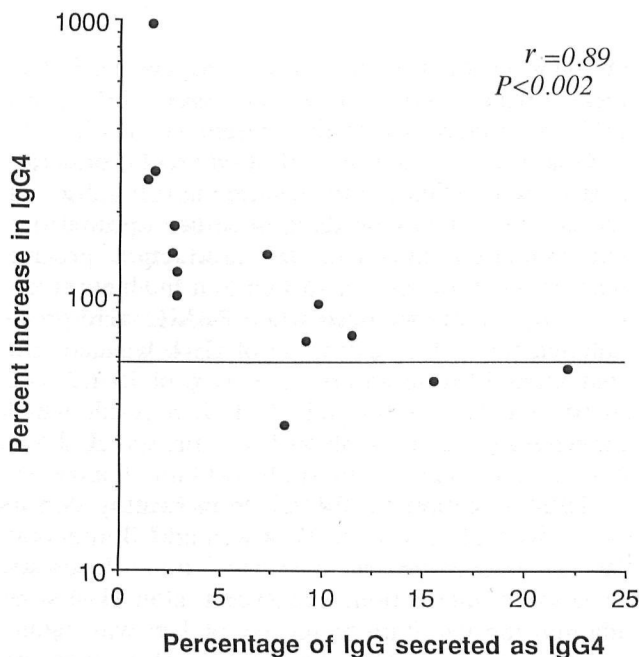


Fig. 2. Effect of IL-4 on synthesis of IgG4. The 14 experiments used PBMC from 14 different normal donors. Spontaneous IgG4 and IgG production was estimated in a culture assay in which PBMC were preincubated for 48 h in medium, washed, and recultured in fresh medium for an additional 10 days. Percentage of total IgG secreted as IgG4 was calculated. For testing effect of IL-4, PBMC were preincubated in 200 U/ml IL-4, washed, and recultured in 100 U/ml IL-4 for 10 days. Data were expressed as means of triplicate cultures for each treatment and percent increase in IgG4 production was calculated.

Table 1. Effect of IFN- γ on IL-4-induced enhancement of IgG4

Experiment	IgG4 (ng/ml)		
	Medium*	IL-4 [†]	IL-4+IFN- γ **
1	2.3 $\pm$ 0.5	4.1 $\pm$ 0.6	1.5 $\pm$ 0.3
2	1.4 $\pm$ 0.3	3.4 $\pm$ 1.2	1.8 $\pm$ 0.3
3	0.4 $\pm$ 0.1	13.6 $\pm$ 4.1	4.0 $\pm$ 1.5

Results are presented as the mean $\pm$ SD of triplicate cultures; each experiment employed PBMC from different donors.

* PBMC were preincubated 48 h in medium, washed, and recultured in medium. After 10 days, CSN were collected and IgG4 quantified.

[†] Cells were preincubated in 200 U/ml IL-4 and cultured in 100 U/ml IL-4.

** Cells were preincubated in 200 U/ml IL-4 and recultured in 100 U/ml IL-4 plus 100 U/ml IFN- γ .

(Fig. 2). IL-4 had little effect on PBMC from subjects with a high IgG4 spontaneous release. The three donors in whom IL-4 did not induce any IgE synthesis had a high IgG4 spontaneous release.

Suppression of IgE and IgG4 production by IFN- γ

IFN- γ decreased the spontaneous IgG4 production in 8/11 experiments. In two of the three experiments shown in Table 1 (donors 1 and 2), IL-4 failed to enhance IgG4 synthesis above spontaneous levels when 100 U/ml IFN- γ was added simultaneously to the cultures. In the other (donor 3), the level of IgG4 was much lower in the presence of both IFN- γ and IL-4 than in the presence of IL-4 alone. IFN- γ suppressed IL-4-enhanced IgG4 secretion by an average of 64% $\pm$ 17% above the level of secretion in response to IL-4 alone.

Inhibition of SAC-induced enhancement of Ig secretion by IL-4

PBMC were preincubated with SAC for 48 h in the presence or absence of 100 U/ml IL-4. The results of three representative experiments are shown in Table 2. Preincubation with SAC increased the secretion of both total IgG and IgG4 from 10-fold to over 100-fold, depending on the donor. Addition of 200 U/ml IL-4 with SAC during the preincubation inhibited the SAC-induced increase in both IgG and IgG4 secretion by at least 80%. However, when 100 U/ml of IL-4 was added at day 0, the level of IgG and IgG4 secretion increased, and IgG4 synthesis returned to nearly that induced by SAC alone in one of the three experiments. IgA and IgM levels were also increased by stimulation with SAC, and this SAC-induced stimulation was also inhibited in the presence of 200 U/ml of IL-4 (not shown).

Conversely, IgE synthesis was not increased by the addition of SAC (Table 3). When SAC and IL-4 were added together in the preincubation and 100 U/

Table 2. Effect of SAC and SAC+IL-4 on the production of IgG and IgG4

Exp.	IgG (ng/ml)				IgG4 (ng/ml)			
	Med.	SAC	SAC+IL-4	Sac*+IL-4	Med.	SAC	SAC+IL-4	SAC*+IL-4
1	47±5	359±52	47±14	102±14	0.4±0.1	11±1	1.1±0.4	10±2
2	89±3	9276±364	769±92	984±79	1.7±0.4	81±7	18±2	41±7
3	71±14	905±92	187±36	218±41	1.9±0.8	52±7	8±2	10±3

Cultures of PBMC were preincubated in medium, 0.01% (v/v) SAC, or SAC+200 U/ml IL-4 for 48 h. After preincubation (day 0), cells were washed and recultured in fresh medium. No additional SAC was added at this time.

* These cultures received an additional 100 U/ml IL-4 on day 0.

Table 3. Effect of preincubation in SAC, IL-4, or SAC+IL-4 on production of IgE

Exp.	IL-4 added at day 0 (100 U/ml)	IgE (ng/ml)			
		Preincubation			
		Medium	Medium+IL-4	SAC	SAC+IL-4
1	-	≤0.15	≤0.15	0.4±0.1	≤0.15
	+	1.2±0.5	15.8±2.5	4.4±1.0	0.3±2.8
2	-	≤0.15	0.4±0.2	≤0.15	≤0.15
	+	1.3±0.4	7.6±1.8	1.6±0.2	≤0.15
3	-	≤0.15	≤0.15	≤0.15	≤0.15
	+	4.8±1.2	13.2±2.6	0.9±0.3	2.5±0.7

PBMC were preincubated for 48 h in medium, 200 U/ml IL-4, 0.01% SAC, or 0.01% SAC+200 U/ml IL-4. After washing, the cells were recultured in fresh medium with or without 100 U/ml IL-4. Results are presented as the mean ± SD of triplicate cultures for each treatment. Each experiment was performed with PBMC from different donors.

ml of IL-4 was added at day 0, IgE secretion was suppressed by at least 80%, as compared with the control performed in the absence of SAC.

Discussion

The study presented here shows that the production of IgE and IgG4 is regulated by the same cytokines, for similar doses and incubation times. IL-4 enhances the production of both immunoglobulins, whereas IFN- γ blocks it. Although IL-4 induces a similar effect on the production of both immunoglobulin isotypes, differences between their production were observed when a polyclonal B-cell activator was used in combination with IL-4.

The role of IL-4 in the regulation of IgG4 synthesis is not well understood. Nüsslein & Spiegelberg (21) and Ishizaka et al. (15) have demonstrated an increase in IgG4 secretion, but no increase of IgG1, IgG2, or IgG3, from PBMC in response to IL-4. Lundgren et al. (18) have shown evidence that IL-4 promotes isotype switching to both IgE and IgG4 among human splenic B cells. Gascan et al. have shown that IL-4, when added together with an extra signal provided by T-cell clone supernatants or an anti-CD40 mAb, can induce IgG4 switching in purified B cells (12, 13). In contrast, Flores-Romo et al. (11) have shown that IL-4 is ineffective at increas-

ing IgG4 production among purified, cycling B cells from tonsils; among these cells, IgG1, IgG2, and IgG3 were increased in the presence of IL-4.

To investigate the role of IL-4 on IgG4 production and compare effects with its action in IgE induction, we analyzed IgG4 production under optimal and suboptimal conditions for IgE production: preliminary experiments demonstrated that the highest levels of IgE were produced when PBMC were preincubated for 48 h in 200 U/ml of IL-4, washed, and reincubated for an additional period of 10–12 days in the presence 100 U/ml of IL-4, a result which agreed with results published by Chrétien et al. (5). We demonstrated in this study 1) that enhancement of IgG4 secretion by PBMC from healthy donors was observed only when IL-4 was added in a similar, two-step culture regimen with similar doses and duration of incubation, and 2) that, after IL-4 stimulation, the variation in release of IgE was significantly correlated with that of IgG4. Thus, these results indicate that IL-4 affects the levels of both IgG4 and IgE in a very similar manner.

We tested various methods for measuring IgG4 concentrations, using several antibodies, either monoclonal or polyclonal. The protocol reported in the present paper gave the best results concerning reproducibility and specificity, as well as the lower limit of sensitivity. Most of the reagents used were

from the same source as those used by Ishizaka et al. (15).

An original finding of the present study is the variable effect of IL-4 on IgG4 production according to its spontaneous level. Enhanced IgG4 secretion was observed only when spontaneous IgG4 secretion was relatively low ($<7\%$ of total IgG secretion). If the spontaneous IgG4 level was over 7% of total IgG, IL-4 was unable to induce a further increase in secretion. Interestingly, in this case, IL-4 was also less effective or unable to stimulate IgE secretion. Chrétien et al. (5) have also reported that increased IgE synthesis by IL-4 was found only in 77% of normal donors. It has been demonstrated that IL-4 alone is not sufficient to induce IgE synthesis by PBMC of normal donors but requires other stimulating agents. Alternatively, or additionally, specific suppressor signals for IgE or IgG4 production, respectively, could contribute to the preference for one of the two isotypes.

Since IFN- γ inhibits IL-4-induced IgE synthesis (5, 24), we investigated the role of IFN- γ on spontaneous and IL-4-enhanced IgG4 secretion. We confirm the observation made by Ishizaka et al. (15) that IFN- γ suppresses the IL-4-induced enhancement of IgG4 secretion. We have extended this latter observation to show that IFN- γ also suppresses spontaneous IgG4 secretion in a manner similar to that of IgE.

There have been controversial reports of the effect of IL-4 on various isotypes according to the various *in vitro* systems used. Jelinek & Lipsky (16) and Splawski et al. (29) have reported that IL-4 strongly suppresses B-cell proliferation and Ig production in response to SAC and IL-2. IL-4 inhibition of IL-2-induced effects on B cells have also been reported by Defrance et al. (7) and Llorent et al. (17). In contrast, Gordon et al. (14) have demonstrated that IL-4 synergistically increases proliferation and IgM production in response to anti-IgM⁺ and anti-CD40 mAb. Thus, the effect of IL-4 on a given population of B cell appears to be different, depending upon the presence of additional signals.

To characterize better the modulation of the production of IgE and IgG4 by IL-4, we studied the effect of this cytokine on the production of both immunoglobulins by PBMC activated by SAC. SAC particles constitute a polyclonal B-cell mitogen. When preactivated *in vitro* with SAC, highly purified B cells differentiate into Ig-secreting cells in response to factors provided by activated T cells (6). We have shown in this report that IL-4 affects the synthesis of IgG4 and IgE differently when added in the presence of SAC, since IL-4 suppresses SAC-induced IgG4 synthesis, whereas IL-4 induced IgE synthesis is suppressed by the addition of SAC, indicating that under certain experimental conditions, IL-4 can sup-

press spontaneous Ig synthesis including IgG4, but not IgE. These results indicate that in the presence of additional signals, IL-4 may selectively promote the synthesis of one isotype (IgE) and block the synthesis of another (IgG4). Nüsslein & Spiegelberg (21) have suggested that IL-4 fails to enhance IgE after SAC stimulation because of SAC-induced production of IFN- γ in the cell cultures. Paradoxically, we have shown that IFN- γ suppresses the production of IgG4, the secretion of which is strongly enhanced by SAC. Additional IL-4 added after the preincubation step in SAC alone was unable to stimulate the production of IgE. This situation following SAC stimulation appears to be analogous to that observed among donor PBMC which had an elevated spontaneous secretion of IgG4. In both groups, IL-4 was unable to stimulate further production of IgG4, and the response of IgE to IL-4 was diminished or absent. If SAC mimicks the binding of antigen to surface Ig, it would suggest that high levels of antigen promote IgG4 production and suppress IgE; this is the strategy employed when allergic patients undergo hyposensitization. The blocking of this effect by IL-4 suggests that agents other than IL-4 are also important in the induction of either a protective state or an allergic reaction. It is also possible that preactivated B cells are less responsive to IL-4 than are nonactivated cells because preactivated B cells are already switched, as described previously for the IgE synthesis (26).

These results demonstrate that the stimulation of PBMC by IL-4 in the presence of SAC affects the synthesis of IgG4 and IgE differently. Since IL-4, in the presence or absence of SAC, can, respectively, downregulate or upregulate the synthesis of IgG4, this suggests that IL-4 can induce the selective synthesis of IgG4 or IgE, depending upon the presence of costimulatory signals.

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