

RECOMBINANT INTERLEUKIN 4 SUPPRESSES THE  
PRODUCTION OF INTERFERON  $\gamma$  BY  
HUMAN MONONUCLEAR CELLS

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IL-4 and INF- $\gamma$  are multifunctional lymphokines with profound effects on the immune response. In some cases they display similar effects, such as increasing T cell-mediated cytotoxicity (1), while in other situations, such as the regulation of Ig isotype expression, antagonistic effects have been demonstrated (2).

Recent studies in mice clearly indicate that the *in vivo* synthesis of IgE is highly dependent upon the balance between the production of IL-4 and INF- $\gamma$ . Administration of anti-IL-4 neutralizing antibody blocks the primary, the secondary, and most of the ongoing IgE responses (3), whereas the injection of anti-INF- $\gamma$  neutralizing antibody has the reverse effect (4). There is strong evidence that the isotype selection is dictated by the selective activation of either Th1 or Th2 CD4<sup>+</sup> T cell subsets. Indeed, Th2 cells were shown to produce IL-4 and IL-5, but not INF- $\gamma$ , whereas Th1 cells produce IL-2, INF- $\gamma$ , and lymphotoxin, but not IL-4 (5). In humans, this dichotomy of Th cells is not as apparent, since the majority of T cell clones producing IL-4 also secrete INF- $\gamma$  and IL-2 (6). However, several studies have indicated that the *in vitro* synthesis of human IgE requires the presence of IL-4 and that it is suppressed by INF- $\gamma$  (7, 8). This suggests that, like in the murine model, human IgE synthesis is also dependent upon the balance between IL-4 and INF- $\gamma$ . We demonstrate here that IL-4 suppresses the *in vitro* production of INF- $\gamma$  by human PBMC and that there is a parallelism between this suppression and the IgE synthesis by allogeneic stimulated PBMC. Hence, in addition to triggering cellular interactions leading to IgE synthesis, IL-4 also blocks the synthesis of a strong inhibitor of the IgE response.

Materials and Methods

*Reagents.* PHA was obtained from Wellcome Diagnostics (Beckenham, UK) and PWM was from Gibco Laboratories (Grand Island, NY). Human rIL-4 was purchased from Genzyme (Boston, MA). Cycloheximide was obtained from Sigma Chemical Co. (St. Louis, MO).

Neutralizing anti-IL-4 mAb (IgG1,  $\kappa$ ) hybridoma culture supernatants were a kind gift from Dr. C. Heuser (CIBA-GEIGY, Switzerland).

*Cell Preparation and Culture Conditions.* Mononuclear cells were isolated from heparinized venous blood of healthy volunteers by centrifugation over a Sepacell gradient (Sepratech

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Co., Oklahoma City, OK). Cells were cultured in HB101 culture medium (Hana Biologics Inc., Alameda, CA) supplemented with penicillin G (100 U/ml), streptomycin (100 µg/ml) (Sigma Chemical Co.), L-glutamine (2 mM/liter) (Gibco Laboratories), sodium pyruvate (10 mM/liter), and Hepes (10 mM/liter).

PBMC ( $1.5 \times 10^6$  in 1 ml) were cultured in 48-well culture plates (Costar, Cambridge, MA) in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air. Culture supernatants from mitogen-stimulated cells (PHA 1%; PWM 1%; vol/vol) were harvested after 3 d. Two-way mixed lymphocyte cultures (MLC), containing equal numbers of PBMC from two unrelated donors, were harvested after 6 d, except when otherwise indicated.

*RIAs.* The IFN-γ levels in cell-free culture supernatants were detected by a commercially available solid-phase RIA kit (Centocor Co., Malvern, PA). IgE was measured by a solid-phase RIA using two mAbs, exactly as previously described (9). The net synthesis of IgE and of IFN-γ was determined by subtracting the values measured in the culture supernatants of cycloheximide-treated cells from those of untreated cells.

*Northern Blot Analysis.* Total cytoplasmic RNA was purified from  $10^7$  MLR mononuclear cells by the guanidine-thiocyanate method (10) and subjected to electrophoresis on a formaldehyde-denaturing agarose gel. After transfer to a nitrocellulose membrane (Schleicher & Schuell, Inc., Keene, NH), the samples were hybridized (11) with a 1.4-Kb nick-translated Bam HI/Bam HI fragment of the human IFN-γ cDNA sequence derived from pSVT69 (12). After autoradiography, the IFN-γ probe was washed off and the membrane was rehybridized with a human γ-actin cDNA probe.

## Results

In the course of our studies on the regulation of IgE synthesis by PBMC from healthy adults, we noticed that IL-4 not only triggered the synthesis of IgE but also suppressed the production of IFN-γ by these cells. In 21 consecutive experiments where the levels of IFN-γ in the unstimulated cultures ranged from 5 to 45 U/ml, IL-4 induced a suppression ranging from 70 to 100%. These observations prompted us to examine the effect of IL-4 on the synthesis of IFN-γ by PBMC stimulated with mitogens or with allogeneic cells. The data in Tables I and II clearly indicate that IL-4 strongly suppressed the production of IFN-γ induced by optimal concen-

TABLE I  
*Effect of IL-4 on the Spontaneous and Mitogen-induced IFN-γ Production*

Added to cultures	IFN-γ		
	Exp. 1	Exp. 2	Exp. 3
		<i>U/ml</i>	
-	7	20	34
IL-4	1	3	5
PWM	370	530	2,700
PWM + IL-4	80	250	480
PWM + IL-4 + anti-IL-4 mAb	470	450	ND
PWM + IL-4 + anti-Lol P1 mAb	75	260	ND
PHA	1,000	1,680	1,500
PHA + IL-4	300	430	380
PHA + IL-4 + anti-IL-4 mAb	1,100	1,490	ND
PHA + IL-4 + anti-Lol P1 mAb	360	425	ND

Shown are the mean values of four replicate cultures; the coefficient of variation was <20%. The isotype-specific control mAb anti-Lol P1, IgG1,κ (8) does not alter the IL-4 effect.

TABLE II  
Effect of IL-4 on IFN- $\gamma$  Production during MLC

Added to MLC	IFN- $\gamma$		
	Exp. 1	Exp. 2	Exp. 3
-	124	184	120
IL-4	30	36	8
IL-4 + anti-IL-4 mAb	96	139	ND
IL-4 + anti-Lol P1 mAb	28	36	ND

Shown are the mean values of four replicate cultures; the coefficient of variation was <20%. The isotype-specific control mAb anti-Lol P1, IgG1, $\kappa$  (8) does not alter the IL-4 effect.

trations of PWM, PHA, or by allogeneic cells. This effect was blocked by a neutralizing mAb to IL-4. In all these experiments, IL-4 was used at a concentration of 300 U/ml, which is optimal for the induction of IgE synthesis. However, the IL-4-induced suppression of IFN- $\gamma$  is dose dependent. In the representative experiment shown in Fig. 1, increasing concentrations of IL-4 were added to a MLC. As seen, IL-4 had a reciprocal and dose-dependent effect on the synthesis of both IFN- $\gamma$  and IgE. The IgE levels were low due to the short duration (6 d) of the culture. As another approach to analyze the mechanism of IFN- $\gamma$  suppression by IL-4, time course experiments were performed. As shown in Fig. 2, IL-4 must be present from the very beginning of the MLC in order to exert maximal suppression of IFN- $\gamma$  production and to induce IgE synthesis. It is of note that neither IL-1, IL-2, IL-6, nor TNF- $\alpha$ , tested over a wide concentration range, could inhibit the IFN- $\gamma$  production by allogeneic stimulated PBMC (data not shown).

Finally, we have demonstrated that IL-4 inhibits the expression of IFN- $\gamma$  mRNA in allogeneic stimulated cells. In the experiment shown in Fig. 3, cells were analyzed by Northern blot hybridization for the expression of IFN- $\gamma$  mRNA after a 3 d stimulation in MLC, in the presence (lane 1) or absence (lane 2) of 300 U/ml of IL-4.

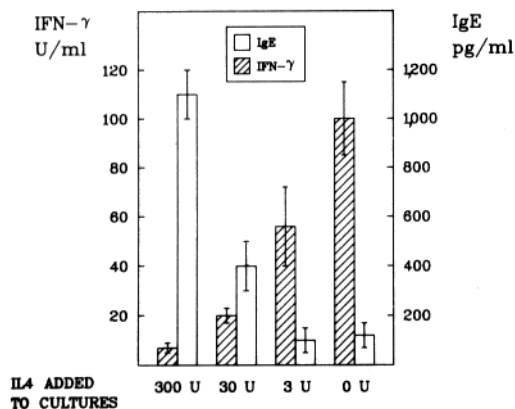


FIGURE 1. Dose-dependent effect of IL-4 on the synthesis of IFN- $\gamma$  and IgE during MLC. MLC was performed as described in Materials and Methods. IL-4, at the indicated concentrations, was added at the start of the cultures. Culture supernatant was harvested after 6 d.

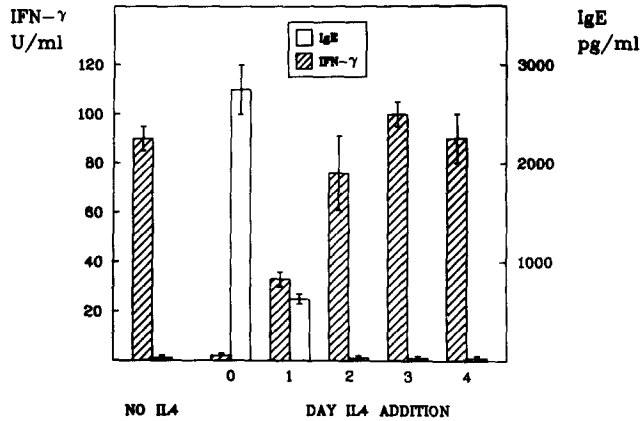


FIGURE 2. Time-dependent IL-4 effect on the synthesis of IFN- $\gamma$  and IgE during MLC. IL-4 (300 U/ml) was added to MLC at the indicated time-points. The levels of IFN- $\gamma$  and of IgE were determined after a 12-d culture period.

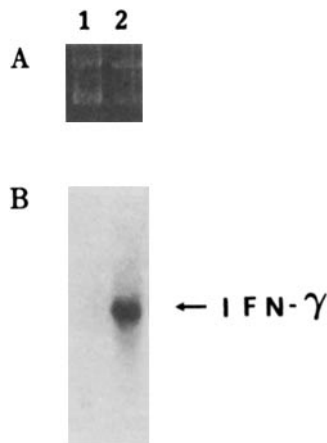


FIGURE 3. Effect of IL-4 on IFN- $\gamma$  mRNA. (A) Ethidium bromide staining of the 18 S and 28 S ribosomal RNA bands from the equalized RNA samples used to prepare the RNA blot. (B) Northern blot analysis of IFN- $\gamma$  mRNA in the presence (lane 1) or absence (lane 2) of IL-4 (300 U/ml). The data shown are representative of four separate experiments.

After normalization according to the human  $\gamma$ -actin mRNA level, which was not influenced by IL-4 treatment, the accumulative IFN- $\gamma$  mRNA level displayed an inhibition of 75% (as determined by densitometer scanning) in the presence of 300 U/ml IL-4 (data not shown).

### Discussion

Our observations demonstrate that IL-4 inhibits the *in vitro* production of IFN- $\gamma$  by human PBMC. The suppression is observed both at the protein and at the mRNA level; it is dose and time dependent, and it is blocked by a neutralizing mAb to IL-4. The dose-response and the kinetic studies confirm the prevalent concept of an inverse relationship between the production of IgE and of IFN- $\gamma$ .

The data from the time course experiments may further suggest that the selection of the IgE isotype expression occurs early in the course of an immune response. Indeed, IL-4 has to be present at the initiation of the lymphocyte activation in order to exert its maximal suppressive effect on the production of IFN- $\gamma$ , indicating that,

when lymphocytes are activated in such a way that IL-4 is produced first, this would prevent the subsequent production of IFN- $\gamma$ . Therefore, the balance between these two antagonistic lymphokines may be significantly influenced by the chronological order of activation of their respective gene.

We are currently investigating the molecular mechanism of these observations and we are testing whether the IL-4-induced inhibition of the accumulation of IFN- $\gamma$ -specific mRNA in allogeneic stimulated cells is due to a reduced transcription rate or to an increased mRNA degradation.

The observed effect of IL-4 cannot be attributed to inhibition of the lymphocyte activation, since the addition of IL-4 to cells stimulated by mitogens or by allogeneic cells enhanced the DNA synthesis, as measured by [ $^3$ H]thymidine uptake (data not shown).

IL-2 has been reported to increase the production of IFN- $\gamma$  by T cells stimulated with mitogens or antigen (13). We observed that IL-4 still suppressed the IFN- $\gamma$  production in MLR cultures supplemented with IL-2 (data not shown). This indicates that the IL-4 effect is not due to the inhibition of the IL-2-mediated IFN- $\gamma$  induction.

The suppressive activity of IL-4 on the production of IFN- $\gamma$  might occur in vivo as well. Indeed, in a recent study on murine Leishmaniasis, Heinzl et al. (14) reported that susceptible strains of mice (BALB/c) express high tissue levels of IL-4 mRNA and low levels of IFN- $\gamma$  mRNA, whereas the resistant C57BL/6 strain expresses high levels of IFN- $\gamma$  and low levels of IL-4. As expected, BALB/c mice display a strong IgE response after Leishmania infection, whereas C57BL/6 do not. IFN- $\gamma$  was shown to play a critical role in the resistance to this parasite (15, 16). Relevant to the present data is the observation that treatment of susceptible mice with anti-IL-4 mAb significantly enhanced their resistance to the parasite. As suggested by the authors, this might be due to the neutralization of the harmful effects of IL-4 in this infection or to the restoration of IFN- $\gamma$  production. The latter possibility is supported by the present observations that IL-4 suppresses the production of IFN- $\gamma$ .

Since IFN- $\gamma$  plays a key role in protective immunity against several pathogens with an obligatory or facultative intracellular cycle, the observation that IFN- $\gamma$  production is suppressed by IL-4 may be relevant in clinical conditions where an increased IgE production (as a marker of high IL-4 and low IFN- $\gamma$  production) is associated with either chronic parasitic disease or increased susceptibility to infections.

### Summary

rIL-4 inhibits the production of IFN- $\gamma$  by PBMC stimulated with mitogens or allogeneic cells. The suppression is observed at the protein and at the mRNA level; it is dose and time dependent, and it is abolished by a neutralizing mAb to IL-4. It is suggested that the balance between the production of IL-4 and IFN- $\gamma$  may be significantly influenced by the chronological order of activation of their respective gene.

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