

Serum IgE level is negatively correlated with the ability of peripheral mononuclear cells to produce interferon gamma (IFN γ): evidence of reduced expression of IFN γ mRNA in atopic patients

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Summary

Background In patients with atopic diseases such as bronchial asthma and atopic dermatitis, an elevated serum IgE level is common. Several studies showed that interleukin-4 (IL-4) and interferon-gamma (IFN γ) are important for regulation of IgE production.

Objectives The study was designed to examine the pathogenesis of an elevated serum IgE level at the production levels of TH₁-type and TH₂-type cytokines.

Methods The production of interleukin-2 (IL-2), IL-4, interleukin-5 (IL-5) and IFN γ by peripheral blood mononuclear cells (PBMCs) stimulated with phytohemagglutinin (PHA) was analysed in 20 individuals with various serum IgE levels. The amount of IFN γ mRNA in the stimulated PBMCs was analysed using a quantitative polymerase chain reaction method.

Results Cytokine production was analysed simply as a function of serum IgE level. The serum IgE level was negatively correlated with the amount of IFN γ secreted by PBMCs ($n = 20$, $R = -0.724$, $P < 0.005$) when logarithmically transformed data were analysed, but were not or were only weakly correlated with those of IL-4, IL-2, or IL-5 secreted by these cells ($n = 20$). For investigation of the cause of reduced IFN γ production in individuals whose serum IgE level are high, the amount of IFN γ mRNA was determined. The levels of IFN γ mRNA expression in the stimulated PBMCs correlated well with the amount of IFN γ secreted by the PBMCs ($n = 8$, $R = 0.947$, $P < 0.001$).

Conclusions Reduced IFN γ production ability due to reduced IFN γ mRNA expression in PBMCs is associated with an elevated serum IgE level in atopic patients.

Keywords: atopic dermatitis, IgE, interferon-gamma, IL-4, IL-5, IL-2, mRNA

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Introduction

Patients with atopic diseases characteristically exhibit elevated serum IgE levels [1,2]. The basis for the increased IgE synthesis in such patients has been investigated [3–6] but not completely clarified. It has been shown that the class switch to IgE and production of IgE in B cells are regulated

by cytokines produced by T cells [7–12]. In particular, stimulation with interleukin-4 (IL-4) is necessary for expression of germ-line C ϵ transcripts in B cells [7], and induces the class switch to IgE together with additional signals such as CD40-CD40 ligand interaction [13]. In contrast, interferon-gamma (IFN γ) inhibits *in vivo* and *in vitro* IgE synthesis in mice as well as IL-4 induced IgE synthesis by human peripheral blood mononuclear cells (PBMCs) *in vitro* [7–12].

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Several investigators have shown that PBMCs from patients with atopic dermatitis (AD) produce less IFN γ , but more IL-4 than do PBMCs from healthy subjects upon mitogen stimulation such as with concanavalin A [3], phytohemagglutinin (PHA) [4,6], calcium ionophore plus phorbol myristate acetate (PMA), and PHA plus PMA [5]. Patients with Buckley's syndrome (hyper-IgE syndrome) [14], a distinct clinical entity, also show defective production of IFN γ by circulating T cells [15].

Murine helper T cells are divided into two subsets, TH₁ and TH₂ cells. The former produce interleukin-2 (IL-2) and IFN γ , and the latter produce IL-4 and interleukin-5 (IL-5) [16]. Such a dichotomy is also evident in human T cells, albeit to a less polarized extent than in murine T cells [17]. Hence, the reduced IFN γ production and elevated IL-4 production in atopic patients has been discussed mainly in terms of imbalance of TH₁ and TH₂ cells. However, to our knowledge, there are few reports of studies in which IL-2 and IL-5 production in addition to IL-4 and IFN γ production were assayed simultaneously. Hence in this study we investigated the relationship between serum IgE level and production of TH₁-type and TH₂-type cytokines by PHA-stimulated PBMCs from 20 individuals with various serum IgE levels.

Tang *et al.* reported that reduced IFN γ production by PBMCs from patients with AD was associated with neither a

reduced population of cells producing IFN γ [18] nor reduced IFN γ mRNA expression, and suggested that a post-translational defect in IFN γ secretion is responsible for reduced IFN γ production [19]. Hence we undertook this study to determine whether reduced IFN γ production is due to reduced expression of IFN γ mRNA or a post-translational defect as suggested by Tang *et al.*

In the present study, we clearly showed that the serum IgE level was negatively correlated with the level of IFN γ production by PHA-stimulated PBMCs, and that the reduced IFN γ production by PHA-stimulated PBMCs from individuals with high serum IgE level was due to reduced IFN γ mRNA expression in them.

Materials and methods

Patients and control subjects

Peripheral blood was obtained from 12 atopic patients (age 1–16 years) and eight control subjects (age 7 months–16 years). Patients with AD were diagnosed according to the criteria of Hanifin [20]. The diagnosis of asthma was made according to the criteria of the American Thoracic Society. Table 1 summarizes serum IgE levels of patients and control subjects. None of the patients had undergone treatment with systemic steroids. Informed consent was obtained from all subjects or from their parents.

Cell cultures

PBMCs were isolated from heparinized blood samples by Ficoll gradient centrifugation (Pharmacia), washed three times in phosphate buffered saline and resuspended at 2×10^6 cells/mL in RPMI1640 medium supplemented with 10% fetal calf serum heat-inactivated at 56°C for 30 min, 15 mM HEPES, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Cells were stimulated with 10 μ g PHA in a volume of 1 mL in a round-bottom tube (Falcon 2059) at 37°C in a 5% CO₂ humidified atmosphere. After 24-, 48- and 72-h stimulation, cells were harvested and stored at –80°C until use. The supernatants were subjected to cytokine assays. Simultaneously, 2×10^5 cells per 0.2 mL culture medium were added to 96-well round-bottom microtitre plates. After 24-h PHA stimulation, 0.5 μ Ci methyl-[³H]-thymidine (Amersham) was added and incubated for further 4 h, and thymidine incorporation was measured.

IgE assay

Plasma samples from the heparinized blood were kept at –30°. Plasma IgE concentrations were determined by

Table 1. Subjects analysed in this study

Case	Sex	Age	Disease	Serum IgE (IU/mL)
1	F	1 year 6 months	Control	2.5
2	M	3 years	Control	2.8
3	M	11 months	Control	6.0
4	F	16 years	Control	14.5
5	M	3 years	Control	21.3
6	M	7 months	Control	27.2
7	F	2 years	Control	85.2
8	M	5 years	Control	186
9	M	10 years	BA	336
10	M	10 years	BA	700
11	F	3 years	AD	728
12	F	13 years	BA	1400
13	M	9 years	BA	1460
14	M	3 years	AD, BA	4037
15	F	11 years	AD, BA	4433
16	F	8 years	AD, BA	7957
17	M	1 year	AD	10126
18	F	6 years	AD, BA	10397
19	F	11 years	AD	10565
20	M	16 years	AD, BA	36847

BA, bronchial asthma; AD, atopic dermatitis; F, female; M, male.

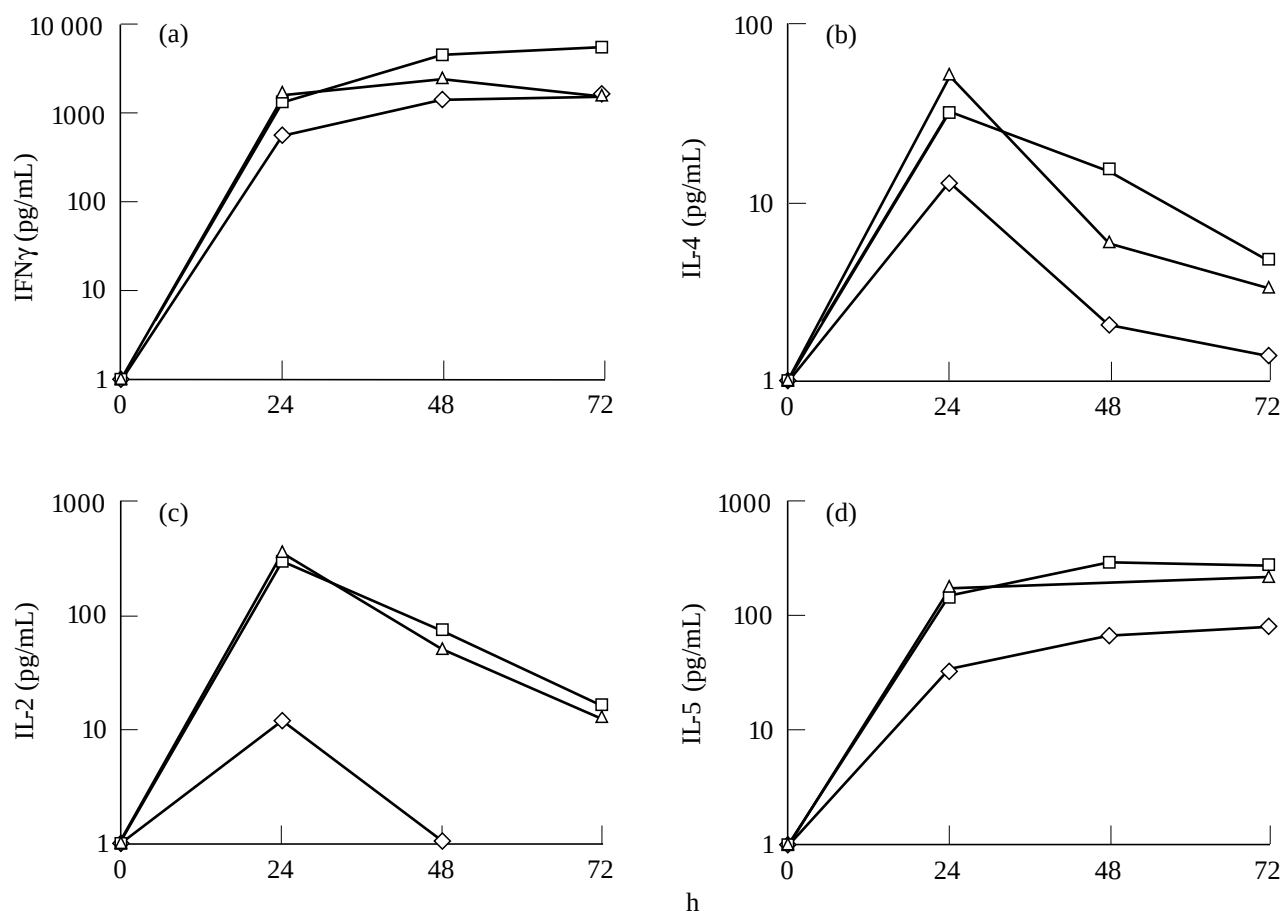


Fig. 1. Time courses of cytokine secretion by 24-, 48- and 72-h PHA-stimulated PBMCs. The levels of serum IgE and cytokine secretion were plotted logarithmically. We used PBMCs from a severe AD patient (serum IgE level 2500 IU/mL, $\diamond$ — $\diamond$), a mild AD patient (serum IgE level 150 IU/mL, $\square$ — $\square$) and a control subject (serum IgE level 9 IU/mL, $\triangle$ — $\triangle$).

chemiluminescent enzyme immunoassay. The values were regarded as serum IgE concentrations.

Assays for cytokines

IFN- γ , IL-2, IL-4, and IL-5 were assayed using a human IFN- γ ELISA kit (Ohtsuka, Japan), human IL-2 ELISA kit (Ohtsuka), The Cytoscreen human IL-4 ELISA kit (Bio Source International), and IL-5 human ELISA system (Amersham), respectively.

RNA extraction and reverse transcription

Total cellular RNA was extracted from the stimulated PBMCs using Isogen (Nippon Gene, Japan). Two microgram of total RNA was reverse transcribed at 37°C for 60 min with 200 units of MMLV reverse transcriptase (GIBCO BRL), and 5 pmol of an IFN- γ specific antisense primer

(5'-GATGCTCTTCGACCT CGAAACAGCAT-3') and 10 pmol of oligo d(T) primer in 20 μ L of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT, 1 mM dNTPs and 20 U of RNasin (Pharmacia LKB Biotech).

Quantitative PCR for human IFN- γ mRNA

One microlitre aliquot of the above first strand cDNA solution with one of 10-fold dilution series of human IFN- γ PCR MIMICs (Clontech) was subjected to a competitive PCR with human IFN- γ Amplifier Set (Clontech), according to the manufacturer's instructions. The PCR product yields were analysed by electrophoresis on a 2.0% agarose gel and visual inspection of EtBr-stained agarose gel. We estimated the amount of IFN- γ mRNA at the amount of MIMICs of which band had the same intensity as that of the target human IFN- γ cDNA.

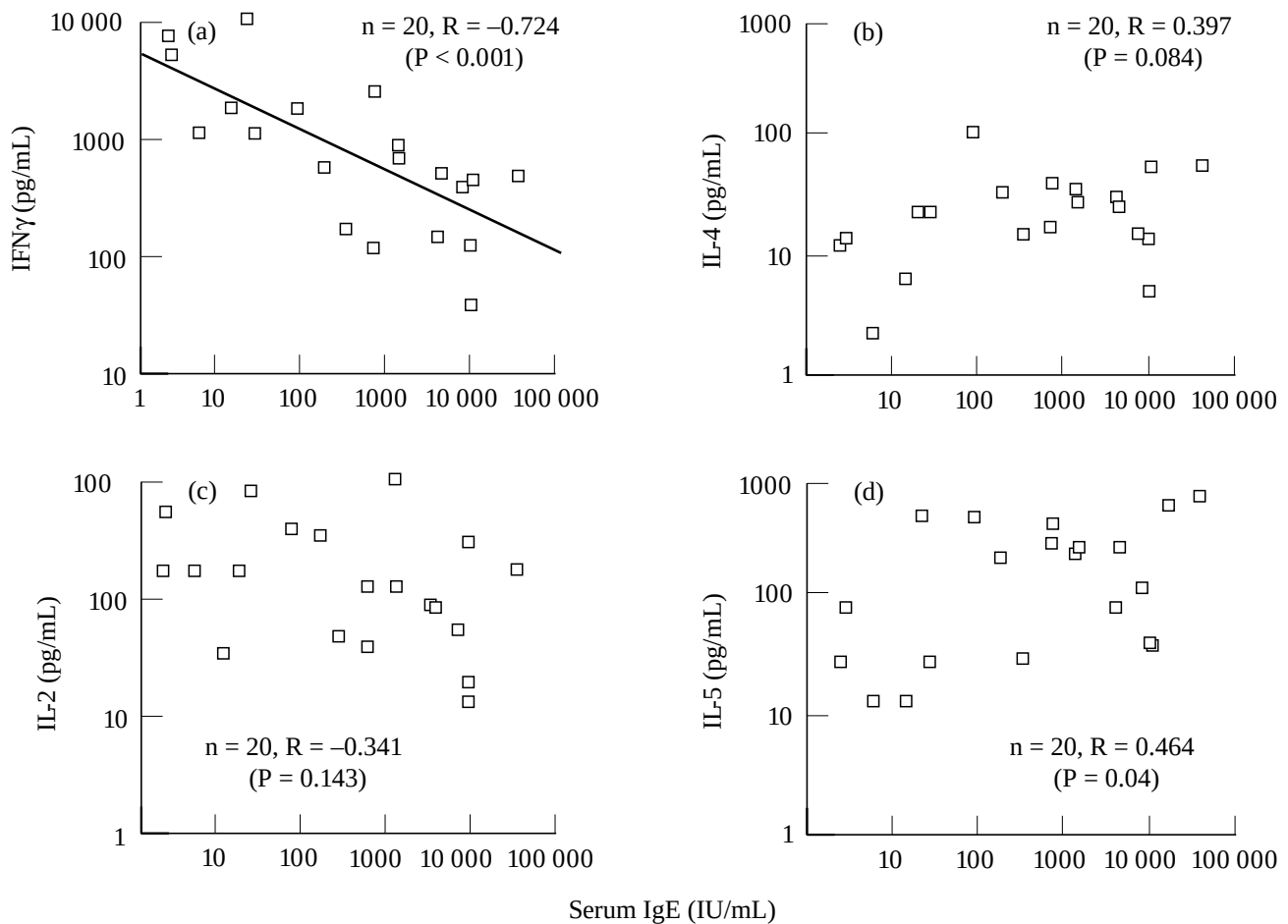


Fig. 2. Correlation between levels of serum IgE and cytokine secretion from PHA-stimulated PBMCs. Both variables were plotted logarithmically.

Statistical analysis

Pearson's correlation coefficient (R) was used to calculate the correlation between two random variables of logarithmically transformed data.

Results

Time courses of levels of cytokine secretion by PHA-stimulated PBMCs

We investigated the levels of IFN γ , IL-4, IL-2 and IL-5 secretion by PHA-stimulated PBMCs from three individuals at 24, 48 and 72 h after the start of the stimulation. As shown in Fig. 1(b),(c), the secretion of IL-4 and IL-2 peaked at 24 h of PHA stimulation for all individuals. The IFN γ (Fig. 1a) and IL-5 (Fig. 1d) secretion levels at 24 h of PHA stimulation were almost the same as those at 48 and 72 h. Hence we used 24-h PHA stimulation to assay the secretion levels of these cytokines.

Correlation between serum IgE level and secretion level of IFN γ from PHA-stimulated PBMCs

We investigated the relationship between serum IgE level and level of IFN γ secretion by PHA-stimulated PBMCs from 20 individuals with various serum IgE levels (Table 1). The level of IFN γ secretion by non-PHA stimulated PBMCs was under the detection limit of our assay (<7.5 pg/mL). The cytokine secretion levels were analysed simply as functions of serum IgE level. As shown in Fig. 2(a), the serum IgE level was negatively correlated with the amount of IFN γ secreted by the stimulated PBMCs ($n = 20$, $R = -0.724$, $P < 0.001$) when both variables were plotted logarithmically. The reduced IFN γ secretion was not due to a poor lymphocyte proliferative response to PHA stimulation since the level of IFN γ secretion was not correlated with the magnitude of the lymphocyte proliferative response ($n = 20$, $R = 0.385$, $P > 0.1$, data not shown).

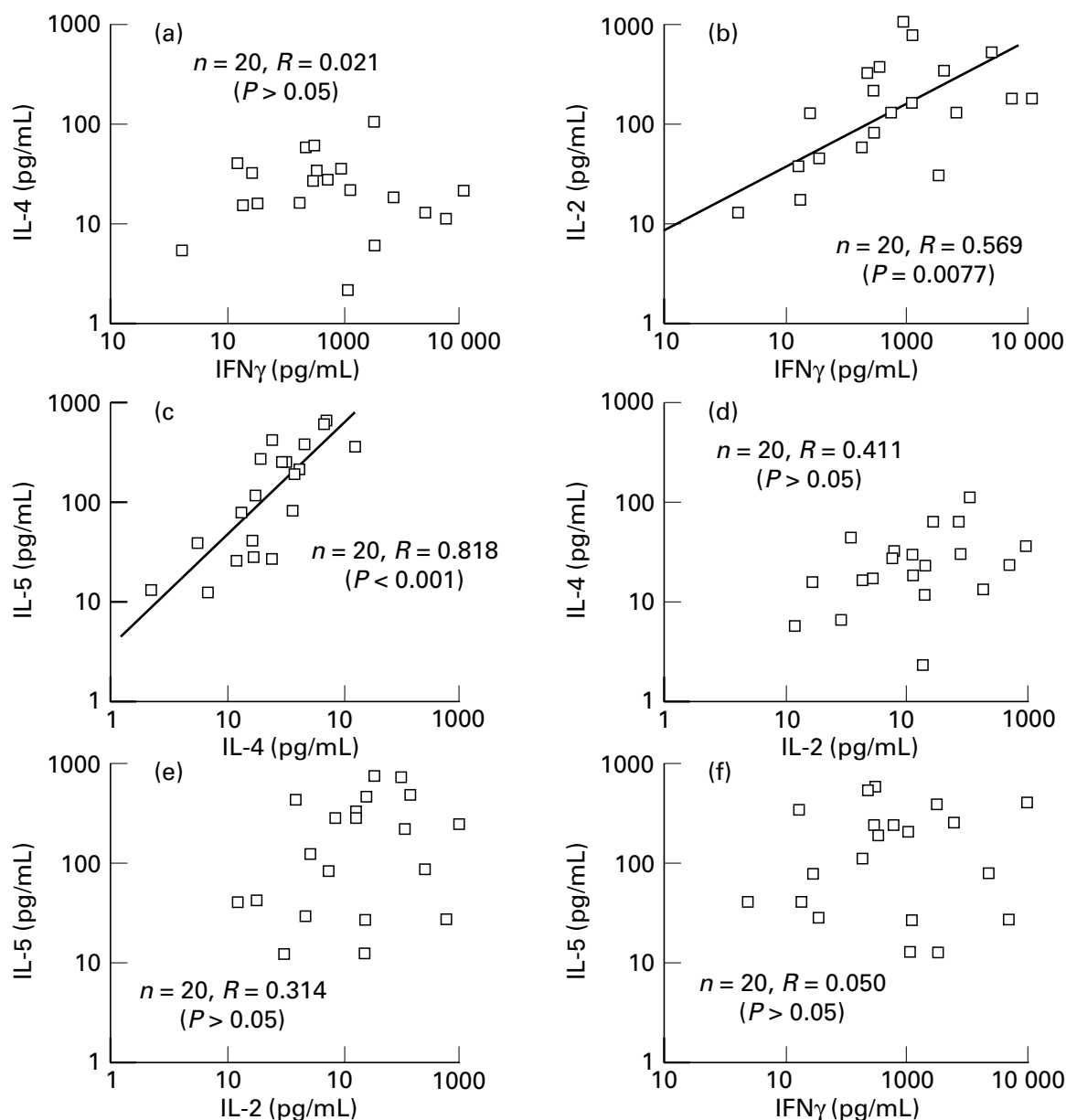


Fig. 3. Correlation between levels of cytokine secretion from PHA-stimulated PBMCs. Both variables were plotted logarithmically.

Correlation between serum IgE level and secretion levels of other cytokines by PHA-stimulated PBMCs

Serum IgE level and secretion levels of IL-4, IL-5, or IL-2 by stimulated PBMCs were logarithmically plotted in Fig. 2(b–d). No or weak correlation was observed between serum IgE level and secretion levels of IL-4 ($n=20$, $R=0.397$, $P=0.084$), IL-5 ($n=20$, $R=0.464$, $P=0.04$), and IL-2 ($n=20$, $R=-0.341$, $P>0.1$).

Correlation between secretion levels of cytokines

Figure 3 shows the relationship between secretion levels of

two of IFN γ , IL-4, IL-2, IL-5. Although IL-4 inhibits IFN γ production [21,22], no correlation between the levels of IL-4 and IFN γ was observed (Fig. 3a). Weak but significant correlation was detected between secretion levels of TH $_1$ -type cytokines, IFN γ and IL-2 (Fig. 3b, $R=0.569$, $P=0.007$) and tight correlation was observed between those of TH $_2$ -type cytokines, IL-4 and IL-5 (Fig. 3c, $R=0.818$, $P<0.001$).

Expression of IFN γ mRNA in PBMCs stimulated by PHA

In order to examine whether the reduced IFN γ secretion by

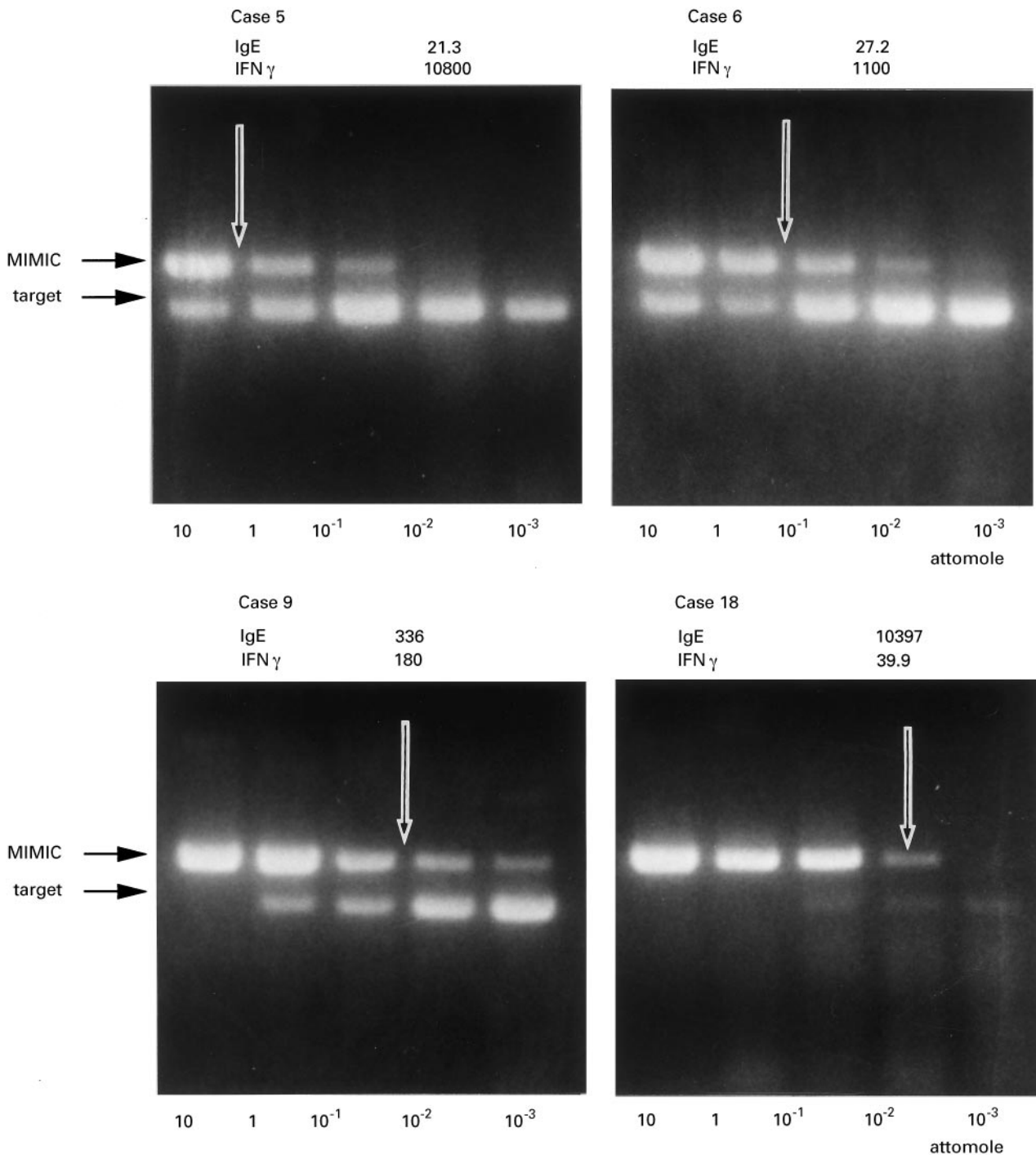


Fig. 4. Competitive PCR with PCR-MIMICs to quantify IFN γ mRNA. One-twentieth of the first-strand cDNA solution reverse transcribed from 2 μ g of total cellular RNA was mixed with the amount of human IFN γ PCR-MIMICs shown under photographs, and amplified with Human IFN γ Amplimer Set. Arrows with white edges indicates equivalent points at where amounts of products of target cDNA (IFN γ) and PCR-MIMICs were almost the same.

the stimulated PBMCs from individuals with high serum IgE levels was due to the suppressed expression of the IFN γ gene or not, we performed quantitative PCR with PCR-MIMICs. The efficiency of the reverse transcription in each sample was

checked by amplification of mitochondrial acetoacetyl-CoA thiolase cDNA [23]. Since the levels of IFN γ secretion by the stimulated PBMCs ranged from 39.9 to 10800 pg/mL, a 10-fold dilution series of MIMICs was used for evaluation of the

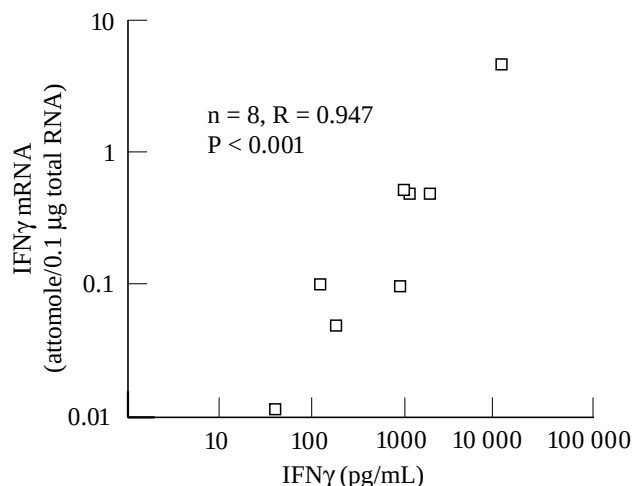


Fig. 5. Correlation between level of IFN γ secretion by the PHA-stimulated PBMCs and amount of IFN γ mRNA in them. Both variables were plotted logarithmically.

amount of IFN γ mRNA. Typical results of the competitive PCRs are shown in Fig. 4. In case 5 PBMCs, an equivalent point at which the amount of a target IFN γ cDNA was almost the same as that of MIMICs was estimated to be 5 attomole between 10 attomole and 1 attomole, whereas such an equivalent point was estimated to be 10^{-2} attomole in case 18 PBMCs. Since the level of IFN γ secretion and the amount of IFN γ mRNA in case 5 PBMCs were about 270-fold and 500-fold, those in case 18 PBMCs, respectively, the level of IFN γ secretion was expected to be closely related to the level of IFN γ mRNA expression. Figure 5 shows the correlation between levels of IFN γ protein and IFN γ mRNA in eight cases ($n = 8$, $R = 0.947$, $P < 0.001$). Our results indicated that reduced IFN γ secretion in individuals with high serum IgE levels was due to reduced IFN γ gene transcription.

Discussion

In the present study the serum IgE level was negatively correlated with the level of IFN γ production by the PHA-stimulated PBMCs and the production was apparently regulated at the transcriptional level.

In mice, helper T cells are divided into two functionally distinct subpopulations, TH₁ and TH₂ cells. The former produce IL-2 and IFN γ , and the latter produce IL-5 and IL-4 [16]. The same concept applies human T cells, albeit to a less polarized extent than to murine T cells [17, 24–25]. We investigated the correlation between serum IgE level and the levels of secretion of not only IFN γ and IL-4 but also IL-2 and IL-4 by PHA-stimulated PBMCs from 20 individuals with various serum IgE levels.

Several investigators have reported finding that PBMCs from AD patients produced less IFN γ but more IL-4 than

ones from healthy controls, upon stimulation of the PBMCs with mitogens [3–6]. Tang *et al.* divided subjects into groups with severe AD, mild AD, and healthy controls and reported that patients with the mild AD showed no difference in the level of IL-4 and IFN γ secretion by PHA-stimulated PBMCs compared with healthy controls, whereas patients with severe AD showed elevated IL-4 secretion and decreased IFN γ secretion compared with mild AD patients and healthy controls [4]. Previous studies focused on the differences between atopic patients and controls [3–6]. In the present study, we did not divide the subjects into such groups, and determined the levels of cytokine production by the PHA-stimulated PBMCs as functions of the serum IgE level. Recently, Tang *et al.* observed similar correlations between serum IgE level and the level of IFN γ production by PHA-stimulated PBMCs from asthmatic patients and healthy controls [6]. This means that the correlation between the level of IFN γ production and serum IgE level is reproducible. Our data suggest that even among atopic patients, the lower the level of IFN γ production by PBMCs from an individual the higher the serum IgE level of the individual is. Based on the present results, together with the fact that IFN γ can suppress IL-4-induced IgE production, the level of IFN γ production by PBMCs was high in the individuals with low serum IgE levels, possibly preventing overproduction of IgE. In individuals with high serum IgE levels, insufficient IFN γ production may result in lack of suppression of IgE production. Interestingly, the level of IFN γ production *in vitro* by circulating T cells from hyper IgE syndrome (Buckley's syndrome) patients was also found to be low [14].

There are a few reports about IFN γ mRNA expression in the PBMCs of atopic patients with high serum IgE levels [19,26,27]. Tang *et al.* reported that the level of IFN γ mRNA expression in PMA/Ca-stimulated PBMCs from patients with acute exacerbation of severe AD and from healthy controls were almost the same [19], whereas the level of IFN γ secretion by PHA-stimulated PBMCs from the patients was lower than that of healthy controls [18]. The observation at the IFN γ secretion level by Tang *et al.* [4] is relevant to our results, but the cause of the reduced IFN γ secretion in the PBMCs indicated by our results here is quite different from that suggested by Tang *et al.* [19]. Tang *et al.* suggested that reduced secretion of IFN γ was related not to abnormal regulation of transcription, but rather to a post-translational defect of IFN γ secretion. Since our aim in the present study has been to find the primary cause of high serum IgE level in atopic patients, it was important to determine whether the reduced IFN γ production was due to a defect at the transcriptional level or a post-translational defect as proposed by Tang *et al.* We showed that the level of IFN γ secretion was strongly correlated with the level of IFN γ mRNA expression in PHA-stimulated PBMCs. In general, IFN γ production is considered to be regulated

primarily by the rate of gene transcription [28–30] and differences in cytokine mRNA levels reflect variations in the number of cytokine-producing cells rather than increased transcription in each cell [31,32]. Our strategy of using total PBMCs did not allow clear demonstration of whether the reduced IFN γ mRNA expression reflected a decrease in the TH $_1$ -like cell count or an intrinsic transcriptional defect in TH $_1$ -like cells.

In the present study, the IL-4 secretion level was not correlated with the serum IgE level. Even when our subjects were divided into atopic and control groups, no significant difference in IL-4 secretion level was found between them (data not shown), whereas several investigators have reported that the level of IL-4 production by PBMCs from atopic patients was higher than that from control subjects [3,4]. These contrasting results may reflect differences in subjects studied because most of the atopic subjects in the previous studies were phenotypically severe, whereas ones with the present study included less phenotypically severe.

The levels of IL-4 and IL-5 secretion by the stimulated PBMCs were tightly correlated with each other ($n=20$, $R=0.818$, $P<0.001$), suggesting that TH $_2$ -type cytokines are cosecreted by the stimulation. The level of secretion of another cytokine, IL-13, which is also produced by TH $_2$ cells, was also not correlated with the serum IgE level but was tightly correlated with that of IL-4 (unpublished observation). However, the levels of IL-2 and IFN γ secretion were not so tightly correlated ($n=20$, $R=0.569$, $P=0.0077$). Hence, this may imply that a defect in IFN γ -specific production exists in TH $_1$ -like cells from high serum IgE level individuals. As discussed above, a limitation of our strategy of using total PBMCs is that we could not determine whether the results are due to imbalances in T-cell subpopulations having different abilities to produce cytokines or as yet uncharacterized intrinsic defects in IFN γ production. Recently, Jung *et al.* [33] reported that the proportion of IFN γ producing cells of CD4 $^+$ cells but not of CD8 $^+$ cells of atopic patients was significantly decreased as determined at the single cell level by cytometric analysis. They also demonstrated that IL-2 production by the CD4 $^+$ T cells was decreased whereas IL-4 production by the CD4 $^+$ T cells was unchanged, suggesting a deficiency in the ability of atopic T cells to produce TH $_1$ -type cytokines on stimulation with phorbol ester, ionomycin, or anti-CD3 monoclonal antibodies. In a preliminary study in which we used PBMCs depleted of CD4 $^+$ cells or CD8 $^+$ cells we found that the levels of IFN γ secretion by PBMCs depleted of CD8 $^+$ cells (CD4 $^+$ cell dominant population) from individuals with high serum IgE levels were relatively lower than those from individuals with normal serum IgE levels, compared with the levels of IFN γ secretion by PBMCs depleted of CD4 $^+$ cells (CD8 $^+$ cell dominant population). Renz *et al.* reported inhibition of

IgE production and normalization of airway responsiveness by sensitized CD8 $^+$ T cells in a mouse model of allergen-induced sensitization and suggested that CD8 $^+$ T cells play an important role in the negative regulation of IgE production and IFN γ may be a relevant mediator of the functions of CD8 $^+$ cells [34]. Further analysis is needed to determine whether CD4 $^+$ and/or CD8 $^+$ cells fail to produce IFN γ in response to PHA stimulation in some atopic patients.

In this study, we showed that the serum IgE level is negatively correlated with the level of IFN γ production by PHA-stimulated PBMCs. Since PHA stimulates T lymphocytes nonspecifically, the reduced IFN γ production in total PBMCs may reflect transcriptional suppression in a rather large population of T cells than allergen-specific T cells. We are planning to find out whether IFN γ production is also reduced in the case of antigen-specific stimulation of PBMCs.

Acknowledgements

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