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Differential spontaneous expression of mRNA for IL-4, IL-10, IL-13, IL-2 and interferon-gamma (IFN-γ) in peripheral blood mononuclear cells (PBMC) from atopic patients

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SUMMARY

Distinct cytokine-producing T cell subsets are well known to play a major role in IgE production and to be differentially regulated in allergic patients, although the characterization of the type 1/type 2 cytokine pattern in PBMC during allergic responses remains to be clearly defined. The aim of this study was to determine whether different cytokine profiles are observed directly in PBMC of atopic donors. We attempted to study several cytokines (IL-2, IFN-γ, IL-4, IL-10 and IL-13) using not only ELISA but also polymerase chain reaction (PCR) techniques, because the frequency of cytokine-producing cells in peripheral blood is very low. All the patients were selected during their acute symptomatologic phase. Data showed a significantly higher production of IL-4 ($P = 0{\cdot}05$) and IL-10 ($P < 0{\cdot}005$) as determined by ELISA in phytohaemagglutinin (PHA)/phorbol myristate acetate (PMA)-stimulated mononuclear cells of atopic donors compared with controls, although spontaneous IL-4 production without stimulation was never detected within either atopic or control groups. The reverse-transcriptase (RT)-PCR technique appeared to be advantageous in that it allowed the detection of the spontaneous expression of cytokine mRNA in cells without stimulation. We found a clear expression of IL-4 mRNA spontaneously in all atopic patients, whereas normal donors in most cases did not show specific signals ($P < 0{\cdot}0001$). Less differences between atopic subjects and controls were found in IL-10 mRNA expression. Although the technique of RT-PCR amplification used in this study is semiquantitative, a reproducible and significant ($P < 0{\cdot}001$) enhancement of IL-10 mRNA expression was observed in atopic donors. A heterogeneous expression of IL-13 mRNA was observed in individuals from the two groups studied, although mean levels in atopic donors were slightly enhanced compared with controls ($P = 0{\cdot}02$). Furthermore, we did not observe any alteration in the expression of the type 1-derived cytokines such as IFN-γ and IL-2. In addition, we showed a lack of correlation between the expression of serum IgE (total or specific) and spontaneous IL-4 mRNA expression. This study showed a tendency of PBMC from atopic donors to express a type 2-like cytokine pattern, with IL-4 as the most discriminatory cytokine. Additionally, as the level of serum IgE has a low predictive value in allergic disease, and as the elevated expression of IL-4 that we found was not correlated with serum IgE, we could strongly suggest that the measurement of IL-4 in blood mononuclear cells may be of great value in the analysis of allergic responses in atopic donors.

Keywords atopy human cytokine profile polymerase chain reaction blood mononuclear cells

INTRODUCTION

Atopic allergic diseases are characterized by an increased ability of B lymphocytes to form IgE antibodies directed against allergens. Cytokines are produced by many cell types and have important biological and regulatory functions [1–6]. IL-4 and IL-13, which seems to share many properties with IL-4, are crucial for switching B lymphocytes to IgE production [1,7–8], whereas IFN-γ antagonizes this effect [1,9]. Three types of T $CD4^+$ helper clones were originally described in mice: Th1 cells which produce IL-2, IFN-γ and tumour necrosis factor-beta (TNF-β), Th2 cells which produce IL-4, IL-5, IL-10, IL-13, and Th0 cells which produce both Th1- and Th2-type cytokines [1,5,10]. Th2 cells cause eosinophilia and provide B cell help, especially for IgE production, whereas Th1 is responsible for DTH and IgG2a production [5].

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Table 1. Patient characteristics

	Age (years)	Sex (M/F)	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)	Ratio CD4/CD8	Total IgE (U/ml)	Specific IgE (RAST+)
Allergic	32·5 ± 10 (16–51)	11/7	33·9 ± 6·4	6·27 ± 2·1	57·9 ± 6	1·69 (0·85–3·18)	329 (36–1064)	18/18
Control	35 ± 85 (24–52)	4/11	30·8 ± 5·8	4·43 ± 1·2	64·1 ± 5·8	1·73 (1·02–3·22)	<100	—

Studies investigating the site of the allergic reaction showed a high proportion of T cells with an expression of mRNA for IL-4, IL-3, IL-5 and granulocyte-macrophage colony- stimulating factor (GM-CSF), suggesting a Th2 profile [11–13], or a spontaneous cytokine production with an increase in IL-4 production but not IFN-γ and IL-2 [14]. Several studies showed that the great majority of allergen-specific $CD4^+$ T cell clones derived from PBMC of atopic donors displayed a high production of IL-4 and IL-5, and little or no production of IFN-γ and IL-2 [15–18]. The investigation of PBMC is advantageous, in that it analyses the effects of cytokines produced by multiple cell types on Th cell function, and includes both autocrine and paracrine regulation. In this regard, however, conflicting results have been reported on cytokine production by PBMC from atopic patients. Previous studies have demonstrated that mononuclear cells did not release cytokines spontaneously, but had to be stimulated with combinations of T cell polyclonal activating agents [19–21] for a long period of culture. In contrast, Walker *et al.* showed that purified T cells from PBMC of atopic patients secrete IL-4 spontaneously [14]. IFN-γ production was reported to be normal in atopic patients [22,23], while another group showed a decrease in IFN-γ production in atopic patients [20]. Although the cytokines were all measured by immunoassay techniques, the experimental conditions of these studies were different. In addition, no study has yet investigated the expression of IL-10 and IL-13 directly in PBMC of allergic patients.

The aim of the present study was to characterize more fully the cytokine pattern directly in human peripheral blood during allergic responses. Our study focused on the simultaneous analysis of the expression of IL-2, IFN-γ, IL-4, IL-10 and IL-13 in PBMC of allergic donors. By use of the polymerase chain reaction (PCR) amplification technique, we could detect spontaneous expression of cytokines without cell activation, in contrast to ELISA techniques. During this study, we also attempted to establish a relationship between the IgE-regulating cytokines' mRNA expression in atopic subjects and serum IgE levels.

PATIENTS AND METHODS

Patients

PBMC from 18 atopics were used in this study. The characteristics of the patients are indicated in Table 1. All patients showed allergic symptoms (asthma and/or allergic rhinitis). Serum IgE levels (CAP system; Pharmacia Diagnostics, Uppsala, Sweden) were elevated in most patients (range 120–1064 U/ml), and six patients had low IgE levels (<100 U/ml). Specific IgE were determind using the RAST system (Pharmacia), and ranged between 3 U/ml and >100 U/ml. None of the patients had received oral steroid therapy or immunotherapy for at least 3 months before testing. PBMC from 15 normal healthy donors without symptoms or history of allergy were also studied; their serum IgE levels were <100 U/ml, and no specific IgE response was observed in the RAST. None of the donors had received corticosteroids.

Preparation of PBMC

Heparinized peripheral venous blood (20 ml) was collected and mononoclear cells were obtained by density separation over Ficoll–Hypaque (Lymphoprep; Gibco Life Technologies, Cergy Pontoise, France). Mononuclear cells at the interface were carefully transferred with a Pasteur Pipette, then washed with RPMI 1640. Cells were suspended at a density of $2{\cdot}5 \times 10^6$ cells/ml in RPMI 1640 supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin, 2 mM glutamine and 10% heat-inactivated fetal calf serum (FCS) (all from Gibco). One millilitre of this PBMC suspension was transferred to each well of 24-well plates. Cell cultures were carried out in the presence or not of phytohaemagglutinin (PHA; 10 μg/ml) plus phorbol myristate acetate (PMA; 10 ng/ml), and incubated for specified periods at 37°C in a humidified atmosphere containing 5% CO_2. We have chosen polyclonal activation, as in previous experiments we showed that PBMC did not release cytokines spontaneously using ELISA detection, but had to be stimulated with combinations of T cell polyclonal activating agents. The doses chosen were those reported by our previous studies as giving optimal effects on cytokine expression without toxic or other action [24]. Culture supernatants were collected to quantify cytokine release. For mRNA analysis, culture conditions were the same.

RNA extraction

After incubation for an appropriate time, cells were isolated by brief centrifugation and then stored in 1 ml Trizol (Gibco) at −80°C until further processing. RNA extraction was performed using phenol/chloroform extraction and ethanol precipitation following the manufacturer's instructions. The RNA content of the solution was quantified using the optical density (OD) at 260 nm measured on a Genequant spectrophotometer (Pharmacia LKB, St Quentin en Yvelines, France) and the RNA aliquots were stored at –80°C until analysed. The ratio 260/280 nm was always more than 1·8.

Reverse-transcription and PCR

cDNA was synthesized from oligo-dT-primed RNA by reverse-transcription (RT) with M-MLV superscript reverse transcriptase (GIBCO). The total RNA mixture was incubated with 200 U M-MLV reverse transcriptase, 20 U RNasin, 0·5 mM dNTPs, 25 μg/ml primer dT, 10 mM dithiothreitol, 50 mM Tris–HCl pH 8·3, 75 mM KCl and 3 mM $MgCl_2$, in a final volume of 20 μl for 50 min at 42°C.

The final cDNA product was stored at –20°C for subsequent cDNA amplification by PCR.

Reaction mixtures for PCR of β-actin and cytokines contained 2 μl sample, 0.5 mM dNTP, 0·5 U Taq polymerase, 1 μg of each primer, 50 mM Tris–HCl, 3 mM $MgCl_2$, 75 mM KCl, 0·01% gelatin in a final volume of 100 μl. PCR was performed on a thermal cycler (Techne; OSI, Maurepas, France). Reaction times for β-actin were 94°C 1 min, 65°C 1 min, and 72°C 1 min for 28 cycles, followed by 10 min extension at 72°C. Reaction times for cytokine PCR were as follows: for IL-4 and IL-10, 94°C 1 min, 65°C 1 min, and 72°C 1 min; for IL-2, IL-13, IFN-γ, 94°C 1 min, 55°C 1 min, and 72°C 1 min for 38–40 cycles followed by 10 min extension at 72°C for all cytokines.

Primer sequences for the internal control, β-actin, were 5′-TAC ATG GCT GGG GTG TTG AA-3′ for the downstream primer, and 5′-AAG AGA GGC ATC CTC ACC CT-3′ for the upstream primer (Eurogenetech, Liege, Belgium). Primer sequences for cytokines were as follow: for IL-4, 5′-CTT CCC CCT CTG TTC TTC CT-3′ for the 5' primer, and 5′-TTC CTG TCG AGC CGT TTC AG-3′ for the 3′ primer (Eurogenetech); for IL-10, 5′-ATG CCC CAA GCT GAG AAC CAA GAC CCA-3′ for the 5' primer, and 5′-TCT CAA GGG GCT GGG TCA GCT ATC CCA-3′ for the 3′ primer (Eurogenetech); for IL-13, 5′-TGC CTC CCT CTA CAG CCC TCA-3′ for the 5' primer, and 5′-CAG TTG AAC CGT CCC TCG CG-3′ for the 3′ primer (Beite Kaito, Saint Maur, France); for IL-2, 5′-AAC TCC TGT CTT GCA TTG CAC TA-3′ for the 5′ primer, and 5′-TTG CTG ATT AAG TCC CTG GGTC-3′ for the 3′ primer (Beite Kaito); for IFN-γ, 5′-AGT TAT ATC TTG GCT TTT CA-3′ for the 5′ primer, and 5′-ACC GAA TAA TTA GTC AGC TT-3′ for the 3′ primer (Eurogenetech). The primer sequences were chosen in two different exons. These primer sequences were confirmed to specifically amplify the corresponding cytokine cDNA by Sourthern blot analysis using a fluorescein-labelled internal probe. cDNA products were visualized by gel electrophoresis in 2% agarose after ethidium bromide staining. A cDNA positive control, a negative control and a DNA ladder were run with all PCR reactions. Amplifications resulted in 200–400 bp product, as determined by electrophoresis on 2% TEA agarose gel containing bromide ethidium (BET).

Dot-blot analysis

PCR products were denatured and vacuum dot blotted onto Hybond-N + membrane (Amersham, Les Ulis, France). Specific probes were 3′-end labelled with fluorescein-11-dUTP using the ECL 3′-oligolabelling reagents (RPN 2130; Amersham). The sequences of internal probes were as follow: for β-actin, 5′-CCA ACT GGG ACG ACA TGG AGA AAA - 3′; for IL-2, 5′-GGC CAC AGA ACT GAA ACA TCT-3′; for IL-4, 5′-CTC GGT GCT CAG AGT CTT CTG CTC T-3′; for IL-10, 5′-CAG GTG AAG AAT GCC TTT AAT AAG CTC CAA CAG AAA GGC ATC TAC AAA GCC ATG AGT GAC TTT GAC ATC-3′; for IL-13, 5′-GGC AGC ATG GTA TGG AGC AT-3′; for IFN-γ, 5′-ATT TGG CTC TGC ATT ATT TTT CT GT-3′.

Following hybridization to the dot blots and incubation with anti-fluorescein–horseradish peroxidase (HRP) conjugate, the detection of the bound peroxidase was performed using hydrogen peroxide and luminol (RPN 2105; Amersham). The luminescence was detected by autoradiography on blue light-sensitive film (Hyperfilm-ECL; Amersham). The amount of each spot was determined by densitometry analysis (Vilbert Lourmat, Torcy, France). All of the cytokine PCR products were analysed comparatively to the amount of β-actin detected in the same mRNA sample. All samples were checked in the same test run for each cytokine analysed. Separate cycle course experiments confirmed linearity of amplification for β-actin and cytokine cDNA over 20–35 cycles and 30–45 cycles, respectively. For each PCR, linearity of amplification relative to cDNA dilutions was over 1/5–1/20 for IL-4 and IFN-γ, 1/5–1/40 for IL-13 and IL-2, 1/5–1/80 for IL-10 and β-actin.

Cytokine measurement by ELISA

Cytokine protein amounts in cell culture supernatants were determined using either specific ELISA kits for IL-2 (R&D Systems, Abingdon, UK) and IL-10 (Immunotech, Marseille, France) or ELISA house methods for IL-4 and IFN-γ as described elsewhere [24]. The sensitivity of these assays is 6 pg/ml for IL-2, 3 pg/ml for IL-10, 7 pg/ml for IL-4 and 10 pg/ml for IFN-γ.

Statistical analysis

Assays for cytokine quantification were performed in duplicate. Each data point is the average of two determinations, which varied within 2% of the average. Statistical analysis was performed using the non-parametric statistic Mann–Whitney *U*-test. *P* values <0·05 were considered significant. The coefficient of correlation of Pearson (*r*) was used to calculate the correlation between cytokine expression and serum IgE of logarithmically transformed data. All the analyses were performed using a computer package (Statistica release 4.5A for Windows; Statsoft, Tulsa, OK).

RESULTS

In vitro *cytokine production*

Production of cytokines in unstimulated and PHA/PMA- stimulated PBMC cultures from atopic patients and control subjects was measured at 24 h of incubation. Results show that mean levels of secreted IL-4 in stimulated cultures were higher in allergic subjects than in controls (P = 0·05) (Fig. 1a). IL-4 production was not enhanced when the culture period was increased to 72 h (data not shown). Spontaneous IL-4 production without stimulation was never detected (data not shown).

Mean levels of IL-10 in stimulated cultures were higher in atopic patients than in normal donors (statistical significance P = 0·002) (Fig. 1b). A slight spontaneous secretion of IL-10 could be detected in culture supernatants (range 100–300 pg/ml), and no significant differences were obtained between atopic subjects and controls (data not shown).

For the two groups of donors, comparable amounts of IL-2 and IFN-γ were synthesized following 24 h of stimulation, whatever the degree of allergenic sensitivity (P = 0·37 and P = 0·83, respectively) (see Fig. 1c,d).

These data indicate that secretion of IL-4 and IL-10 by PHA/PMA-stimulated PBMC was higher in atopic patients than in controls, while no differences were noticed for IL-2 and IFN-γ.

Expression of cytokine mRNA in PBMC cultures from atopic patients and healthy controls

Because the frequency of cytokine-producing cells in peripheral blood is very low, which probably explains why no spontaneous secretion of cytokines could be detected by ELISA, we studied the expression of cytokine genes in PBMC with a semiquantitative

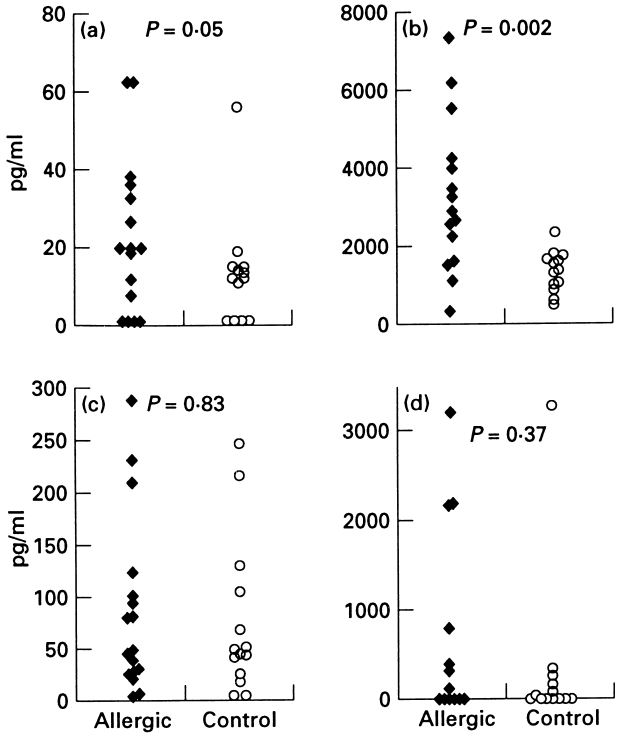

Fig. 1. Cytokine production by PBMC from atopic and normal subjects. PBMC were stimulated or not *in vitro* with phytohaemagglutinin (PHA) plus phorbol myristate acetate (PMA) for 24 h; then the culture supernatants were collected and the synthesis of IL-4 (a), IL-10 (b), IFN-γ (c) and IL-2 (d) was determined by ELISA. The values indicated as dots are from assays performed on three aliquots harvested from a single supernatant of one individual donor. The *P* values related to the differences between the two groups are indicated.

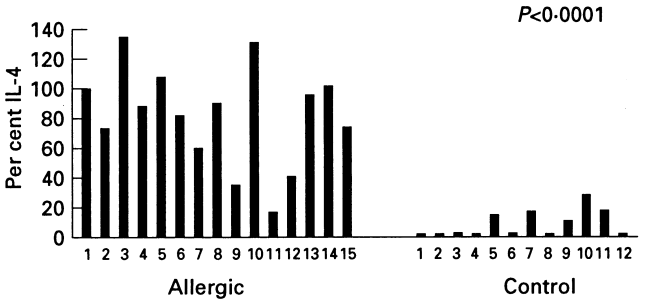

Fig. 2. IL-4 mRNA expression in normal and atopic subjects. IL-4 mRNA expression was analysed in PBMC from atopic donors compared with controls. The cytokine polymerase chain reaction (PCR) products were dot-blotted, hybridized with a specific probe, and the intensity of the signals was determined by densitometry and normalized to β-actin signal. The percentage of signal intensity obtained from individual subjects is presented.

PCR technique. Cytokine-specific cDNA were normalized to the intensity of the β-actin product as a standard marker.

Data revealed spontaneous IL-4 mRNA expression in all atopic patients, whereas normal donors in most cases did not show specific signals ($P < 0{\cdot}0001$) (Fig. 2). Representative examples of autoradiographs showing signals from PBMC of atopic and normal donors after PCR amplification and specific hybridization are shown in Fig. 3.

Although the technique of RT-PCR amplification used in this study remained semiquantitative, IL-10 mRNA expression appeared to be significantly enhanced in atopic patients compared with control subjects ($P < 0{\cdot}001$) (Fig. 4a).

As IL-13 has been reported to be involved in IgE production, we next examined its expression in mononuclear cells (Fig. 4b). Heterogeneous expression of IL-13 was observed in individuals from the two groups studied. Mean levels of IL-13 expression were slightly enhanced in atopic donors compared with controls, although the difference was not considered highly significant ($P = 0{\cdot}02$).

Data shown in Figs 3 and 5 indicate that the expression of mRNA for IFN-γ and IL-2 in atopic patients did not differ at all from control subjects ($P = 0{\cdot}31$ and $P = 0{\cdot}43$, respectively, for IFN-γ and IL-2).

Using RT-PCR techniques, our results show a clear enhancement of spontaneous IL-4 mRNA expression in atopic patients over controls, whereas IL-10 and IL-13 were less significantly enhanced. In addition, mRNA expression of the type 1-derived cytokines (IL-2 and IFN-γ) in the same experimental conditions did not differ between the two groups.

Cytokine expression versus *serum IgE*

Analysis of the amount of IL-4 mRNA expression revealed a lack of positive correlation with serum IgE levels (Fig. 6a), but rather a tendency to a negative correlation ($r = -0{\cdot}60$, $P = 0{\cdot}016$). Given that IgE production is considered to involve the production of type 2 cytokines, we were surprised with the lack of positive correlation of IL-4 mRNA expression relative to serum IgE. As IL-4-induced IgE production is to a large extent negatively regulated by Th1 population and especially by IFN-γ [9], we decided to analyse IFN-γ mRNA expression as well as the other cytokines in the same experimental conditions. As presented in Fig. 6b, no correlation at all was observed between IFN-γ and IgE. Results shown in Table 2 demonstrate an inverse correlation between IL-2 mRNA expression and serum IgE ($r = -0{\cdot}69$, $P = 0{\cdot}008$), and no correlation was observed with the other cytokines (IL-10 and IL-13). Similar results were observed when cytokine mRNA expression was compared with specific serum IgE (data not shown).

DISCUSSION

For a better understanding of the potential mechanisms underlying the induction of IgE responses, we examined the spontaneous cytokine pattern directly in PBMC. The striking finding in this study was that allergic donors could be differentiated on the basis of spontaneous IL-4 mRNA expression. The increased expression of IL-4 mRNA in atopic patients that we observed is consistent with the findings obtained in atopic dermatitis indicating increased IL-4 mRNA levels by Northern analysis after PMA/Ca ionophore stimulation [25], and those recently reported by Tang & Kemp that showed spontaneous IL-4 mRNA expression in children with atopic dermatitis [26]. However, we did not find any spontaneous IL-4 secretion whatever the degree of allergenicity or IgE levels. While the ELISA method that we used displayed a threshold of 7 pg/ml, one could suggest that the sensitivity of the method remained not sufficient for the detection of cytokine production. Several reports have indicated data that are somewhat controversial. The lack of spontaneous IL-4 secretion in PBMC was also

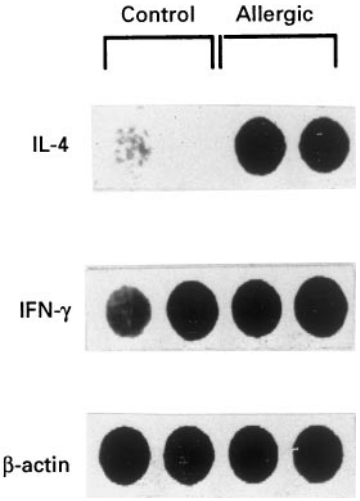

Fig. 3. Differential expression of mRNA for IL-4 and IFN-γ in PBMC from atopic subjects. The cytokine polymerase chain reaction (PCR) products were dot-blotted, hybridized with a specific probe, and the resulting autoradiographs were shown comparatively to those of β-actin signals.

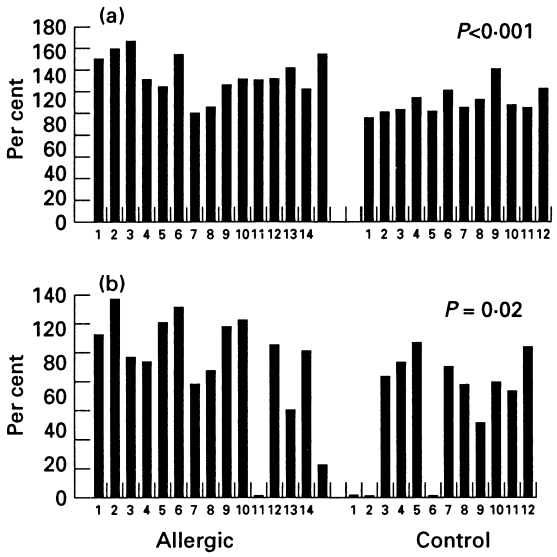

Fig. 4. Differences in the expression of mRNA for IL-10 and IL-13 in normal and atopic patients. The expression of mRNA for IL-10 (a), IL-13 (b) in PBMC from normalized atopic donors is shown as percentages relative to the signals of β-actin. The results from individual subjects are presented.

found by other groups [20,21,26–28]. However, another study showed spontaneous IL-4 production in isolated bronchoalveolar T cells and PBMC [14]. These discrepancies could be explained by the poor sensitivities of the IL-4 assays used, the low frequency of cytokine-producing cells in peripheral blood, and the use of different experimental conditions. In our study, we can effectively rule out the possibility of a lack of correlation between gene transcription and active synthesis of the relevant protein, since the increased spontaneous mRNA expression for IL-4 as detected by PCR is compatible with the secretion of this cytokine ($P < 0{\cdot}05$) after PHA/PMA activation that leads to appropriate intracellular pathways of activation.

A less but significant increase in IL-10 mRNA expression was obtained in atopic patients, and this was supported by an increase in IL-10 secretion after polyclonal stimulation. Although IL-13 was considered as a Th0/Th2 product [7], and was strongly suspected to have a major role in IgE and IgG4 production by human B cells in an IL-4-independent manner during *in vitro* studies [29], no study has yet investigated the spontaneous expression of IL-13 in allergic patients. Our results indicated that IL-13 mRNA expression was slightly enhanced in atopic patients, although this did not reach a high statistical significance.

In contrast, IFN-γ mRNA expression as well as IFN-γ protein secretion in mononuclear cells from atopic subjects remained unchanged compared with control healthy subjects. These data are consistent with the study of Robinson *et al.*, indicating that the expression of mRNA for IFN-γ in bronchoalveolar T lymphocytes was not modified in atopic asthma [12]. Normal production of IFN-γ in atopic patients was also described by other groups [22,23]. In contrast, other studies showed a decrease in IFN-γ production [20,22,30].

Pathogenic concepts of allergic diseases include a central role for differential cytokine production by $CD4^+$ T cell subsets. In particular, Th0/Th2-like profile seems to be involved in the regulatory responses to helminth parasites and in IgE production [4]. The *in vivo* evidence of these T cell subsets in atopic populations is not yet clearly demonstrated. In the site of the reaction, expression of both IL-4 and IL-5 in bronchoalveolar lavage T lymphocyte populations and in bronchial biopsies was observed in atopic subjects with asthma [12,13,31]. Accordingly, expression of mRNA for IL-3, IL-4, and IL-5 was shown to be present in skin biopsies in atopic subjects [11,32]. IL-10 mRNA

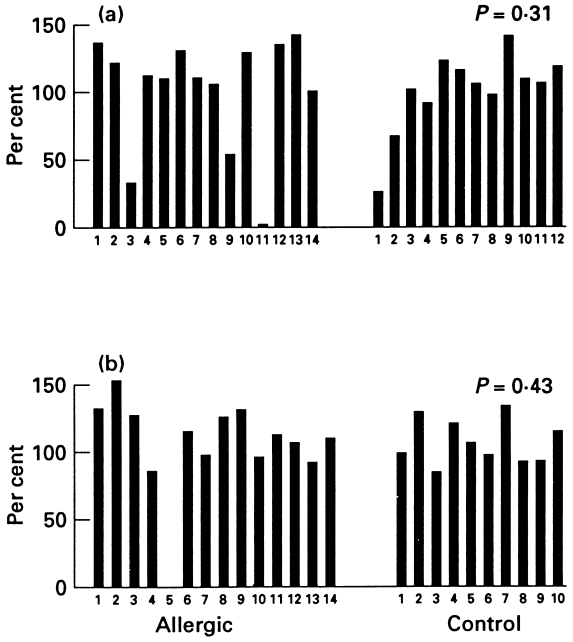

Fig. 5. Differences in the expression of mRNA for IFN-γ and IL-2 in normal and atopic patients. The expression of mRNA for IFN-γ (a) and IL-2 (b) in PBMC from normalized atopic donors is shown as percentages relative to the signals of β-actin. The results from individual subjects are presented.

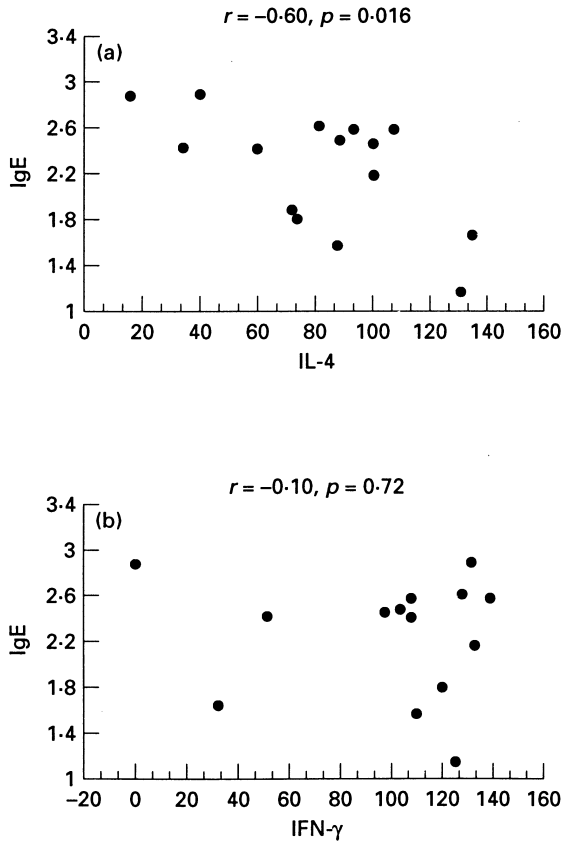

Fig. 6. Analysis of correlation of serum IgE with IL-4 and IFN-γ mRNA expression. Levels of IL-4 and IFN-γ mRNA expression were normalized to β-actin expression by mononuclear cells. The scatterplots are presented. (a) IL-4. (b) IFN-γ.

Table 2. Serum IgE levels *versus* cytokine

	Coefficient, *r*	*P*
IL-2	−0·69	0·008
IL-10	0·35	0·18
IL-13	−0·23	0·4

Analysis of correlation between serum IgE and the expression of mRNA for IL-2, IL-10 and IL-13. *P* values and coefficients *r* were indicated for each cytokine.

expression was investigated only in atopic dermatitis patients, and an over-expression of this cytokine was observed in this case [33].

Our results demonstrated a tendency of PBMC from atopic donors to express a Th2-like pattern with a particularly strong expression of IL-4. Elevated expression of IL-4 occurred in the abscence of a decreased expression of IFN-γ and IL-2, and this does not accord well with the classification of the antagonistic Th1/Th2 subpopulations. In addition, IL-13, which is strongly suspected to have a major role in allergic responses, was found to be not clearly enhanced. The functional classification of T cell clones *in vitro* may therefore be not sufficiently flexible to account for the environment of these cells *in vivo* during the disease, and ignored the influence of paracrine regulatory mechanisms during cell–cell interactions in blood. In fact, the classified Th2 cytokines can also be produced by Th1 cells such as IL-10 [34], and by non-T cells, including monocytes/macrophages, B cells, basophils/mast cells, eosinophils [35–37]. Production of IL-4 and IL-13 by mast cells was recently demonstrated *in vivo* in allergic rhinitis by Bradding *et al.* [37]. In addition, $CD8^+$ T cells and γ/δ T cells were shown to express IL-4 and to control the IgE response [38,39]. On the other hand, $CD4^+$ T cells, $CD8^+$ T cells and natural killer (NK) cells represent the possible source of IFN-γ. The differential implication of these cell types in the regulation of the cytokine profile in blood of atopic patients remains to be established.

Whether our results imply that IL-4 and IL-10 play a major role in pathogenic mechanisms of IgE-induced allergy remains to be determined, and probably depends on the relative contribution of each cytokine and the expression of the corresponding receptors. IL-4 is crucial for switching B lymphocytes to IgE production [4], and IL-13 also has an important role in IgE synthesis [7,40]. It is intriguing to speculate on the role of IL-10 in the pathogenesis of allergic diseases. IL-10 induces B cell proliferation and production of the isotypes IgM, IgG and IgA, with no apparent effect on IgE synthesis [41]. In addition, the Th2 cytokines are well known to be able to induce mast cell growth [3,42], and were reported to be present at the site of the reaction during the late phase of allergic reactions [33,43,44].

In this study, all patients were selected during their acute symptomatologic phase. There is an immediate question: does the cytokine pattern of the allergic patients' blood determine the atopic state of the patients or the development of the allergic disease? Given that IgE production is largely considered to involve the production of Th2 or type 2 cytokines, we were surprised with the lack of positive correlation of IL-4 mRNA expression relative to residual serum IgE, but rather a tendency to a negative correlation, whereas comparatively no correlation at all was observed with IL-10, IL-13 and IFN-γ. These data are supported by the results of the Aarden's group that have already shown no correlation between IgE and IL-4 production after 3 days *in vitro* culture of lymphocytes stimulated with anti-CD2 and rIL-2 [45]. In relative discordance, other groups have shown that high and low IgE responders are under the control of IL-4 expression [20], and that IL-4 production in atopic children correlated with serum IgE [46], although analysis of IL-4 mRNA expression was not reported in these papers. Additionally, an inverse correlation between serum-specific IgE levels and IFN-γ production was also reported in allergic PBMC [47]. There are a number of possible explanations for these results. It is now clear that measurement of IgE in the serum as the only screening for allergic diseases is unreliable, as it was found to have a low predictive value of 50%, because some asymptomatic subjects have relatively high IgE levels and symptomatic subjects can have low levels [48]. It is well known that the late response is associated with an influx into the site of the reaction of inflammatory cells such as eosinophils [49]. An increase in mononuclear cells such as T lymphocytes has also been observed [49], although its significance has for a long time been largely ignored. Thus one could suggest that blood mononuclear cells producing IL-4 may have a significant role in promoting local IL-4-producing cells as well as IgE production, by inducing the switch mechanism events.

Another explanation is the possible involvement of another IgE-inducing factor in atopic donors. It was effectively shown that the control of IgE synthesis *in vitro* requires not only IL-4 and/or IL-13 but also a second signal involving the interaction CD40L/

CD40 and CD23/CD21 between T and B cells [50]. More recently, it was shown that the coexpression of both IL-4 and CD40L on mast cells could also promote the local differentiation of IgE-producing B lymphocytes [51] and the development of allergic reactions. On the other hand, IL-4 (and IL-10) could induce growth of mast cells and their progenitors [3,42] that may be important in the progression of allergic disease.

Taken together, our results have provided novel and direct evidence *in vivo* for an increase in mRNA expression for IL-4 and IL-10 spontaneously in PBMC from symptomatic atopic donors, suggesting rather a type 2-like cytokine profile, with IL-4 as the most stringent discriminatory cytokine. Expression of the type 1 cytokines was not modified. Additionally, a lack of positive correlation between serum IgE and IL-4 mRNA expression was observed. These data further substantiate that IL-4 is a critical factor during allergic diseases, and strongly suggest that the determination of IL-4 mRNA expression in blood mononuclear cells may be significant in the analysis of clinical allergic responses in atopic donors.

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REFERENCES

1 Romagnani S. Lymphokine production by human T cells in disease states. Annu Rev Immunol 1994; **12**:227–57.
2 Benbernou N, Matsiota-Bernard P, Jolivet C *et al.* Tumour necrosis factor, IL-1 and IL-6 in bronchoalveolar washings and their *in vitro* production during *Nippostrongylus brasiliensis* infection. Clin Exp Immunol 1992; **88**:264–8.
3 Romagnani S, Del Prete G, Maggi E *et al.* Role of interleukins in induction and regulation of human IgE synthesis. Clin Immunol Immunopathol 1989; 5**0**:s13–s23.
4 Romagnani S. Regulation and deregulation of human IgE synthesis. Immunol Today 1990; **11**:316–21.
5 Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989; **7**:145–73.
6 Benbernou N, Matsiota-Bernard P, Guenounou M. Antisense oligonucleotides to IL-4 regulate IgE and IgG2a production by spleen cells from *Nippostrongylus brasiliensis* infected rats. Eur J Immunol 1993; **23**:659–63.
7 McKenzie ANJ, Gulpepper JA, de Waal Malefyt R *et al.* Interleukin-13, a novel T cell-derived cytokine that regulates monocyte and B cell function. Proc Natl Acad Sci USA 1993; **90**:3735–9.
8 de Vries JE. Novel fundamental approaches to intervening in IgE-mediated allergic diseases. Dermatol 1994; **102**:141–4.
9 Maggi E, Parronchi P, Manetti R *et al.* Reciprocal regulatory effects of IFN-γ and IL-4 on the *in vitro* development of human Th-1 and Th-2 clones. J Immunol 1992; **148**:2142–7.
10 Mosmann TR, Cherwinski H, Bond MW *et al.* Two types of murine helper T cell clone. J Immunol 1986; **136**:2348–57.
11 Kay AB, Ying S, Varney V *et al.* Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. J Exp Med 1991; **173**:775–8.
12 Robinson DS, Qutayba H, Ying S *et al.* Predominant Th-2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992; **326**:298–304.
13 Durham SR, Ying S, Varney VA *et al.* Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 1992; **148**:2390–4.
14 Walker C, Bode E, Boer L *et al.* Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. Am Rev Respir Dis 1992; **146**:109–15.
15 Wierenga EA, Snoek M, Henk M *et al.* Human atopen-specific types 1 and 2 T helper cell clones. J Immunol 1991; **147**:2942–9.
16 Parronchi P, Macchia D, Piccini MP *et al.* Allergen-bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. Proc Natl Acad Sci USA 1991; **88**:4538–43.
17 van der Heijden FL, Wierenga EA, Bos JD *et al.* High frequency of IL-4-producing $CD4^+$ allergen-specific T lymphocytes in atopic dermatitis lesional skin. J Invest Dermatol 1991; **97**:389–94.
18 Yssel H, Johnson KE, Schneider PV, Wideman J, Terr A, Kastelein R, de Vries JE. T cell activation-inducing epitopes of the house dust mite allergen Der pI. J Immunol 1992; **148**:738–45.
19 Pène J. Heterogeneity of atopy demonstrated by differences in cytokine release by peripheral blood T cells. Ann Allergy 1993; **71**:322–6.
20 Pène J, Rivier A, Lagier B *et al.* Differences in IL-4 release by PBMC are related with heterogeneity of atopy. Immunology 1994; **81**:58–64.
21 Röken M, Mùller KM, Saurat JH *et al.* Lectin-mediated induction of interleukin 4 producing $CD4^+$ T cells. J Immunol 1991; **146**:577–81.
22 Tang MLK, Kemp AS, Varigos G. IL-4 and interferon-gamma production in children with atopic disease. Clin Exp Immunol 1993; **92**:120–4.
23 Paganelli R, Scala E, Capobianchi MR *et al.* Selective deficiency of interferon-gamma production in the hyper-IgE syndrome. Relationship to *in vitro* IgE synthesis. Clin Exp Immunol 1991; **84**:28–33.
24 Benbernou N, Esnault S, Potron G *et al.* Regulatory effects of pentoxyfilline on T helper cell-derived cytokine production in human blood cells. J Cardiovasc Pharmacol 1995; 2**5**: (Suppl. 2a):575–9.
25 Brown MA, Li SH, Chan SC *et al.* Interleukin 4 mRNA expression by normal and atopic T lymphocytes. Clin Res 1989; **37**:406A.
26 Tang MLK, Kemp AS. Spontaneous expression of IL-4 mRNA in lymphocytes from children with atopic dermatitis. Clin Exp Immunol 1994; **97**:491–8.
27 Jujo K, Renz H, Abe J *et al.* Decreased interferon gamma and increased interleukin-4 production in atopic dermatitis promotes IgE synthesis. J Allergy Clin Immunol 1992; **90**:323–31.
28 Rousset F, Robert J, Andary M *et al.* Shifts in interleukin-4 and interferon-g production by T cells of patients with elevated serum IgE levels and modulatory effects of these lymphokines on spontaneous IgE synthesis. J Allergy Clin Immunol 1991; **87**:58–63.
29 Punnonen J, Aversa GG, Cocks BG *et al.* Interleukin-13 and interleukin-4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc Natl Acad Sci USA 1993; **90**:3730–4.
30 Tang MLK, Varigos G, Kemp AS. Reduced interferon-gamma (IFN-γ) secretion with increased IFN-γ mRNA expression in atopic dermatitis: evidence for a post-transcriptional defect. Clin Exp Immunol 1994; **97**:483–90.
31 Del Prete GF, De Carli M, D'Elios MM *et al.* Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. Eur J Immunol 1993; **23**:1445–9.
32 Varney VA, Hamid QA, Gaga M *et al.* Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. J Clin Invest 1993; **92**:644–51.
33 Ohmen JD, Hanifin JM, Brian JN *et al.* Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. J Immunol 1995; **154**:1956–63.
34 DelPrete G, DeCarli M, Almerigogna F *et al.* Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and

inhibits their antigen-specific proliferation and cytokine production. J Immunol 1993; **150**:353–60.
35 Seminario MC, Gerald JG. The role of eosinophils in the pathogenesis of asthma. Curr Opin Immunol 1994; **6**:860–4.
36 Romagnani S. Regulation of the development of type 2 T-helper cells in allergy. Curr Opin Immunol 1994; **6**:838–46.
37 Bradding P, Feather IH, Wilson S *et al.* Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. J Immunol 1993; **151**:3853–65.
38 Ferrick DA, Schrenzei MD, Mulvania T *et al.* Differential production of interferon-γ and interleukin-4 in response to Th1- and Th2-stimulating pathogens by $\gamma\delta$ T cells *in vivo*. Nature 1995; **373**:255–7.
39 McMenamin C, Holt PG. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted $CD8^+$ T cell-mediated but MHC class II-restricted $CD4^+$ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. J Exp Med 1993; **178**:889–93.
40 Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 1994; **15**:19–26.
41 Rousset F, Garcia E, DeFrance T *et al.* Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 1992; **89**:1890–5.
42 Thompson-Snipes L, Dhar V, Bond MW *et al.* Interleukin 10: a novel stimulatory factor for mast cells and their progenitors. J Exp Med 1991; **173**:507–12.
43 Masuyama K, Jacobson MR, Rak S *et al.* Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Clin Exp Immunol 1994; **82**:192–9.
44 Emery BE, White MV, Igarashi Y *et al.* The effect of IL-4 on human nasal mucosal responses. J Allergy Clin Immunol 1992; **90**:772–8.
45 Van Der Pouw Kraan CTM, Aalberse RC, Aarden LA. IgE production in atopic patients is not related to IL-4 production. Clin Exp Immunol 1994; **97**:254–9.
46 Tang MLK, Coleman J, Kemp AS. Interleukin-4 and interferon-gamma production in atopic and non-atopic children with asthma. Clin Exp Allergy 1995; **25**:515–21.
47 Dorion BJ, Burks AW, Harbeck R *et al.* The production of interferon-gamma in response to a major peanut allergy, Ara h II correlates with serum levels of IgE anti-Ara h II. J Allergy Clin Immunol 1994; 9**3** (1 Pt 1):93–99.
48 Backer V, Ulrik CS, Wendelboe D *et al* .Distribution of serum IgE in children and adolescents aged 7–16 years in Copenhagen, in relation to factors of importance. Allergy 1992; **47**:484–9.
49 Bascom R, Pipkorn U, Lichtenstein LM *et al.* The influx of inflammatory cells into nasal washings during the late response to antigen challenge: effect of corticosteroid pretreatment. Am Rev Respir Dis 1988; **138**:406–10.
50 Zhang K, Clark EA, Saxon A. CD40 stimulation provides an IFN-gamma-independent and IL-4-dependent differentiation signal directly to human B cells for IgE production. J Immunol 1991; **146**:1836–42.
51 Gauchat JF, Henchoz S, Mazzel G *et al.* Induction of human IgE synthesis in B cells by mast cells and basophils. Nature 1993; **365**:341–3.