

The profiles of interleukin (IL)-2, IL-6, and interferon-gamma production by peripheral blood mononuclear cells from house-dust-mite-allergic patients: a role for IL-6 in allergic disease

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We have developed a model to measure cytokine production by peripheral blood mononuclear cells (PBMC) *in vitro*. In this report, we examine the production of interleukin-2 (IL-2), IL-6, and interferon-gamma (IFN- γ) by PBMC of house-dust-mite (*Dermatophagoides pteronyssinus*)-allergic subjects. When stimulated with specific allergen (*D. pteronyssinus*), PBMC of patients produced significant levels of IL-2 and high levels of IL-6, but little or no IFN- γ . Nonatopic control PBMC also produced IL-6, although at lower levels, but no IL-2 or IFN- γ . A ubiquitous antigen, streptokinase/streptodornase (SKSD), induced high levels of IL-2 in patients, but only low levels of IFN- γ and IL-6. Nonatopic controls produced similar levels of IL-2 and IL-6, but high levels of IFN- γ to SKSD. IL-2 and IFN- γ levels induced by the T-cell mitogen phytohaemagglutinin (PHA) were similar in patient and control groups, but IL-6 levels were significantly lower in the patients. IgE synthesis *in vitro* was shown only in atopic PBMC cultures stimulated with specific allergen. The major points can be summarized as 1) IL-2 production by atopic patients in response to allergen; 2) IL-6 production to allergen by both atopic and nonatopic patients, but significantly increased in atopic patients; and 3) defective IFN- γ production by atopic patients to both allergen and antigen. These findings suggest that IL-6 may be important in the immune response to inhaled allergens such as *D. pteronyssinus*, possibly by creating a cytokine environment favourable to a T_H2 response, and that atopic patients exhibit a generalized defect of IFN- γ production, not related to the response to allergen.

S. M. McHugh, A. B. Wilson, J. Deighton,
P. J. Lachmann, P. W. Ewan

Molecular Immunopathology Unit, MRC Centre,
Hills Road, Cambridge CB2 2QH, UK

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Dr S. M. McHugh
Molecular Immunopathology Unit
MRC Centre
Hills Road
Cambridge CB2 2QH
UK

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Immunoglobulin isotype selection is closely regulated by cytokines produced by antigen-specific T cells and accessory antigen-presenting cells (4). Interleukin (IL)-4 plays a central role in inducing IgE synthesis (12, 26, 30), and this effect is inhibited by interferon-gamma (IFN- γ) (24). IL-4-induced IgE expression has been shown to be augmented by IL-5 and IL-6 (29).

The role of IL-6 in immune response regulation and in allergic disease is less clear. IL-6 is a pleio-

tropic cytokine, classically associated with the induction of immunoglobulin in B cells, acute-phase proteins in liver cells, and cytokine T-cell differentiation and activation of haemopoietic stem cells (14). Dysregulation of IL-6 has been implicated in the pathogenesis of several conditions including rheumatoid arthritis, where it is found with other monokines in the joints (13), and in Alzheimer's disease, where it has been implicated in amyloidogenesis (28). As a terminal B-cell differentiation fac-

tor, IL-6 is likely to be involved in disorders where there is aberrant regulation of immunoglobulin, such as hypergammaglobulinaemia (25), parasitic infections (2), and allergic disease where there is overproduction of IgE.

There is now convincing evidence that the different functional phenotypes of T-helper (T_H) cells originally described in the mouse (19) also exist in man (30). These cells are distinguished by their pattern of cytokine secretion. In man, T_H2 cells principally produce IL-4, IL-5, and IL-6, whereas T_H1 cells secrete mainly IFN- γ , while IL-2 is probably secreted by both T_H1 and T_H2 cells. Additionally, it is now widely recognized that an intermediate or precursor subset also exists, the T_H0 phenotype. These cells can secrete an overlapping combination of cytokines which individually contribute to the discrete T_H1 and T_H2 extremes (7). A predominantly T_H2 response leads to IgE production and activation of mast cells and eosinophils, whereas a T_H1 response inhibits IgE production.

Studies using allergen-specific T-cell clones (TCC) suggest that in atopic subjects the T-cell response to allergen is abnormal, being predominantly T_H2 in type, whereas the response in nonatopic subjects to both allergen and bacterial antigens is mainly T_H1 (1, 22). This rather clear-cut pattern has emerged from several studies. Other data, however, suggest that the picture may not be as clear-cut as this. It has recently been shown that bacterial antigen-specific T-cell clones from atopic subjects behave abnormally in being able to produce T_H1 and T_H2 cytokines on stimulation with antigen (20), whereas earlier studies showed that the normal response of such clones from nonatopic subjects was to produce T_H1 cytokines only (1). In addition, murine T-cell clones have been described which produce both T_H1 and T_H2 cytokines, showing that not all clones need be predominantly T_H1 or T_H2 cytokine producers (7).

We have chosen to study cytokine production from peripheral blood mononuclear cells (PBMC), rather than TCC. This model avoids the problems of selection inherent in studies of clones, and may give a more representative picture of T-cell responses *in vivo*. It also includes a variety of cell types, particularly antigen-presenting cells, and may thus more accurately reflect the local cytokine milieu generated during response to allergen *in vivo*.

The main abnormality described in allergic disease is excess IL-4 production, in response to allergen, and most studies with TCC focus on IL-4 and IFN- γ production stimulation. There have been relatively few studies on the role of IL-6. In this study, we have investigated the profile of production of IL-6, IFN- γ , and IL-2 by PBMC from patients allergic to *D. pteronyssinus*, and have also measured

in vitro IgE synthesis. Our findings suggest an important role for IL-6 in allergic disease.

Material and methods

Subjects

Eleven subjects, five male and six female, who had house-dust-mite (HDM) (*D. pteronyssinus*)-allergic rhinitis, and six nonatopic controls, three male and three female, were included in this study. Full characterization of the subjects appears elsewhere (18).

Generation of culture supernatants for cytokine assays

PBMC were stimulated with varying concentrations of either a T-cell mitogen, phytohaemagglutinin (PHA)-L (lectin from *Phaseolus vulgaris*, Sigma), or a recall antigen SKSD (streptokinase/streptodornase, Lederle Laboratories, Gosport, UK), or specific allergen *D. pteronyssinus* (PharmalgenTM, ALK, Hørsholm, Denmark). Preparation of PBMC, culture conditions, and methods for supernatant generation have been described previously (18). Concentrations of stimuli giving maximal proliferative responses were those used to generate supernatants for cytokine measurement: 10 μ g/ml PHA, 10 μ g/ml SKSD, and 1000 BU/ml *D. pteronyssinus* (18). The culture medium used in all PBMC assays was RPMI-1640 (Flow Laboratories, Irvine, UK) supplemented with 100 units/ml penicillin (Sigma Chemical Co, Poole, UK), 100 μ g/ml streptomycin (Sigma), 2 mM L-glutamine (Sigma), 20 mM N-2-hydroxyethylpiperazine-N'-2 ethanesulphonic acid (HEPES, Merck Ltd, Poole, UK) (standard RPMI), and 10% heat-inactivated AB⁺ normal human serum (Department of Haematology, Addenbrookes Hospital, Cambridge, UK).

Determination of IL-2 levels

IL-2 levels were determined by a CTLL bioassay by the method of Gillis et al. (6), as previously described (18). Briefly, cells were washed three times in standard RPMI (without HEPES) supplemented with 2×10^{-5} mol/l 2-ME (Sigma) and 1% AB⁺ NHS, and resuspended in the same medium at a concentration of 4×10^4 cells/ml. Next, 100- μ l aliquots of recombinant human IL-2 (Cetus Diagnostics, Emeryville, CA, USA) standards were prepared in standard RPMI + 2-mercaptoethanol + 10% AB⁺ NHS, and plated out in triplicate on 96 U-well microtitre plates (Nunc). Then 100- μ l aliquots of culture supernatants and internal standards were added in duplicate, and 100 μ l CTLL cell suspension was added to each well, giving a final concentration of 2×10^4 cells/

well. Plates were incubated for 24 h in a 5% CO₂ humidified incubator at 37°C, harvested, and counted as below. The specificity of the cell line for IL-2 was established by titrating monoclonal anti-rIL-2 (British Biotechnology Ltd, Oxford, UK) in culture supernatants and in IL-2-spiked medium. Recombinant human IL-4, IL-5, IL-6, and IFN- γ , as well as the stimuli used (PHA and SKSD) were not reactive in this assay. Good qualitative and quantitative results were obtained between samples tested in this bioassay and in a commercially available enzyme-linked immunosorbent assay (ELISA) (British Biotechnology Ltd QuantikineTM IL-2 ELISA; data not shown). The sensitivity of this assay was 0.004–> 10 U/ml ($\approx$ 0.5–> 1250 pg/ml).

IL-6 bioassay with MH60 BSF2 cell line

IL-6 was measured in culture supernatants by the method of Matsuda et al. (17). Briefly, the IL-6-dependent murine B-cell hybridoma clone, MH60 BSF2 (kindly donated by Dr T. Hirano, Osaka, Japan), was cultured at $2\text{--}4 \times 10^4$ cells/ml in 100- μ l aliquots with an equivalent volume of the test samples for 48 h. Samples were serially diluted 1:10 (neat to 1:10 000) to obtain readings on the standard curve, because high amounts of IL-6 (> 1 ng/ml) were found to be toxic or inhibitory to the MH60 cell line. Cells were pulsed with 2 μ l (0.7 μ Ci) of 6-³H-labelled thymidine (Amersham International, Amersham, UK), diluted to 10 Ci/mmol and 10 μ g/ml thymidine, for the last 6 h of culture, and harvested (Titertek cell harvester; Skatron AS, Lier, Norway) onto glass fibre filter paper (Whatman GFA; Whatman International Ltd, Maidstone, UK), dried, and prepared for liquid scintillation.

β -radioactivity was measured in a scintillation counter (LKB 1216 Rackbeta, LKB Instruments Ltd, South Croydon, UK). IL-6 reactivity of the MH60 BSF2 cells was calibrated against recombinant human IL-6 in the dose range 0.003–1000 pg/ml. Various other recombinant human cytokines and the stimuli used in culture were tested to establish potential synergy, inhibition, or toxicity: these included rIL-2 (Cetus), rIFN- γ (Biogen Research Corporation, Biogen Ltd, London, UK), rIL-4 (British Biotechnology Ltd, Oxford, UK), and rTNF- β (Genentec, Inc, San Francisco, CA, USA). The specificity of the cell line for IL-6 was established by titrating monoclonal anti-rIL-6 (British Biotechnology Ltd, Oxford, UK) in culture supernatants and in IL-6-spiked medium. The lower level of sensitivity of this assay was 0.1–0.3 pg/ml. For maintenance of MH60 BSF2 cultures, the serum concentration was reduced to 1% and the medium supplemented with 2-ME (2×10^{-5} mol/l). Cultures were split and fed every 3–4 days with medium containing 150 pg/ml

rhIL-6 (British Biotechnology Ltd, Oxford, UK). The results from positive samples were tested in a commercially available ELISA (British Biotechnology IL-6 Quantikine ELISA), excellent qualitative and reasonable quantitative results being obtained.

Inhibition ELISA for IFN- γ

Ninety-six-well micro-ELISA plates (Dynatech Immulon M129B) were coated with 0.2 μ g/ml recombinant human IFN- γ (specific activity 1.3×10^6 U/ml, 100 μ g/ml; Biogen) in carbonate-bicarbonate buffer, pH 9.6, overnight at 4°C, with a working volume of 100 μ l. Plates were washed four times with phosphate-buffered saline (PBS) containing 0.5% Tween 20 (PBS-T) and blocked with 200 μ l 5% heat inactivated FCS in PBS-T for 1 h at room temperature. After one wash with PBS-T, 50- μ l aliquots of standards (rIFN- γ diluted in culture medium) and samples (culture supernatants) were added, immediately followed by 50 μ l/well of a 1:50 000 dilution of monoclonal antihuman IFN- γ (20-G7 donated by Celltech) in PBS-T + 5% FCS, with the relevant controls. The plates were shaken to mix the well contents and left overnight at 4°C. After three washes with PBS-T and one with distilled water, 100 μ l/well of goat antimouse IgG, conjugated with alkaline phosphatase (Sigma) diluted 1:750 in PBS-T + 5% FCS, was added and incubated for 4 h at room temperature. Plates were washed three times with distilled water, and 100 μ l/well of the enzyme substrate *p*-nitrophenyl phosphate at 1 mg/ml in diethanolamine buffer (pH 9.8) was added and the colour allowed to develop for 2 h at 37°C in the dark. Plates were read at 405 nm in an automated plate reader (Bio-Rad 3550). Results were analysed with Microplate Manager software (Bio-Rad). The lower level of sensitivity of this assay was between 0.03 and 0.1 ng/ml.

ELISA for detection of in vitro IgE production

Micro-ELISA plates (Dynatech Immulon M129 B) were coated with mouse monoclonal antihuman IgE (Southern Biochemical Associates) at 1 μ g/ml in carbonate-bicarbonate buffer, pH 9.6, overnight in a moist box at 4°C, with a working volume of 100 μ l/well. Plates were washed three times in wash buffer (PBS-T with 0.2% human serum albumin and 10 μ M NaN₃), and 100 μ l/well of IgE standards (serum pool calibrated against the World Health Organization second international reference preparation of human serum IgE 75/502), diluted in culture medium, and samples (culture supernatants) were added and incubated for 3 h at room temperature. Plates were washed three times in wash buffer, and 100 μ l/well of biotinylated antihuman IgE (The Binding

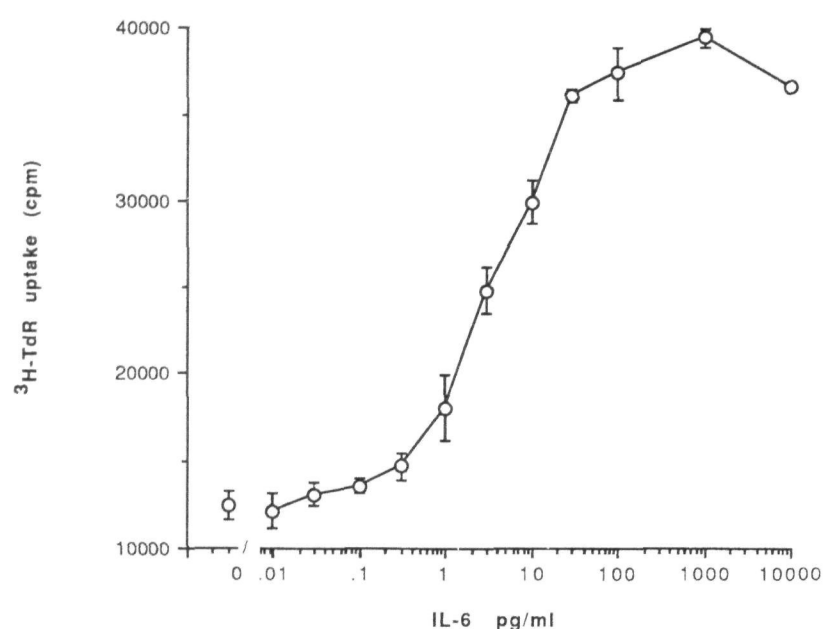


Fig. 1. Representative standard curve for IL-6 bioassay with MH60 BSF2 cell line showing ³H-labelled thymidine uptake against IL-6 concentration (pg/ml). Each point is mean $\pm$ SE of triplicate samples.

Site) diluted 1:500 in wash buffer was added and incubated for 90 min at room temperature. After two washes with wash buffer and one with distilled water, 100 μ l/well avidin-alkaline phosphatase conjugate (Sigma) diluted 1:500 in wash buffer was added and incubated for 90 min at room temperature. Plates were washed twice with wash buffer and once with distilled water, and then 100 μ l/well of the enzyme substrate *p*-nitrophenyl phosphate at 1 mg/ml in diethanolamine buffer (pH 9.8) was added and the colour allowed to develop for 1 h at 37°C in the dark. Plates were read at 405 nm. The lower level of

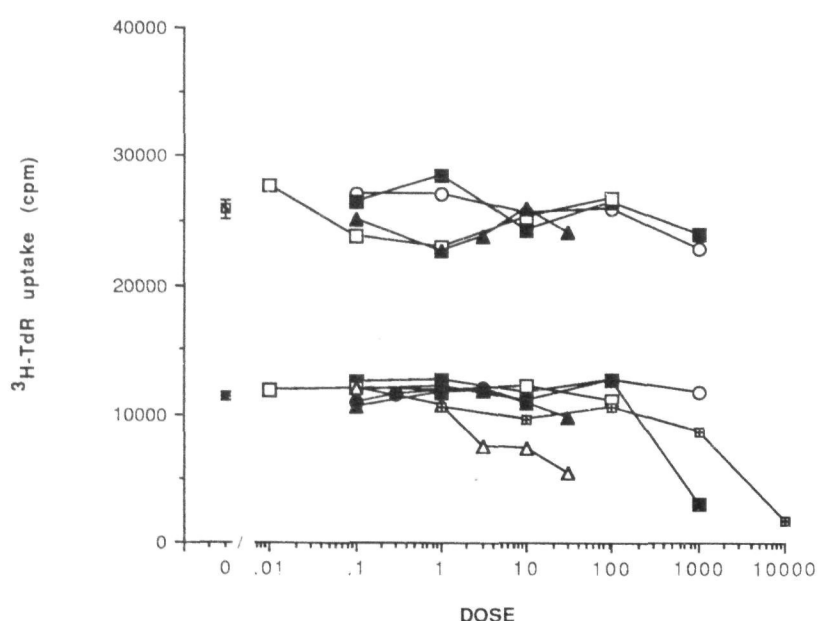


Fig. 2. Effect of various cytokines and stimuli on MH60 cell proliferation, to test for additive/synergistic/inhibitory effects in presence (top traces) or absence (lower traces) of 10 pg/ml IL-6. $\circ$ = IFN- γ (U/ml); $\blacksquare$ = IL-4 (U/ml); $\square$ = IL-2 (U/ml); $\blacktriangle$ = TNF- β (U/ml); $\triangle$ = SKSD (μ g/ml); $\bullet$ = PHA (μ g/ml); $\boxplus$ = *D. pteronyssinus* (BU/ml); $\square$, $\times$ = respective controls.

sensitivity of this assay was approximately 0.41 IU/ml (90 pg/ml). Only supernatants generated at time zero and after incubation for 9 days (maximum time) were tested (seven patients and four controls).

Results

IL-6 assay calibration

Fig. 1 shows a representative standard curve for recombinant human IL-6 titrated against the MH60 BSF2 cell line. Half-maximal tritiated thymidine (³H-labelled TdR) incorporation was detected at 6 pg/ml, in close agreement with Matsuda et al. (17). Fig. 2 shows that human rIFN- γ , rIL-4, rIL-2, and rTNF- β did not interfere with the IL-6 assay, and

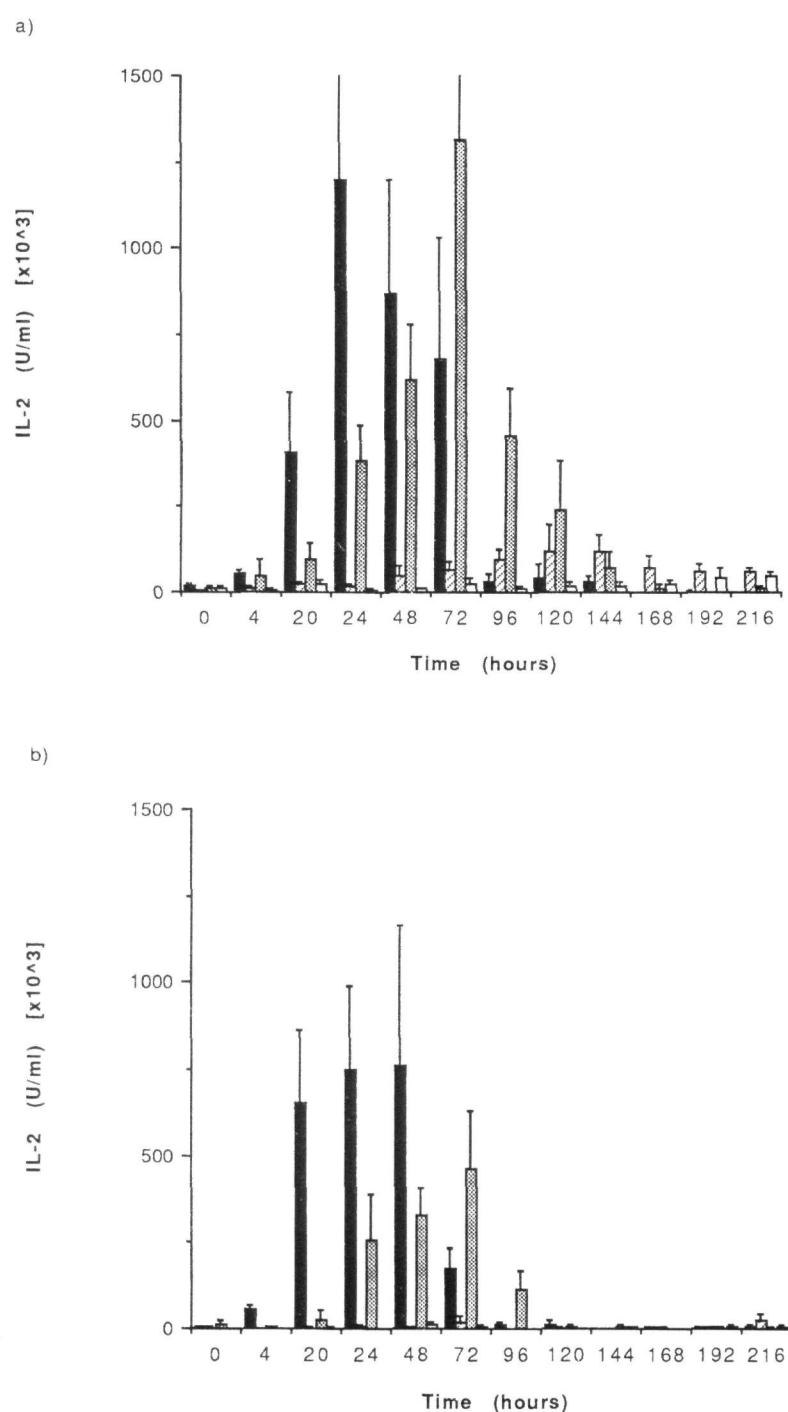


Fig. 3. Temporal profiles of IL-2 production in (a) allergic patients and (b) nonatopic controls. Columns represent group means $\pm$ SE from 11 patients and six controls with minimum of four and maximum of 16 time points per group. $\blacksquare$ = PHA, $\boxtimes$ = *D. pteronyssinus*, $\boxplus$ = SKSD, $\square$ = unstimulated.

Table 1. IL-2, IL-6, and IFN- γ production by PBMC of house-dust-mite-allergic and control subjects

	Stimulant				
	PHA	SKSD	<i>D. pter</i>	None	
Patients (<i>n</i> = 11)	0.771 (0.335–1.775)	1.106* (0.368–3.328)	0.153* (0.104–0.226)	0.021 (0.006–0.069)	IL-2 (U/ml)
Controls (<i>n</i> = 6)	0.555 (0.215–1.433)	0.295 (0.085–1.022)	0.025 ^s (0.009–0.071)	0.014 (0.005–0.044)	
Patients (<i>n</i> = 11)	22293 [†] (15957–31146)	2752 [†] (1434–5282)	31729* (19323–52097)	102 (41–257)	IL-6 (pg/ml)
Controls (<i>n</i> = 6)	35748 (23933–53393)	7343 (4825–11176)	19644 (16013–24098)	694 (200–2404)	
Patients (<i>n</i> = 11)	12.515 (5.782–27.091)	1.211 ^s (0.597–2.459)	1.328 ^s (0.859–2.051)	0.962 (0.414–2.233)	IFN- γ (ng/ml)
Controls (<i>n</i> = 6)	15.476 (5.049–47.377)	2.958 (0.660–13.267)	1.933 ^s (1.080–3.459)	1.393 (0.883–2.197)	

Peak cytokine production (geometric means $\pm$ 95% confidence intervals).
* Significantly higher than corresponding nonatopic controls (*P* < 0.05).
[†] Significantly lower than corresponding nonatopic controls (*P* < 0.05).
^s Not significantly different from unstimulated cultures (all others were significantly greater than the corresponding unstimulated cultures: *P* < 0.05).

that PHA, SKSD, and *D. pteronyssinus* had no effect at the concentrations used in the stimulated cultures (PHA and SKSD 10 μ g/ml, *D. pteronyssinus* 1000 BU/ml). The cell line was highly specific for IL-6, with all bioactivity at 6 pg/ml IL-6 being ablated at an anti-IL-6 antibody concentration of 10 ng/ml. There was also no residual bioactivity in PHA-derived supernatants measured at 32850 pg/ml (1/10000 dilution) when treated with 10 ng/ml anti-IL-6.

Cytokine profiles

Profiles for IL-2, IL-6, and IFN- γ production are shown in Figs. 3, 4, and 5, respectively, for both patient and control groups. Similar levels of IL-2 were recorded in both groups after PHA stimulation, but the IL-2 responses to SKSD and *D. pteronyssinus* were raised in the patient group, in terms of both duration of response and amount produced (Fig. 3, Table 1).

The onset of IL-6 production to PHA in both groups occurred within 4 h of stimulation and increased rapidly to maximal levels at around 96 h (Fig. 4). Unlike IL-2, the levels of IL-6 remained high to the end of the experimental period of 8–9 days. The nonatopic controls produced substantially more IL-6 to PHA than the patients (Table 1). In contrast, patients produced more IL-6 to *D. pteronyssinus* than controls, exceeding the levels generated after PHA stimulation, although showing a similar temporal profile. It was of interest that SKSD almost completely failed to induce IL-6 in the patient group, and levels were only slightly raised in the nonatopic controls (Table 1).

IFN- γ profiles (Fig. 5) for PHA were similar in the patient and control groups, rising at 24–48 h to peak at 72 h. However, patients exhibited only a modest increase in IFN- γ levels to SKSD, initially rising at 72–96 h and peaking around 120 h, whereas the onset of IFN- γ in the controls occurred at around 48 h, rising by 120 h to a sustained maximum at

Table 2. Levels of IgE detected in supernatants over culture period

a) Subject details		
Initials	<i>D. pter</i> IgE RAST units	Total IgE kU/l (PRIST)
Patients		
DM	4	34
MS	2	3360*
MD	3	25
HF	4	9
GW	4	12
GB	4	76
SC	3	110
Nonatopic		
LB	0	60
JD	0	3
CH	0	14
SC	0	4
b) <i>In vitro</i> IgE production		
Stimulant	Patients (<i>n</i> = 6)	Nonatopic controls (<i>n</i> = 4)
<i>D. pter</i>	0.150 $\pm$ 0.050	0.080 $\pm$ 0.066
SKSD	0.096 $\pm$ 0.058	0.066 $\pm$ 0.098
None	0.116 $\pm$ 0.025	0.111 $\pm$ 0.039

a) Subject details giving serum levels of *D. pteronyssinus*-specific and total IgE levels (* not included for *in vitro* IgE production). b) IgE (kU/l) produced *in vitro* (amount at end of culture – amount at time zero). Figures shown are arithmetic means $\pm$ SE. Geometric mean level of total IgE (PRIST) for donors contributing to AB⁺ NHS used was 21.0 (95% CI 6.4–69.0) kU/l, and it was present in final pool at concentration of 5.4 kU/l. All donors to this pool had zero scores for *D. pteronyssinus* RAST.

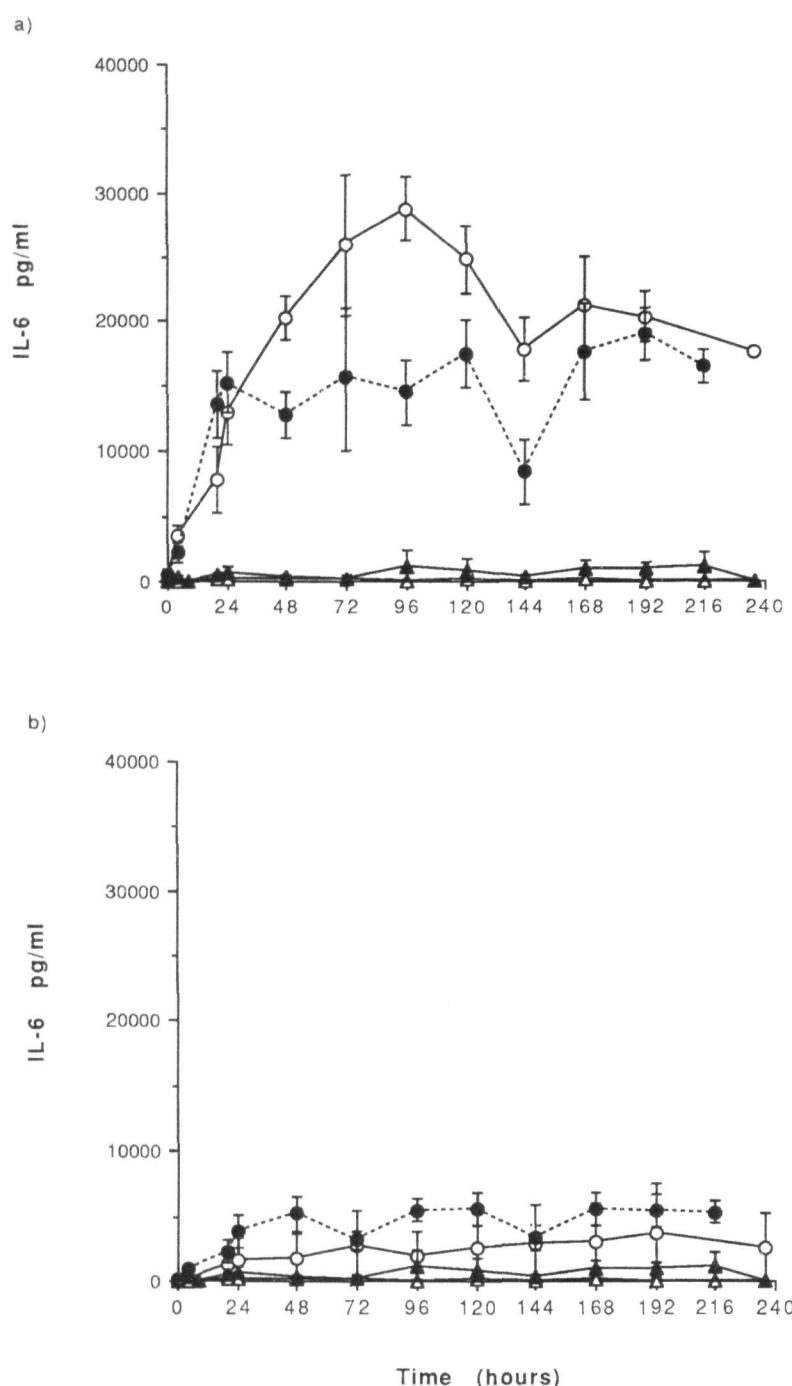


Fig. 4. Temporal profiles of IL-6 production for (a) *D. pteronyssinus* and (b) SKSD. Symbols show group means + SE from 11 patients and six controls with minimum of four and maximum of 16 time points per group. ○ = stimulated PBMC of atopic patients and ● = nonatopic controls. Unstimulated PBMC of ▲ = atopic patients and △ = nonatopic controls.

about double the levels recorded in the patients (Table 1). Neither patients nor nonatopic controls produced IFN- γ in response to *D. pteronyssinus*. However, it is interesting to note that the amounts detected after stimulation with *D. pteronyssinus* in the patient group were significantly below the levels spontaneously produced by parallel unstimulated PBMC from the same subjects, for the first 72 h of culture (Fig. 6), implying consumption of IFN- γ from the carrier AB + human serum or an inhibition of production.

In vitro IgE production

Table 2 shows the levels of IgE detected in supernatants over the culture period. Low levels of spon-

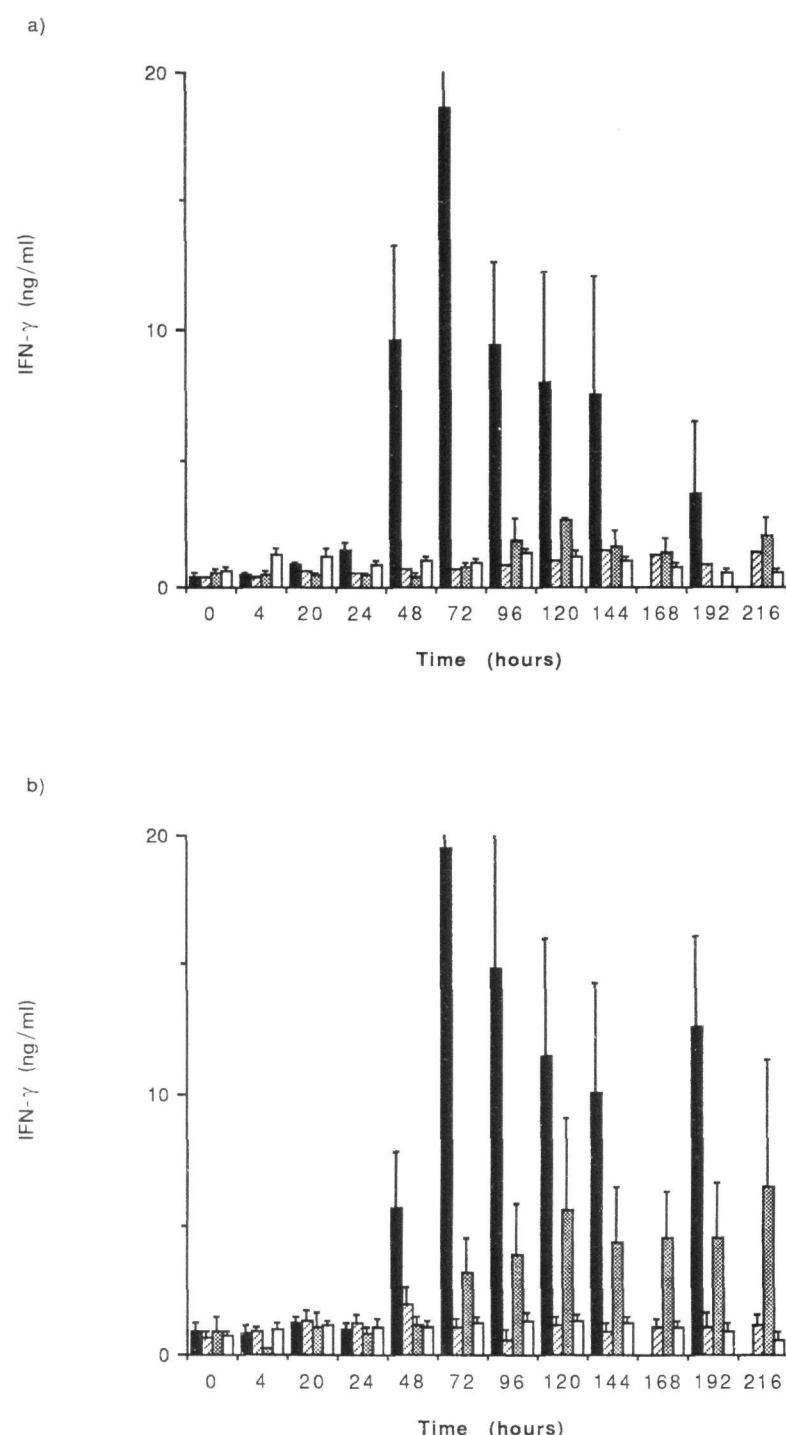


Fig. 5. Temporal profiles of IFN- γ production in (a) allergic patients and (b) nonatopic controls. Columns represent group means + SE from 11 patients and six controls with minimum of four and maximum of 16 time points per group. ■ = PHA, ▨ = *D. pteronyssinus*, ▩ = SKSD, □ = unstimulated.

taneous IgE production occurred in most subjects, including 3/4 nonatopic controls. However, higher levels of IgE were detected in 5/7 HDM-sensitive patients, but in only one control after stimulation with *D. pteronyssinus*. The nonspecific ubiquitous antigen, SKSD, did not induce IgE production in most HDM-sensitive patients and evoked similar or lower amounts of IgE than those spontaneously produced in the nonatopic controls. One patient produced large amounts (up to 3.2 kU/l) of IgE under all conditions and is not included in Table 2. This may be because he had eczema as well as rhinitis and asthma, and had a very high total IgE (> 1000 kU/l). IgE in the AB⁺ normal human serum used as the carrier in these assays was present at a level of 5.4 kU/l (i.e., 0.54 kU/l when present at 10%).

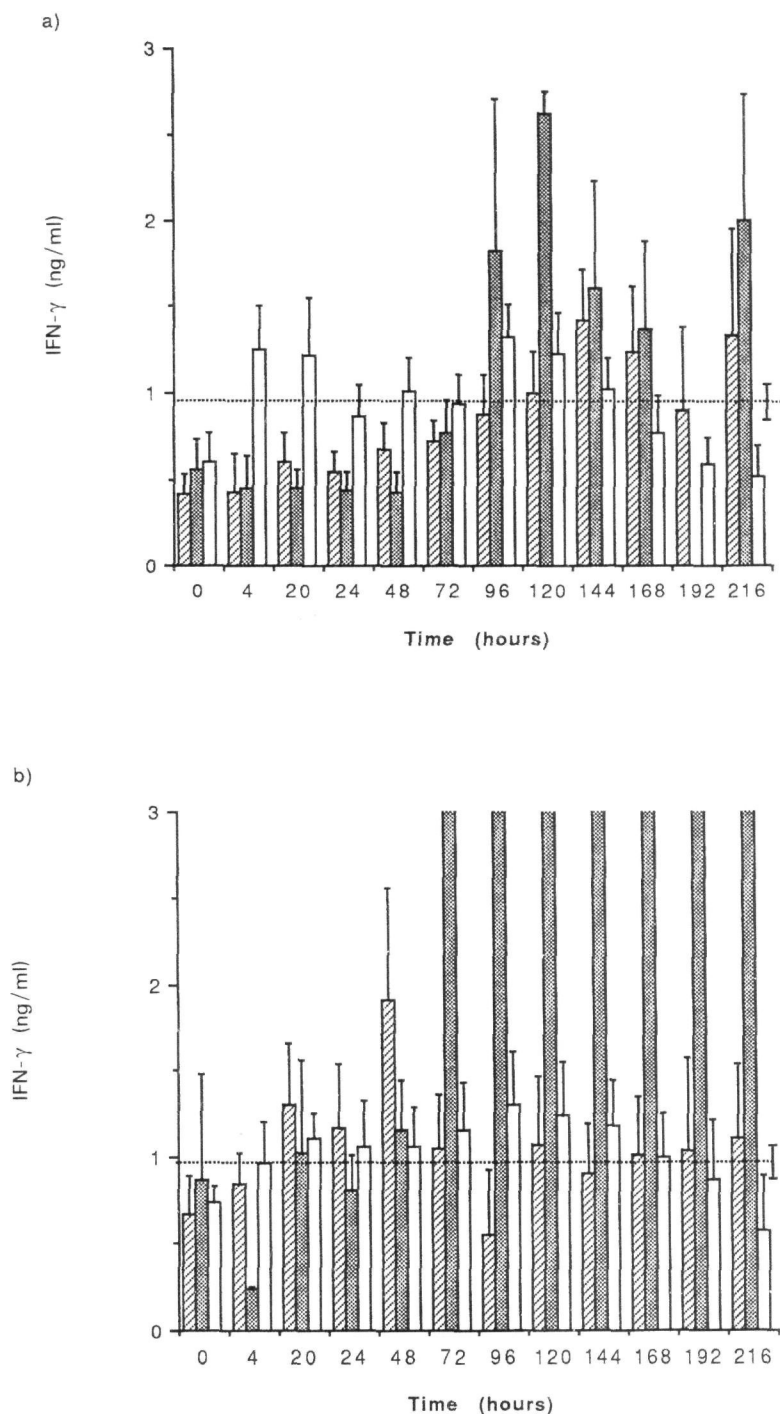


Fig. 6. Temporal profiles of IFN- γ production in (a) allergic patients and (b) nonatopic controls showing reduction in IFN- γ in antigen-stimulated cultures below spontaneous production and below levels found in AB + NHS of culture medium (mean $\pm$ SE; $n = 45$). Columns represent group means $\pm$ SE from 11 patients and six controls with minimum of four and maximum of 16 time points per group. $\square$ = *D. pteronyssinus*, $\square$ = SKSD, $\square$ = unstimulated.

Discussion

We have shown that early and then sustained production of large amounts of IL-6 was a feature of the immune response by PBMC from atopic patients in response to a specific allergen, but not to a ubiquitous antigen. On the other hand, IL-2 production in atopic subjects was raised to both allergen and antigen, whereas IFN- γ production to both was suppressed. These findings confirm our previous work showing impaired IFN- γ production (3, 18), but they also suggest that IL-6 is important in the regulation of allergic immune reactions.

Recent evidence suggests that IL-6 plays an obligatory role in IL-4-dependent IgE synthesis (29). It has also been shown that, in addition to its role in inhibiting IL-4-induced IgE synthesis, IFN- γ inhibits the growth of an IL-6-dependent human myeloma cell line. This effect was demonstrated *in vitro* at concentrations in the range 100–1000 mM/l ($\approx$ 6–60 mg/ml) (9). Thus, the high levels of IL-6 and low levels of IFN- γ we found in atopic subjects after allergen stimulation would facilitate IgE isotype selection and high levels of immunoglobulin production, driven by IL-6.

The source of the IL-6 in allergen-stimulated atopic PBMCs is of interest. The amount detected exceeded that produced in mitogen-driven cultures. Since PHA is a potent and specific T-cell mitogen, this suggests that the IL-6 induced by allergen is derived from other mononuclear cells in culture, particularly monocytes and possibly B cells (14), as well as from T cells. The time course of IL-6 production in our cultures, with very early onset and sustained production, is compatible with production initially from monocytes and subsequently from allergen-specific T cells.

Vercelli et al. (29) have shown an obligatory role for IL-6 in IL-4-induced IgE production and have also shown that endogenous, possibly monocyte-derived, IL-6 is sufficient for this process in human PBMC cultures. In addition, there is now considerable evidence that monocytes are required in culture for the production of IL-4-induced IgE (15, 24), although this is not always the case (23, 24). It is possible that in situations where monocytes were not required, there was already sufficient endogenous IL-6 for IL-4-induced IgE production. Other cells not present in significant number in our cultures, such as eosinophils and mast cells, may also produce IL-6 (and other T_H2 cytokines) *in vivo*, and contribute to the cytokine milieu at sites of allergic inflammation. IL-6 from monocytes and other cells may thus be important, contributing both directly and indirectly (via induction of IL-4) to a T_H2 -type environment, which potentiates IgE production and related allergic manifestations.

Peak IL-6 production on allergen stimulation was much higher in atopic than nonatopic subjects; nevertheless, considerable amounts of IL-6 were produced by the nonatopic subjects. However, in atopic subjects, allergen-induced IL-6 production exceeded that in mitogen-driven cultures, whereas in nonatopic subjects, mitogen induced much more IL-6 than did antigen (Fig. 4). It is not clear why allergen induced IL-6 production in nonatopic subjects. It is possible that the IL-6 produced by nonatopic subjects would not necessarily lead to induction of IL-4, since in these subjects the response to allergen would not be of the T_H2 phenotype.

Recent studies have shown that memory T cells (CD45RO+) are preferentially recruited to the sites of allergic inflammation in the skin after antigen challenge (5). It has also been shown that memory CD4+ T cells can be activated by anti-CD2 antibodies to release lymphokines and proliferate, but that naive (CD45RA+) CD4+ T_H cells can be activated by this route only in the presence of exogenous IL-6 (11). Memory T cells appear to secrete their own IL-6 upon CD2 stimulation, thus suggesting an essential role for IL-6 in the activation of CD4+ T cells via the CD2 molecule.

The profile of cytokines produced after antigen or mitogen challenge is complex. The traditional view that IL-2 production precedes, but is coregulated with, IFN- γ production (8, 10) appears to hold for mitogen- and antigen-stimulated cells in nonatopic subjects. However, this does not seem to be true for cells from allergic patients, in which vigorous IL-2 responses to SKSD and *D. pteronyssinus* were followed by attenuated or inhibited IFN- γ production, although responses after mitogen stimulation appeared to be normal (11). This confirms our previous preliminary findings of IFN- γ secretion from stimulated PBMC (18) and is consistent with data from allergen-specific T-cell clones which appear to secrete little or no IFN- γ (30). However, studies with TCC suggest that impaired IFN- γ production occurs only in allergen-specific TCC in atopic subjects, whereas antigen-specific TCC from atopic subjects produce high levels of IFN- γ (7). Our data suggest a broader defect in the IFN- γ response in atopic subjects, because this was impaired in response to both allergen and antigen stimulation.

The cytokine response we observed to allergen stimulation in this system is compatible with an impaired T_H1 response (IFN- γ), and an increased T_H2 response (assuming part of the IL-6 is derived from T_H2 cells). These findings suggest that the small but significant increase in IL-2 production is T_H2 derived. There is evidence suggesting that, for a sustained IL-2 response, costimulation via the CD28 receptor may be essential, because CD28 costimulation enhances survival of T cells activated via the T-cell receptor and increases IL-2 production (27). This suggests that the enhanced IL-2 production detected in atopic subjects may be mediated by abnormal costimulatory pathways in T_H2 cells. Our findings are compatible with the T_H-phenotype observed in studies with atopic allergen-specific TCC (17, 30).

We have chosen to work with PBMC, whereas other groups studying cytokine production by T cells have focused on T-cell clones (1, 17, 30). Our PBMC model has certain advantages. It avoids the problems of selection inherent in the use of clones and therefore may better reflect the repertoire of T-cell

responses to allergen. This may be important because it is becoming clear that the distinction between T_H1 and T_H2 cells is not absolute: both cell types may exist in an individual in response to the same stimulus (21), and clones have been described which secrete both T_H1 and T_H2 cytokines (20). Such clones have been classified as T_H0 cells and may represent either a precursor subset of T_H cells or a phenotypically stable intermediate subset (7, 20, 30).

In the PBMC model, more cell types, including antigen-presenting cells, are present in the culture, making it more like the situation *in vivo* than in studies with T-cell clones. It is the interaction of a variety of cytokines, irrespective of their source, at sites of inflammation which determines the biologic effect. This model allows us to study the effect of different stimuli, such as allergen, antigen, and mitogen, and our results show that the profile of the cytokine response to these various stimuli is complex. A potential problem with T-cell clones is that the cytokine environment during their generation can greatly modify the cytokine secretion pattern of the resulting clones. Thus, the addition of IL-4 early in bulk culture can change the cytokine secretion from a T_H1 to a T_H2 profile, whereas addition of IFN- γ can lead to suppression of T_H2 cytokine secretion (16). A disadvantage of the PBMC model is the small number of antigen-specific cells per well, which might be a problem when measuring very small amounts of cytokine production. It can also be argued that PBMC are not representative of the cells at the site of inflammation. However, this also applies to TCC, and PBMC represent the pool from which cells are recruited and to which memory T cells return. Data from this model may provide additional information, as shown here for IL-6, to complement existing data from T-cell clones.

Our studies of IgE production showed that only atopic PBMC produced higher levels of IgE after specific allergen stimulation. It is interesting that both patients and nonatopic controls produced less IgE after stimulation with SKSD than the amounts spontaneously released in unstimulated cultures. Thus, it appears that not only is there an increase in specific IgE production to allergen in atopic subjects, but there is also a concomitant switch away from IgE production when cells are stimulated with recall antigen. These findings need to be reexamined with larger sample sizes and longer culture periods with the necessary controls to distinguish *de novo* IgE synthesis.

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