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IL-13 production by allergen-stimulated T cells is increased in allergic disease and associated with IL-5 but not IFN-γ expression

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SUMMARY

Interleukin-13 (IL-13) shares many, but not all, of the properties of the prototypic T-helper type 2 (Th2) cytokine IL-4, but its role in allergen-driven T-cell responses remains poorly defined. We hypothesized that allergen stimulation of peripheral blood T cells from patients with atopic disease compared with non-atopic controls results in elevated IL-13 synthesis in the context of a 'Th2-type' pattern. Freshly isolated peripheral blood mononuclear cells (PBMC) obtained from sensitized atopic patients with allergic disease, and non-atopic control subjects, were cultured with the allergens *Phleum pratense* (Timothy grass pollen) or *Dermatophagoides pteronyssinus* (house dust mite) and the non-allergenic recall antigen *Mycobacterium tuberculosis* purified protein derivative (PPD). Supernatant concentrations of IL-13, along with IL-5 and interferon-γ (IFN-γ) (Th2- and Th1-type cytokines, respectively) were determined by enzyme-linked immunosorbent assay (ELISA). Allergen-induced IL-13 and IL-5 production by T cells from patients with allergic disease was markedly elevated ($P=0{\cdot}0075$ and $P=0{\cdot}0004$, respectively) compared with non-atopic controls, whereas IFN-γ production was not significantly different. In contrast to allergen, the prototypic Th1-type antigen *M. tuberculosis* PPD induced an excess of IFN-γ over IL-13 and IL-5 production, and absolute concentrations of cytokines were not affected by the presence or absence of atopic disease. Addition of exogenous recombinant IFN-γ or IL-12, cytokines known to inhibit Th2-type responses, significantly inhibited allergen-driven production of both IL-13 and IL-5, but not T-cell proliferation, whereas exogenous IL-4 did not significantly affect production of IL-13 or IL-5. We conclude that allergen-specific T cells from atopic subjects secrete elevated quantities of IL-13 compared with non-atopic controls, in the context of a Th2-type pattern of cytokine production.

INTRODUCTION

Interleukin-13 (IL-13) is a 132-amino acid cytokine expressed by T cells,[1–5] mast cells[6] and Epstein–Barr virus (EBV)-transformed B cells.[4] Properties attributed to this cytokine include the up-regulation of major histocompatibility complex (MHC) class II and low-affinity IgE receptor (CD23) expression on monocytes,[7] induction of IgE and IgG4 switching in B cells,[8] and up-regulation of vascular cell adhesion molecule-1 (VCAM-1) expression on endothelial cells.[9] Furthermore, since IL-13 is able to down-regulate expression of interferon-α (IFN-α) and IL-12 by monocytes,[7] it may indirectly favour the establishment of T-helper type 2 (Th2)-type responses *in vivo*. IL-13 therefore has the potential to contribute to the pathogenesis of allergic inflammatory disorders, and indeed elevated expression of IL-13 mRNA has been demonstrated in bronchoalveolar lavage mononuclear cells following local endobronchial allergen challenge of sensitized atopic asthmatics.[10]

Although many of the properties of IL-13 are similar to those of IL-4 and there is some evidence for structural conservation and receptor sharing,[11,12] there remain important differences between these two cytokines. Firstly, unlike IL-4, IL-13 appears not to have growth factor activity for T cells, and does not appear to act directly on T cells to favour development of Th2-type cells.[13] Secondly, whereas IL-4 is a prototypic Th2 cytokine expressed primarily by CD45RO$^+$ 'memory' T cells,[3] IL-13 expression has been demonstrated in human Th0, Th1 and Th2 clones[1] and, furthermore, significant levels of production can be detected in CD45RA$^+$ as well as CD45RO$^+$ cells.[3–5] Further important differences are evident in the kinetics of IL-4 and IL-13 expression in T cells,[1, 3] with expression of the latter occurring earlier, and tending to persist longer following stimulation. Thus, despite the similarities in

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biological properties and the consequent implication that IL-13 may play a significant role in driving the inappropriate IgE responses that characterize allergy, it remains to be determined whether allergen-specific T cells of patients with allergic disorders are predisposed to produce IL-13, as has been shown for IL-4 and IL-5 in studies involving T-cell clones.[14] In order to address this question, we investigated the patterns of cytokine production by peripheral blood mononuclear cells (PBMC) obtained from sensitized patients with symptomatic atopic disease and non-atopic normal controls in response to stimulation with the allergens *Phleum pratense* (*Phl p*) and *Dermatophagoides pteronyssinus* (*Der p*), and the prototypic Th1-antigen *Mycobacterium tuberculosis* purified protein derivative (PPD).[15,16] We also investigated whether allergen-driven IL-13 secretion can be modulated by the presence of exogenous IFN-γ, IL-12 or IL-4, cytokines known to favour development of Th1-type (IFN-γ and IL-12) and Th2-type (IL-4) responses, respectively.[17–20] We hypothesized that *Phl p*-stimulated peripheral blood T cells express elevated quantities of IL-13 along with IL-5 in patients with atopic disease but not non-atopic controls, whereas PPD stimulation favours production of elevated quantities of the Th1-type cytokine IFN-γ compared with both IL-13 and IL-5 in both subject groups. We also hypothesized that allergen-dependent production of IL-13 and IL-5 is increased by exogenous IL-4, and decreased by IL-12 or IFN-γ.

MATERIALS AND METHODS

Subject populations

Peripheral venous blood was collected from patients with a history of symptomatic seasonal rhinitis out of the pollen season, when patients were not receiving medication. All subjects had strong positive skin prick tests (wheal 5 mm $\geq$ negative control at 15 min following testing) to *Phl p* ('Soluprick'; ALK, Horshølm, Denmark) in the presence of negative diluent and positive histamine controls, and elevated concentrations of serum IgE specific for mixed grass pollen. Non-atopic control subjects had negative skin prick tests to a range of 12 common aeroallergens and no history of symptoms indicative of allergic disease. In certain experiments, peripheral blood was obtained from atopic patients suffering from perennial rhinitis and/or asthma, who were skin prick test positive to *Der p*.

Preparation of cells

PBMC were isolated from heparinized blood samples by density gradient centrifugation over Ficoll–Hypaque (Pharmacia, Uppsala, Sweden), washed twice in HEPES-buffered RPMI (Chester Beatty Laboratories, London, UK) and resuspended in RPMI (Gibco, Paisley, UK) supplemented with 5% human AB serum (Sigma, Poole, UK), 100 IU/ml penicillin/streptomycin (Gibco) and 2 mM L-glutamine (Gibco).

Cell cultures

For proliferation assays, PBMC were incubated at $0{\cdot}5 \times 10^6$ cells/ml (200 μl volume) in a minimum of three replicate cultures with 20 μg/ml *Phl p* ('Aquagen' extract; a kind gift of ALK), 10 μg/ml PPD or medium only. Cellular proliferation was measured on day 7 by adding 0·5 μCi of tritiated methylthymidine (Amersham Int., Amersham, UK) per well for the last 16 hr of culture, and assaying label incorporation by liquid scintillation spectroscopy. For cytokine production, PBMC were incubated in quadruplicate 200 μl volumes at 2×10^6 cells/ml in the presence of 20 μg/ml *Phl p*, 10 μg/ml PPD or medium. Supernatants were harvested after 6 days incubation and stored at $-20°$ pending cytokine measurements. To determine the effects of IFN-γ, IL-12 and IL-4 on allergen-induced IL-13 and IL-5 production, PBMC from four *Der p*-sensitive atopic donors were incubated for 6 days with 2·5 μg/ml *Der p* (ALK) in the presence of recombinant IL-12 (0·5 ng/ml; R&D Systems, Abingdon, UK), IFN-γ (1·0 ng/ml; R&D systems) or IL-4 (50 ng/ml; R&D Systems). *Der p*-induced proliferation in parallel cultures was measured on day 7 as described above.

Cytokine assays

IL-5 concentrations in culture supernatants were determined by means of a specific sandwich enzyme-linked immunosorbent assay (ELISA) sensitive above 6·5 pg/ml, as described previously.[21] A commercially available kit, sensitive above 2 pg/ml, was used for determination of IFN-γ (R&D Systems). IL-13 was also measured using a commercially available ELISA, sensitive above 0·5 pg/ml (CMB, Amsterdam, the Netherlands). Specific cytokine production in response to antigen was calculated by subtracting concentrations measured in unstimulated cultures (i.e. background) from those measured in allergen- or PPD-stimulated cultures.

RESULTS

IL-13, IL-5 and IFN-γ production and cellular proliferation in response to *Phl p* (grass pollen) and PPD stimulation of PBMC were measured in patients suffering form severe seasonal rhinitis and non-atopic normal controls. *Phl p*-induced IL-13 production by PBMC (Fig. 1a) was elevated in seasonal rhinitics ($P = 0{\cdot}0075$) relative to control subjects, although IL-13 production in response to *Phl p* stimulation was detectable in supernatants from all but one of the non-atopic control subjects. When PBMC were stimulated with PPD, IL-13 production was detectable in all subjects regardless of the presence or absence of atopic disease (Fig. 1b).

In order to compare the patterns of IL-13 production with those of a well-established pro-allergic 'Th2-type' cytokine, concentrations of IL-5 were determined in the same supernatants. IL-13 displayed similar patterns of expression to IL-5: secretion by *Phl p*-stimulated PBMC (Fig. 1c) was significantly elevated in rhinitics ($P = 0{\cdot}0004$), and although PPD-induced IL-5 production was detectable in supernatants from the majority of subjects in both groups (Fig. 1d) this was not affected by the presence or absence of atopic disease. Although IFN-γ production in response to both *Phl p* and PPD was not significantly different between rhinitic or non-atopic subjects, PPD stimulation induced much higher degrees of expression of this cytokine than did *Phl p* (Fig. 1e, f).

Although PPD induced measurable secretion of IL-13 and IL-5 in a majority of individuals, this was in the context of high IFN-γ production. The ratios of IL-13/IFN-γ and IL-5/IFN-γ production were specifically elevated only in *Phl p*-stimulated PBMC from rhinitics. Furthermore, the IL-13/IFN-γ and IL-5/IFN-γ ratios in response to *Phl p*

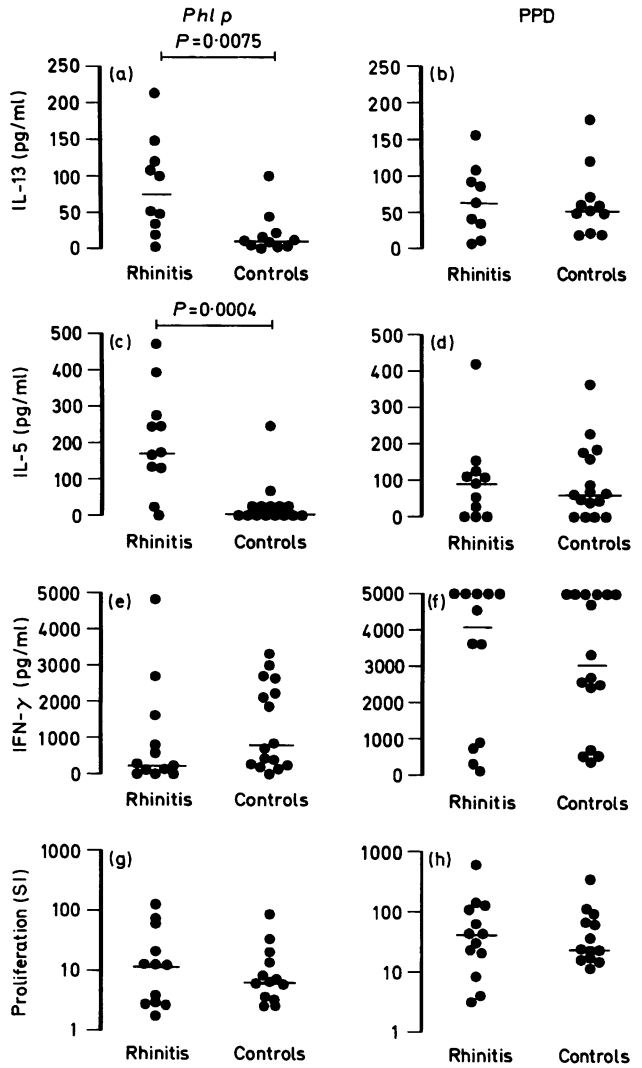

Figure 1. IL-13, IL-5 and IFN-γ production in response to stimulation with 20 μg/ml *Phl p* (Timothy grass pollen) or 10 μg/ml *M. tuberculosis* PPD. PBMC were stimulated for 6 days at 2×10^6 cells/ml. The upper sensitivity limit for the IFN-γ ELISA was 5000 pg/ml. Statistically significant differences are indicated, as determined by the Mann–Whitney *U*-test.

stimulation of PBMC obtained from non-atopic subjects approximated to those observed in response to PPD (Table 1). Finally, cellular proliferation in response to both *Phl p* and PPD was not significantly different in both rhinitic and non-atopic control groups, although in both groups PPD tended

Table 1. Ratios of IL-13 and IL-5 to IFN-γ production in response to Timothy grass pollen (*Phl p*) and PPD stimulation of PBMC from patients with atopic rhinitis and non-atopic controls

Cytokine ratio*	Phl p		PPD	
	Rhinitic	Non-atopic	Rhinitic	Non-atopic
IL-13/IFN-γ	0·30	0·014	0·015	0·016
IL-5/IFN-γ	0·81	<0·001	0·022	0·018

* Ratios calculated using median values of cytokine production.

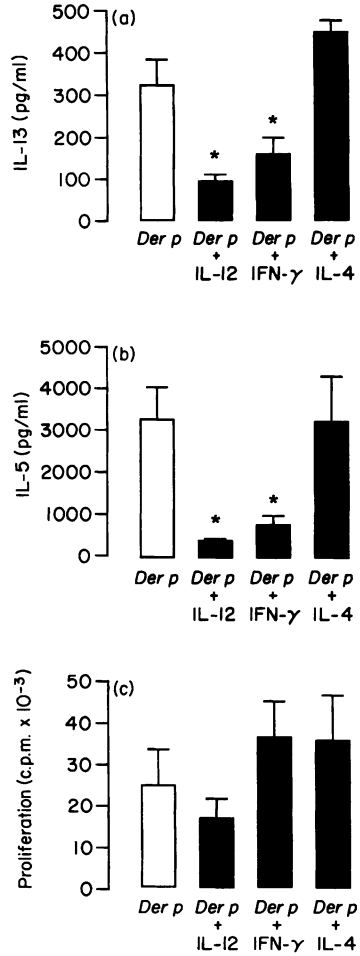

Figure 2. Effects of IL-12, IFN-γ and IL-4 on allerge-induced (a) IL-13 production, (b) IL-5 production, and (c) PBMC proliferation. For cytokine measurements, PBMC were stimulated with 2·5 μg/ml *Der p* for 6 days at 5×10^6 cells/ml, in the presence of 0·5 ng/ml IL-12, 1 ng/ml IFN-γ or 50 ng/ml IL-4. To measure proliferation, PBMC were stimulated with 25 μg/ml of *Der p* at $0{\cdot}5 \times 10^6$ cells/ml. Data represent mean $\pm$ SEM of four separate experiments. **P0·05 versus Der p*-only stimulated cultures (paired *t*-test).

to induce a higher degree of cellular proliferation than did allergen (Fig. 1g, h).

In order to determine whether allergen-stimulated secretion of IL-13 could be modulated by cytokines known to inhibit or promote the development of T cells producing Th2-type cytokines such as IL-4 or IL-5, we supplemented cultures of *Der p*-stimulated PBMC from sensitized symptomatic atopic donors with recombinant IL-12, IFN-γ or IL-4. Both IL-12 and IFN-γ significantly inhibited allergen-driven IL-13 (Fig. 2a) and IL-5 production (Fig. 2b), although neither of these recombinant cytokines was found to have significant inhibitory effects on allergen-induced proliferation by PBMC (Fig. 2c) when added at the same concentration. In contrast, IL-4 did not significantly modulate either IL-5 or IL-13 secretion or proliferation in response to allergen.

DISCUSSION

In this study, we have demonstrated that exposure of peripheral blood T cells from subjects with allergic rhinitis to allergen

ex vivo results in elevated production of IL-13 and IL-5, compared with T cells from non-atopic controls. Furthermore, this was associated with a markedly increased ratio of IL-13 or IL-5 to IFN-γ production. In contrast, exposure of T cells to *M. tuberculosis* PPD resulted in equivalent secretion of both IL-13 and IL-5 in both the atopic patients and the controls, with a relatively low ratio of IL-13 or IL-5 to IFN-γ production. Moreover, we report that, like IL-5, allergen-induced IL-13 expression can be modulated by exogenous IL-12 or IFN-γ, but not IL-4.

These data are novel, since although IL-13 is sometimes referred to as a Th2-like cytokine,[13] until now there has existed little evidence to support this statement beyond the fact that since many of its properties are similar to IL-4, it is perceived to be pro-allergic in character. Indeed, despite evidence that IL-13 mRNA and protein expression is up-regulated following local endobronchial challenge in asthma,[10] a previously published study into the association between IL-13 production and that of IL-4 and IFN-γ provided evidence that this cytokine was expressed equally by Th1, Th0 and Th2 clones.[1] In contrast, we chose to analyse expression in a simple primary culture model, involving a single antigenic stimulation of freshly isolated PBMC *ex vivo*. Depletion experiments confirm that $CD4^+$ T cells are the source of IL-5 in these cultures (data not shown), and there can be little doubt that T cells are also the source of IL-13 secreted in response to allergen or PPD stimulation. Such methodology, we believe, may provide a truer reflection of allergen-induced cytokine synthesis by T cells *ex vivo*, permits valid inter-group comparisons, and minimizes possible confounding effects arising from long-term *in vitro* culture of T lymphocytes or non-specific stimulation with T-cell mitogens.

Under these conditions we observed elevated T-cell IL-5 and IL-13 production in response to stimulation with relevant allergen, but not PPD, in individuals with allergic disease. Although IL-5 and IL-13 secretion in response to PPD stimulation of PBMC could often be detected in the present study, this accompanied substantial IFN-γ secretion. Thus, high ratios of IL-13/IFN-γ and IL-5/IFN-γ production were confined to allergen-driven T-cell responses in sensitized rhinitic patients. We speculate that it is the ratio of IL-5 or IL-13 to IFN-γ production that may be one important factor in determining the development of atopic disease. For example, IFN-γ inhibits IL-13-induced IgE switching in human fetal B cells,[8] and the inhibition of cutaneous allergen-induced late phase reactions following immunotherapy was associated with increased numbers of IFN-γ mRNA-positive cells in the skin, without any reduction in numbers of IL-5 or IL-4 mRNA-positive cells.[22] It should be noted that the absolute amounts of IL-5 and IL-13 production induced by PPD and *Phl p*, although appearing similar in Fig. 1, are not directly comparable since PPD induced greater proliferative responses than *Phl p*, probably reflecting a greater frequency of PPD-specific, compared with *Phl p*-specific, T cells.

Our data suggest that T-cell IL-13 expression is associated more with that of IL-5 than IFN-γ in response to physiological (i.e. antigen-mediated) stimulation. We did not make comparisons with IL-4 production in this study because we have found that the concentrations of this cytokine in primary cultures are much lower than those of IL-5, making reproducible detection difficult. It should be noted, however, that using a highly sensitive bioassay, elevated IL-4 production by PBMC derived from seasonal rhinitics compared with non-atopic controls in response to pollen stimulation has been demonstrated previously.[23] Nevertheless, we feel justified in focusing on IL-5 since Th2 clones and cell lines, at least at the population level, tend to co-express IL-4 and IL-5.[14, 20] Furthermore, T-cell expression of mRNA for both of these cytokines is consistently up-regulated in allergic inflammation,[24,25] and IL-5 is believed to have a highly significant role in the pathogenesis of allergic disorders. Finally, unlike IL-13, both IL-4 and IL-5 are preferentially expressed by $CD45RO^+$ 'memory' T cells.[26]

IL-12 and IFN-γ have both previously been shown to inhibit the development of 'Th2-type' allergen-specific T cells *in vitro*.[17–19] Conversely, it is well established that the presence of IL-4 during primary cultures favours the outgrowth of Th2-like cell lines and clones.[20] In the present study, allergen-driven IL-13 and IL-5 production by T cells from atopic subjects could be inhibited by the addition of exogenous IFN-γ or IL-12, further supporting the concept that IL-13 is preferentially expressed by Th2-type cells, although it is possible that at least some of the effects of IL-12 may have been mediated through up-regulation of IFN-γ expression by natural killer (NK) cells, as has been described previously.[18] Although IL-12 has been shown to inhibit mitogen-induced IL-13 expression by $CD45RA^+$ and $CD45RO^+$ T cells,[3] this is the first evidence that antigen-induced IL-13 production may be modulated by this cytokine. Moreover, whereas a previous study failed to observe any effect of IFN-γ on IL-13 production by $CD45RA^+$ T cells stimulated with mitogens,[3] we found that IFN-γ could modulate allergen-driven IL-13 production. It is unlikely that inhibition of IL-13 and IL-5 production by IL-12 and IFN-γ could be attributed to non-specific inhibition of T cells, since allergen-stimulated cellular proliferation was not significantly inhibited by these cytokines.

In conclusion, expression of IL-13 by allergen-stimulated T cells from sensitized atopic subjects closely mirrored that of IL-5, consistent with a role for IL-13 in the pathogenesis of allergic inflammation. This might include regulation of IgE synthesis or eosinophil recruitment to the sites of allergic inflammation through up-regulation of VCAM-1 expression on endothelium.[9] Further studies will be needed to assess the relative contributions of allergen-driven T-cell-derived IL-4 and IL-13 to these processes.

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