Clin Exp Immunol 1994; 95:66–72

Production and secretion of interferon-gamma (IFN-γ) in children with atopic dermatitis

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(Accepted for publication 15 September 1993)

SUMMARY

IFN-γ is known to be a major inhibitor of IgE synthesis *in vitro*. Recent studies demonstrating reduced production of IFN-γ in children and adults with atopic dermatitis and elevated serum IgE suggest a similar role for this cytokine *in vivo*. The reasons for this reduced IFN-γ production are not known. One possibility is that atopic individuals have a reduced population of cells producing IFN-γ *in vivo*. Using a fluorescence-labelled antibody to detect intracellular IFN-γ, the percentage of IFN-γ-producing cells was determined in children with atopic dermatitis and in non-atopic controls. Children with atopic dermatitis had a greater percentage of IFN-γ-producing cells in unstimulated cultures compared with controls, indicating *in vivo* activation of lymphocytes in the atopic group. This could reflect the significant degree of inflammation present in these children, or the presence of bacterial infection or colonization. Although secretion of IFN-γ after stimulation with phorbol myristate acetate (PMA)/Ca was significantly lower in children with atopic dermatitis compared with controls, the percentage of IFN-γ-producing cells in the stimulated cultures from this group was equivalent to controls. This demonstrates that the reduced ability of atopic children to secrete IFN-γ *in vitro* does not relate to a lack of IFN-γ-producing cells, but to a difference in the regulation of IFN-γ production beyond the stage of signal transduction.

Keywords atopy childhood IFN-γ intracellular

INTRODUCTION

Previous studies have demonstrated an important role for IFN-γ in the regulation of IgE synthesis *in vitro*. Whilst IL-4 is the sole inducer of class switching to IgE production in B lymphocytes [1,2], IFN-γ is the key inhibitor of IL-4-induced IgE synthesis [3]. Reports of reduced IFN-γ secretion in atopic adults with elevated IgE levels suggest that IFN-γ has a role in the regulation of IgE synthesis *in vivo* [4]. A similar deficiency of IFN-γ secretion was recently documented in highly atopic children with atopic dermatitis, and was shown to correlate with severity of disease [5]. Furthermore, the symptomatic and clinical improvement observed with the use of subcutaneous recombinant IFN-γ in cases of severe recalcitrant atopic dermatitis implicates a role for reduced IFN-γ secretion in the pathogenesis of disease [6–8].

The mechanisms underlying this reduced *in vitro* secretion of IFN-γ in atopic individuals are unclear. One possibility is that there is a reduced population of T lymphocytes capable of producing IFN-γ. Previous studies addressing this possibility have employed methods of clonal analysis and have revealed conflicting results [9,10]. Romagnani *et al.* [9] reported no significant difference in the proportion of IFN-γ-producing T cell clones (TCC) generated from the peripheral blood of subjects with atopic dermatitis and controls, whereas Del Prete *et al.* [10] documented a significantly lower proportion of IFN-γ-producing $CD4^+$ TCC in the peripheral blood of adults with atopic dermatitis compared with controls. There have been no studies investigating the percentage of IFN-γ-producing cells directly from the peripheral blood of atopic patients without the manipulations required for clonal analysis. Using fluorescence cytometry to detect cells containing intracellular IFN-γ, we examined the proportion of IFN-γ-producing mononuclear cells in the peripheral blood of children with atopic dermatitis and age-matched, non-atopic controls.

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MATERIALS AND METHODS

Reagents

Reagents used were: mouse IgG1 anti-human IFN-γ MoAb (Endogen, Boston, MA); mouse IgG1 control kindly supplied by Dr R. Boyle (Department of Microbiology, University of Melbourne, Australia); mouse IgG1 anti-human mitochondria antibody (MAB1284; Chemicon, Temecula, CA); R-PE-conjugated sheep $F(ab)_2$ anti-mouse IgG, (DDAPE; Silenus, Melbourne, Australia); Jurkat cell line kindly provided by Dr G. Varigos (Royal Children's Hospital, Melbourne, Australia);

CEM-CCRF and CEM-VLB100 T cell lines generously supplied by Dr J. R. Zalcberg (Department of Medical Oncology, Heidelberg Repatriation Hospital, Melbourne, Australia); Nonidet-P40 (BDH Chemicals, Poole, UK); human IFN-γ ELISA kit (CSL Ltd, Melbourne, Australia); Ficoll–Hypaque (Pharmacia, Uppsala, Sweden); phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 (Sigma Chemical Co., St Louis, MO); Cyclosporin A (Sandoz, North Ryde, Australia); RPMI 1640 medium, fetal calf serum (FCS), L-glutamine, penicillin and streptomycin (Flow, Rickmansworth, UK); Quanticlone IgE immunoradiometric assay kit (Kallestad Diagnostics Inc, Chaska, MN).

Patients

Heparinized venous blood samples were obtained from:

1 *Thirteen children with severe atopic dermatitis (AD)* who had elevated levels of serum IgE. These children had an acute exacerbation of AD, with a total clinical severity score $\geqslant 7$ and involvement of at least 20% of their body surface area. The total clinical severity score was defined as the sum of the individual scores, graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe), for each of six parameters (pruritis, erythema, oedema/papulation, lichenification, scaling, and erosion/weeping) [11]. The extent of skin involvement was estimated using the rule of nines [11]. Levels of IgE were elevated above the normal limits for age in all cases (0–0·2 years <6 U/ml, 0·2–0·5 years <45 U/ml, 0·5–0·7 years <20 U/ml, 0·7–1 year <25 U/ml, 1–2 years <35 U/ml, 2–4 years <130 U/ml, 4–7 years <150 U/ml, >7 years <200 U/ml). The mean serum IgE level was 5888 U/ml, and the mean age was 3·5 years. All patients were treated with topical steroids with or without emolient wet dressings. None had received oral steroid therapy for at least 1 month before testing.

2 *Thirteen age-matched non-atopic controls.* These were children admitted for elective surgery at the Royal Children's Hospital (herniotomies, orchidopexies, tonsillectomies, insertion of tympanic membrane aeration tubes, plastic surgical procedures, and orthopaedic procedures) who had a negative history of atopic disease and plasma IgE levels within normal limits for age (0–0·2 years <6 U/ml, 0·2–0·5 years <45 U/ml, 0·5–0·7 years <20 U/ml, 0·7–1 year <25 U/ml, 1–2 years <35 U/ml, 2–4 years <130 U/ml, 4–7 years <150 U/ml, >7 years <200 U/ml). The mean serum IgE was 35 U/ml and the mean age was 4·1 years. They were otherwise well, free of acute infection and not on any medication at the time of testing. Heparinized venous blood was obtained immediately on induction of anaesthetic.

Cell cultures

Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood by density gradient centrifugation over Ficoll–Hypaque. Heparinized blood (5 ml) was layered on 3 ml of Ficoll–Hypaque and centrifuged at 700 ***g*** for 20 min. The low density PBMC layer was aspirated and washed three times in sterile PBS. PBMC were cultured at a concentration of 2×10^6 cells/ml in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Cultures were stimulated with 50 ng/ml PMA and 2·0 μM calcium ionophore (PMA/Ca). Where Cyclosporin A (CsA) was added to PMA/Ca-stimulated cultures, this was added before addition of PMA/Ca. A dose of 100 ng/ml CsA was used, as this achieved $>95\%$ inhibitory effect on intracellular IFN-γ in preliminary dose response experiments in adults. PBMC were harvested at 6 h as detection of intracellular IFN-γ was greater at 6 h of culture ($13{\cdot}3 \pm 3{\cdot}8\%$ (mean ± s.e.m.)) than at 24 h ($4{\cdot}9 \pm 1{\cdot}1\%$). Cells were collected by gentle pipetting and adequate retrieval was confirmed by examination on an inverted light microscope. The supernatants from 6 h and 72 h cultures were stored at −70C for measurement of IFN-γ. We have found that the kinetics of IFN-γ secretion are similar in atopic and control children, with progressive accumulation of IFN-γ following stimulation to reach maximal levels at 72 h [5].

Detection of intracellular IFN-γ

The method used to detect intracellular IFN-γ is as described by Sander *et al.*, with a modification of the detergent used for permeabilization [12]. As we were unable to obtain adequate positive staining for mitochondria using 0·1% saponin in PBS, we elected to use PBS containing 0·05% Nonidet-P40 (0·05% NP40). Briefly, PBMC were washed in sterile PBS and resuspended at a concentration of 10×10^6 in sterile PBS. Aliquots of 5×10^5 cells were fixed with 0·5 ml of cold 4% paraformaldehyde (16·9 g/*l* $NaH_2PO_4 \times H_2O$, 3·85 g/*l* NaOH, 40 g/*l* paraformaldehyde, 5·4 g/*l* glucose, pH 7·4) for 5 min at room temperature. After washing with PBS the cells were permeabilized with 0·5 ml PBS containing 0·05% NP40 for 5 min at room temperature. After two washes in PBS, the cells were incubated in 0·5 ml blocking solution (PBS containing 1% bovine serum albumin (BSA)) for 30 min at room temperature. The suspension was then spun down and the blocking solution tipped off. Fifty microlitres of either mouse anti-human IFN-γ antibody, mouse anti-human mitochondria antibody, or mouse IgG1 antibody were added to the cell pellet at a concentration of 10 μg/ml in 1% BSA and incubated for 1 h at 4°C. After two washes in sterile PBS, 50 μl of R-PE-conjugated sheep anti-mouse IgG diluted 1:40 in 5% heat-inactivated human AB serum were added and incubated at 4°C for 40 min. After two washes in sterile PBS, the cells were resuspended in PBS and analysed on a FACSTAR fluorescence-activated cell sorter using Consort 30 software (Becton Dickinson). Five thousand events were collected for each analysis. The lymphocyte population was gated on forward and side scatter characteristics and histograms of fluorescence intensity were generated for each set of antibodies. Specificity for detection of IFN-γ-producing cells was demonstrated using several approaches. Analysis of Jurkat cells known to produce IFN-γ upon stimulation but not constitutively [13] revealed a uniform increase in fluorescence intensity following stimulation with PMA/Ca (Fig. 1a), which was inhibited by the presence of CsA. In contrast, when two T cell lines, CEM-CCRF [14] and CEM-VLB100 [15], and a pre-B cell line, NALM-6 [16] which did not secrete IFN-γ following stimulation with PMA/Ca were examined, no IFN-γ^+ cells were found (Fig. 1b). Analysis of cultured PBMC from normal controls and children with atopic dermatitis revealed a proportion of IFN-γ^+ cells following stimulation with PMA/Ca rather than a uniform shift in fluorescence intensity as seen with Jurkat cells (Fig. 1c and d). The percentage of IFN-γ^+ PBMC was quantified by subtracting the percentage of cells staining with the isotype control antibody from the percentage staining with specific antibody. The isotype-specific IgG1 antibody was used as a control for non-specific binding of antibody. The anti-mitochondrial antibody

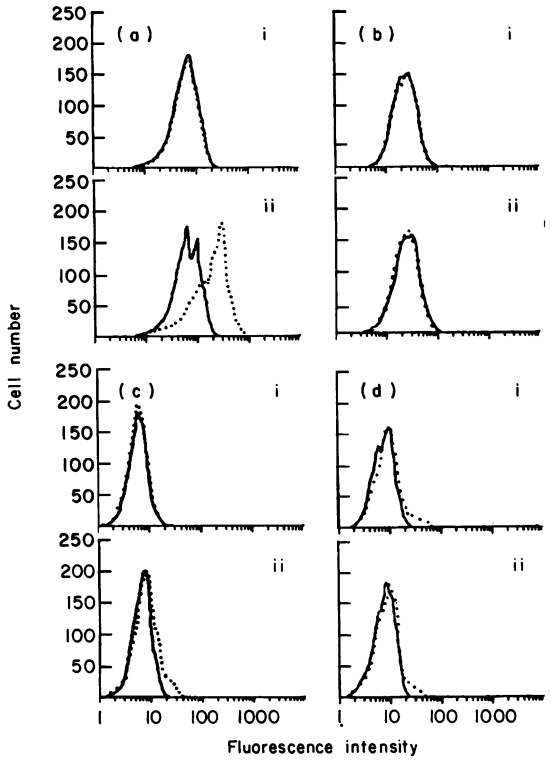

Fig. 1. Representative fluorescence intensity profiles for (a) Jurkat cells, (b) CEM-CCRF, CEM-VLB100, NALM-6 cell lines, (c) lymphocytes from non-atopic controls, and (d) lymphocytes from patients with atopic dermatitis which were examined for intracellular IFN-γ after 6 h of culture. In each case, fluorescence profiles for (i) unstimulated and (ii) phorbol myristate acetate (PMA)/Ca-stimulated cultures are presented in separate panels. ——, isotype-specific control antibody; . . . . . , anti-hu IFN-γ antibody.

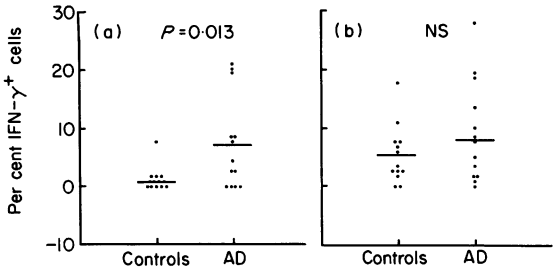

Fig. 2. Peripheral blood mononuclear cells (PBMC) from children with atopic dermatitis (AD) and age-matched controls were cultured for 6 h with or without phorbol myristate acetate (PMA)/Ca. (a) Children with AD had a significantly greater percentage of IFN-γ^+ cells in unstimulated cultures than controls. (b) The percentage of IFN-γ^+ cells in stimulated cultures was not significantly different in the two groups.

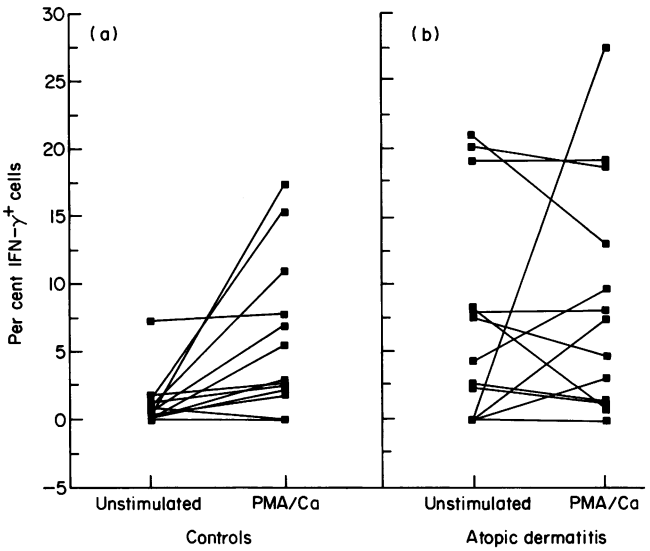

Fig. 3. Shifts in the percentage of IFN-γ^+ cells following *in vitro* stimulation with phorbol myristate acetate (PMA)/Ca were examined. (a) The percentage of IFN-γ^+ cells in peripheral blood mononuclear cell (PBMC) cultures from controls increased significantly ($P=0{\cdot}012$) following stimulation. (b) There was no significant increase in the percentage of IFN-γ^+ cells in PBMC cultures from children with atopic dermatitis (AD) following stimulation.

was used as a positive control to confirm adequate permeabilization of cells.

Cytokine assays

Supernatants were stored at −70°C. IFN-γ was measured on a human IFN-γ ELISA kit (CSL) using two MoAbs directed to IFN-γ according to the manufacturer's instructions. Purified human IFN-γ supplied with the kit was used as a standard. Sensitivity of the assay was 1 U/ml. Plates were read on a spectrophotometer at wavelength 450 nm. Levels of IFN-γ (U/ml) were determined from the optical density readings at 450 nm (OD_{450}) using BIOMEK 1000 EIA data reduction software (Beckman). All samples were run in duplicate.

IgE assay

Plasma samples were stored at −70°C until processed for IgE measurements. Plasma IgE levels were determined by immunoradiometric assay using Quanticlone IgE IRMA kits according to the manufacturer's instructions.

Statistical analysis

Analyses involving percentages of IFN-γ-producing cells were carried out using Student's t-test. The independent t-test was used to assess comparisons between groups, and the paired t-test was used to test the significance of shifts in paired samples. As data regarding secreted IFN-γ in supernatants were non-parametric, pairwise comparisons between groups were carried out using the Mann–Whitney non-parametric U-test statistic, and the significance of shifts in paired samples was assessed using the Wilcoxon sign test.

RESULTS

Percentage of IFN-γ^+ cells in unstimulated and PMA/Ca-stimulated cultures

Representative fluorescence profiles for unstimulated and stimulated cultures from normal controls and children with atopic dermatitis are presented in Fig. 1c and d. Children with AD had a significantly greater proportion of IFN-γ^+ cells in unstimulated cultures compared with controls (mean AD 7·2%, mean controls 1%, $P=0{\cdot}013$) (Fig. 2a). Nine of the 13 children with

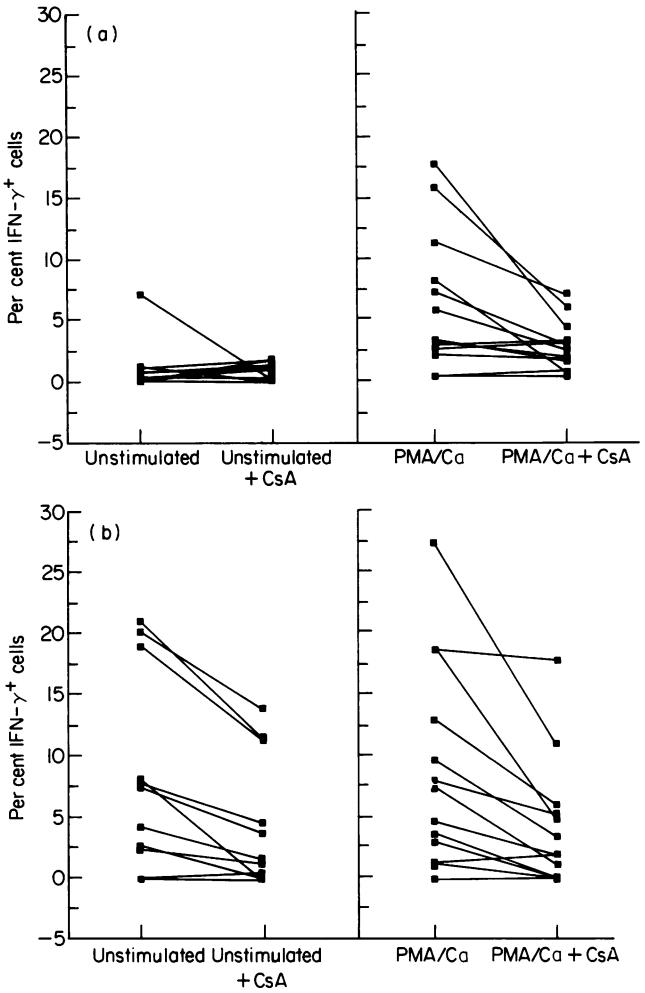

Fig. 4. Effect of Cyclosporin A (CsA) on the percentage of IFN-γ^+ cells. (a) Addition of 100 ng/ml CsA to stimulated cultures from controls resulted in a significant reduction of IFN-γ^+ cells ($P=0{\cdot}043$). (b) Addition of 100 ng/ml CsA to peripheral blood mononuclear cell (PBMC) cultures from children with atopic dermatitis (AD) induced a significant reduction of IFN-γ^+ cells in both unstimulated ($P=0{\cdot}005$) and phorbol myristate acetate (PMA)/Ca-stimulated cultures ($P=0{\cdot}009$).

AD had more than 2% IFN-γ^+ cells in unstimulated cultures, compared with only one of 13 controls. The percentage of IFN-γ^+ cells in cultures stimulated with PMA/Ca was not significantly different in the two groups (mean AD 8·9%, mean controls 5·2%) (Fig. 2b). In the atopic group, the percentage of IFN-γ^+ cells did not change significantly following stimulation ($P>0{\cdot}05$). In contrast, there was a significant increase in the percentage of IFN-γ^+ cells on stimulation in the controls ($P=0{\cdot}012$) (Fig. 3a, b).

Effect of CsA on the percentage of IFN-γ^+ cells

CsA (100 ng/ml) was added to both unstimulated and stimulated cultures to determine if the intracellular IFN-γ in unstimulated cells from the atopic children was newly synthesized *in vitro*, as well as to investigate whether the increase in intracellular IFN-γ seen with stimulation represented newly synthesized IFN-γ. Addition of CsA to the stimulated cultures from controls resulted in a significant reduction in the proportion of IFN-γ^+ cells (5·2% to 2·5%, $P=0{\cdot}043$) (Fig. 4a). The percentage of IFN-γ^+ cells in unstimulated cultures from controls remained unchanged in the presence of CsA (1% to 1·1%). In the single control subject with a significant proportion of IFN-γ^+ cells in unstimulated culture, addition of CsA resulted in a reduction from 7% to 0% IFN-γ^+ cells. In the atopic children, addition of CsA resulted in a significant reduction in the proportion of IFN-γ^+ cells in both unstimulated (7·2% to 3·8%, $P=0{\cdot}005$) and stimulated cultures (8·9% to 4·2%, $P=0{\cdot}009$) (Fig. 4b). The percentage of IFN-γ^+ cells in CsA-treated cultures was not significantly different in the atopic children and controls for both unstimulated and stimulated cultures.

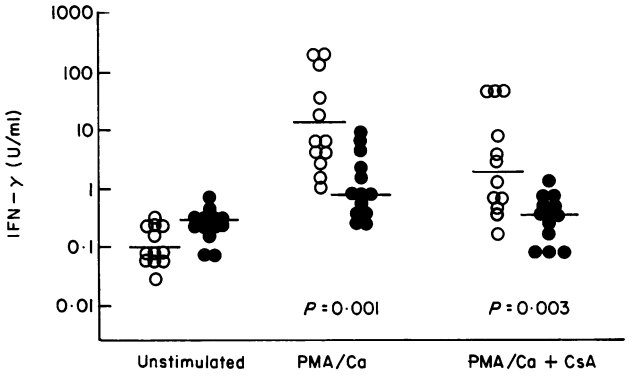

Fig. 5. Secretion of IFN-γ in peripheral blood mononuclear cell (PBMC) cultures at 6 h was measured by ELISA. There was no detectable IFN-γ in unstimulated cultures from children with atopic dermatitis (AD) (●) or controls (○). Children with AD secreted significantly less IFN-γ in phorbol myristate acetate (PMA)/Ca-stimulated cultures than controls ($P=0{\cdot}001$). Addition of 100 ng/ml Cyclosporin A (CsA) to PMA/Ca-stimulated cultures significantly inhibited secretion of IFN-γ in both children with AD ($P=0{\cdot}023$) and controls ($P=0{\cdot}002$), but secretion of IFN-γ remained significantly greater in controls ($P=0{\cdot}003$).

IFN-γ secretion in supernatants at 6 h and 72 h

Secretion of IFN-γ into supernatants at 6 h was determined for both groups (Fig. 5). There was no detectable IFN-γ secretion in unstimulated cultures from either atopic children or controls. Stimulation with PMA/Ca resulted in a significant increase of IFN-γ secretion in both the atopic children ($P=0{\cdot}006$) and controls ($P=0{\cdot}004$). However, only five of 13 children with AD secreted detectable amounts ($\geqslant 1$ U/ml) of IFN-γ in stimulated cultures at 6 h compared with 12 of 13 controls. Secretion of IFN-γ in stimulated cultures from controls was significantly greater than in atopic children ($P=0{\cdot}001$). Addition of CsA to stimulated cultures significantly inhibited secretion of IFN-γ in both the atopic ($P=0{\cdot}023$) and control ($P=0{\cdot}002$) groups, but secretion of IFN-γ in PMA/Ca+CsA treated cultures from controls remained significantly higher than that in the atopic group ($P=0{\cdot}003$).

At 72 h, secretion of IFN-γ in stimulated cultures was significantly lower in the atopic children compared with controls ($P=0{\cdot}04$; geometric mean for AD 39·0 U/ml, 95%CI 8·2–186·6; geometric mean for controls 346·7 U/ml, 95%CI 151·4–794·3). In contrast to the findings at 6 h, all controls ($n=8$) and all but one of the atopic children ($n=10$) had detectable levels of IFN-γ at 72 h. There was no detectable IFN-γ in unstimulated cultures from either the atopic children or controls.

DISCUSSION

The principal finding of this study was that children with active AD had a significantly greater percentage of PBMC containing intracellular IFN-γ (IFN-γ^+ cells) in unstimulated cultures compared with age-matched non-atopic controls. Previous studies have demonstrated that intracellular IFN-γ is not present in unstimulated PBMC from healthy adults, and that activation of T lymphocytes with either specific antigen or non-specific stimuli such as PMA/Ca is required for the detection of intracellular IFN-γ [17–20]. Our finding that control children had $< 1\%$ IFN-γ^+ cells in unstimulated cultures which increased to 5·2% following stimulation with PMA/Ca is similar to these previous reports. Thus, the presence of a significant percentage of IFN-γ^+ cells in children with AD without stimulation *in vitro* implies that previous activation has occurred *in vivo*. The fact that *in vitro* stimulation did not result in a further significant increase of IFN-γ^+ cells in these children would also support this possibility. The *in vivo* activation of PBMC may be a feature of the extensive inflammation present in these children, as they had active dermatitis involving greater than 20% of their skin surface area. Active atopic dermatitis is associated with a marked inflammatory infiltrate of CD4$^+$ T helper cells within the dermatitis lesions [21] as well as marked lymph node hypertrophy with proliferation of the paracortical T cell areas in lymph nodes [22]. As T cells are the predominant source of IFN-γ [23], the generation of IFN-γ^+ cells could occur at either of these sites, with the cells entering the circulation from lymph nodes via lymphatics or directly from involved skin. The putative activating antigens are unknown, although food and inhalant allergens have been suggested to be involved [24–26]. *Staphylococcus aureus* may also contribute to this activation, as *Staph. aureus* colonization/infection is a persistent finding in atopic dermatitis [27], and at least one staphylococcal antigen (enterotoxin A) is known to be a potent inducer of IFN-γ production *in vitro* [28,29].

In PMA/Ca-stimulated cultures, the non-atopic children and the children with AD were found to have 5·2% and 8·6% IFN-γ^+ PBMC, respectively. These percentages are similar to previous studies in healthy adults, where the proportion of PBMC containing intracellular IFN-γ determined by fluorescence cytometry or microscopy was found to be between 2·8% and 9·5% at 6 h following non-specific stimulation with phytohaemagglutinin (PHA), PMA/Ca or OKT3 [17,18,20]. Our finding that addition of 100 ng/ml CsA to stimulated cultures resulted in a 55% and 52% decrease in the mean proportion of IFN-γ^+ cells in atopic dermatitis children and controls, respectively, implies the presence of active transcription of IFN-γ rather than passive uptake as has been suggested by Kabilan *et al.* [20]. A similar level of inhibition was found in a previous study, where 60 ng/ml of CsA induced a 50% reduction in the percentage of IFN-γ^+ cells [18]. In addition, if the intracellular IFN-γ largely represented internalized IFN-γ, one might have expected to find a greater percentage of IFN-γ^+ cells at 24 h, when there is more secreted IFN-γ in the culture supernatant rather than at 6 h as was found in our study.

Despite the presence of a significant percentage of PBMC producing intracellular IFN-γ in unstimulated cultures from children with AD, there was no detectable spontaneous secretion of IFN-γ into supernatants at either 6 h or 72 h. Furthermore, stimulation with PMA/Ca induced secretion of IFN-γ in cultures from children with AD without a corresponding increase in the percentage of IFN-γ^+ cells. One possible explanation for these findings is that an ongoing stimulus is required for secretion of IFN-γ, so that removal of PBMC from *in vivo* stimuli resulted in cessation of secretion. An alternative explanation is that although PBMC from children with AD are able to produce intracellular IFN-γ, they have a reduced ability to secrete this cytokine. Previous studies in healthy adults have demonstrated a heterogeneity in the ability of PBMC containing intracellular cytokine to subsequently secrete it. Kabilan *et al.* found that only 20% of PHA-stimulated lymphocytes which produced intracellular IFN-γ subsequently secreted it, and that amongst IFN-γ-secreting cells, there was a variability in the amount secreted per cell [20]. It has also been suggested that T cells can focus lymphokines produced intracellularly directly to antigen-presenting cells with little release into the environment [30]. Whatever the reasons for the discrepancy between intracellular and secreted IFN-γ in children with AD, the finding that secretion of IFN-γ in stimulated cultures from children with AD was significantly lower than in controls despite the presence of an equivalent percentage of IFN-γ-producing cells implies that the reduced IFN-γ secretion does not relate to a lack of PBMC capable of producing IFN-γ, but rather to a reduced production or secretion of IFN-γ per cell. The finding that CsA induced a similar magnitude of reduction in the percentage of IFN-γ^+ cells in cultures from children with AD and controls also indicates that similar proportions of PBMC in these two groups were transcribing and producing intracellular IFN-γ. As stimulation with PMA and calcium is known to bypass surface receptor activation and to activate protein kinase C and calcium release directly [31], it seems likely that any defect of IFN-γ production or secretion in PBMC from children with AD lies beyond the stage of signal transduction from surface receptor. Studies of the regulation of IFN-γ mRNA transcription will help to further localize the level at which IFN-γ production is impaired.

It has recently been postulated that there is a deficiency of allergen-specific T cells producing IFN-γ and a preponderance of allergen-specific T cells producing IL-4 in the peripheral blood of adults with atopic dermatitis [32–34]. However, this has not been directly demonstrated. Although studies of *Dermatophagoides pteronyssinus*-specific TCC generated from the peripheral blood of six atopic adults, three of whom had atopic dermatitis, have documented a preponderance of clones producing IL-4 with little or no IFN-γ [32–34], the subjects tested had elevated specific IgE antibodies to *Der p I*, and it might be expected that any allergen-specific TCC would produce greater amounts of IL-4 compared with IFN-γ. The importance of specific inhaled allergens such as *Der p I* in AD remains uncertain, and a role for other stimuli such as *Staph. aureus* is possible. Studies of non-specific T cell clones generated from AD patients without the use of an allergen have revealed conflicting results. T lymphocytes expanded from active AD lesions were found to produce significant amounts of IL-4 with little or no IFN-γ [35], but a similar abnormality has not been confirmed for clones generated from the peripheral blood [9,10]. One study reported a deficiency of IFN-γ-producing clones generated from the peripheral blood of AD subjects, whilst another found no significant difference between atopic patients and controls [9,10]. In these studies of specific or non-specific clones, expansion of T cells was achieved by stimulation with IL-2 alone or in combination with IL-4 [9,10,30–33]. Cloning

with either IL-2 or IL-4 has been shown to preferentially stimulate cells to produce IL-4 with little or no IFN-γ, compared with cloning in the presence of IFN-γ [36–38]. Furthermore, the cytokine profiles of these clones were determined by secretion, and as demonstrated in this and previous studies [20], cytokine secretion into supernatant may not correlate with intracellular production. There have been no studies directly examining the proportions of IL-4 or IFN-γ-producing cells within the peripheral blood or skin lesions of patients with atopic dermatitis. Direct examination for IFN-γ mRNA expression in bronchoalveolar lavage cells from asthmatics and controls showed an equivalent number of mRNA$^+$ cells in the two groups [39]. Similar studies of cutaneous lesions and draining lymph nodes from individuals with atopic dermatitis would obviously be of interest.

ACKNOWLEDGMENTS

We thank Dr G. Varigos, Director Department of Dermatology, Royal Children's Hospital, for facilitating access to patients under his care. M.T. is a recipient of a National Health and Medical Research Council medical postgraduate scholarship.

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