

Original article

Increased sensitivity to IL-4 in cystic fibrosis patients with allergic bronchopulmonary aspergillosis

Background: Allergic bronchopulmonary aspergillosis (ABPA) is characterized by a heightened Th2 CD4⁺ T-cell response to *Aspergillus fumigatus* allergens and a hyper-immunoglobulin (Ig)E state compared with cystic fibrosis patients without ABPA. We hypothesize that one reason for this response is increased sensitivity to interleukin (IL)-4 in ABPA resulting in increased expression of CD23 and CD86 and leading to a positive amplification mechanism that increases Th2 CD4⁺ T cell responses.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from seven ABPA CF and 19 non-ABPA CF patients and 16 nonatopic controls and stimulated with rIL-4 (range 0.1–10 ng/ml) and rIL-13 (range 1–10 ng/ml) for 48 h. The number of CD23 molecules and percentages of CD23⁺ B cells were quantified by flow cytometry. Both phorbol 12-myristate 13-acetate (PMA)/ionomycin (IO) and antigen stimulated, toxoid and Asp f2/f3/f4, PBMC were examined for cytoplasmic cytokine synthesis enumerated by cytokine staining using flow cytometry to measure Th2 and Th1 CD3⁺ T cells.

Results: The numbers of CD23 molecules on B-cells were significantly elevated at time 0 in ABPA CF patients compared with both non-ABPA CF patients and nonatopic controls. Following IL-4 stimulation *in vitro*, the numbers and percentages of CD23 expression on B cells were significantly up-regulated in ABPA CF patients compared with non-ABPA CF patients and controls. The IL-13 stimulation up-regulated CD23 expression; however, there was no significant difference in ABPA CF patients compared with non-ABPA CF patients and controls. The percentages of interferon (IFN)- γ ⁺ CD3⁺ T cells following PMA/IO stimulation were significantly decreased in both ABPA and non-ABPA CF patients compared with controls. There were no significant differences of IL-4⁺ and IL-13⁺ CD3⁺ T cells between ABPA and non-ABPA CF patients. When tetanus toxoid stimulated T cells were examined, both ABPA and non-ABPA CF patients had significantly decreased IFN- γ ⁺ CD3⁺ T cells compared with controls. In Asp f2/f3/f4 stimulated T cells, ABPA CF patients had significantly increased IL-4⁺ CD3⁺ T cells compared with non-ABPA CF patients and controls.

Conclusions: ABPA CF patients have increased sensitivity to IL-4 but not to IL-13 up-regulation of CD23 molecules compared with non-ABPA CF patients. There were decreased percentages of IFN- γ ⁺ and IL-2⁺ Th1 T cells in CF patients compared with nonatopic controls but similar percentages of IL-4⁺ Th2 T cells in all three groups. However, ABPA CF patients had increased frequency of *Aspergillus*-stimulated Th2 T cells. This indicated that there is skewing of Th2 T cells in ABPA CF patients. Thus, in CF ABPA patients there is increased Th2 T cells and increased sensitivity to IL-4.

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Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease mediated by an allergic late-phase inflammatory response to *Aspergillus fumigatus* (Af) antigens characterized by markedly elevated Af-specific and total immunoglobulin (Ig)E levels and eosinophilia, manifested by wheezing, pulmonary infiltrates and

bronchiectasis and fibrosis that occurs in 7–15% of patients with cystic fibrosis (CF) (1). The diagnosis of ABPA in patients with CF is especially difficult because of the high prevalence of *A. fumigatus* bronchial colonization, elevated total and *Aspergillus*-specific IgE antibodies, pulmonary infiltrates, bronchiectasis and atopy. In both

ABPA and atopic CF patients, elevated IgE and IgG anti-*A. fumigatus* antibodies, elevated total serum IgE concentrations, and Th2 CD4⁺ T cells are present (1). Previously we reported that there was an interleukin (IL)-4 dependent autocrine Th2 CD4⁺ T cell response to an *Aspergillus* allergen, Asp f1 (2). In addition, ABPA patients' B cells spontaneously synthesized and secreted IgE suggesting *in vivo* IL-4 and/or IL-13 stimulation (3). The IL-4 up-regulates cellular activity via binding to IL-4 receptor (IL-4R) found on a variety of cells, including B, NK, subpopulation of T, mast cells, and endothelial cells (4–6). Recently, mutations of IL-4 receptor alpha chain (IL-4R α) in atopic individuals associated with elevated IgE levels have been reported (7, 8). These mutations increase IL-4 and IL-4R interactions, which may result in a gain-of-function of IL-4R α . The IL-4 induction of the low affinity IgE receptor CD23 and soluble CD23 augments B cell IgE synthesis (9–14). In addition, T cell CD23 and B cell CD21 cognate ligation augments B cell IgE synthesis. CD86 on B cells is also an important costimulatory molecule for augmentation of IgE synthesis (15–26). The ligand for CD86 is CD28 on T-cells. Several studies have previously observed CD86 to be critical in promoting Th2 CD4⁺ T cell responses, Th2 cytokine production, eosinophilic airway inflammation, and airway hyperresponsiveness after antigen challenge. The CD86 expression on B-cells is up-regulated in response to IL-4 in atopic patients. In the present study, CD23 and CD86 expression was observed to be increased in ABPA subjects. The IL-13R is also a heterodimer, consisting of IL-4R α and IL-13R α chains (27–31). Interleukin-4 stimulates both the IL-4R and the IL-13R receptors; whereas both IL-4 and IL-13 stimulate the IL-4 α /IL-13R α receptor. The IL-13, like IL-4, increases CD23 expression, IgE isotype switching, and IgE synthesis. In the present study, we hypothesized that there may be an increased quantitative Th2 response in ABPA compared with atopic non-ABPA CF patients. The results indicate that ABPA CF patients have increased sensitivity to IL-4 stimulation and have increased frequency of *A. fumigatus* specific Th2 cells.

Methods

Patients

The CF patients were enrolled in the study from the Cardinal Glennon Children's Hospital Cystic Fibrosis Clinic. Studies were approved by the Institutional Review Board at St Louis University, and informed consent was obtained. The diagnosis of ABPA was based on the presence of the following criteria recommended by the Cystic Fibrosis Society:

- asthma (recurrent wheezing),
- pulmonary infiltrates unresponsive to antibiotics and resolved with corticosteroids,
- elevated total serum IgE > 1000 IU/ml,
- positive *A. fumigatus* immediate prick skin test,
- positive precipitating antibodies to *A. fumigatus* and

- elevated IgE and IgG anti-*A. fumigatus* antibodies by enzyme-linked immunosorbant assay (ELISA) or UniCAP.

The diagnosis of ABPA in CF patients can be very difficult. Criteria no. 2 is important in the diagnosis; namely, pulmonary infiltrates unresponsive to antibiotics and that resolved with systemic corticosteroid treatment. Bronchiectasis and eosinophilia were not required for a diagnosis. Adult nonatopic controls without CF and without asthma were obtained from laboratory and hospital personnel. The study was approved by the St Louis University Institutional Review Board. The ABPA CF patients were studied during periods of remission and flares.

IL-4 induction of B-cell CD23 expression

Peripheral blood mononuclear cells (PBMC) were isolated from venous blood by Ficoll-Hypaque density centrifugation as previously described (32). The PBMC were cultured at 1×10^6 cells/ml in 1 ml of Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% fetal calf serum (FCS) for 48 h at 37°C in a 6% CO₂ humidified atmosphere. The cultures were stimulated with rhuIL-4 (PeproTech, Inc, Rocky Hill, NJ) at 0.5, 1.0, 5.0, 10 and 25 ng/ml and with rhuIL-13 (Biosource International, Camarillo, CA) at 1, 5, and 10 ng/ml. After 48 h, the cells were washed and analyzed by flow cytometry.

Recombinant *A. fumigatus* allergens

Three major recombinant allergens (Asp f 2, f 3, and f 4) from *A. fumigatus* have been used in the study. A cDNA library was constructed from *A. fumigatus* in λ ZAP II vector and the protein expressed in a pET 23b (+) vector as described before (33–35). After IPTG induction, the cells containing the expressed proteins were sonicated and the recombinant proteins were purified using Ni-NTA agarose column (Qiagen, Inc., Valencia, CA). The purity, molecular size, and the reactivity of the allergens with known sera were ascertained as described (35). All the recombinant allergens were stored at –70°C.

Th2 and Th1 cells

Both total and antigen-specific Th2 and Th1 T cells were measured by analysis of cytoplasmic interferon (IFN)- γ and IL-4 as previously described (32). The PBMC were cultured at 1×10^6 cells/ml in 1 ml of RPMI and 10% FCS for 8 h stimulated with media or with phorbol ester 50 ng/ml [phorbol 12-myristate 13-acetate (PMA), Sigma, St Louis, MO] and ionomycin (IO) 1 μ g/ml (Sigma). Brefeldin 10 μ g/ml (Sigma) was added to block the secretion of stimulated cytokines. The PBMC were washed with phosphate-buffered saline (PBS) containing 2% FCS and analyzed for cytoplasmic cytokines by flow cytometry.

For antigen specific T cells, PBMC 1×10^6 cells/ml were cultured for 7 days stimulated either with medium, tetanus toxoid 4 LF/ml (Connaught Laboratories, Swiftwater, PA) or Asp f2/f3/f4 10 μ g/ml. After 7 days, the supernatants were harvested and frozen at –20°C until analysis of IFN- γ and IL-4. The PBMC was resuspended in RPMI and stimulated with PMA/IO for 8 h. After culture, the PBMC were washed and cytoplasmic IFN- γ and IL-4 were measured by flow cytometry.

Flow cytometry

The PBMC prior to culture and after culture were analyzed for induction of cell surface CD23 and CD86 expression on B-cells, and

T-cells analyzed for induction of cytoplasmic cytokine expression as previously described (32). For cultures stimulated with IL-4 and IL-13, PBMC were washed and stained with murine monoclonal antibody to CD23-PE, CD86-FITC and CD20 per CP (Becton Dickinson, San Jose, CA). The PBMCs were washed and fixed with 1% PBS buffered paraformaldehyde. Forward and side-scatter was performed to gate on the live lymphocyte population and were further gated on CD20⁺ cells for analysis using the CellQuest MacIntosh program (Becton Dickinson). A minimum of 10 000 cells were counted. Quantibrite PE flow cytometry beads (Becton Dickinson) were used to quantitate the number of CD23 receptors per B-cell for each experiment. The beads contain a given number of PE molecules per bead. A linear equation was calculated from which the number of CD23 receptors per cell is extrapolated. Both the percentages of B-cells expressing CD23 and CD86 and the total number of CD23 receptors expressed per B-cell were measured. Staining of cytoplasmic cytokines was performed as previously described. After culture, the PBMC were washed with PBS with 2% FCS. The PBMC were made permeable with CytoPerm/Cytofix (BD Pharmingen, San Diego, CA), washed twice with Perm/Wash buffer (BD Pharmingen), stained with murine monoclonal antibodies to IFN- γ FITC, IL-2 FITC, IL-4 PE, IL-13 PE, and CD3 PerCP (Becton Dickinson). A minimum of 25 000 cells were counted with gating on CD3 + T cells.

IgE levels

Total serum IgE levels were measured by UniCAP according to manufacturer's instructions (Pharmacia, New York, NY). The IgE and IgG antibodies to unfractionated *A. fumigatus* extract were measured by UniCAP according to manufacturer's instructions. IgE antibodies to recombinant *A. fumigatus* allergens were measured by ELISA according to previously described methods (35). Five micrograms per ml of Asp f2, Asp f3, and Asp f4 antigens in PBS pH 7.4 was used coated on the ELISA plates. Serum dilutions were allowed to react with the antigen and the IgE antigen binding was demonstrated by color reaction as described before. The color was read as O.D. values and the data evaluated by comparing the different groups.

HLA-DR genotypes

Low resolution HLA-DR typing was performed by the HLA Laboratory at St Louis University Health Sciences Center.

Statistics

The data was expressed as the geometric mean $\pm$ SE. Statistical analysis using one way analysis of variance (ANOVA) was performed on the three groups, followed by a *post hoc* procedure, Scheffe test to determine difference between pairs using SPSS software (SPSS Inc, Chicago, IL).

Results

The mean ages of ABPA and non-ABPA CF patients were comparable, 12 ± 1 years vs 15 ± 1 years (Table 1). The male to female sex ratio was predominantly male in the ABPA group, 6 : 1, compared with 11 : 8 in the non-ABPA CF group. The ABPA CF patients had significantly increased serum total IgE levels compared

Table 1. Characteristics of cystic fibrosis patients with allergic bronchopulmonary aspergillosis (ABPA)

Test	ABPA CF 7	Non-ABPA CF 19	Control 16	P-value*
Age, years	12 $\pm$ 1	15 $\pm$ 1	41 $\pm$ 13	
Sex, M : F	6 : 1	11 : 8	8 : 8	
FEV-1, % predicted	61 $\pm$ 5	85 $\pm$ 7	—	
Medications at time of study, % of patients				
Inhaled corticosteroids	76%	5%	0%	<0.01
LABA	14%	0%	0%	<0.04
LTRA	19%	5%	0%	
Systemic corticosteroids				
Daily	33%	0%	0%	0.01
QOD	5%	0%	0%	
Itraconazole	52%	0%	0%	<0.01
IgE, IU/ml	1859 $\pm$ 626	87 $\pm$ 25	52 $\pm$ 22	<0.01
Range	104–8079	6–294	33–310	
Af ST+, %	100	32	0	
IgE anti-Af, μ g/l	45.7 $\pm$ 11.0	3.2 $\pm$ 2.1	0 $\pm$ 0	<0.001
IgG anti-Af, μ g/l	78.5 $\pm$ 15.4	36.2 $\pm$ 9.3	43.4 $\pm$ 12.1	<0.01
IgE anti-Asp f2, OD	1.75 $\pm$ 0.41	0.04 $\pm$ 0.02	0	<0.01
IgE anti-Asp f3, OD	0.69 $\pm$ 0.28	0.01 $\pm$ 0.01	0	<0.02
IgE anti-Asp f4, OD	1.31 $\pm$ 0.40	0.01 $\pm$ 0.01	0	<0.01
HLA-DR types, % of patients				
HLA-DR2+ and/or DR5+	80%	12%	—	<0.05
HLA-DR4+ and/or DR7+	20%	70%		
HLA-DQ2+	20%	29%		

* P-value comparing ABPA vs non-ABPA. Statistical analysis using one way analysis of variance (ANOVA) was performed on the three groups, followed by a *post hoc* procedure, Scheffe test to determine difference between pairs.

Data expressed as mean $\pm$ SE.

M, male; F, female; LABA, long-acting- β 2-agonist; LTRA, leukotriene receptor antagonist; QOD, every other day; Af, *Aspergillus fumigatus*; ST, skin test positive; Ig, immunoglobulin.

with non-ABPA CF patients, 1859 ± 626 vs 87 ± 25 ($P < 0.01$). The ABPA CF patients' serum IgE levels ranged from 104 to 8079 IU/ml while non-ABPA CF patients serum IgE levels ranged from 6 to 294 IU/ml. One ABPA represented the low IgE of 104 IU/ml after being treated with corticosteroids and itraconazole. His peak IgE level was 263 IU/ml and he has not had a subsequent flare. During flares of ABPA, patients were treated with systemic corticosteroids. In order to determine whether different stages of ABPA and medications affected the results of the studies, ABPA patients were studied at different times. The majority of ABPA patients were treated with inhaled corticosteroids, 76%, compared with only 5% of non-ABPA CF patients. In the ABPA CF patients, studies were performed 33% of the time when patients were on daily corticosteroids and 5% of the time when patients were on alternate day corticosteroids. Fifty-two percent of the studies in ABPA patients were performed when the patients were taking itraconazole. The number of ABPA patients in this study is small, but no discernible differences were observed attributed to medications. Of the non-ABPA CF patients, 32% were Af skin test positive and 68% were Af skin test negative.

Serum IgE levels were slightly increased in Af skin test positive compared with Af skin test negative CF patients, 125 ± 29 vs 52 ± 22 ($P = \text{NS}$).

The results of *Aspergillus* specific IgE against various allergens in the two groups of sera studied are presented in Table 1. All recombinant allergens showed higher levels of specific IgE in ABPA CF patients compared with CF without ABPA. The levels of IgE were strikingly different against Asp f2, f3, and f4 and may be of value in differentiating the two groups. Serum IgE anti-Af antibody levels were significantly higher in ABPA CF compared with non-ABPA CF patients, 45.7 ± 11.0 vs 3.2 ± 2.1 ($P < 0.001$). When used together these three allergens identified ABPA in the CF patients with $>90\%$ specificity and sensitivity. All three allergens have an intrinsic property eliciting a Th2 type response in the patients. ABPA CF patients had significantly elevated IgG anti-Af antibodies compared with non-ABPA CF patients, 78.5 ± 15.4 vs 36.2 ± 9.3 ($P < 0.01$); however, both Af ST+ and ST- CF patients had similar IgG anti-Af antibody titers.

The presence of HLA-DR2 and DR-5 has been associated with risk for development of ABPA. Eighty percent of ABPA CF patients had the presence of HLA-DR2 and/or HLA-DR5 compared with 12% of non-ABPA CF patients. The HLA-DR4 and DR7 may also be associated with development of ABPA. An additional 20% of CF ABPA and 70% of non-ABPA CF patients were either HLA-DR4+ or DR7+. HLA-DQ2 has been reported to be protective against the development of ABPA. However, 20% of ABPA and 29% of non-ABPA CF patients were HLA-DQ2+.

On day 0, the numbers of CD23 molecules per B-cell were significantly increased in ABPA CF patients compared with non-ABPA CF patients, 3406 ± 323 vs 2038 ± 186 molecules as seen in Fig. 1 ($P < 0.01$). Similarly, the numbers of CD23 molecules per CD86+ B-cell were significantly increased in ABPA vs non-ABPA CF patients, 3303 ± 337 vs 1962 ± 199 ($P < 0.02$). The percentages of CD23+ B-cells and CD23+CD86+ B-cells were significantly increased in ABPA patients compared with non-ABPA CF patients, $87 \pm 4\%$ vs $73 \pm 7\%$ ($P < 0.01$), and $48 \pm 5\%$ vs $27 \pm 4\%$ ($P < 0.01$), respectively.

Following IL-4 stimulation, the slope of increase of the numbers of CD23 molecules on B-cells and on CD86+ B-cells was significantly increased in ABPA compared with non-ABPA CF patients, 773 ± 97 vs 379 ± 73 ($P < 0.01$) and 934 ± 119 vs 548 ± 96 ($P < 0.01$), respectively. In contrast, following IL-13 stimulation as seen in Fig. 2, there was no significant difference in ABPA vs non-ABPA CF patients in the rate of increase of the number of CD23 molecules on B-cells and on CD86+ B-cells. However, when compared with Af skin test negative CF patients, ABPA patients did have significantly increased CD23 expression on B-cells ($P < 0.03$).

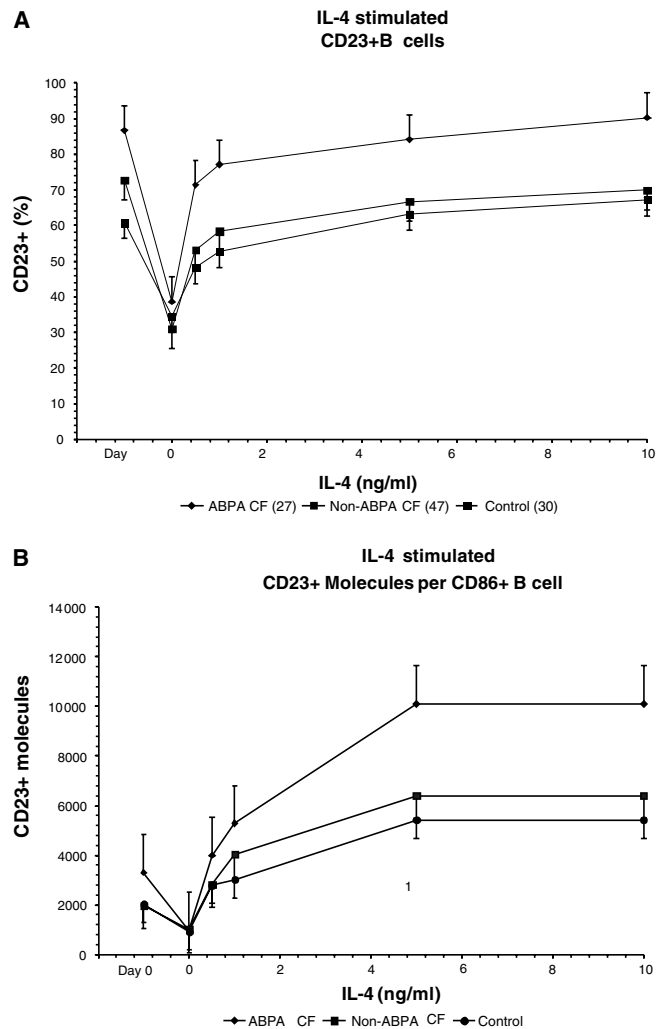


Figure 1. Interleukin (IL)-4 induction of CD23+ molecules on CD20+ and on CD86+ CD20+ B-cells. Allergic bronchopulmonary aspergillosis (ABPA) cystic fibrosis (CF) patients had significantly increased number of CD23 molecules per B-cell (A) and per CD86+ B-cell (B) on day 0 compared with non-ABPA CF patients and nonatopic control patients ($P < 0.01$). Following IL-4 stimulation, ABPA CF patients had a significantly increased rate of CD23+ expression per B-cell (A) and per CD86+ B-cell (B) compared with non-ABPA CF and nonatopic control patients ($P < 0.01$). Data presented as mean $\pm$ SE. Statistical analysis using one way analysis of variance (ANOVA) was performed on the three groups, followed by a *post hoc* procedure, Scheffe test to determine difference between pairs.

Both PMA/IO and antigen stimulated PBMC cells were examined for cytoplasmic cytokine synthesis. In PMA/IO stimulated cultures, IFN- γ , IL-2, IL-4 and IL-13 positive CD3+ T-cells were similar in ABPA compared with non-ABPA CF patients, as seen in Fig. 3. However, both ABPA and non-ABPA CF patients had decreased IFN- γ + CD3+ T-cells compared with non-atopic normal controls, 9.3 ± 0.8 and

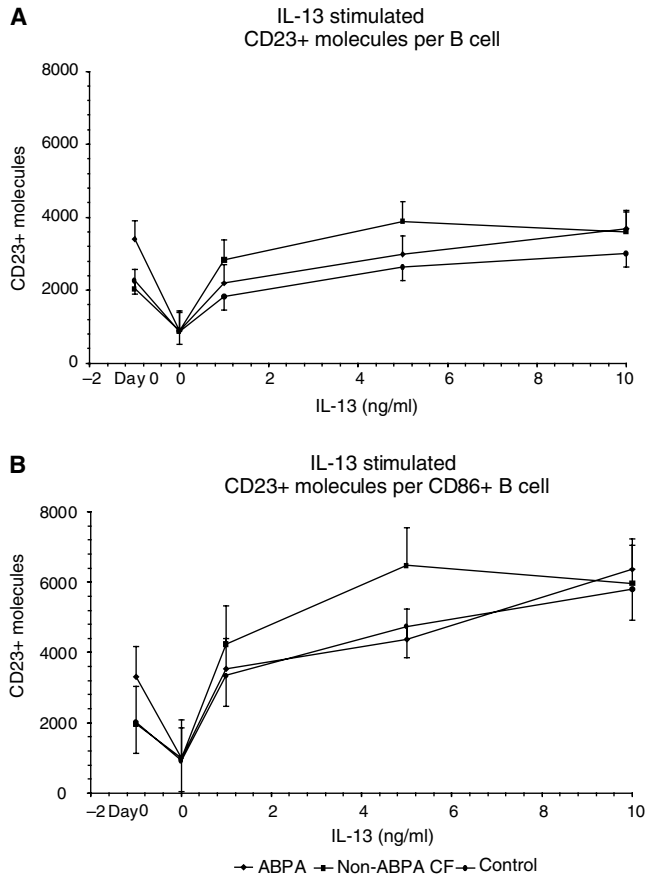


Figure 2. Interleukin (IL)-13 induction of CD23+ molecules on CD20+ and on CD86+CD20+ B-cells. Following IL-13 stimulation allergic bronchopulmonary aspergillosis (ABPA) cystic fibrosis (CF) patients had no significantly increased rate of CD23+ expression per B-cell (A) and per CD86+ B-cell (B) compared with non-ABPA CF and nonatopic control patients ($P < 0.01$). Data presented as mean $\pm$ SE. Statistical analysis using one way analysis of variance (ANOVA) was performed on the three groups, followed by a *post hoc* procedure, Scheffé test to determine difference between pairs.

$10.4 \pm 1.0\%$ vs $27.8 \pm 2.2\%$ ($P < 0.01$). In addition, IL-2+ CD3+ T cells tended to be decreased in ABPA and non-ABPA CF patients compared with controls. There was a trend toward increased IL-4+ CD3+ T-cells in ABPA compared with non-ABPA patients, $1.2 \pm 0.1\%$ vs $0.9 \pm 0.1\%$.

Antigen-specific Th1 and Th2 cells were examined following a culture with either tetanus toxoid or Asp f2/f3/f4 (Fig. 3). In cultures stimulated with tetanus toxoid, there was no significant difference of IFN- γ + and IL-4+ CD3+ T-cells in ABPA compared with non-ABPA CF patients. However, tetanus toxoid stimulated IFN- γ + CD3+ T-cells were significantly decreased compared with normal controls, 5.8 ± 1.2 and $5.3 \pm 1.3\%$ vs $12.0 \pm 1.4\%$ ($P < 0.01$). In Asp f2/ f3/f4 stimulated cultures, there was no significant difference of IFN- γ +

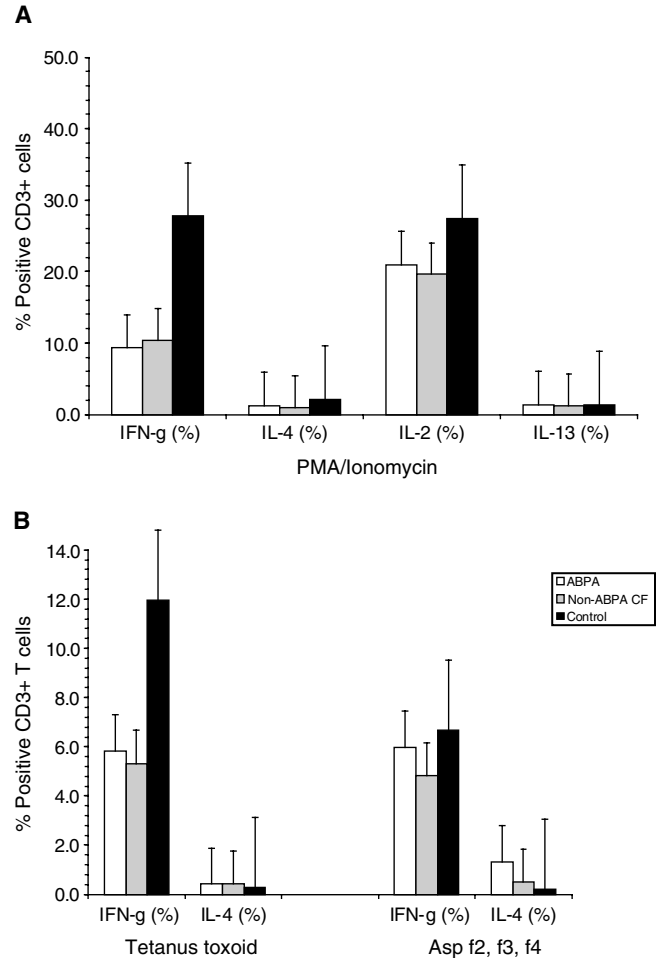


Figure 3. Th1 and Th2 CD3+ T cells. Following PMA and ionomycin stimulation (A), allergic bronchopulmonary aspergillosis (ABPA) cystic fibrosis (CF) patients had comparable percentages of interferon (IFN)- γ and IL-4 CD3+ T cells compared with non-ABPA CF patients. However, both ABPA and non-ABPA CF patients had significantly decreased percentages of IFN- γ CD3+ T cells compared with normal controls ($P < 0.01$). Following tetanus toxoid stimulation (B), both ABPA and non-ABPA CF patients had significantly decreased IFN- γ CD3+ T cells compared with normal control ($P < 0.01$) but not different from each other. However, ABPA CF patients had significantly increased Asp f2/3/4/ stimulated IL-4 CD3+ T cells compared with non-ABPA CF patients and normal controls ($P < 0.02$). Data presented as mean $\pm$ SE. Statistical analysis using one way analysis of variance (ANOVA) was performed on the three groups, followed by a *post hoc* procedure, Scheffé test to determine difference between pairs.

CD3+ T-cells in ABPA compared with non-ABPA CF patients, though they tended to be decreased compared with controls. However, in Asp f2/ f3/f4 stimulated cultures, IL-4+ CD3+ T-cells were significantly increased in ABPA compared with non-ABPA CF patients and controls, $1.3 \pm 0.2\%$ vs $0.5 \pm 0.1\%$ vs $0.2 \pm 0.1\%$ ($P < 0.02$), respectively.

Discussion

A central question is why only 7–15% of CF patients develop ABPA when as many as 60% become colonized with *A. fumigatus*. From previous studies, a Th2 cytokine response to *A. fumigatus* antigens was demonstrated (1). However, this was also demonstrated in atopic *A. fumigatus* sensitive atopic non-ABPA CF patients (2). Recently, Chauhan et al. (36, 37) demonstrated HLA restriction, HLA-DR2 (DRB1*1501 and 1503) in ABPA patients. Furthermore, the presence of HLA-DQ2 appeared to protect against development of ABPA in HLA-DR2 positive asthmatics. However, not all ABPA patients were HLA-DR2 or DR5 positive. Thus, certain HLA restrictions may facilitate development of ABPA. However, it does not fully address the development of ABPA.

In the present study, sensitivity to IL-4 stimulation was examined by up-regulation of CD23 expression. Previous investigators have reported single nucleotide mutations or polymorphisms of the IL-4 alpha receptor (IL-4R α) that alter its function (7, 8). Some of these polymorphisms are associated with up-regulation with IL-4 stimulation. However, recently Wang et al. (38) were unable to demonstrate hypersensitive induction of CD23 with either the Q576R and Y575F mutant cells. This suggested that the hypersensitive induction of CD23 expression from allergic and ABPA patients may be due to some other alteration in the IL-4 signaling pathway. Our observation that IL-4 produced a far greater induction of CD23 expression compared with IL-13 suggests that the IL-4R α is probably playing a role in ABPA in CF patients.

The IL-4R is a heterodimer, consisting of the unique IL-4 R α chain and a common gamma chain. The IL-13

receptor consists of IL-13R α and IL4R α and is stimulated by both IL-4 and IL-13. In ABPA CF patients, there was significantly increased sensitivity to IL-4. As IL-4 stimulation also induces increased expression of a number of receptors in addition to up-regulating B-cell CD23 expression and IgE synthesis, increased IL-4R activity would then favor increased allergen induced inflammatory responses as seen in ABPA (1). The IL-13, like IL-4, increases CD23 expression, IgE isotype switching, and IgE synthesis. Interleukin-13 stimulation up-regulated CD23 expression in both ABPA and non-ABPA CF patients, but there was no significant difference between the groups. However, IL-13 does not activate and skew Th2 responses (27–31). This would indicate that the IL-4R α chain is critical for these results.

Though there was a trend for increased frequency of IL-4+ CD3+ T-cells in ABPA, it was not significantly increased. However, there was a significantly increased frequency of *A. fumigatus* stimulated Th2 cells. The increased sensitivity to IL-4 perhaps with skewing of *A. fumigatus* Th2 T-cells may be part of the immunopathogenesis in atopic individuals developing ABPA. The results suggest that targeting IL-4 perhaps through sIL-4R may be important therapeutically.

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