

August 1994, part 1 • Volume 94 • Number 2

***Asp f* I CD4 + T_{H2}-like T-cell lines in allergic bronchopulmonary aspergillosis**

Alan P. Knutsen, MD [\[MEDLINE LOOKUP\]](#)
Kathleen R. Mueller, BS [\[MEDLINE LOOKUP\]](#)
Alan D. Levine, PhD [\[MEDLINE LOOKUP\]](#)
Bella Chouhan, PhD [\[MEDLINE LOOKUP\]](#)
Patricia S. Hutcheson, BA [\[MEDLINE LOOKUP\]](#)
Raymond G. Slavin, MD [\[MEDLINE LOOKUP\]](#)

• St. Louis, Mo.

Sections

- [Abstract](#)
- [Methods](#)
- [Results](#)
- [Discussion](#)
- [References](#)
- [Publishing and Reprint Information](#)
- [Articles with References to this Article](#)

Abstract

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease caused by bronchial colonization with *Aspergillus fumigatus* (*Af*) characterized by elevated serum total and *Af*-specific IgE levels and eosinophilia. In order to examine T-cell reactivity to *Af* antigens, six T-cell lines were established from the peripheral blood of patients with ABPA to *Asp f* I, an 18 kd protein purified from *Af* extracts. The *Asp f* I-specific T-cell lines, analyzed by flow cytometry, were 100% CD3+CD4+. Lymphoproliferative responses of the T-cell lines were specific for *Asp f* I stimulation, 28,999 cpm (stimulation index = 12.2), and showed no response to tetanus toxoid stimulation, 2178 cpm (stimulation index = 1.1) ($p < 0.001$). Furthermore, *Asp f* I-stimulated lymphoproliferation was inhibited in two experiments by monoclonal anti-interleukin (IL)-4 antibody in a dose-response fashion, 78% and 84% inhibition at 5% concentration of anti-IL-4. In contrast, anti-IL-2 antibody did not inhibit *Asp f* I-stimulated proliferation. *Asp f* I-stimulated T-cell lines synthesized predominantly IL-4 (mean, 21.5 ng/ml) after 48 hours of culture, and nondetectable quantities of interferon- γ and IL-2. In summary, *Asp f* I-specific T-cell lines established from patients with ABPA were characterized as being CD4+ T_{H2}-like in their cytokine synthesis pattern, and secreted IL-4 behaved in an autocrine fashion, stimulating proliferation. (J ALLERGY CLIN IMMUNOL 1994;94:215-21.)

ABPA	Allergic bronchopulmonary aspergillosis
<i>Af</i>	<i>Aspergillus fumigatus</i>
APCs	Antigen-presenting cells

FITC	Fluorescein isothiocyanate
IL	Interleukin
mAb	Monoclonal antibody
PBMCs	Peripheral blood mononuclear cells
SI	Stimulation index
TCS	T-cell supernatant

Key words: [Allergic bronchopulmonary aspergillosis](#), [Aspergillus fumigatus](#), [Asp f I](#), [interleukin-4](#), [CD4+ T_{H2} T-cell lines](#)

In 1952, Hinson et al.¹ first described allergic bronchopulmonary aspergillosis (ABPA), a hypersensitivity lung disease caused by *Aspergillus fumigatus* (AF), which is characterized by asthma, recurrent pulmonary infiltrates, blood and pulmonary eosinophilia, immediate Af skin reactivity, Af precipitins, elevated total serum IgE levels, elevated IgE, IgA, and IgG anti-Af antibodies, bronchiectasis, and pulmonary fibrosis.²⁻⁷ Subsequently, Mearns et al.⁸ reported that patients with cystic fibrosis were also susceptible to ABPA. Because of the markedly elevated serum IgE levels and eosinophilia, a T_{H2}-like T-cell response to *Aspergillus* antigens has been hypothesized. In murine models Mosmann et al.^{9, 10} reported that CD4+ T_{H2} T cells induce B-cell IgE isotope switching and synthesis and eosinophil differentiation and activation. In a murine model of ABPA, Kurup et al.¹¹ have reported that anti-interleukin (IL)-4 and anti-IL-5 antibodies inhibited specific and polyclonal IgE antibody formation and eosinophilia responses to *Aspergillus fumigatus* (Af) stimulation, respectively.

In this study we generated CD4+ T-cell lines to an immunodominant Af antigen, Asp f I, from peripheral blood mononuclear cells (PBMCs) of patients with ABPA. Analysis of the Asp f I-specific CD4+ T-cell lines characterized a T_{H2} T-cell cytokine pattern, and lymphoproliferation was IL-4-dependent.

Methods

Patients

Five patients with ABPA and one atopic Af-sensitive patient with cystic fibrosis were evaluated. The diagnosis of ABPA was based on criteria outlined by Laufer et al.¹² At the time the patients donated blood, ABPA was in remission (stage 2). Elevated serum IgE levels were observed in patients with ABPA (Table I). One patient (NC) did not fulfill the clinical criteria for ABPA but had elevated serum IgE, a positive skin test response to Af, and IgE and IgG anti-Af antibodies. The St. Louis University Medical Center Institutional Review Board approved these studies.

TABLE I. Phenotype profile of Asp f I T-cell lines from patients with ABPA

Study	Patients with CF				Patients with asthma	
	TH	PN	SK	NC	KM	CM
Diagnosis of ABPA	+	+	+	Atopic*	+	+
Serum IgE (IU/ml)	3800	1950	2091	634	7000	450
Phenotype						

CD3+ (%)	97	99	100	100	99	97
CD4+ (%)	100	99	98	100	100	100
CD8+ (%)	0	0	0	0	0	0
CD25+ (%)	96			99	97	97
DR+ (%)	91			100	97	95
TCR (a/b)+ (%)	99			99	100	99
CD45RO+ (%)	100			100		
CD3 +CD23+ (%)	1.3			0.7	1.3	1.6

*Af skin test results were positive; IgE and IgG anti-Af results were positive.

Generation of *Asp f* I-specific T-cell lines

T-cell lines with specificity for *Asp f* I were established from PBMCs with modifications of methods previously described for alloreactive T cell lines.¹³ PBMCs, obtained from peripheral blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density centrifugation, were cultured in RPMI-1640 media (Gibco, Grand Island, N.Y.) supplemented with 10% pooled normal human AB negative serum in 24-well flat-bottom plates (Linbro; Flow Laboratories, Inc., McLean, Va.) at 4.0×10^6 cells/well in a final volume of 2 ml with 5 µg/ml of *Asp f* I for 10 days in a 5% CO₂ humidified atmosphere at 37° C. *Asp f* I was obtained from crude culture extracts of *Af* and purified by column chromatography with a monoclonal antibody (mAb) (gift of Dr. M. Chapman). On the tenth day of incubation, the dividing “blast” T cells were isolated by Ficoll-Hypaque density centrifugation and resuspended in RPMI containing 10% AB serum at 1.0×10^6 cells/ml with 20% autologous irradiated (5000 r) Epstein-Barr virus-transformed B cells (antigen-presenting cells [APCs]), 25 U/ml rIL-2, and 5 µg/ml of *Asp f* I in a final volume of 2 ml for 7 days. Every week, fresh autologous irradiated APCs, 5 µg/ml of *Asp f* I, recombinant IL-2, and media were added.

In vitro lymphoproliferative responses by *Asp f* I T cells

T-cell lines were rested for 7 to 14 days, washed, and purified by Ficoll-Hypaque density centrifugation. The T cells (1×10^5) and 20% irradiated autologous APCs were cultured in flat-bottomed microtiter plates in RPMI-1640 supplemented with 20% pooled normal AB negative heat-inactivated human serum, penicillin/streptomycin, L-glutamine, and N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid and stimulated with either *Asp f* I 5 µg/ml, tetanus toxoid (Connaught Laboratories, Inc., Swiftwater, Pa.), or medium in a final volume of 0.2 ml for 3 days at 37° C in a 5% CO₂ humidified atmosphere.¹⁴ Tritiated thymidine (1 µCi) (ICN Radiochemicals, Irvine, Calif.) was added to each well for the final 6 hours of culture. The cultures were harvested onto glass filter paper, and the tritiated thymidine incorporated into DNA was counted in a Beckman beta scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.). Data were expressed as geometric mean counts per minute of stimulated (E) minus unstimulated (C) cultures and as stimulation index (SI) calculated as E divided by C. Lymphoproliferative studies were replicated a minimum of two times, and a representative experiment was presented. In some experiments anti-IL-4 or anti-IL-2 mAbs (diluted vol/vol, kindly provided by Alan Levine) were added to cultures to determine whether proliferation was inhibited.

Phenotypic analysis of *Asp f* I T cells by flow cytometry

The cell surface markers of the *Asp f* I T-cell lines were determined by reacting mAbs conjugated either with fluorescein isothiocyanate (FITC) or phycoerythrin and analyzed on a flow cytometer.^{14, 15} T cells were mixed with optimal concentrations of FITC-conjugated mAb directed against one surface antigen and another phycoerythrin-conjugated mAb directed against the second antigen. Negative controls (murine IgG2a-FITC and IgG2a-phycoerythrin) were run to produce only a small percent of positive fluorescence. The lymphocyte region was selected, and FITC fluorescence was measured with a 530 nm bandpass filter, and phycoerythrin fluorescence was measured with a 575 nm bandpass filter. Samples were displayed and analyzed in the form of two-parameter histograms. Murine mAbs used were CD3 (Leu 4), CD4 (Leu 3a), CD8 (Leu 2a), CD25 (IL2R), CD45RO (UCHL-1), CD23 (IOB8), and CD20 (B1); they were obtained from Becton-Dickinson

Immunocytometry Systems (San Jose, Calif.) and Coulter Corporation (Hialeah, Fla.) and preconjugated with either FITC or phycoerythrin as needed. Sequential phenotype analysis was performed to determine the stability of the T-cell lines, and the latest analysis was presented.

***Asp f* I-stimulated T cell cytokine synthesis**

In these studies *Asp f* I-specific T-cell lines were used to assay for cytokine synthesis pattern. *Asp f* I-specific T-cell lines were rested and washed, then T cells (1×10^6 cells/ml) and 20% irradiated autologous APCs were cocultured with *Asp f* I (5 µg/ml) or media in a final volume of 2 ml for 48 hours. The T-cell supernatant (TCS) was obtained by centrifugation of the cultures and analyzed for IL-4, interferon- γ (IFN- γ), and IL-2 cytokines. *Asp f* I-stimulated T-cell cytokine synthesis studies were replicated a minimum of two times, with the exception of the SK T-cell line, and representative experiments were presented.

Measurement of T-cell cytokines

The concentration of *Asp f* I-stimulated TCS IL-4 was measured by induction of soluble CD23 (sCD23) from monocytes. PBMCs (10^5 cells) were cultured in triplicate with TCS in 0.1 ml RPMI, 10% AB serum, glutamine, sodium pyruvate, and penicillin/streptomycin in flat-bottom 96-well plates for 72 hours at 37° C. The cells were removed by centrifugation, and the conditioned media was analyzed for sCD23 production by a two-site ELISA. The capture antibody, Epstein-Barr virus cell surface antigen 4 (IgM isotype), was used to coat Immulon II plates (Nunc Inc., Naperville, Ill.) at 1 µg/well.¹⁶ After blocking with phosphate-buffered saline containing 2% fetal calf serum, the monocyte-conditioned media was added. sCD23 was detected by indirect enzyme immunochemistry with anti-CD23 mAb BU38 (The Binding Site Inc., San Diego, Calif.) and horseradish peroxidase linked to rabbit anti-mouse IgG₁ (Southern Biotechnology Association, Birmingham, Ala.). Each assay was calibrated against a recombinant human IL-4 standard.¹⁷ The specificity of this bioassay for IL-4 was determined by parallel analysis of replicate supernatants in the presence of neutralizing rabbit anti-human IL-4 antisera (F199). Human monocytes did not synthesize sCD23 in response to human IL-2 at any concentration tested. The assay detected IL-4 at 0.3 ng/ml with a standard deviation less than 10%.

TCS IL-2 was analyzed with the IL-2-dependent cytotoxic lymphoid line. Murine cytotoxic lymphoid line (10^4 cells) were incubated with TCS in a final volume of 0.1 ml RPMI containing 10% fetal calf serum, glutamine, sodium pyruvate, and penicillin/streptomycin in flat-bottom 96-well plates for 48 hours at 37° C. Cultures were pulsed for the final 6 hours of incubation with 1 µCi of tritiated thymidine, and thymidine uptake was measured by liquid scintillation counting. An internal standard of human recombinant IL-2 (Biosource International, Camarillo, Calif.) was run in each assay. The murine cytotoxic lymphoid line cells did not proliferate in response to human IL-4 stimulation at any concentration tested. TCS IFN- γ was measured with commercial ELISAs (Genzyme, Cambridge, Mass.).

Statistics

Paired Student's *t* test was used for statistical analysis.

Results

T-cell line phenotype analysis

As seen in ■Table I, analysis of the *Asp f* I-specific T-cell lines revealed that they were phenotypically CD3+ CD4+ T cells. Markers for T-cell activation markers revealed that they coexpressed CD25+ and HLA DR+. The cells expressed the CD45RO+ marker for memory CD4+ T cells, and the T-cell receptor was α/β . CD23 was expressed in only low concentrations, ranging from 0.7% to 1.6%.

Lymphoproliferation responses

The *Asp f* I-specific T-cell lines were responsive only to *Asp f* I stimulation (■Table II). The mean lymphoproliferative response was 28,999 cpm (SI = 12.2) to *Asp f* I stimulation, whereas no stimulation was

seen with the irrelevant antigen tetanus toxoid, 2178 cpm (SI = 1.1) ($p < 0.001$). As a control to determine whether *Asp f* I is mitogenic, two tetanus-specific T-cell lines did not proliferate to *Asp f* I stimulation (data not shown). Anti-IL-4 or anti-IL-2 mAbs were added to parallel cultures to determine whether lymphoproliferation was IL-4-dependent and/or IL-2-dependent. Anti-IL-4 inhibited *Asp f* I-stimulated lymphoproliferation in a dose-dependent manner in two experiments, 78% and 84% (■ Fig. 1). However, anti-IL-2 mAb only minimally inhibited *Asp f* I-stimulated lymphoproliferation, 7% and 10%.

FIG. 1. In this representative experiment, monoclonal anti-IL-4 antibody (provided by Alan Levine) inhibited *Asp f* I-stimulated lymphoproliferation of antigen-specific T-cell lines in a dose-response manner. Maximum inhibition was 84% at 5% anti-IL-4 antibody concentration (diluted vol/vol) in this experiment and 78% in a second experiment (data not shown).

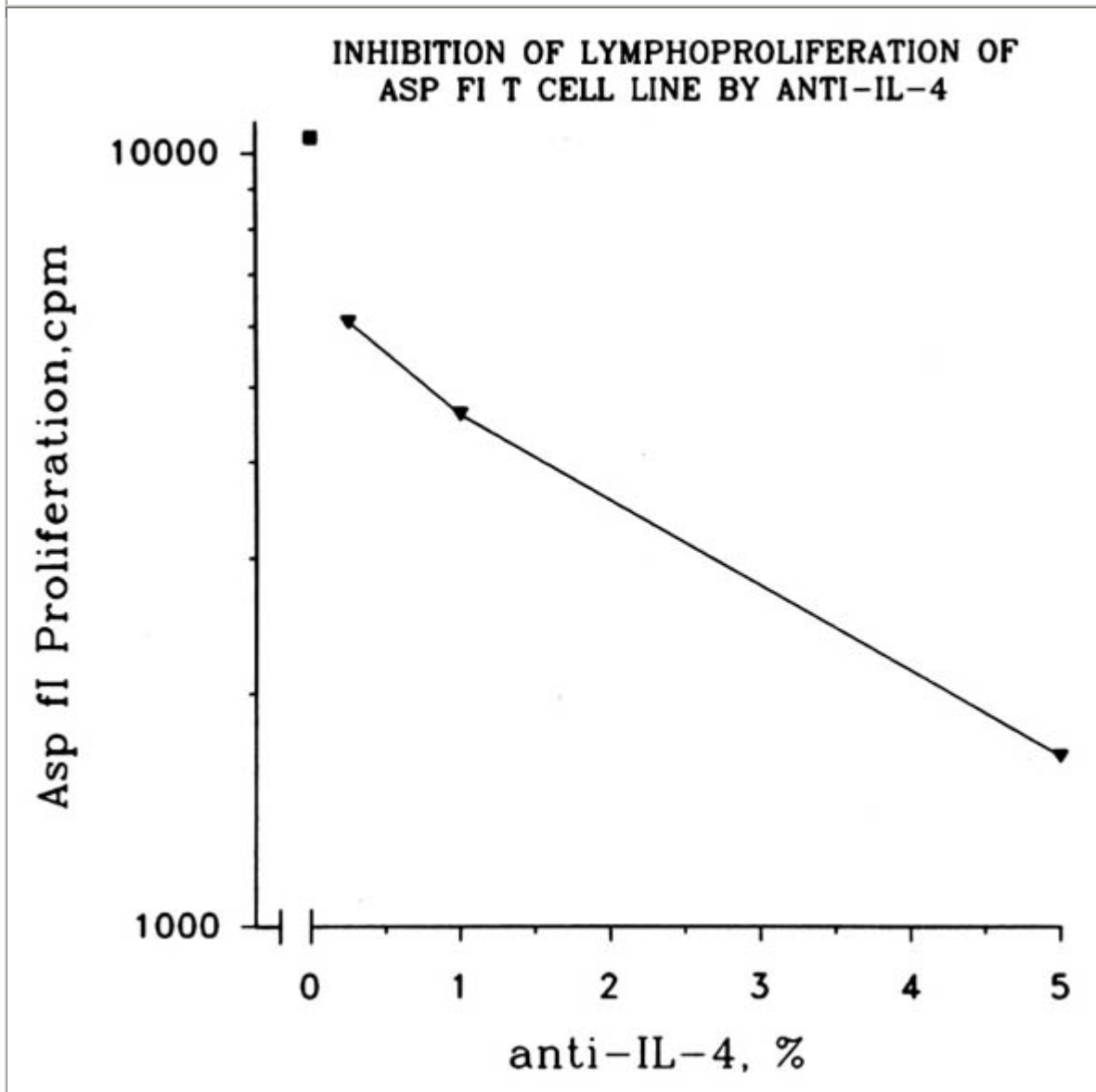


TABLE II. Lymphoproliferative responses of *Asp f* I T-cell lines from patients with ABPA

T-cell line	Media (cpm)	Lymphoproliferative responses, E-C	
		<i>Asp f</i> I (cpm)	Tetanus toxoid (cpm)
TH	1,876	37,481 (21.0)	-1,137 (0.4)
PN	6,912	16,219 (3.3)	1,009 (1.1)

NC	1,237	24,481 (20.8)	27 (1.0)
KM	17,295	57,168 (4.3)	10,712 (1.6)
CM	926	9,644 (11.4)	278 (1.3)
Mean $\pm$ SD	5,649 $\pm$ 6,950	28,999 $\pm$ 18,867*	2,178 $\pm$ 4,832

T-cell lines were incubated with media, *Asp f* I, or tetanus toxoid for 72 hours. Stimulation indexes are in parentheses.

* $p < 0.01$ for *Asp f* I versus tetanus toxoid stimulation.

As seen in ■Table III, *Asp f* I-stimulated T-cell lines synthesized and secreted into the supernatant significant quantities of IL-4, 21.5 ± 11.3 ng/ml. Only one T-cell line had detectable supernatant IFN- γ , 1.2 ng/ml (sensitivity 0.08 ng/ml), and IL-2 was not detectable (sensitivity 0.3 U/ml). Nonstimulated (medium) T-cell lines also synthesized amounts of IL-4 similar to amounts synthesized by stimulated cultures (data not shown). Subsequently, one T-cell line (SK) was rested for 2 to 17 days after IL-2 and *Asp f* I stimulation; and supernatant was sampled serially, and IL-4 was measured. As seen in ■Fig. 2, IL-4 was secreted into the supernatant in large amounts for up to 10 days of resting, 20 ng/ml, and was detectable for up to 17 days. Thus the T-cell lines were not rested for a sufficient time in the previous experiments.

FIG. 2. *Asp f* I-specific T-cell line was rested for 2 to 17 days after IL-2 and *Asp f* I stimulation; supernatant was sampled serially, and IL-4 was measured. Significant amounts of IL-4 were synthesized for up to 10 to 14 days of rest.

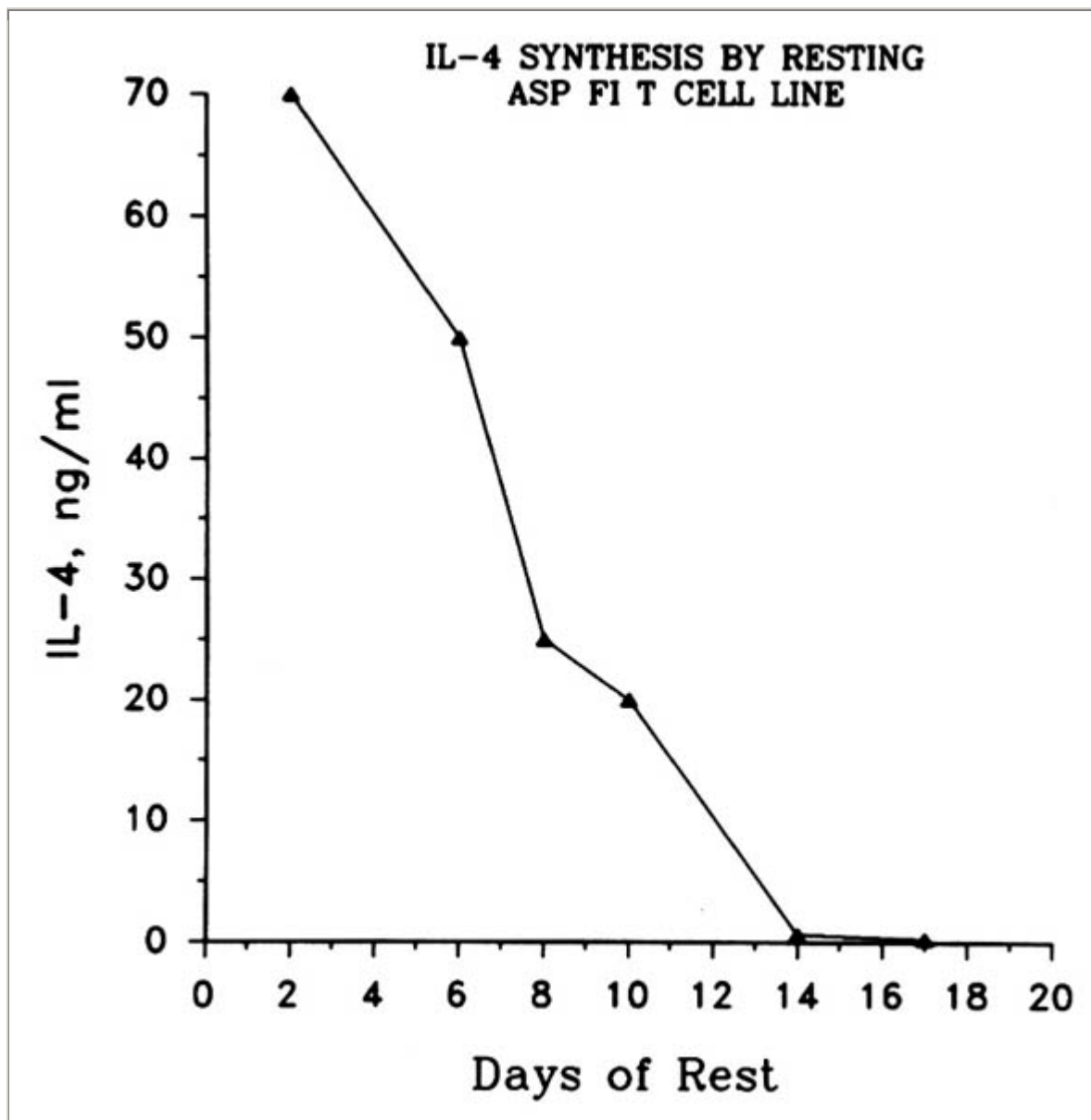


TABLE III. Cytokine synthesis of *Asp f I* T-cell lines from patients with ABPA

T-cell line	IL-4 (ng/ml)	Cytokine synthesis	
		IFN- γ (ng/ml)	IL-2 (U/ml)
TH	0.3	<0.1	ND
SK	70.0	1.2	ND
NC	6.6	<0.1	<0.3
KM	13.8	<0.1	ND
CM	16.6	<0.1	<0.3
Mean $\pm$ SD	21.5 $\pm$ 27.9	<0.1	<0.3

T-cell lines were rested for 7 to 14 days (SK was not rested), washed, and stimulated with *Asp f I* for 48 hours; supernatant was harvested for cytokine analysis.

ND, Not done.

Discussion

The pathogenesis of ABPA is still not completely understood. We hypothesized that ABPA in many aspects appears to be a late-phase asthmatic reaction as a response to continual antigen exposure by a living organism within the bronchi of genetically susceptible individuals with asthma or cystic fibrosis. In biopsy specimens of patients with ABPA, there is a marked interstitial infiltration of macrophages, lymphocytes—often with granuloma formation—and activated eosinophils below the cylindrical epithelium of the bronchial wall.¹⁸ In addition, the bronchi contain thick tenacious mucus with fibrin, eosinophils, Charcot-Leyden crystals, and *Af* mycelia.¹⁹ Typically, *Af* does not invade the bronchial wall, though we have demonstrated *Af* hyphae within the bronchial interstitium with mAbs. We and others²⁰ have demonstrated secretion of eosinophil mediators in the pulmonary interstitial spaces and from bronchoalveolar lavage specimens of patients with ABPA. Eosinophil degranulation has been demonstrated in response to *Af* stimulation via IgE, IgA, and IgG anti-*Af* antibodies.^{21, 22} Thus IgE-mediated eosinophil and mast cell degranulation by *Af* allergens within the bronchi appear to play a major role in the pathogenesis of the bronchospasm and inflammation found in ABPA.

Because T lymphocytes regulate B-cell IgE synthesis and eosinophil differentiation and activation, investigators have evaluated T-cell activity in ABPA. CD4+T_{H2}-like T cells secrete cytokines that enhance B-cell IgE synthesis and secretion and stimulate maturation and activation of eosinophils. Specifically, IL-4 causes B-cell IgE isotype switching; and other T-cell cytokines, such as the ligand for the B-cell receptor CD40, enhance IgE secretion.²³⁻³¹ Granulocyte-macrophage colony-stimulating factor, IL-3, and IL-5, also secreted by CD4+T_{H2} T cells, promote eosinopoiesis and activation of eosinophils.^{9, 10, 23, 31} Furthermore, IL-4 stimulates endothelial cell expression of the adhesion molecule vascular cell adhesion molecule-1. Vascular cell adhesion molecule-1 and its ligand CD49, present on eosinophils, basophils, and lymphocytes, would enhance transendothelial migration of these cells.^{32, 33}

In this study we generated CD4+ T-cell lines in response to *Asp f* I stimulation. Arruda et al.³⁴ have previously reported that *Asp f* I is an immunodominant allergen in patients with ABPA. We demonstrated that the cytokine synthesis pattern of the *Asp f* I-specific T-cell lines was T_{H2}-like in that IL-4 secretion, but not IFN- γ and IL-2 secretion, was observed. This is similar to the findings in atopic individuals: antigen-specific T-cell clones with a T_{H2} T-cell cytokine pattern have been reported with increased frequency. Our studies indicate that the frequency of CD4+ T_{H2} *Asp f* I-specific T cells is probably greatly increased in ABPA, perhaps to a greater degree than in other atopic states, since our findings were obtained when T-cell lines were used. Furthermore, we observed that *Asp f* I-stimulated proliferation was an IL-4-dependent, autocrine response similar to that reported by Vella et al.³⁵

We previously observed increased CD4+ CD23+ T lymphocytes in a bronchoalveolar lavage specimen of a patient with ABPA.³⁶ However, CD4+ CD23+ T cells comprised only a very minor population of the CD4+ T lymphocytes in the *Asp f* I-specific T-cell lines. Recently, Bonnefoy et al.³⁷ reported that CD23 might be an important ligand for the B-cell CD21 receptor regulating IgE synthesis. To determine whether this small T-cell population regulates IgE synthesis in ABPA further investigation is needed.

The increased frequency of *Af* antigen-specific CD4+ T_{H2} T cells in ABPA suggests genetic susceptibility. Because HLA class II antigens play an important role in IgE-specific allergen antibody responses,³⁸ HLA specificity needs to be evaluated for ABPA.

We thank the patients who participated in this study, and Dr. Rejent, Director of the St. Louis University Cystic Fibrosis Center. We also thank Dr. Martin Chapman and Carla Arruda for their generous gift of the anti-*Asp f* I monoclonal antibody.

References

1. Hinson KFW, Moon AJ, Plummer NS. Bronchopulmonary aspergillosis: a review and report of eight new cases. *Thorax* 1952;7:317-33.

2. Forman SR, Fink JN, Moore VL, Wang J, Patterson R. Humoral and cellular immune responses in *Aspergillus fumigatus* pulmonary disease. *J ALLERGY CLIN IMMUNOL* 1978;62:131-6.
3. Brummund W, Resnick A, Fink JN, Kurup VP. *Aspergillus fumigatus*-specific antibodies in allergic bronchopulmonary aspergillosis and aspergilloma: evidence for a polyclonal antibody response. *J Clin Microbiol* 1987;25:5-9.
4. Kaufman HF, Beaumont F, Sluiter HJ, de Vries K. Immunologic observations in sera of a patient with allergic bronchopulmonary aspergillosis by means of the enzyme-linked immunosorbent assay. *J ALLERGY CLIN IMMUNOL* 1984;74:741-6.
5. Greenberger PA, Patterson R. Application of enzyme-linked immunosorbent assay (ELISA) in diagnosis of allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 1982;99:288-93.
6. Wang JLF, Patterson R, Rosenberg M, Roberts M, Cooper BJ. Serum IgE and IgG antibody activity against *Aspergillus fumigatus* as a diagnostic aid in allergic bronchopulmonary aspergillosis. *Am Rev Respir Dis* 1978;117:917-27.
7. Gutt L, Greenberger PA, Liotta JL. Serum IgA antibodies to *Aspergillus fumigatus* in various stages of allergic bronchopulmonary aspergillosis. *J ALLERGY CLIN IMMUNOL* 1986;78:98-101.
8. Mearns M, Longbottom J, Batten J. Precipitating antibodies to *Aspergillus fumigatus* in cystic fibrosis. *Lancet* 1967;1:538-9.
9. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348-57.
10. Mosmann TR, Coffman RL. Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv Immunol* 1989;46:111-47.
11. Kurup VP, Choi H, Resnick A, Kalbfleisch J, Fink JN. Immunopathological response of C57BL/6 and C3H/HeN mice to *Aspergillus fumigatus* antigens. *Int Arch Allergy Appl Immunol* 1990;91:145-54.
12. Laufer P, Fink JN, Bruns WT, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *J ALLERGY CLIN IMMUNOL* 1984;73:44-8.
13. Sligh JM, Mueller KR, Knutsen AP, Slavin RG. Cytotoxicity of allogenic T-cell lines from patients with cystic fibrosis [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:288.
14. Knutsen AP, Slavin RG. In vitro T cell responses in patients with cystic fibrosis and allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 1989;113:428-35.
15. Knutsen AP, Mueller KR, Hutcheson PS, Slavin RG. T- and B-cell dysregulation of IgE synthesis in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Clin Immunol Immunopathol* 1990;55:129-38.
16. Sugden B, Marsh K, Yates J. A vector that replicates as a plasmid and can be efficiently selected in B-lymphoblasts transformed by Epstein-Barr virus. *Mol Cell Biol* 1985;5:410-3.
17. Carr C, Aykent S, Kimack N, Levine AD. Disulfide assignments in recombinant mouse and human interleukin 4. *Biochemistry* 1991;30:1515-23.
18. Slavin RG, Gleich GJ, Hutcheson PS, Kephardt GM, Knutsen AP, Tsai CC. Localization of IgE to lung germinal lymphoid follicles in a patient with allergic bronchopulmonary aspergillosis. *J ALLERGY CLIN IMMUNOL* 1992;12:1006-8.
19. Slavin RG, Bedrossian CW, Hutcheson PS, et al. A pathologic study of allergic bronchopulmonary aspergillosis. *J ALLERGY CLIN IMMUNOL* 1988;81:718-25.

- 20.** Kaufman HF, Koeter GH, van der Heide S, de Monchy JGR, Klopogge E, de Vries K. Cellular and humoral observations in a patient with allergic bronchopulmonary aspergillosis during a nonasthmatic exacerbation. *J ALLERGY CLIN IMMUNOL* 1989;83:829-38.
- 21.** Cromwell O, Moqbel R, Fitzharris P, et al. Leukotriene C4 generation from human eosinophils stimulated with IgG-*Aspergillus fumigatus* antigen immune complexes. *J ALLERGY CLIN IMMUNOL* 1988;82:535-43.
- 22.** Abu-Ghazaleh RI, Fujisawa T, Mestecky J, Kyle RA, Gleich GJ. IgA-induced eosinophil degranulation. *J Immunol* 1989;142:2393-400.
- 23.** Vercelli D, Geha RS. Regulation of IgE synthesis in humans: a tale of two signals. *J ALLERGY CLIN IMMUNOL* 1991;88:285-95.
- 24.** Jabara HH, Ackerman SJ, Vercelli D, et al. Induction of interleukin-4 dependent IgE synthesis and interleukin-5 dependent eosinophil differentiation by supernatants of a human helper T cell clone. *J Clin Immunol* 1988;8:437-46.
- 25.** Del Prete GFE, Maggi E, Parronchi P, et al. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol* 1988;140:4193-8.
- 26.** Thyphronitis G, Tsokos GC, June CH, Levine AD, Finkelman FD. IgE secretion by Epstein-Barr virus-infected purified human B-lymphocytes is stimulated by interleukin-4 and suppressed by interferon-gamma. *Proc Natl Acad Sci U S A* 1989;86:5580-4.
- 27.** Pene JF, Rousset F, Briere F, et al. IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. *J Immunol* 1988;141:1218-24.
- 28.** Pene J, Chretien I, Rousset F, Briere F, Bonnefoy JY, de Vries JE. Modulation of IL-4-induced human IgE production in vitro by IFN-gamma and IL-5: the role of soluble CD23 (s-CD23). *J Cell Biochem* 1989;39:253-64.
- 29.** Parronchi P, Tiri A, Macchia D, et al. Noncognate contact-dependent B cell activation can promote IL-4 dependent in vitro human IgE synthesis. *J Immunol* 1990;144:2102-8.
- 30.** Zhang K, Clark EA, Saxon A. CD40 stimulation provides an IFN-gamma-independent and IL-4-dependent differentiation signal directly to human B cells for IgE production. *J Immunol* 1991;146:1836-42.
- 31.** Pene J, Rousset F, Briere F, et al. Interleukin-5 enhances interleukin-4-induced IgE production by normal human B cells: the role of soluble CD23 antigen. *Eur J Immunol* 1988;18:929-35.
- 32.** Schleimer RP, Sterbinsky SA, Kaiser J, et al. Interleukin-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. *J Immunol* 1992;148:1086-92.
- 33.** Thornhill MH, Kyan-Aung V, Haskard DO. IL-4 increases human endothelial cell adhesiveness for T cells but not for neutrophils. *J Immunol* 1990;144:3060-5.
- 34.** Arruda LK, Platts-Mills TAE, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the mitogillin family of cytotoxins. *J Exp Med* 1990;172:1529-32.
- 35.** Vella AT, Hulsebosch MD, Pearce EJ. *Schistosoma masoni* eggs induce antigen-responsive CD44-hi T helper 2 cells and IL-4-secreting CD44-lo cells. *J Immunol* 1992;149:1714-22.
- 36.** Knutsen AP, Slavin RG. Allergic bronchopulmonary mycosis in cystic fibrosis. *Semin Respir Infect* 1992;7:179-92.
- 37.** Bonnefoy J-Y, Pochon S, Aubry J-P, et al. A new pair of surface molecules involved in human IgE regulation. *Immunol Today* 1993;14:1-2.

38. Higgins JA, Lamb JR, Marsh SGE, et al. Peptide-induced nonresponsiveness of HLA-DP restricted human T cells reactive with *Dermatophagoides* spp. (house dust mite). J ALLERGY CLIN IMMUNOL 1992;90:749-56.

Publishing and Reprint Information

- From St. Louis University Medical Center, St. Louis and Monsanto Corporate Research, St. Louis.
- Supported in part by grant from the National Institutes of Health.
- Received for publication July 7, 1993.
- Revised Dec. 17, 1993.
- Accepted for publication Feb. 3, 1994.
- Reprint requests: Alan P. Knutsen, MD, Pediatric Research Institute, St. Louis University Medical Center, 1465 S. Grand Blvd., St. Louis, MO 63104.
- Copyright © 1994 by Mosby—Year Book, Inc.
- 0091-6749/94 \$3.00 + 0 **1/1/54891**

Articles with References to this Article

TOP

This article is referenced by these articles:

HLA-DRB1 alleles control allergic bronchopulmonary aspergillosis–like pulmonary responses in humanized transgenic mice

Journal of Allergy and Clinical Immunology, The
September 2007 • Volume 120 • Number 3

Sherri Koehm, Raymond G. Slavin, Patricia S. Hutcheson, Theodore Trejo, Chella S. David, Clifford J. Bellone

Association of polymorphisms in the collagen region of SP-A2 with increased levels of total IgE antibodies and eosinophilia in patients with allergic bronchopulmonary aspergillosis

Journal of Allergy and Clinical Immunology, The
May 2003 • Volume 111 • Number 5

Shweta Saxena, MSc^{a,b}, Taruna Madan, PhD^a, Ashok Shah, MD^c, Kambadur Muralidhar, PhD^b, Puranam Usha Sarma, PhD^a

Allergic bronchopulmonary aspergillosis

Journal of Allergy and Clinical Immunology, The
November 2002, part 1 • Volume 110 • Number 5
Paul A. Greenberger, MD

Purification of a major allergen, Asp f 2 binding to IgE in allergic bronchopulmonary aspergillosis, from culture filtrate of *Aspergillus fumigatus*

Journal of Allergy and Clinical Immunology, The
June 1997, Part 1 • Volume 99 • Number 6

Banani Banerjee, PhD^a, Viswanath P. Kurup, PhD^a, Paul A. Greenberger, MD^b, Donald R. Hoffman, PhD^c, Deepu S. Nair^a, Jordan N. Fink, MD^a
Milwaukee, Wis., Chicago, Ill., and Greenville, N.C.