

Role of T-helper type 2 cytokines in down-modulation of *Fas* mRNA and receptor on the surface of activated CD4⁺ T cells: molecular basis for the persistence of the allergic immune response

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ABSTRACT The mechanisms responsible for persistence of T lymphocytes at the sites of allergic inflammation are not completely understood. Activated T cells, usually expressing *Fas* on their surface, undergo activation-induced apoptotic death, thus limiting the dangerous consequences of a persistent immune reaction. We have previously shown that pulmonary T lymphocytes from untreated asthmatic subjects do not express surface *Fas* receptors nor do they contain *Fas* mRNA, yet they display normal levels of *Fas* ligand. This is not an inherited defect and is confined to mucosal T cells. To gain insights into the mechanism responsible for these findings, we performed a set of experiments with both purified *Dermatophagoides pteronyssinus* allergen and recombinant human cytokines: interleukin 2 (IL-2), IL-4, IL-5, transforming growth factor β_1 , interferon γ , and granulocyte-macrophage colony-stimulating factor (GM-CSF). *In vitro* exposure of purified CD4⁺ lymphocytes to allergen yielded only transient up-regulation of surface *Fas* but did not influence susceptibility to *Fas*-mediated cell death. T-helper type 2 cytokines (IL-4, IL-5, and GM-CSF) had a dose-dependent and specific inhibitory effect on *Fas* mRNA, suggesting a new fundamental biological role in the survival of inflammatory cells during allergen exposure.—Spinuzzi, F., Agea, E., Fizzotti, M., Bassotti, G., Russano, A., Droetto, S., Bistoni, O., Grignani, F., Bertotto, A. Role of T-helper type 2 cytokines in down-modulation of *Fas* mRNA and receptor on the surface of activated CD4⁺ T cells: molecular basis for the persistence of the allergic immune response. *FASEB J.* 12, 1747–1753 (1998)

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IN RECENT YEARS it has become evident that T cells orchestrate the allergic mucosal immune response by secreting the so-called T-helper (Th)² type 2 cyto-

kines [i.e., interleukin 4, (IL-4), IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF)], thus determining the local recruitment and accumulation of other inflammatory cells such as neutrophils, eosinophils, and mast cells (1). However, while the attention of immunologists was focused for long time in explaining the inductive phase of the allergic response, it has not yet been searched why this response cannot be turned off in allergic individuals. The immune system has evolved multiple nonoverlapping mechanisms for controlling potentially harmful reactions. Induction of programmed cell death (apoptosis) through an interaction of *Fas* (CD95) with its ligand (*FasL*) is now recognized as the major factor regulating a normal immune response (2–4). In fact, in some situations specific immune responses have little or no protective value and harmful consequences become dominant (5–11). The allergic pulmonary immune response in susceptible individuals, for instance, displays the characteristics of a harmful response to otherwise inoffensive airborne particulate antigens. The recognition of aerodispersed allergens and the initiation of allergic inflammation take place immediately after *in vivo* airway exposure in susceptible individuals. This implies a rapid and simple recognition mechanism that may be mediated by local resident cells, such as $\gamma\delta$ T lymphocytes and/or CD1⁺ dendritic cells (12). The former recognize allergens directly or in association

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[†] Abbreviations: bp, base pair; FEV₁, forced expiratory volume in 1 s; FITC, fluorescein; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IgM, immunoglobulin; IL, interleukin; mAb's, monoclonal antibodies; PBMC, peripheral blood mononuclear cells; RT-PCR, reverse transcription-polymerase chain reaction; TGF- β_1 , transforming growth factor; Th, T-helper.

with CD1, and subsequently expand at the surface of inflamed airways (13–15). Thus, allergen-specific T lymphocytes (with both $\gamma\delta$ and $\alpha\beta$ T cell receptors) are the main effector cells that prime the local ongoing allergic immune response (16, 17). However, the respiratory (as well as the gastrointestinal) tract each day eliminates an enormous quantity of foreign substances without experiencing any inflammatory activity. Therefore, a physiological mechanism should exist that enables phagocytic resident cells to block any possible T cell reactivity. In an experimental mouse model, it was recently shown that the *Fas*/*FasL* pathway is involved in the elimination of inhaled particulate antigens from the respiratory tract (18). Similarly, normal human intraepithelial gut T lymphocytes express *Fas* at their surface and could be eliminated via *FasL*⁺ antigen-presenting-cell interaction (19).

We have investigated a possible down-modulation of this pathway on the respiratory tract of asthmatic subjects. Unlike that seen in normal subjects, T lymphocytes with both $\alpha\beta$ and $\gamma\delta$ T cell receptors in asthmatics never express the *Fas* molecule at their surface. They also have a persistently down-modulated *Fas* mRNA (20). The aim of the present study was to try to determine how such an effect could be ascribed to Th2-type cytokines, so that the allergic immune response can take place and persist in target organs through the combined effect of IL-4, IL-5, and GM-CSF on the recruitment and survival of resident immunocompetent cells.

MATERIALS AND METHODS

Patients and controls

Fifteen untreated asthmatic subjects (8 adults and 7 children, age range: 8–45 years, all diagnosed as being allergic to *Der-matophagoides pteronyssinus*) and 10 age- and sex-matched controls were recruited for the study. At the time of enrollment, all asthmatic subjects had stable pulmonary function with a forced expiratory volume in 1 s (FEV₁) ranging from 72 to 98% of that predicted for their age and height. The methacholine test was performed starting with 0.095 mg/ml concentrations. Concentration-response curves were plotted for each challenge test as percentage fall in FEV₁ against the methacholine concentration and were characterized by their threshold value (PC₂₀). None were smokers or had had upper respiratory tract infections 8 wk prior to investigations. Informed consent was obtained from the children's parents or directly from adult patients, and the research was conducted in accordance with local ethical committee guidelines. Peripheral blood mononuclear cells (PBMC) were isolated from patients and controls over a Ficoll-Hypaque density gradient and stained for *Fas* receptor expression on CD3⁺ T cells using a double color cytofluorometric assay, as described in full below.

Cell surface marker analysis

For cytofluorometric analysis, PBMC suspensions were stained with the following monoclonal antibodies (mAb's): phyco-

erythrin-conjugated anti-CD3 (OKT3, Ortho, Raritan, N.J.), fluorescein (FITC) -conjugated anti-*Fas* (Serotec, Oxford, U.K.). For staining, 5,000 to 10,000 cells were resuspended in 50 μ l of saline, incubated at 4°C for 30 min, washed, and analyzed by flow cytometry (FACScan, Becton-Dickinson). For the analysis of two-color cytofluorometric data, an electronic gate was set on the lymphocyte population based on the forward-angle vs. right-angle light scatter histogram. Quadrant markers in fluorescence histograms were set using matched isotype controls. The Lysis II program (Becton-Dickinson) was used to optimize gating of lymphocytes, providing an objective means of excluding debris (noncellular events due to particulate matter) and other cells from the lymphocyte gate.

Source of purified CD4 T cells

CD4⁺ peripheral blood T cells from normal donors and asthmatic subjects were enriched by negative immunomagnetic bead selection using a mixture of the following mAb's coated with magnetic beads (Immunotech, Marseille, France): anti-CD8, anti-CD16, anti-CD19. Positively coated cells were removed by magnetic sorting and enriched CD4⁺ cells were recovered by negative selection.

All samples were >95% CD4⁺ cells, as revealed by immunofluorescent staining with FITC anti-CD4 mAb (Immunotech). Plastic-coated anti-CD3 mAb (ATCC, Rockville, Md.) was used as an activator for all cultured samples. Purified recombinant human cytokines were added at fixed times (basal, 24, 48, and 72 h) to culture wells. Harvested cells were used for subsequent RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR).

Evaluation of activation-induced programmed cell death

PBMC were resuspended in RPMI-1640 (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum, 2 mM/l of L-glutamine, 10 mM/l of HEPES, 50 U/l of penicillin, and 50 μ g/l of streptomycin at 10⁶ cells/ml. All samples were incubated for 24 h with either medium alone, purified allergen (DP1, 10 μ g/ml; ALK-Neo-Abellò, Madrid, Spain), or anti-CD3 (ATCC) plus recombinant human IL-2 (Serotec; 20 IU/ml), then washed and exposed to an immunoglobulin M (IgM) anti-*Fas* mAb (clone CH11, Immunotech; 100 ng/ml). After an additional 18 h, samples were washed, centrifuged at 200 $\times$ g for 10 min, and dissolved in hypotonic lysing buffer (100 mM/l NaCl, 10 mM/l tris, 1 mM/l EDTA, 1% sodium dodecyl sulfate, 200 μ g/ml of proteinase K, pH 7.5). Quantitative analysis of spontaneous and anti-*Fas*-induced activation death was done by cytofluorometry with a fluorochrome solution containing propidium iodide.

Fas and *FasL* mRNA analysis

RT-PCR analysis was carried out on RNA extracted by the guanidine isothiocyanate-acid phenol method using 1 $\times$ 10⁶ enriched CD4⁺ T cells from all patients and controls. First strand cDNA synthesis was performed using total RNA, random hexadeoxynucleotide primers and Moloney murine leukemia virus reverse transcriptase (Gibco-BRL) according to the manufacturer's protocol. Primers for *Fas*, *FasL*, and β -actin PCR amplification as follows: *Fas* 5' sense primer, GTGGGA-TCCCACTTCGGAGGATT GCTCAACAACC; *Fas* 3' antisense primer, GTGCTGC AGTATGTTGGCTCTTCAGCG CTAATA; *FasL* 5' sense primer CAAGTCCCACTGA AGGTCCATGCC; *FasL* 3' antisense primer, CAGAGAGAGCTCAGATACGTT-GAC; β -actin 5' sense primer TGACGGGGTCA CCCACA-CTGTGCCCCATCTA; β -actin 3' antisense primer, CTAGAA GCATTGCGGTG GACGATGGAGGG. The size of amplified

TABLE 1. Clinicophysiological characteristics of patients' population at the time of study enrollment

Patient no.	Age (years)	Disease onset (months)	PC ₂₀ ^a	FEV ₁ (% of predicted)	Treatment
1	8	4	6	88	None
2	12	6	4.5	82	None
3	25	3	8	80	β ₂ -agonist ^b
4	35	8	5.2	85	None
5	10	3	6.5	78	None
6	15	4	4.6	80	None
7	28	6	3.8	87	β ₂ -agonist
8	42	6	4.5	72	β ₂ -agonist
9	38	8	3.5	75	β ₂ -agonist
10	22	4	2.5	80	None
11	19	5	6	79	None
12	13	6	5.5	78	β ₂ -agonist
13	30	4	7.5	70	β ₂ -agonist
14	12	4	8	98	None
15	45	8	4.5	82	β ₂ -agonist

^a Provocation concentration dose of methacoline (mg/ml) producing a 20% fall in basal FEV₁. ^b The indicated drugs were usually used as needed.

fragments were: *Fas* 1167 base pair (bp); *FasL* 350 bp; β-actin 661 bp. After amplification, one-tenth of PCR product was run on 2% agarose gel and stained with ethidium bromide.

RESULTS AND DISCUSSION

Clinical and physiological characteristics of the patient population are summarized in **Table 1**. Because the majority of subjects were newly diagnosed as asthmatics, none had been treated with drugs (corticosteroids, theophylline, anti-histaminics, sodium-nedocromil) presumably interfering with the immune system.

Table 2 shows that *Fas* receptor is present on the surface of CD3⁺ T lymphocytes from PBMC of asthmatic patients and controls, both basally and after activation. These data rule out the possibility of an inherited defect of *Fas* expression, but raise the question of whether this previously described mucosal defect (20) could be due to environmental factors, such as the nature of the stimulating agent (notably allergen) and/or the cytokine milieu in which the allergic

inflammation occurs. Recent genetic studies and the clinical course of many allergic diseases suggest a multifactorial genetic pattern of inheritance involving bronchial hyperreactivity, elevated IgE levels, and IL-4 production, all mapped on chromosome 5q31-q33 (21–23). We therefore find it unlikely that an additional perturbation in chromosome 10, where the human *Fas* gene is located (24), would be necessary for clinical development of an allergic disease. On the other hand, one might assume that the nature of a stimulating allergen per se in association with other well-described genetic abnormalities may be crucial. To address this point, we performed *in vitro* experiments with purified undenatured allergen DP1 (*Dermatophagoides pteronyssinus* antigen 1) to test its ability to stimulate surface *Fas* expression on T cells.

Purified allergen (10 µg/ml) was added to bulk cultures using the same procedure generally adopted for cloning peripheral blood T cells. As seen in **Fig. 1**, allergen stimulation for 48 and 72 h was significantly less efficient than anti-CD3 + rIL-2 treatment in inducing *Fas* expression on CD3⁺ T cells. However, incubation with purified allergen, after a transitory initial induction of *Fas* molecule, persistently reduced its expression during the culture period. Thus, it seems that a purified allergen is not a good stimulus for *Fas* receptor expression. In parallel, we assessed the functional status of activation-induced *Fas* molecule by adding, at the end of 24 h culture with both allergen and anti-CD3 + IL-2, a stimulating IgM anti-*Fas* mAb. After an additional 18 h incubation, harvested cells were stained with propidium iodide to assess the percentage of apoptotic cells. As expected (25), only anti-CD3-preactivated T cells showed a significant percentage of apoptotic nuclei, whereas allergen-primed cells did not differ substantially from

TABLE 2. Coexpression of *Fas* receptor on the surface of resting and activated peripheral blood T lymphocytes from asthmatics and controls (NC)^a

	Asthmatics	NC
Resting		
CD3 ⁺ Fas ⁺ (%)	9.2 ± 3	8.7 ± 3.2
Activated		
CD3 ⁺ Fas ⁺ (%)	29.5 ± 4.6	32.3 ± 5.8

^a Cells were double-stained with PE-anti-CD3 and FITC-anti-*Fas* mAb's basally and after 24 h *in vitro* activation with anti-CD3 mAb and IL-2 (20 IU/ml). The percentage ± SD of double-stained cells was calculated on each sample from both asthmatics patients and controls.

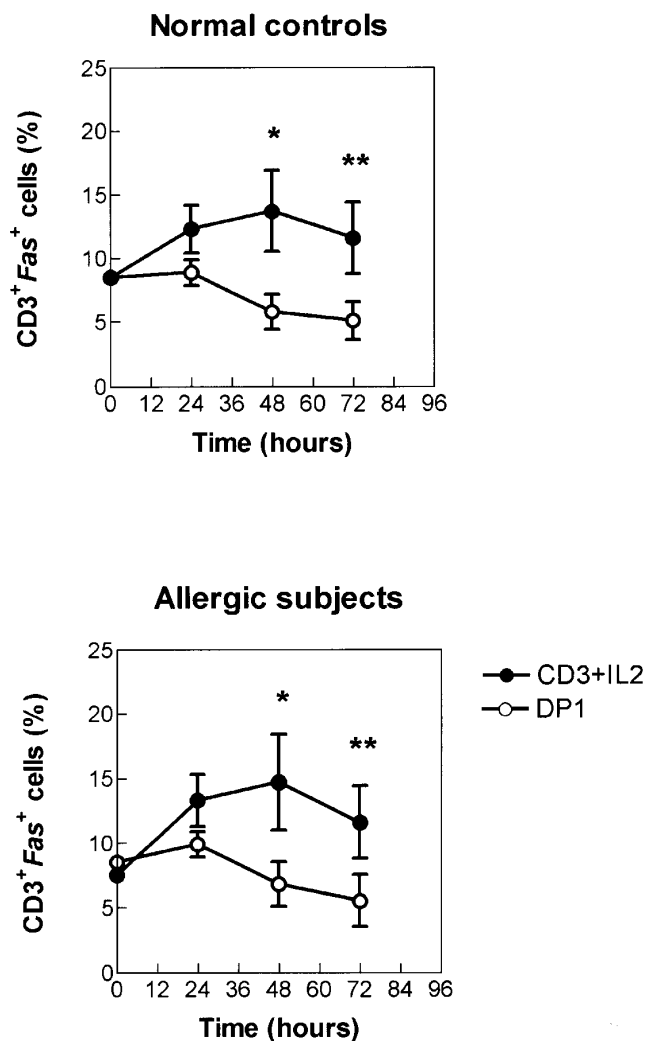


Figure 1. *In vitro* induction of Fas receptor. After *in vitro* activation with anti-CD3 + rIL-2 (Genzyme, Geneva, Switzerland; 20 IU/ml) or purified undenatured DP1 allergen (10 µg/ml), time course experiments were performed on bulk cultures from PBMC of atopic patients and controls to study the *in vitro* induction of surface Fas receptor. Two $\times 10^6$ /ml PBMC were seeded in plastic culture wells in the presence of anti-CD3 (ATCC, Rockville; 25 ng/ml) + rIL-2 or DP1 for 72 h. Cells were harvested at regular intervals, washed in PBS, and stained for the coexpression of CD3 and Fas antigens by double-color immunofluorescence and flow cytometry. * $P < 0.01$ and ** $P < 0.05$ with respect to cultures stimulated with allergen.

the unstimulated controls (Fig. 2). The same results were observed in both normal and asthmatic subjects, thus confirming the acquired nature of the previously described defect in Fas expression on the surface of pulmonary T cells and showing that allergens, though activating T cells, do not contribute to the triggering of the activation-induced cell death. On the other hand, we have previously demonstrated that pulmonary T lymphocytes from asthmatic subjects, while not expressing *in vivo* the Fas receptor, can acquire the molecule on their surface after *in vitro* culture with anti-CD3 mAb (20). In these experimental conditions, coculturing with IL-2 maintained

the expression of the molecule, whereas IL-4 incubation caused a down-modulation of the receptor only in allergic patients. Therefore, a coordinate effect of Th2-type cytokines on the kinetics of the molecule may be hypothesized. In fact, IL-5 is closely related to clinical expression of asthma, whereas IL-4 is related to overproduction of IgE and is derived not only from T cells, but also from mast cells, basophils, and eosinophils (26). However, only the production of many other cytokines, particularly IL-5, GM-CSF, IL-13, and chemokines may allow for the subsequent multiple cellular and molecular interactions responsible for the induction of chronic airway inflammation (27, 28).

To address this, experiments were performed with purified peripheral blood CD4⁺ T cells exposed *in vitro* to various cytokine mixtures. Results obtained in both asthmatic patients and controls seem to indicate that only Th2-type cytokines (IL-5, GM-CSF, and IL-4) can substantially inhibit Fas mRNA on cultured T cells (Fig. 3). This effect is dose dependent and specific, since it was not observed after incubation with IL-2, interferon γ (IFN- γ), or transforming growth factor β_1 (TGF- β_1) (Fig. 4) and did not affect other mRNAs, such as that for FasL. In addition, the cytokine concentration must be maintained during culture by adding, at regular intervals, almost half of the initial dose in order to mimic conditions presumably occurring *in vivo* in the inflamed tissues. Addition of Th2 cytokines only at the start of the culture determined a transitory inhibition of Fas mRNA that was promptly reversed during the next 24 h (data not shown). Similar results have been also observed in

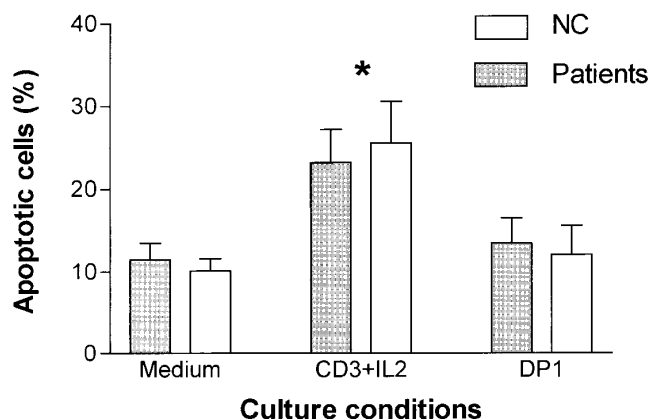


Figure 2. Fas-induced programmed cell death. Functional activity of membrane Fas receptor was investigated by inducing programmed cell death in allergen- and anti-CD3-activated T cells. Bulk cultures were stimulated for 24 h with purified allergen (DP1) or anti-CD3 + IL-2, then washed and exposed to an IgM anti-Fas mAb (clone CH11, Immunotech; 100 ng/ml). After an additional 18 h, cells were stained with propidium iodide to assess the percentage of apoptotic nuclei. Lymphocytes from asthmatic patients and controls showed a significant (* $P < 0.05$) percentage of hypodiploid DNA compared to those unstimulated or exposed to DP1 allergen.

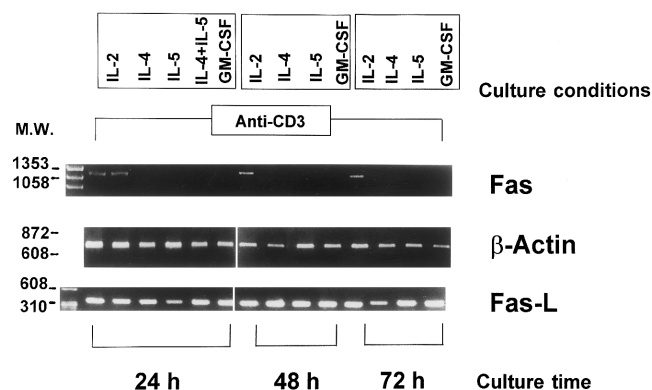


Figure 3. Modulation of *Fas* and *FasL* mRNA by Th2-type cytokines. CD4⁺ peripheral blood T lymphocytes were enriched by negative magnetic selection using a mixture of anti-CD8, anti-CD16, and anti-CD19 mAb (Immunotech), followed by incubation with anti-mouse IgG conjugated to magnetic beads (Dynal A.S., Oslo, Norway). PBMC, depleted of adherent cells by 6 h incubation on plastic petri dishes at 37°C in humidified atmosphere at 5% CO₂, were incubated on ice with mAb for 45 min. After washing, cells were mixed with an optimal concentration of sheep anti-mouse IgG coated with magnetic beads (1 mg of suspension for 10⁷ cells) and passed over a magnet (Dynal) for two consecutive cycles. Recovered cells consisted of >95% CD4⁺ lymphocytes and were used for experimental procedures. Plastic plates (Nunc) were coated overnight with anti-CD3 mAb (ATCC; 25 µg/ml) and used for culturing CD4⁺ cells with cytokines. Control wells consisted of stimulated cells plus IL-2 (50 IU/ml), and Th2-type cytokines were added at the following concentrations: 400 IU/ml IL-4 (Genzyme, Geneva, Switzerland); 5 ng/ml IL-5 (Celbio, Milano, Italy); 50 µg/ml GM-CSF (Schering-Plough, Milano, Italy). All cytokines were renewed at regular intervals (24 h) during culture (72 h) by adding one-half of the initial concentration. At the end of the culture, cells were harvested and subjected to RNA extraction and RT-PCR.

atopic nonasthmatic controls (F. Spinozzi, unpublished observations).

Both IL-2 and IL-4 share a γ -chain signaling component that has been found to up-regulate Bcl-2 and Bcl-x expression, thus preventing apoptosis in T lymphocytes (29–31). The regulation of *Fas* receptor seems to follow a different pattern, as demonstrated in the present study. In fact, IL-2, like other Th1-type cytokines, when added to *in vitro* cultures persistently up-regulates *Fas* mRNA. In T cells, this pathway is induced by repeated activation, is potentiated by IL-2, and is not yet prevented by constitutive expression of Bcl-2 or Bcl-x_L (32). Moreover, targeted disruption of the IL-2 gene does not lead to profound immunodeficiency but to uncontrolled accumulation of activated lymphocytes and manifestations of autoimmunity (33). Mice lacking the high-affinity IL-2 receptor show the same phenotypic abnormalities (34). These results suggest that IL-2 is a necessary feedback inhibitor of lymphocyte responses, and its role as a growth factor can be replaced by other cytokines (32). The control of potentially harmful T cell and macrophage reactions by immunosuppressive cytokines is impor-

tant to limit the consequences of immune responses, because mice with targeted disruptions of TGF- β develop uncontrolled leukocyte activation and tissue injury (35). On the other hand, GM-CSF and IL-5 obtained by BAL from asthmatic patients have been shown to prolong eosinophil survival *in vitro*, presumably by interfering with surface *Fas* expression, which increases in parallel with cytokine withdrawal (36, 37). Similarly, the anti-apoptotic properties of IL-4 have been described in various experimental models (38, 39). Finally, transgenic mice expressing IL-4 in the lung show an impressive increase in the percentages of pulmonary lymphocytes, eosinophils, and neutrophils (40). The extreme sensitivity of mucosal T cells from asthmatic patients to the biological effects of IL-4, which is presumably produced in large amounts during allergic inflammation, can explain the fact that normal pulmonary T cells were relatively insensitive to the inhibitory effect of IL-4 on *Fas* molecule expression (20).

The complex cellular and biochemical interactions that govern the lung inflammatory response in allergic asthma have been clarified in recent years by the demonstration that T lymphocytes play a fundamental role in orchestrating both the early recognition and the subsequent immune response to allergens (1, 12, 26–28). It is possible to hypothesize that this main biological function of Th2 cytokines in the inflamed airways of allergic subjects could be due to a prolonged survival of resident immunocompetent cells; the biochemical mechanism of this phenomenon could be driven by the down-regulation of *Fas* mRNA, as demonstrated here with *in vitro* cultured T lymphocytes. The subsequent failure to express *Fas* receptor protects these cells, and probably eosino-

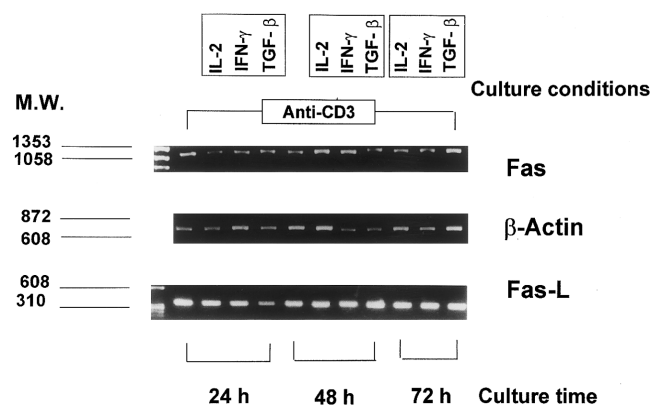


Figure 4. Modulation of *Fas* and *FasL* mRNA by Th1-type cytokines. Purified CD4⁺ cells (obtained as described above) were cultured on anti-CD3-coated plastic wells and incubated with IL-2 (50 IU/ml), IFN- γ (Genentech, San Francisco, Calif.; 10 IU/ml), and TGF- β (British Biotechnology, Abington, U.K.; 10 µg/ml). All cytokines were renewed at regular intervals (24 h) during culture time (72 h) by adding one-half of the initial concentration. At the end of the culture, cells were harvested and processed for RNA extraction and RT-PCR.

phils, from activation-induced death and determines the self-perpetuation of mucosal inflammatory response during allergen exposure. In an experimental mouse model, the elimination of particulate inhaled antigens from the airways occurred through induction of programmed cell death via *Fas/FasL* interaction in pulmonary T cells (18). A similar mechanism may exist in normal subjects, as suggested by our phenotypic and functional studies (20). Allergic subjects may have defective activation-induced death; thus, the clinical remission of symptoms invariably occurs with allergen avoidance (27) or by therapeutical interventions able to induce programmed cell death of allergen-specific T cells, such as corticosteroids (41, 42). Novel therapeutic strategies to restore the *Fas/FasL* interactions at the mucosal level are needed for a more targeted molecular approach to the therapy of respiratory allergic diseases. **[F]**

REFERENCES

- Romagnani, S. (1994) Lymphokine production by human T cells in disease states. *Annu. Rev. Immunol.* 12, 227–257
- Abbas, A. K. (1996) Die and let live: eliminating dangerous lymphocytes. *Cell* 84, 655–657
- Nagata, S., and Golstein, P. (1995) The Fas death factor. *Science* 267, 1449–1455
- Lynch, D. H., Ramsdell, F., and Alderson, M. R. (1995) Fas and FasL in the homeostatic regulation of immune responses. *Immunol. Today* 16, 569–574
- Carson, D. A., Ribeiro, J. R. (1993) Apoptosis and disease. *Lancet* 341, 1251–1254
- Mysler, E., Bini, P., Drappa, J., Ramos, P., Friedman, S. M., Krammer, P. H., and Elkon, K. B. (1994) The APO-1/Fas protein in human systemic lupus erythematosus. *J. Clin. Invest.* 93, 1029–1034
- Drappa, J., Vaishnaw, A. K., Sullivan, K. E., Chu, J. L., Elkon, and K. B. (1996) The Canale Smith syndrome: an inherited autoimmune disorder associated with defective lymphocyte apoptosis and mutations in the Fas gene. *N. Engl. J. Med.* 335, 1643–1649
- Vaishnaw, A. K., McNally, J. D., and Elkon, K. B. (1997) Apoptosis in the rheumatic diseases. *Art. Rheum.* 40, 1917–1927
- Giordano, C., Stassi, G., de Maria, R., Todaro, M., Richiusa, P., Papoff, G., Ruberti, G., Bagnasco, M., Testi, R., and Galluzzo, A. (1997) Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* 275, 960–963
- Chervonsky, A. V., Wang, Y., Wong, S. F., Visintin, I., Flavell, R. A., Janeway, C. A., and Matis, L. A. (1997) The role of Fas in autoimmune diabetes. *Cell* 89, 17–24
- Kong, L., Ogawa, N., Nakabayashi, T., Liu, G. T., D'Souza, E., McGuff, H. S., Guerrero, D., Talal, N., and Dang, H. (1997) Fas and Fas ligand expression in the salivary glands of patients with primary Sjögren's syndrome. *Art. Rheum.* 40, 87–97
- Spinazzi, F., Agea, E., Bistoni, O., Forenza, N., and Bertotto, A. (1998) $\gamma\delta$ T cells, allergen recognition and airway inflammation. *Immunol. Today* 19, 22–26
- Bendelac, A. (1995) CD1: presenting "unusual" antigens to "unusual" T lymphocytes. *Science* 269, 185–186
- De Libero, G. (1997) Sentinel function of broadly reactive $\gamma\delta$ T cells. *Immunol. Today* 18, 22–26
- Spinazzi, F., Agea, E., Bistoni, O., Forenza, N., Monaco, A., Falini, B., Bassotti, G., De Benedictis, F., Grignani, F., and Bertotto, A. (1995) Local expansion of allergen-specific CD30⁺ Th2-type $\gamma\delta$ T cells in bronchial asthma. *Mol. Med.* 1, 821–826
- Mosmann, T. R., and Sad, S. (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* 17, 138–146
- Wen, L., Barber, D. F., Pao, W., Wong, F. S., Owen, M. J., and Hayday, A. (1998) Primary $\gamma\delta$ cell clones can be defined phenotypically and functionally as Th1/Th2 cells and illustrate the association of CD4 with Th2 differentiation. *J. Immunol.* 160, 1965–1974
- Milik, A. M., Buechner-Maxwell, V. A., Sonstein, J., Kim, S., Seitzman, G. D., Beals, T. F., and Curtis, J. L. (1997) Lung lymphocyte elimination by apoptosis in the murine response to intratracheal particulate antigen. *J. Clin. Invest.* 99, 1082–1091
- De Maria, R., Boirivant, M., and Cifone, M. G. M., et al. (1996) Functional expression of Fas and Fas ligand on human gut lamina propria T lymphocytes. A potential role for the acid sphingomyelinase pathway in normal immunoregulation. *J. Clin. Invest.* 97, 316–322
- Spinazzi, F., Fizzotti, M., Agea, E., Piattoni, S., Droetto, S., Rusano, A., Forenza, N., Bassotti, G., Grignani, F., and Bertotto, A. (1998) Defective expression of Fas messenger RNA and Fas receptor on pulmonary T cells from patients with asthma. *Ann. Int. Med.* 128, 363–369
- Sandford, A., Weir, T., and Paré, P. (1996) The genetics of asthma. *Am J. Resp. Crit. Care Med.* 153, 1794–1765
- Marsh, D. G., Neely, J. D., Breazeale, D. R., Ghosh, B., Freidhoff, L. R., Ehrlich-Kautzky, E., Schou, C., Krihnaswamy, G., and Beaty, T. H. (1994) Linkage analysis of IL-4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 264, 1152–1156
- Postma, D. S., Bleeker, E. R., Amelung, P. J., Holroyd, K. J., Xu, J., Panhuysen, C. I. M., Meyers, D. A., and Levitt, R. C. (1995) Genetic susceptibility to asthma-bronchial hyperresponsiveness co-inherited with a major gene for atopy. *N. Engl. J. Med.* 333, 894–900
- Behrmann, I., Walczak, S., and Krammer, P. H. (1994) Structure of the human APO-1 gene. *Eur. J. Immunol.* 24, 3057–3062
- Wong, B., Arron, J., and Choi, Y. (1997) T cell receptor signals enhance susceptibility to Fas-mediated apoptosis. *J. Exp. Med.* 186, 1939–1944
- Kay, A. B., Ying, S., Humbert, M., Barkans, J., Corrigan, C. J., Pfister, R., Menz, G., Larché, M., Robinson, D. S., and Durham, S. R. (1997) The molecular immunopathology of atopic and nonatopic asthma. *Prog. Allergy Clin. Immunol.* 4, 396–399
- Holgate, S. T. (1997) The cellular and mediator basis of asthma in relation to natural history. *Lancet* 350 (Suppl. II), SII5–SII9
- Ricci, M., Matucci, A., and Rossi, O. (1997) Pathogenetic mechanisms and genetic aspects of bronchial asthma. *ACI Int.* 9/5, 141–148
- Akbar, A. N., Borthwick, N. J., Wickremasinghe, R. G., Panayiotidis, P., Pilling, D., Bofill, M., Krajewski, S., Reed, J. C., and Salmon, M. (1996) Interleukin-2 receptor common gamma-chain signaling cytokines regulate activated T cell apoptosis in response to growth factor withdrawal: selective induction of anti-apoptotic (Bcl-2, Bcl-x_L) but no pro-apoptotic (Bax, Bcl-x_S) gene expression. *Eur. J. Immunol.* 26, 294–299
- Vella, A., Teague, T. K., Ihle, J., Kappler, J., and Marrack, P. (1997) Interleukin 4 (IL-4) or IL-7 prevents the death of resting T cells: stat-6 is probably not required for the effect. *J. Exp. Med.* 186, 325–330
- Vella, A. T., Dow, S., Potter, T. A., Kappler, J., and Marrack, P. A. (1998) Cytokine-induced survival of activated T cells in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* 95, 3810–3815
- Van Parijs, L., and Abbas, A. K. (1998) Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science* 280, 243–248
- Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Ferrel, A. C., and Horak, I. (1993) Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 75, 253–261
- Suzuki, H., Kundig, T. M., Furlonger, C., Wakeham, A., Timms, E., Matsuyama, T., Schmits, R., Simard, J. J., Ohashi, P. S., and Griesser, H. (1995) Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science* 268, 1472–1476
- Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Allen, R., Sidman, C., Proetzel, G., and Calvin, D. (1992) Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature (London)* 359, 693–697
- Adachi, T., Motojima, S., Hirata, A., Fukuda, T., and Makino, S. (1995) Eosinophil viability-enhancing activity in sputum from

- patients with bronchial asthma, contribution of interleukin-5 and granulocyte-macrophage colony-stimulating factor. *Am. J. Resp. Crit. Care Med.* 151, 618–623
37. Druilhe, A., Cai, Z., Hailé, S., Chouaib, S., and Pretolani, M. (1996) Fas-mediated apoptosis in cultured human eosinophils. *Blood* 87, 2822–2830
 38. Migliorati, G., Nicoletti, I., Pagliacci, M.C., D'Adamio, L., and Riccardi, C. (1993) Interleukin-4 protects double-negative and CD4 single-positive thymocytes from dexamethasone-induced apoptosis. *Blood* 81, 1352–1358
 39. Spinozzi, F., Nicoletti, I., Agea, E., Belia, S., Moraca, R., Migliorati, G., Riccardi, C., Grignani, F., and Bertotto, A. (1995) IL-4 is able to reverse CD2-mediated negative apoptotic signal to CD4-CD8- $\alpha\beta$ and/or $\gamma\delta$ T cells. *Immunology* 86, 379–384
 40. Rankin, J. A., Picarella, D. E., Geba, G. P., Temann, U. A., Prasad, B., DiCosmo, B., Tarallo, A., Stripp, B., Whitsett, J., and Flavell, R. A. (1996) Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. *Proc. Natl. Acad. Sci. USA* 93, 7821–7825
 41. Spinozzi, F., Agea, E., Bistoni, O., Travetti, A. M., Migliorati, G., Moraca, R., Nicoletti, I., Riccardi, C., Paoletti, C., and Vaccaro, R. (1995) T lymphocytes bearing the $\gamma\delta$ T-cell receptor are susceptible to steroid-induced programmed cell death. *Scand. J. Immunol.* 41, 504–508
 42. Spinozzi, F., Agea, E., Bistoni, O., Forenza, N., Monaco, A., Bassotti, G., Nicoletti, I., Riccardi, C., Grignani, F., and Bertotto, A. (1996) Increased allergen-specific, steroid-sensitive $\gamma\delta$ T cells in bronchoalveolar lavage fluid from patients with asthma. *Ann. Int. Med.* 124, 223–227

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