Local Expansion of Allergen-Specific CD30$^+$Th2-Type $\gamma\delta$ T Cells in Bronchial Asthma

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ABSTRACT

Background: T lymphocytes infiltrating airways during the allergic immune response play a fundamental role in recruiting other specialized cells, such as eosinophils, by secreting interleukin 5 (IL-5), and promoting local and systemic IgE synthesis by producing IL-4. Whether these presumed allergen-specific T cells are of mucosal or systemic origin is still a matter of conjecture.

Materials and Methods: Immunophenotype, IL-4 production, and in vitro proliferative response to specific or unrelated allergens were analyzed in the bronchoalveolar lavage (BAL) fluid lymphocyte suspensions obtained from untreated patients with allergic asthma. Healthy subjects and patients affected by pulmonary sarcoidosis, a granulomatous lung disease characterized by infiltrating Th1 CD4$^+$ lymphocytes, served as controls.

Results: The proportion of $\gamma\delta$ T lymphocytes, mostly CD4$^+$ or CD4$^-$−CD8$^-$, was higher in asthmatic subjects than in controls ($p < 0.05$). Most BAL $\gamma\delta$ CD4$^+$ lymphocytes of asthmatic patients displayed the T cell receptor (TCR)–$\gamma\delta$ Vδ1 chain. While CD30 antigen coexpression on the surface of BAL $\alpha\beta^+$ T lymphocytes was low (ranging from 5 to 12%), about half of pulmonary $\gamma\delta$ T cells coexpressed it. These cells produced IL-4 and negligible amounts of interferon-γ (IFNγ), and proliferated in vitro in response to purified specific but not unrelated allergens. In contrast, control or sarcoidosis $\gamma\delta$ T cells never displayed the CD30 surface molecule or produced significant quantities of IL-4.

Conclusions: These findings not only confirm our previous hypothesis that the allergen-specific Th2-type lymphocytes found in the lungs of asthmatic patients are $\gamma\delta$ T cells belonging to airway mucosal immunocytes, but also strongly support the notion that asthma is a local rather than a systemic disease.

INTRODUCTION

There is considerable evidence that inflammation is crucial to the pathogenesis of allergic bronchial asthma. Studies attempting to quantify the magnitude of the airway inflammatory response have reported increased eosinophils, basophils/mast-cells, and T lymphocytes in bronchoalveolar lavage (BAL) fluid samples (1,2). Recent findings suggest that, among these cells, the T lymphocyte population plays the fundamental role in both recruiting other specialized cells, such as eosinophils, by secreting interleukin 5 (IL-5) (1) and promoting local and systemic synthesis of IgE by producing IL-4 (3,4). Activated T lymphocytes (CD2$^+$CD25$^+$) with such functional activity (Th2-type) have been detected in the lungs of atopic asthmatic subjects, but never in normal controls (5). However, the problem of whether these presumed allergen-specific T cells are of mucosal or systemic origin remains a mat-

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ter of conjecture. Furthermore, their precise phenotype and their role in the induction of airway dysfunction remain largely unknown.

Reports indicate that a substantial proportion of $CD3^+$ intraepithelial lymphocytes in the noses of allergic patients are T lymphocytes which bear the γδ T cell receptor (TCR) heterodimer (6,7). Interestingly, in the presence of IL-4, $CD4^+$TCR-$\gamma\delta^+$ cells can provide in vitro bystander cognate T cell help for IgE production (8,9). Mice deficient in $\alpha\beta^+$ T cells were found to make immunoglobulins of all isotypes with high levels of IgE and IgG1, suggesting a role for γδ T cells in directing isotype switching (10), a finding recently confirmed in humans (11). Furthermore, local endobronchial allergen instillation in sensitized asthmatics leads to an inflammatory infiltrate of the airway mucosa characterized by increased numbers of $CD3^+$ T lymphocytes which lack the CD4 and CD8 molecules, a phenotypical feature usually displayed by γδ T cells (12) and, perhaps, related to those cells ($TCR\delta1^+$) we previously found in the BAL fluids of untreated patients with this pathology (13).

With these findings in mind, we asked whether the detection of Th2-type lymphocytes in BAL fluid of allergic asthma (5) could be accounted for by a local expansion of allergen-specific γδ T cells coexpressing the CD30 surface antigen, a member of the TNF/NGF receptor superfamily present on both $CD4^+$ and $CD8^+$ T cell clones which produce a Th2-type cytokine profile (i.e., IL-4 and IL-5) (14).

MATERIALS AND METHODS

Patients and Controls

Ten subjects (six children and four adults) with mildly symptomatic chronic asthma were included in the study. At the time of enrollment all the asthmatic subjects had stable pulmonary function with a forced expiratory volume in 1 sec (FEV_1) of 70% of that predicted for their age and height. None of the asthmatics were being treated with inhaled or oral corticosteroids, sodium cromoglycate, theophylline, or β_2 agonists. They had increased airway responsiveness to methacholine (concentration producing a decrease of 20% from baseline in $FEV_1 < 8$ mg/ml). All were atopic, as defined by positive skin-prick tests with purified *Dermatophagoides pteronyssinus* (*Der. p*I) allergen extracts (Neo Abellò, Madrid, Spain) and positive for the search of circulating allergen-specific IgE (ELISA; DPC Corp., Los Angeles, CA, U.S.A.). None were smokers or had had upper respiratory tract infections within 8 weeks of investigations. BAL from 10 age-matched healthy nonatopic, nonsmoking volunteers served as control samples. The percentage and absolute number of BAL γδ T cells from patients with asthma were also compared with those calculated in subjects diagnosed as having pulmonary sarcoidosis ($n = 5$), a pathological condition known to be associated with overexpanded lung Th1-type cell population which displays the $CD4^+$ phenotype. Informed consent was given by the children's relatives or directly by the adult patients, and the clinical research was conducted in accord with the local ethical committee guidelines.

Bronchoalveolar Lavage (BAL) Fluid Collection

Premedication and fiberoptic bronchoscopy were performed as previously reported (15). Lavage of the apical segment of the upper right lobe was done with 20-ml aliquots of pre-warmed physiological saline. Recovered BAL cells were spun immediately at $200 \times g$ for 10 min at 4°C, and after resuspension, cytospins were prepared for differential cell counts.

Reagents and Flow Cytometry

Immunophenotyping was carried out by using the following mouse monoclonal antibodies (mAb) separately or in various combinations: phycoerythrin-conjugated anti-CD3 (OKT3; Ortho, Raritan, NJ, U.S.A.), anti-CD4, anti-CD8 (OKT4, OKT8, Ortho), anti CD25 (anti–IL-2 receptor; Becton-Dickinson Co., Mountain View, CA, U.S.A.), purified anti-CD30 (a mouse IgG_1 mAb, BerH2, a generous gift of Prof. Harald Stein, Free University of Berlin), fluorescein-conjugated anti-TCRδ1 (T Cell Sciences, Cambridge, MA), a pan-reactive γδ T cell reagent, anti-γV1(a) and −γV2(a) (T Cell Sciences) which recognize Vγ1 and Vγ2 encoded determinants and identify two major, nonoverlapping γδ T cell subsets that between them account for the total γδ T lymphocyte population both in blood and lymphoid tissues. More than two-thirds of the γδ T lymphocytes in the peripheral blood of healthy individuals are $V\gamma2^+$ cells, while the remainder are of the $V\gamma1^+$ subtype (16). For staining, 5,000 to 10,000 cells were resuspended in 50 μl of saline, incubated at 4°C for 30 min, and washed and analyzed by flow cytometry (FACScan, Bec-

ton-Dickinson). For the analysis of single-color and two-color cytofluorimetric data, an electronic gate was set on the lymphocyte population based on the forward-angle versus 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.

Separation of Enriched γδ T-Cell Populations

BAL mononuclear cells from *Der p*I–sensitive patients were separated into sheep red blood cell rosette-enriched and rosette-depleted subsets, as described in detail elsewhere (17). On the basis of their reactivity with the anti-CD3 mAb OKT3 (>98% by immunofluorescence analysis), the rosetted mononuclear cells were considered highly purified T cell subsets. BAL γδ$^+$ T lymphocytes were negatively selected by incubation with a mixture of anti–TCR-αβ mAb (anti-βF1, T Cell Sciences; and anti-WT31, Becton Dickinson) followed by incubation with magnetic beads–conjugated goat anti-human IgG (Dynal, A. S., Oslo, Norway). This procedure was performed according to the manufacturer's instructions. As several cycles of negative magnetic immunoselection yielded a >85% TCRδ1$^+$ cell population (FACScan analysis) in BAL lymphocyte suspensions, these cells were considered purified γδ T lymphocyte subsets and used for culture and cytokine profile experiments.

Cell Cultures

Mononuclear cells (MC) derived from BAL were washed and suspended in RPMI-1640 (Gibco, Grand Island, NY, U.S.A.) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 10 mM Hepes, 50 U/ml penicillin, and 50 μg streptomycin at 10^6 cells/ml. To assess the allergen specificity of BAL γδ T cells, 1.2×10^5 MNC and 4×10^4 γδ-enriched BAL T cells (the latter cocultured with 8×10^4 autologous adherent cells) from *Der p*I–sensitive patients and, due to the very small percentage of γδ T cells in normal BAL fluid, MC (1.2×10^5) from normal controls were seeded in microplates and cultured in medium alone or in the presence of 1 μg/ml purified *Der p*I (Neo Abellò, Madrid, Spain) or unrelated allergen (purified *Lol p*I, Neo Abellò) from the Graminaceae family. After 60 hr culture, the cells were pulsed for 16 hr with 0.5 μCi [^{3}H]-thymidine ([^{3}H]-TdR), harvested and the radioactivity measured by liquid scintillation. Results are expressed as net cpm [^{3}H]-TdR incorporation and reflect absolute cpm [^{3}H]-TdR uptake minus background cpm (cpm incorporated in the absence of allergen).

Cytokine Profile of Pulmonary Enriched γδ T Cells

One hundred thousand enriched BAL γδ T cells from patients with allergic asthma and from five patients with pulmonary sarcoidosis, and a similar number of enriched total T cells from normal controls were washed in HBSS supplemented with 0.01 M N-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid buffer. Cells were transferred to adhesion slides (BioRad Laboratories, Munich, Germany) and were allowed to adhere electrostatically to the slides for 10 min at room temperature. Excess cells were washed away and the unbound surface area on the adhesive fields was blocked with 2% FCS in HBSS for 10 min at 37°C. Cells were fixed with phosphate-buffered 4% paraformaldehyde at pH 7.4 for 20 min. After subsequent washes with HBSS, the cells were permeabilized with HBSS supplemented with 0.1% saponin (Riedel-de Haën AG, Seelze, Germany) to allow the intracellular entrance of the cytokine-specific mAb. Ten microliters of the mAb (anti-human IFNγ, a mouse IgG2a, and anti-human IL4, a mouse IgG1; Genzyme Corp. Cambridge, MA, U.S.A.), diluted to the final concentration of 1 to 5 μg/ml, were added and left to incubate for 20 min at 37°C. The cells were then washed in HBSS-saponin. Ten microliters of the FITC-conjugated second-step antibody (goat anti-mouse IgG2a and IgG1, Southern Biotechnology Ass., Birmingham, AL, U.S.A.) were added for further 30 min exposure in darkness at room temperature. After extensive washing, the cells were dried on slides at 37°C. Buffered glycerol containing 2% diazobicyclo-octane was used at the mounting medium to reduce UV quenching. Slides were examined by three independent observers on a Leitz Dialux 20 microscope equipped with a 200-W mercury lamp. Results are presented as the percentage of positively stained cells of total MC counted (200 to 800 depending on cell yeld).

Statistical Analysis

Because of the non-normal distribution of samples, the Kruskall-Wallis one-way analysis of

TABLE 1. Absolute counts of BAL γδ T lymphocytes in patients and control subjects

	Lymphocytes ($\times 10^6$/ml)	$CD3^+TCR\delta1^+$ ($\times 10^4$/ml)	$CD4^+\delta V1^+$ ($\times 10^4$/ml)	$TCR\delta1^+30^+$ ($\times 10^4$/ml)
Asthma	1.47 ± 0.4	29.6 ± 2.5[a,b]	15.2 ± 1.3[a,b]	16.9 ± 1.6[c]
Controls	0.80 ± 0.06	0.2 ± 0.1	0.08 ± 0.01	0.01 ± 0.001
Sarcoidosis	9.77 ± 3.1	12.7 ± 3.6	7.8 ± 2	0.002 ± 0.001

[a] $p < 0.0001$ versus controls.
[b] $p < 0.05$ versus sarcoidosis patients.
[c] $p < 0.0001$ versus controls and sarcoidosis patients.

variance was adopted for statistical evaluation. Values of $p < 0.05$ were chosen for rejection of the null hypothesis.

RESULTS

The absolute number of BAL γδ T lymphocytes, mostly $CD4^+$ or $CD4^-CD8^-$, was significantly higher in asthmatics than controls, mainly due to an increase of cells bearing the TCR-γδ Vγ1 chain (Table 1). These values were significantly higher even when compared with those recorded in sarcoidosis patients, which usually display an absolute $CD4^+$ lymphocytosis during the active phase of the disease. A small percentage of αβ (ranging from 5 to 12%), but a significant proportion of BAL γδ T lymphocytes (from 40 to 55%) from asthmatic subjects reacted with the anti-CD30 mAb used in this study. Similar findings were never observed on either normal control or sarcoidosis patient T cells. To ascertain whether this phenoypical pattern represents an expanded Th2-type lymphocyte subpopulation, we carried out experiments to determine if cytokines, such as IL-4 and IFNγ, which functionally exclude the expansion of the reciprocal Th1 and Th2 T-lymphocyte subsets, were detectable in the cytoplasm of BAL γδ T cells. Slide-fixed positive cells were independently enumerated by three observers, unaware of the nature of the employed fluoresceinated probe. The mean percentages of counted cells positively reacting with each of the two cytokines are illustrated in Fig. 1. Anti–IL-4 antibody–positive pulmonary γδ T cells were present only in the slides of allergic asthma patients and virtually absent in the control and sarcoidosis patient counterparts ($p < 0.005$).

The expanded Th2-type γδ T cell population proliferated in vitro in response to specific allergen stimulation (Table 2), whereas $[^3H]$-TdR incorporation was weak with unrelated allergens. Control T cells and Th1 lymphocytes obtained from BAL of

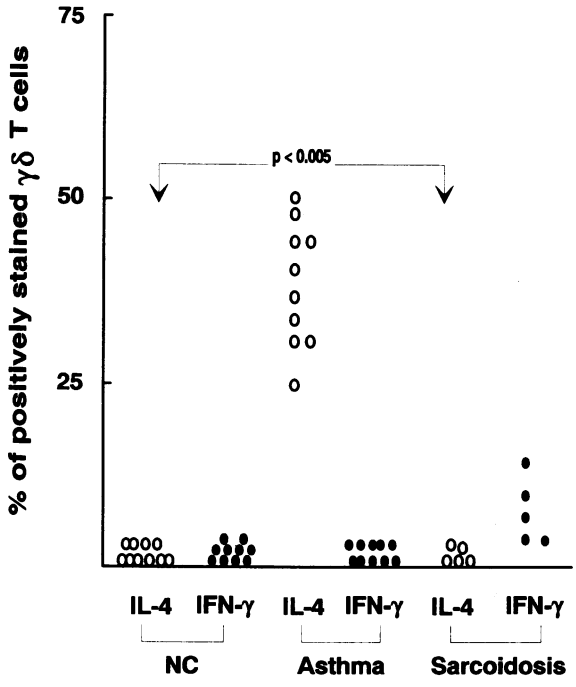

FIG. 1. Percentages of positive-stained (for both anti-IL4 or anti-IFNγ antibodies) enriched γδ T cells from patients with allergic bronchial asthma compared with those of normal subjects and those of untreated sarcoidosis patients

For the preparation of slides, see Materials and Methods. IL-4–positive γδ T lymphocytes in allergic patients ranged from 25 to 50%, and the mean percentage was statistically higher than those calculated in the control groups.

TABLE 2. In vitro response of patient's BAL T lymphocytes to specific or unrelated allergens

	BAL γδ T Cells	BAL Total T Cells
Specific allergen (*Der p*I 1 μg/ml)	5300 ± 520[a]	6950 ± 850
Unrelated allergen (*Lol p*I 1 μg/ml)	350 ± 20	450 ± 50

[a]Results are expressed as net cpm [^{3}H]-TdR incorporation and reflect absolute cpm [^{3}H]-TdR uptake minus background cpm (cpm incorporated in the absence of allergen). Results are representative of all experiments performed in triplicate on enriched γδ T cells and total T cells from three *Der p*I–sensitive patients.

patients with sarcoid alveolitis were always unresponsive to allergen triggering (data not shown).

DISCUSSION

The behavior of cytokine production and nonclassical MHC-related antigen recognition capacity has led to the suggestion that γδ T cells play a complementary role to that of αβ T cells in providing a rapidly induced, but weaker immune response before the αβ T cell response has fully developed (18). However, the finding that the γ-chain of the high-affinity IgE receptor (FcεRI) is a major functional subunit of the TCR-γδ/CD3 complex (19) suggests that γδ T cells might be implicated in allergic inflammation, including asthma, possibly through the recognition of IgE-complexed allergens. The recent identification of a locus in the TCR-α/δ region that controls specific IgE reactions to purified allergens with a recessive genetic inheritance (20) similar to the previously identified chromosome 11q atopy locus (21) would seem to support this proposal.

Interestingly, several lines of evidence suggest that CD30 antigen expression and/or soluble CD30 release are linked to the production of Th2-type cytokines. First, activated Th2, but not Th1 CD4$^+$ and CD8$^+$ T cell clones express membrane CD30 and release its soluble form. Second, peripheral blood T cells activated in vitro with allergens display the surface CD30 molecule, whereas T cells activated with PPD do not. Third, allergen specific CD4$^+$CD30$^+$ T cells secreting Th2-type cytokines are present in the circulation of atopic donors following allergen exposure (13).

Here, we reported that pulmonary CD30$^+$ γδ T lymphocytes are increased in BAL fluid of patients with bronchial asthma, and this should be viewed against the background of our related findings. Furthermore, the in vitro proliferative response to purified allergen stimulation and Th2-type cytokine production strongly support the notion that BAL lymphocytes bearing the γδ TCR play a fundamental role in the local immune response to inhaled allergens. The finding that some γδ T cells recognize unprocessed or native antigen (22,23), a phenomenon that presumably occurs in sensitive patients exposed to aeroallergens, lends supporting evidence for this contention.

The low percentage of BAL CD30$^+$αβ$^+$T cells may reflect a small number of cells and/or a level of surface protein density (as naturally occurs when extracellular CD30 protein is cleaved and secreted in its soluble form) (24), below the limits of our cytofluorimetric assay. This may underscore the relevance of αβ$^+$ T cells during the local immune response to allergens. However, as demonstrated in mice infected with *Nippostrongilus brasiliensis* (25), cytokines produced by γδ T cells may contribute to the local cytokine milieu that influences the differentiation of allergen-specific αβ$^+$CD4$^+$ T cells from Th0 to Th2 lymphocytes.

Taken together, these data favor the hypothesis that native allergen exposure in genetically susceptible (atopic) subjects (19,20) could lead to a local expansion of allergen-specific Th2-type γδ T cells, which in turn amplify the local immune response by secreting IL-4 and IL-5 (17), thus recruiting both Th2-type αβ$^+$ lymphocytes and other specialized cells, such as neutrophils and eosinophils. It is worth noting that a similar scenario may be pharmacologically modified, with rapid clinical benefit, by administering corticosteroids systemically to asthmatic patients and inducing the apoptotic cell death of the expanded pulmonary γδ T-cell subsets as shown by our in vitro and in vivo studies (13).

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REFERENCES

1. MacFadden ER, Gilbert IA. (1992) *N. Engl. J. Med.* **327:** 1928–1937.
2. Corrigan CJ, Kay AB. (1992) T cells and

eosinophils in the pathogenesis of asthma. *Immunol. Today* **13:** 501–506.
3. Del Prete GF. (1992) Human Th1 and Th2 lymphocytes: Their role in the pathophysiology of atopy. *Allergy* **47:** 450–455.
4. Romagnani S. (1990) Regulation and deregulation of human IgE synthesis. *Immunol. Today* **11:** 316–321.
5. Robinson DS, Hamid Q, Ying S, et al. (1992) Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* **326:** 298–304.
6. Okuda M, Pawankar R. (1992) Flow cytometric analysis of intraepithelial lymphocytes in the human nasal mucosa. *Allergy* **47:** 255–259.
7. Okuda M, Pawankar R. (1993) Characteristics of human nasal lymphocytes in perenneal allergic rhinitis in comparison with chronic non-allergic hypertrophic rhinitis. *J. Allergy Clin. Immunol.* **91:** 181.
8. Spits H, Paliard X, Vandekerkhove Y, Van Vlasselaer P, De Vries JE. (1991) Functional and phenotypic differences between $CD4^+$ and $CD4^-$ T-cell receptor $\gamma\delta$ clones from peripheral blood. *J. Immunol.* **147:** 1180–1188.
9. Gascan H, Aversa GG, Gauchat JF, et al. (1992) Membranes of activated $CD4^+$ T cells expressing T cell receptor (TcR) $\alpha\beta$ or TcR $\gamma\delta$ induce IgE synthesis by human B cells in the presence of interleukin-4. *Eur. J. Immunol.* **22:** 1133–1141.
10. Wen L, Roberts SJ, Viney JL, et al. (1994) Immunoglobulin synthesis and generalized autoimmunity in mice congenitally deficient in $\alpha\beta$ (+) cells. *Nature* **369:** 654–658.
11. Horner AA, Jaraba H, Ramesh N, Geha RS. (1995) $\gamma\delta$ T lymphocytes express CD40 ligand and induce isotype switching in B lymphocytes. *J. Exp. Med.* **181:** 1239–1244.
12. Montefort S, Gratziou C, Goulding D, et al. (1994) Bronchial biopsy evidence for leukocyte infiltration and upregulation of leukocyte-endothelial cell adhesion molecules 6 hours after local allergen challenge of sensitized asthmatic airways. *J. Clin. Invest.* **93:** 1411–1421.
13. Spinozzi F, Bertotto A. (1995) Cellular mechanisms in the pathogenesis of bronchial asthma. *Immunol. Today.* **16:** 407–408.
14. Del Prete GF, Maggi E, Pizzolo G, Romagnani S. (1995) CD30, Th2 cytokines and HIV infection: A complex and fascinating link. *Immunol. Today* **16:** 76–80.
15. Spinozzi F, Cimignoli E, Gerli R, et al. (1992) IgG subclass deficiency and sinopulmonary bacterial infections in patients with alcoholic liver disease. *Arch. Intern. Med.* **152:** 99–104.
16. Bertotto A, Gerli R, Spinozzi F, et al. (1993) Lymphocytes bearing the $\gamma\delta$ T cell receptor in acute *Brucella melitensis* infection. *Eur. J. Immunol.* **23:** 1177–1180.
17. Spinozzi F, Agea E, Bassotti G, et al. (1993) Ethanol-specific impairment of T-lymphocyte activation is caused by a transitory block in signal-transduction pathways. *Gastroenterology* **105:** 1490–1501.
18. Cristmas SE. (1992) Cytokine production by T lymphocytes bearing the gamma-delta T cell antigen receptor. *Chem. Immunol.* **53:** 32–46.
19. Qian D, Sperling AI, Lancki DW, et al. (1993) The γ chain of the high-affinity receptor for the IgE is a major functional subunit of the T-cell antigen receptor complex in $\gamma\delta$ T lymphocytes. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 11875–11879.
20. Moffatt MF, Hill MR, Cornélis F, et al. (1994) Genetic linkage of T-cell receptor α/δ complex to specific IgE responses. *Lancet* **343:** 1597–1600.
21. Sandford AJ, Shirakawa T, Moffatt MF, et al. (1993) Localisation of atopy and β-subunit of high-affinity IgE receptor (FcεRI) on chromosome 11q. *Lancet* **341:** 332–334.
22. Schild H, Mavaddat N, Litzenberger C, et al. (1994) The nature of major histocompatibility complex recognition by $\gamma\delta$ T cells. *Cell* **76:** 29–37.
23. Constant P, Davodeau F, Peyrat MA, et al. (1994) Stimulation of human $\gamma\delta$ T cells by nonpeptidic mycobacterial ligands. *Science* **264:** 267–270.
24. Del Prete GF, De Carli M, Almerigogna F, et al. (1995) Preferential expression of CD30 by human $CD4^+$ T cells producing Th2-type cytokines. *FASEB J.* **9:** 81–86.
25. Ferrik DA, Schrenzel MD, Mulvania T, Hsieh B, Ferlin WG, Lepper H. (1995) Differential production of interferon-γ and interleukin-4 in response to Th1- and Th2-stimulating pathogens by $\gamma\delta$ T cells in vivo. *Nature* **373:** 255–257.

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