

## **Virus-specific CD8<sup>+</sup> Cells Can Switch to Interleukin 5 Production and Induce Airway Eosinophilia**

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### **Summary**

Virus infections of the lung are thought to predispose individuals to asthma, a disease characterized by eosinophil infiltration of the airways. CD8<sup>+</sup> T cells are an important part of the host response to virus infection, however, they have no reported role in eosinophil recruitment. We developed a mouse model of virus peptide-stimulated CD8<sup>+</sup> T cell immune responses in the lung. We found that bystander CD4<sup>+</sup> T helper cell type 2 immune responses to ovalbumin switched the virus peptide-specific CD8<sup>+</sup> T cells in the lung to interleukin (IL) 5 production. Furthermore, when such IL-5-producing CD8<sup>+</sup> T cells were challenged via the airways with virus peptide, a significant eosinophil infiltration was induced. In vitro studies indicated that IL-4 could switch the virus-specific CD8<sup>+</sup> T cells to IL-5 production. These results could explain the link between virus infection and acute exacerbation of asthma and, perhaps more importantly, they indicate an IL-4-dependent mechanism that would impair CD8<sup>+</sup> T cell responses and delay viral clearance from the host.

**I**nfiltration and activation of eosinophils in the bronchial mucosa is believed to be a central event in the pathogenesis of asthma (1). The major stimulus for eosinophil accumulation is thought to be allergen-stimulated production of IL-5 by CD4<sup>+</sup> TH2 cells (2, 3). However, virus infection of the upper respiratory tract is also thought to predispose towards and exacerbate bronchial asthma (4, 5). The precise mechanism(s) by which virus infection brings this about has not yet been defined.

Virus infection of the host leads to the activation of both CD4<sup>+</sup> and CD8<sup>+</sup> T cell-mediated immune responses. CD8<sup>+</sup> T cells help control virus replication by the production of IFN- $\gamma$  and lysis of virus-infected cells (6). In fact, some workers have suggested that the IFN- $\gamma$  produced by CD8<sup>+</sup> T cells can suppress CD4<sup>+</sup> TH2 responses in the airways (7, 8).

Recently, it has been demonstrated that CD8<sup>+</sup> T cells activated in the presence of IL-4 can switch to a noncytolytic phenotype that produces the TH2 cytokines IL-4, IL-5, and IL-10 and provides help for B cells (9). This observation suggested the possibility that virus-specific CD8<sup>+</sup> T cells have the potential to develop into IL-5-producing cells and become a major stimulus for eosinophil recruitment into the lung. In the present study, we have investigated whether induction of a local TH2 immune response could modify the pattern of cytokines produced by CD8<sup>+</sup> T cells and determine whether this change in functional phenotype would have pathophysiological consequences.

### **Materials and Methods**

#### *Mice*

To study virus peptide-specific CD8<sup>+</sup> T cell immune responses we made use of transgenic mice expressing an H-2D<sup>b</sup>-restricted TCR specific for the glycoprotein epitope 33-41 of the lymphocytic choriomeningitis virus (LCMV)<sup>1</sup> (10). The TCR transgenic mouse line 318 used in this study is able to respond normally to antigens unrelated to LCMV because the transgenic TCR is expressed only on 50–60% of CD8<sup>+</sup> T cells, and normal numbers of CD4<sup>+</sup> T cells with endogenous TCR are found in the lymphoid organs (11). For the in vitro studies, we made use of a second transgenic mouse line (No. 327) which expresses the LCMV-specific TCR on 100% of the CD8<sup>+</sup> T cells (11).

#### *Immunization*

A TH2 immune response was induced by immunizing 8-wk-old C57BL/6 or LCMV TCR transgenic mice intraperitoneally on days 0 and 14 with 2  $\mu$ g of OVA (Sigma Chemical Co., St. Louis, MO) in 4 mg of alum adjuvant (SERVA, Heidelberg, Germany). Sham-immunized mice received two injections of alum alone.

#### *Virus Peptide-induced Airway Inflammation*

LCMV-specific TCR transgenic mice or nontransgenic C57BL/6 mice were either sham immunized or sensitized to OVA as described

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<sup>1</sup> Abbreviations used in this paper: BAL, bronchoalveolar lavage; LCMV, lymphocytic choriomeningitis virus.

above. 7 d later, mice were anesthetised with a mixture of Hypnorm (Janssen, Baar, Switzerland) and Dormicom (Roche, Basel, Switzerland), and 50  $\mu$ l of LCMV glycoprotein 33-41 peptide (9) (2 mg/ml) was administered to the airways via the intranasal route, the animals were then allowed to recover. 72 h later, animals were anesthetised with urethane (14% wt/vol), the trachea cannulated, and a bronchoalveolar lavage (BAL) performed by five repeated lavages with 0.3 ml of saline. Cytospin preparations were prepared and the cellular composition of the BAL fluid quantified after staining with Dif-Quik (Dade Baxter, Miami, FL) using standard histological criteria.

### Purification of Lung T Cells

To prepare T cells from the lung, BAL was performed five times with 1 ml of sterile PBS to remove resident pulmonary macrophages. The thorax was then opened and the lungs perfused via the right ventricle with 10 ml of PBS containing 500 U/ml of heparin to remove contaminating blood. Lungs were removed, homogenized, and the resulting cell suspension filtered through a 70- $\mu$ m filter, and lymphocytes enriched over a single-step Ficoll gradient. The cells were then labeled with Thy1.2-PE (PharMingen, San Diego, CA) and sorted by flow cytometry.

### Cytokine Production by Lung T Cells

**Anti-CD3-induced Cytokine Production.** FACS<sup>®</sup> (Becton Dickinson & Co., Mountain View, CA) sorted lung T cells (>99.5% pure) were cultured ( $2 \times 10^5$  cells/well) on an anti-CD3-coated (2C11, 50  $\mu$ g/ml) 96-well plate (Nunc, Roskilde, Denmark) in the presence of hIL-2 (200 U/ml) for 72 h. Supernatants were then harvested and the production of cytokines (IL-2, IL-4, IL-5, and IFN- $\gamma$ ) determined by ELISA (PharMingen) as described previously (12). The limits of cytokine detection were 0.2 U/ml (IL-2), 20 U/ml (IL-4), 150 U/ml (IL-5), and 20 U/ml (IFN- $\gamma$ ) (12).

**LCMV Peptide-induced Cytokine Production.** FACS<sup>®</sup> sorted Thy1.2<sup>+</sup> cells (>99.5% pure) derived from LCMV-specific TCR transgenic mice were cultured in 96-well plates ( $2 \times 10^5$ /well) and stimulated with 10  $\mu$ g/ml of LCMV peptide and  $10^5$  irradiated EL-4 target cells/well in the presence of hIL-2 (200 U/ml) for 72 h. Supernatants were harvested and IL-5 and IFN- $\gamma$  levels measured by ELISA (PharMingen).

### LCMV Peptide-specific In Vitro Immune Response Assay

CD8<sup>+</sup> T cells were prepared from the LCMV-specific TCR transgenic mouse line 327, by removal of CD4<sup>+</sup> T and B cells with specific antibodies (GK1.5 and sheep anti-mouse Ig) (12) and magnetic beads (Dynabeads; Dynal, Oslo, Norway). The remaining cell population was then labeled with CD8-FITC (PharMingen) and small CD8<sup>+</sup> T cells were purified (>99.5% purity) by flow cytometry. CD8<sup>+</sup> T cells were cultured (800 cells per well) in 96 U-bottom well plates (Nunc) for 6 d in the presence of the viral peptide (11) (1  $\mu$ g/ml) and IL-2 (200 U/ml) with or without IL-4 (500 U/ml). Cells were harvested from each culture, washed, and recultured ( $10^5$  cells per well) on an anti-CD3-coated plate (50  $\mu$ g/ml) in the presence of hIL-2 (200 U/ml) for 48 h. The supernatants were assayed for the cytokines IL-5 and IFN- $\gamma$  by ELISA. Greater than 99% of cells at the end of the 6-d culture period were surface positive for the transgenic V $\alpha$ 2 and V $\beta$ 8.1 chains (PharMingen antibodies B20.1 and MR5-2, respectively) indicating that only the LCMV-specific cells were activated.

## Results

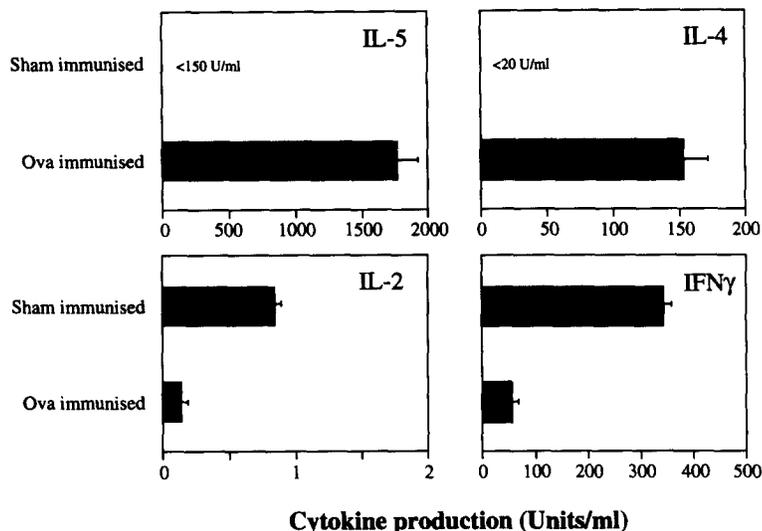
**Induction of a TH2-immune Response in the Lungs of OVA-immunized Mice.** Thy-1.2<sup>+</sup> T cells were purified from the

lungs of OVA- and sham-immunized mice, and their potential for cytokine production determined by stimulation with plate-bound anti-CD3 antibodies. Lung T cells from sham-immunized mice produced IL-2 and IFN- $\gamma$ , with no IL-4 or IL-5 being detected. By contrast, T cells isolated from the lungs of OVA-immunized mice produced significant levels of IL-4 and IL-5, but very little IFN- $\gamma$  or IL-2, indicating that they were "TH2 like" (Fig. 1). Spleen- or lymph node-derived T cells from both OVA- and sham-immunized mice produced IL-2 and IFN- $\gamma$  (data not shown) and no detectable IL-4 and IL-5 when stimulated with anti-CD3 antibodies. This would indicate that there was a selective enrichment of OVA-primed TH2 cells in the lung tissues. Serum analysis of OVA-immunized mice indicated that detectable levels of IgE were induced (data not shown). These IgE levels were maintained for many months, indicating that IL-4 production was ongoing in these immunized mice.

**Effect of TH2 Immunization on Lung CD8<sup>+</sup> T Cell Cytokine Production.** The OVA/alum immunization protocol was applied to the LCMV-specific TCR transgenic mice. The purpose of the experiment was to determine whether the OVA-induced TH2 response in the lung could switch naive LCMV-specific CD8<sup>+</sup> T cells to IL-5 production. T cells were purified from the lungs of OVA-immunized LCMV-specific TCR transgenic mice. In vitro stimulation of the lung T cells from sham-immunized mice with the LCMV peptide resulted in IFN- $\gamma$  production and no detectable IL-5 (Fig. 2). By contrast, lung T cells from the OVA-immunized mice produced IL-5 and reduced amounts of IFN- $\gamma$  (Fig. 2). Importantly, T cells from lymph nodes or spleens of OVA-immunized mice did not produce detectable IL-5 upon viral peptide stimulation in vitro (data not shown), indicating that only the LCMV peptide-specific CD8<sup>+</sup> T cells in the lung had switched phenotype. No cytokines were detected in cultures stimulated with EL-4 target cells alone or cultures of target cells plus the viral peptide.

**Effect of TH2 Immunization on a CD8<sup>+</sup> T Cell-induced Inflammatory Response in the Lung.** It was important to determine whether the switch of LCMV peptide-specific CD8<sup>+</sup> T cells to IL-5 production would lead to eosinophil recruitment if the lungs were subsequently exposed to LCMV peptide. Transgenic mice previously immunized against OVA developed a BAL eosinophilia when intranasally inoculated with the LCMV peptide (Fig. 3 A). However, LCMV peptide challenge of the sham-immunized transgenic mice led to the recruitment of some neutrophils and lymphocytes but no eosinophils (Fig. 3 A). Nontransgenic, OVA-immunized C57BL/6 mice did not develop any inflammatory cell infiltrate upon viral peptide challenge, indicating that the eosinophil infiltration was dependent on the LCMV peptide-specific CD8<sup>+</sup> T cells, and was not due to recognition of the virus peptide by OVA-specific CD4<sup>+</sup> T cells (Fig. 3 B).

These results support the notion that a TH2 immune response to unrelated antigens can modify the functional potential of bystander naive CD8<sup>+</sup> T cells to the degree, that when they meet their specific antigen in the form of class I MHC-associated virus peptide, they can produce IL-5 and recruit eosinophils into the lung.



**Figure 1.** Cytokine production profile of anti-CD3-stimulated, purified lung T cells from sham- or OVA-immunized C57BL/6 mice. Data are shown as the mean + SEM of three separate cultures with  $n = 5$  mice in each group.

*Role of IL-4 in the In Vitro Switch of Viral-specific CD8<sup>+</sup> Cells to IL-5 Production.* We questioned how a TH2 immune response could influence the phenotype of bystander virus-specific CD8<sup>+</sup> T cells. To study this we established an in vitro immune response model using FACS<sup>®</sup>-sorted, LCMV peptide-specific CD8<sup>+</sup> T cells from transgenic mice. By activating the T cells with LCMV peptide in the presence of various costimulatory factors we hoped to identify which cytokine might be relevant to the in vivo switch of CD8 cells to IL-5 production. CD8<sup>+</sup> T cells activated in the presence of IL-2 became well activated, proliferated, and produced IFN- $\gamma$  when restimulated with plate-bound anti-CD3 antibody (Fig. 4). However, if IL-4 was also added to the primary cultures, the resulting CD8 T cells produced IL-5 and reduced amounts of IFN- $\gamma$  when restimulated with anti-CD3 antibody (Fig. 4). The surface phenotype of the 6-d cultured CD8 T cells, both in the presence and absence of IL-4, was

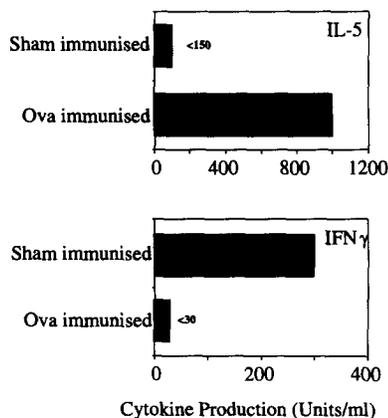
99% positive for the expression of the TCR  $\alpha$  (V $\alpha$ 2) and  $\beta$  (V $\beta$ 8.1) chain transgenes, indicating that all the in vitro-activated CD8 T cells were LCMV peptide specific (data not shown).

## Discussion

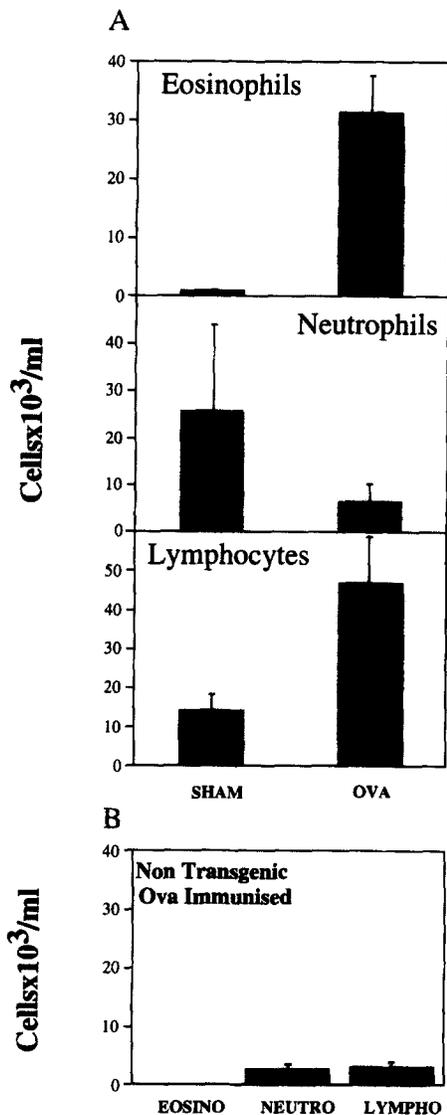
Infiltration and activation of eosinophils in the bronchial mucosa is believed to be a central event in the pathogenesis of asthma (13). There is now strong epidemiological and clinical evidence to suggest that viral infections can exacerbate the symptoms of asthma (14). Paradoxically, a major host response against virus infection is the activation of IFN- $\gamma$ -producing CD8 T cells, which are thought to be antagonistic to eosinophil activity and are proposed to suppress TH2 immune responses in the lung (7). Therefore, the link between viral infection, CD8<sup>+</sup> T cell activation, and eosinophil accumulation in asthmatics remains unexplained.

In our mouse model of LCMV-specific CD8<sup>+</sup> T cell responses we found that the induction of a TH2 immune response to OVA switched the subsequent bystander LCMV-specific CD8<sup>+</sup> T cell response to IL-5 production, leading to the accumulation of eosinophils in the lung. By contrast, the LCMV peptide-specific, CD8 T cell-induced response in non- or sham-immunized transgenic mice resulted in an accumulation of neutrophils, without any detectable eosinophil recruitment. It was clear that the virus peptide-induced inflammatory response in both the sham- and OVA-immunized transgenic mice was due to CD8<sup>+</sup> T cells specific for the LCMV peptide, as peptide provocation of nontransgenic mice failed to induce any inflammatory changes in the lung.

In vitro analysis of the cytokines produced by the LCMV peptide-specific lung CD8<sup>+</sup> T cells revealed the cells from OVA-immunized mice produced IL-5 and reduced levels of IFN- $\gamma$ , whereas CD8<sup>+</sup> T cells from sham-immunized mice secreted large amounts of IFN- $\gamma$  and no detectable IL-5. To our knowledge, this is the first in vivo demonstration that CD8<sup>+</sup> T cells can switch functional phenotype and become



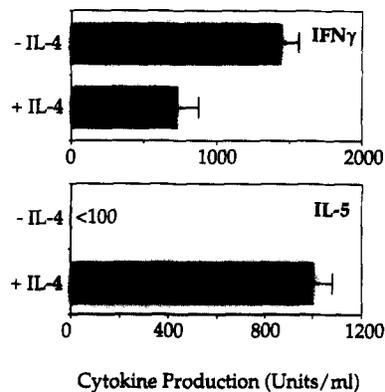
**Figure 2.** Cytokine production profile of LCMV peptide-stimulated, purified lung T cells from sham- or OVA-immunized, LCMV-specific TCR transgenic mice. Each column represents cytokine production (Units/ml) from T cells derived from the lungs of five mice and was reproduced in two separate experiments.



**Figure 3.** Inflammatory cell composition of BAL fluid taken from either sham- or OVA-immunized mice 72 h after LCMV peptide aerosol challenge. (A) OVA- and sham-immunized LCMV-specific TCR transgenic mice or (B) OVA-immunized nontransgenic C57BL/6 mice. Cell number is shown as the mean  $\pm$  SEM of  $n = 6-8$  animals and expressed as cells/ml  $\times 10^3$ .

associated with a pathophysiological outcome, i.e., eosinophil accumulation.

The detection of significant levels of IgE in the serum of



**Figure 4.** Cytokine production by LCMV-specific CD8 T cells activated in the presence or absence of IL-4. FACS<sup>®</sup>-sorted LCMV peptide-specific CD8<sup>+</sup> T cells were stimulated with the LCMV peptide in cultures containing IL-2 in the absence or presence of IL-4 (500 U/ml). Cultured cells were washed and restimulated with plate-bound anti-CD3 antibodies and cytokine production was measured in the supernatants after 48 h. Data are presented as the mean  $\pm$  SEM of three separate cultures and cytokine production expressed as units/ml.

OVA-immunized mice indicated that IL-4 production was induced by the immunization (data not shown) which could possibly influence the CD8<sup>+</sup> T cells. Our *in vitro* experiments demonstrated that IL-4 combined with viral peptide stimulation could switch the cytokine profile of CD8<sup>+</sup> T cells from IFN- $\gamma$  production to IL-5 production. Such an IL-4-dependent switch to a TH2 phenotype has already been reported to operate with naive CD4<sup>+</sup> T cells (15).

In asthmatic individuals, the local TH2 environment exists in the lung which could switch any virus-specific CD8<sup>+</sup> T cell response to IL-5 production (3). Not only would the impaired secretion of IFN- $\gamma$  result in delayed clearance of the virus from the respiratory tract, but virus-induced production of IL-5 by lung CD8<sup>+</sup> T cells would exacerbate the symptoms of asthma by inducing eosinophil infiltration into the airways (16, 17). Subsequent eosinophil activation and release of eosinophil-derived cationic proteins, such as major basic protein would result in a cascade of events leading to the development of airway hyperresponsiveness, a characteristic feature of bronchial asthma (18).

The implications of these results may not be confined to viral infections in asthmatics. In any infectious disease situation where a cytolytic, IFN- $\gamma$ -producing CD8<sup>+</sup> T cell response is required for protection, the switch of the CD8<sup>+</sup> T cells to a TH2 phenotype could leave the host immunocompromised.

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## References

1. Busse, W.W., and C.E. Reed. Asthma: Definition and pathogenesis. 1989. CV Mosby, St. Louis. 969–998.
2. Coffman, R.L., B.W.P. Seymour, S. Hudak, J. Jackson, and D. Rennick. 1989. Antibody to interleukin-5 inhibits helminth induced eosinophilia in mice. *Science (Wash. DC)*. 245: 308–310.
3. Robinson, D.S., Q. Hamid, A. Ying, J. Tsicopoulos, A.M. Barkens, C. Bentley, S.R. Durham, and A.B. Kay. 1992. Predominant Th2 like bronchoalveolar T lymphocyte population in bronchial asthma. *N. Engl. J. Med.* 326:298–300.
4. Beasley, R., E.D. Coleman, Y. Hermon, P.E. Hoist, and T.V. O'Donnel. 1988. Viral respiratory tract infections and acute exacerbation of asthma in adult patients. *Thorax*. 43:679–683.
5. Johnston, S.L., P. Pattermore, S. Smith, G. Sanderson, L. Josephs, P. Bardin, D. Tyrrell, and S. Holgate. 1991. The association of viral infections with longitudinal changes in respiratory symptoms and/or peak flow recordings in school children. *Eur. Respir. J.* 5:109.
6. Fong, T.A., and T.R. Mosmann. 1990. Alloreactive murine T cell clones secrete the Th1 pattern of cytokines. *J. Immunol.* 144:1744–1752.
7. McMEnamin, C., and P.G. Holt. 1993. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8<sup>+</sup> T cell-mediated but MHC class II-restricted CD4<sup>+</sup> T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J. Exp. Med.* 178:889–899.
8. Renz, H., G. Lack, J. Saloga, R. Schwinzer, K. Bradley, J. Loader, A. Lupfer, G.L. Larsen, and E.W. Gelfand. 1994. Inhibition of IgE production and normalisation of airways responsiveness by sensitised CD8 T cells in a mouse model of allergen induced sensitisation. *J. Immunol.* 152:351–360.
9. Erard, F., M.-T. Wild, J.A. Garcia-Sanz, and G. Le Gros. 1993. CD8<sup>+</sup> T lymphocytes can develop into non-cytolytic CD8<sup>-</sup> CD4<sup>-</sup> cells which produce IL-4, IL-5, IL-10, and help B cells. *Science (Wash. DC)*. 260:1802–1805.
10. Pircher, H., K. Burki, R. Lang, H. Hengartner, and R.M. Zinkernagel. 1989. Tolerance induction in double specific T cell receptor transgenic mice varies with antigen. *Nature (Lond.)*. 342:559–561.
11. Kyburz, D., P. Aichele, D.E. Speiser, H. Hengartner, R.M. Zinkernagel, and H. Pircher. 1993. T cell immunity after viral infection versus T cell tolerance induced by soluble viral peptides. *Eur. J. Immunol.* 23:1956–1962.
12. Kopf, M., G. Le Gros, M. Bachmann, M.C. Lamers, H. Bluethmann, and G. Kohler. 1993. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature (Lond.)*. 362: 245–247.
13. Nakajima, H., I. Iwamoto, S. Tomoe, R. Matsumura, H. Tomioka, K. Takatsu, and S. Yoshida. 1992. CD4<sup>+</sup> T lymphocytes and interleukin-5 mediate antigen induced eosinophil infiltration into the mouse trachea. *Am. Rev. Respir. Dis.* 146:374–377.
14. Calhoun, W.J., H.E. Reed, C.A. Stevens, and W.W. Busse. 1991. Experimental rhinovirus 16 infection potentiates airway inflammation only in allergic subjects. *Am. Rev. Respir. Dis.* 143:A47.
15. Le Gros, G., S.Z. Ben-Sasson, R. Seder, F.D. Finkelman, and W.E. Paul. 1990. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4 producing cells. *J. Exp. Med.* 172:921–929.
16. Hamid, Q., M. Azzawi, S. Ying, R. Moqbel, A.J. Wardlaw, C.J. Corrigan, B. Bradley, S.R. Durham, J.V. Collins, P.K. Jeffrey, et al. 1992. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J. Clin. Invest.* 87: 1541–1546.
17. Alwan, W.H., W.J. Kozlowska, and P.J.M. Openshaw. 1994. Distinct types of lung disease caused by functional subsets of antiviral T cells. *J. Exp. Med.* 179:81–89.
18. Coyle, A.J., S.J. Ackerman, and C.G. Irvin. 1993. Cationic proteins induce airway hyperresponsiveness dependent on charge interactions. *Am. Rev. Respir. Dis.* 147:896–900.