

Active Vaccination Against IL-5 Bypasses Immunological Tolerance and Ameliorates Experimental Asthma¹

Marc Hertz,^{2*} Surendran Mahalingam,^{2†} Iben Dalum,^{2*} Steen Klysner,^{*} Joerg Mattes,[†] Anne Neisig,^{*} Søren Mouritsen,^{*} Paul S. Foster,^{3†} and Anand Gautam^{3*}

Current therapeutic approaches to asthma have had limited impact on the clinical management and resolution of this disorder. By using a novel vaccine strategy targeting the inflammatory cytokine IL-5, we have ameliorated hallmark features of asthma in mouse models. Delivery of a DNA vaccine encoding murine IL-5 modified to contain a promiscuous foreign Th epitope bypasses B cell tolerance to IL-5 and induces neutralizing polyclonal anti-IL-5 Abs. Active vaccination against IL-5 reduces airways inflammation and prevents the development of eosinophilia, both hallmark features of asthma in animal models and humans. The reduced numbers of inflammatory T cells and eosinophils in the lung also result in a marked reduction of Th2 cytokine levels. Th-modified IL-5 DNA vaccination reduces the expression of IL-5 and IL-4 by ~50% in the airways of allergen-challenged mice. Most importantly, Th-modified IL-5 DNA vaccination restores normal bronchial hyperresponsiveness to β -methacholine. Active vaccination against IL-5 reduces key pathological events associated with asthma, such as Th2 cytokine production, airways inflammation, and hyperresponsiveness, and thus represents a novel therapeutic approach for the treatment of asthma and other allergic conditions. *The Journal of Immunology*, 2001, 167: 3792–33799.

Interleukin-5 is a proinflammatory cytokine expressed at high levels in asthmatics. Asthma is clinically characterized by episodic airflow obstruction, inflammation of the airways, and enhanced bronchial reactivity to nonspecific spasmogens. The levels of airways obstruction and hyperreactivity (AHR)⁴ often correlate with the degree of airways inflammation, and these clinical features are indicative of asthma severity (1–7). Clinical correlates between the degree of cellular infiltration and disease progression have identified inflammation of the airways as the major contributing factor to pathogenesis and pathobiology. The inflammatory infiltrate in asthma is complex; however, it is now widely recognized that CD4⁺ Th lymphocytes with a Th2 profile (Th2 cells) of cytokine expression play a pivotal role in the clinical expression and pathogenesis of this disorder (8, 9). Th2 cells regulate disease progression and AHR by orchestrating allergic inflammation of the airways through the release of a range of cytokines (IL-4, -5, -9, -10, -13) (10–13). Like Th2 cells, the levels of eosinophils and their inflammatory products in the lung correlate

with disease severity, and accumulation of this leukocyte in the airways is a central feature of bronchial dysfunction during the late-phase asthmatic response (14). Although Th2 cells orchestrate many facets of the allergic response, their role in regulating eosinophilia through the secretion of IL-5 is thought to be a major proinflammatory pathway in asthma.

The central role of IL-5 in regulating eosinophil function (differentiation, expansion, mobilization, and activation) has identified this cytokine as a primary target for therapeutic intervention in asthma. Indeed, the importance of IL-5 in regulating eosinophilia and potentially asthma pathogenesis has been demonstrated in experimental systems that have used animal models of asthma in conjunction with IL-5-deficient mice and inhibitory mAbs (15–17). These studies have highlighted the need to develop advanced methodologies that target IL-5 function for the resolution of both inflammation and AHR in asthma.

The aim of the current investigation was to induce a therapeutic immune response directed against self-IL-5. Although anti-IL-5 mAbs are being used in clinical trials in Ag challenge studies, this cytokine has never been used as a target for active vaccination. Here we describe a novel therapeutic DNA vaccine approach for the treatment of allergy and asthma. Our strategy was to use active DNA vaccination against IL-5 to elicit polyclonal Abs that would neutralize IL-5 produced during recall responses to inhaled allergen and ameliorate disease. This therapeutic approach has the potential advantage not only of providing protection during asthma exacerbation, but also as a longitudinal anti-inflammatory therapy. We have shown previously that by incorporating a strong Th cell epitope within a self-protein, immunological tolerance against self-proteins can be bypassed (18–20). In the current investigation, we extended these studies by using a DNA vaccine encoding murine IL-5 modified to contain a promiscuous foreign Th epitope to break or bypass immunological tolerance to IL-5. In mice, Th-modified IL-5 DNA vaccination induced an immune response directed against native IL-5. Moreover, in our established models of experimental asthma, Th-modified IL-5-vaccinated mice induced an immune response directed against IL-5 that reduced both

*Pharmexa A/S, Hørsholm, Denmark; and [†]Division of Biochemistry and Molecular Biology, John Curtin School of Medical Research, Australian National University, Canberra, Australia

Received for publication April 10, 2001. Accepted for publication August 1, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported in part by the Human Frontiers Science program (to P.S.F.). S.M. is a National Health and Medical Research Council Peter Doherty Postdoctoral Fellow. J.M. is supported by the German Research Association (MA2241/1-1).

² M.H., S.M., and I.D. contributed equally to this work.

³ Address correspondence and reprint requests to Dr. Anand Gautam, Pharmexa A/S, DK-2970 Hørsholm, Denmark. E-mail address: ag@pharmexa.com; or Dr. Paul S. Foster, Leukocyte Signaling and Regulation Laboratory, John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory 0200, Australia. E-mail address: paul.foster@anu.edu.au

⁴ Abbreviations used in this paper: AHR, airways hyperreactivity; BALF, bronchoalveolar lavage fluid; HPRT, hypoxanthine-guanine phosphoribosyltransferase; mIL, mouse IL; Penh, enhanced pause; wt, wild type; PBLN, peribronchial lymph node.

pulmonary lymphocyte infiltration and eosinophilia, reduced the levels of Th2 cytokines, and inhibited the development of AHR. These data substantiate active DNA vaccination against IL-5 as a novel therapeutic approach for the treatment of asthma and potentially other allergic diseases. In addition, our vaccine approach is a platform technology that can be applied to most self-proteins that are aberrantly or overexpressed during chronic disease.

Materials and Methods

Mice

Male C3H/Hen (H-2^b) or C3H/HeJ (H-2^k) mice (6–8 wk old) were obtained from M&B A/S (Ry, Denmark), or the specific-pathogen free facility at the John Curtin School of Medical Research, Australian National University. Experiments were performed according to the Danish Animal Experiments Inspectorate and John Curtin School of Medical Research institutional guidelines for animal care and use.

Modified IL-5 plasmid construction and DNA preparation

Murine IL-5 cDNA (R&D Systems, Minneapolis, MN) was cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA) mammalian expression vector containing the mIL-5 signal peptide and a consensus Kozak sequence (21). The P30 tetanus toxoid (22) helper epitope (FNNFTVSVFWRVVKVSAHLE) was inserted into the first loop region of mIL-5, replacing aa R30 to L41, using sequence overlap extension PCR (sense primer, CTTCTGGCTGCGCGTGCCCAAGGTGAGCGCCAGCCACCTGGAGTGCATTGGAGAGATCTTTCAGGGGC; antisense primer, GCGCTCACCTTGGGCGCAGCCAGCAAGCTCACGGTGAAGTTGTTGAACATCGTCTCATTTGCTTGCAACAGAGC) and standard molecular biology techniques. Plasmid DNA was prepared using endotoxin-free purification kits (Qiagen, Valencia, CA) and was resuspended in sterile 0.9% saline.

DNA vaccination

Mice were anesthetized, and the lower back of each animal was shaved. Vaccination with mIL-5.wt (encodes the wild-type mouse IL-5 cDNA) or mIL-5.2 (encodes the mIL-5.2 Th-modified construct) was then performed by intradermal injections of 100 μ l of DNA solution (1 μ g/ μ l) into each mouse divided into two injections over the lower back. DNA injections were performed six or seven times at 2-wk intervals. At the end of the sixth vaccination, mice were sensitized to OVA to prime for subsequent induction of allergic airway inflammation (described below). Vaccinations were continued (one injection of DNA every 2 wk) during the period of induction of allergic disease of the lung.

ELISA

Anti-mIL-5 titers in sera were determined by direct ELISA. Briefly, pooled sera from vaccinated mice were titrated into 96-well flat-bottom plates (Maxisorp; Nunc, Taastrup, Denmark) precoated with mIL-5 (100 ng/well; R&D Systems). Sera were detected with goat anti-mouse-HRP polyclonal Ab (DAKO, Glostrup, Denmark). Competition ELISAs were performed by adding diluted antisera preincubated with mIL-5 for 1 h to 96-well plates coated with anti-mIL-5 mAb (TRFK5.1 μ g/well; R&D Systems). Inhibition by the antisera was detected by adding biotinylated TRFK4 (R&D Systems) and streptavidin-HRP (Amersham, Hørsholm, Denmark). Serum OVA-specific IgE was detected by isotype-specific ELISA using rat anti-mouse IgE mAb, clone LO-ME-3 (BioSource International, Camarillo, CA). OVA-specific IgE was quantitated against standard mouse IgE.

Induction of allergic airway inflammation

Intranasal model. Mice were sensitized by s.c. injection of 50 μ g of OVA in 0.9% sterile saline mixed 1/1 (v/v) with Adju-Phos (Superfos Biosector, Vedbaek, Denmark) weekly over 3 wk. Four days after the last sensitization the mice were challenged intranasally with 12.5 μ g of OVA in 0.9% sterile saline once a day, every other day, for a total of three challenges. Bronchoalveolar lavage fluid (BALF) was collected 1 day following the last challenge.

Aeroallergen models: sensitization regimen. Mice were sensitized by i.p. injection with 50 μ g of OVA/1 mg of Alhydrogel (CSL, Parkville, Australia) in 0.9% sterile saline. Nonsensitized mice received 1 mg of Alhydrogel in 0.9% saline. On days 12, 14, 16, and 18, all groups of mice were aeroallergen challenged with OVA as previously described (15, 23). Blood was collected on days 13, 15, 17, and 19. Twenty-four hours after the last aeroallergen challenge AHR was measured, and then BALF and lung tissue were collected for analysis of inflammatory infiltrates.

Generation and transfer of Th2 cells and induction of allergic disease of the lung

T cell donor mice (male C3H/HeJ, 6–8 wk of age) were sensitized by i.p. injection of 50 μ g of OVA/1 mg of Alhydrogel in 0.9% sterile saline to prime for CD4⁺ Th2-like cells. Six days following sensitization donor mice were sacrificed, and their spleens were excised. Splenocytes were then disaggregated, contaminating RBC were lysed, and subsequently washed splenocytes were resuspended at 5×10^6 cells/ml in RPMI 1640 culture medium. These cells were then cultured for 4 days at 37°C in the presence of 200 μ g/ml OVA, 20 ng/ml murine IL-4, and 40 μ g/ml anti-IFN- γ (R46A2). CD4⁺ T cells were isolated from cultures using high gradient magnetic MiniMACS separation column (MACS separation) as described previously (23), washed, and resuspended in PBS. CD4⁺ T cells (2×10^6 cells) were adoptively transferred to DNA-vaccinated or unvaccinated naive C3H/HeJ mice or were stimulated in vitro with mitomycin-treated APCs in the presence of OVA to determine Th2 cytokine profiles. Twelve and 36 h later, recipients were exposed to an aerosol of OVA (10 mg/ml) in 0.9% saline twice for 30 min each time (30-min interval between exposures). AHR to β -methacholine was determined, and blood, BALF, and lung tissue were collected for the analysis of inflammatory infiltrates 24 h after the last aerosol.

Characterization of lung morphology and leukocytes in blood, tissue, and BALF

Lung tissue representing the central (bronchi-bronchiole) and peripheral (alveoli) airways was fixed in 10% phosphate-buffered Formalin, sectioned, and stained with Alcian blue-periodic acid-Schiff for the enumeration of mucin-secreting cells or Carbol's chromotrope-hematoxylin for the identification of eosinophils. Eosinophils in blood, BALF, and lung were identified by morphological criteria and quantified as previously described (15, 23).

Measurement of AHR

Responsiveness to β -methacholine was assessed in conscious unrestrained mice by barometric plethysmography using apparatus and software supplied by Buxco (Troy, NY). This system yields a dimensionless parameter known as enhanced pause (Penh), reflecting changes in waveform of the pressure signal from the plethysmography chamber combined with a timing comparison of early and late expirations. Measurement of Penh was performed essentially as previously described (24, 25). Briefly, mice were placed in the plethysmograph chamber and exposed to an aerosol of water (baseline readings) and then to cumulative concentrations of β -methacholine ranging from 3 to 50 mg/ml. The aerosol was generated by an ultrasonic nebulizer and drawn through the chamber for 2 min. The inlet was then closed, and Penh readings were taken for 3 min and averaged. Values were reported as the percent increase over baseline.

Measurement of cytokine production by peribronchial lymph nodes

Cells from the peribronchial lymph nodes were isolated and stimulated with 1 mg/ml OVA in MLC medium for 72 h as described previously (25). The concentrations of IL-4, IL-5, and IL-13 in the cell-free supernatants were measured with ELISA (25). The sensitivity of detection was 0.5 ng/ml for IL-5 and IL-13 and 0.1 ng/ml for IL-4.

RT-PCR analysis

Total RNA was isolated from lungs by standard methods with RNAzol B (Biotech Laboratories, Houston, TX). A RT-PCR procedure was performed as previously described (26) to determine relative quantities of mRNA for various cytokines. The primers and probes for all genes were purchased from Life Technologies (Gaithersburg, MD). Primer and probe sequences for hypoxanthine-guanine phosphoribosyl transferase (HPRT) have been described previously (26). Primer and probe sequences for IL-4, IL-5, IL-10, IL-13, IFN- γ , and HPRT are as follows: IL-4: sense, GAATGT ACCAGGAGCCATATC; antisense, CTCAGTACTACGAGTAATCCA; probe, AGGGCTTCCAAGGTGCTTCGCA; IL-5: sense, GACAAGCA ATGAGACACGATGAGG; antisense, GAACTTGCAGGTAATCCA GG; probe, GGGGGTACTGTGGAATGCTTAT; IL-10: sense, CGG GAAGACAATAACTG; antisense, CATTTCGATAAGGCTTGG; probe, GGACTGCCTTCAGCCAGGTGAAGACTTT; IL-13: sense, CTC CCTCTGACCCCTTAAGGAG; antisense, GAAGGGGCCGTGGCGA AACAG; probe, TCCAATTGCAATGCCATCTAC; and IFN- γ : sense, AACGCTACACACTGCATCTTGG; antisense, GACTCAAAGAGTCT GAGG; probe, GGAGGAACTGGCAAAGGA. The cycle numbers used for amplification of each gene product are: IL-10, 27 cycles; IL-13 and

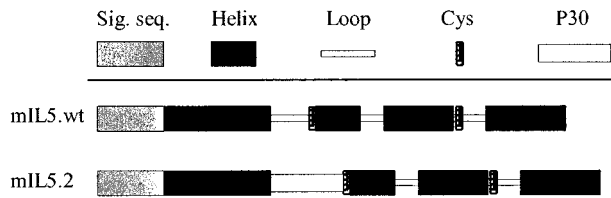


FIGURE 1. Murine Th-modified IL-5 DNA construct design. Murine IL-5 cDNA was cloned into pcDNA3.1 mammalian expression vector (Invitrogen, San Diego, CA) containing the mIL-5 signal peptide. In mIL-5.2, the P30 promiscuous Th epitope (22) was inserted into the first loop region of IL-5.

IFN- γ , 30 cycles; IL-4 and IL-5, 28 cycles; and HPRT, 23 cycles. After the appropriate number of PCR cycles, the amplified DNA was analyzed by gel electrophoresis and Southern blotting and was detected using the ECL detection system as recommended by the manufacturer (Amersham, Arlington Heights, IL). PCR amplification with the HPRT reference gene was performed to assess variations in cDNA or total RNA loading between samples. Mean relative transcript levels per group were determined from cDNA panels as previously described (27). Briefly, values were derived by dividing the mean of the triplicate values measured for the transcript of interest by the mean of triplicate HPRT values for the sample.

Statistical analysis

The significance of differences between experimental groups was analyzed using Student's unpaired *t* test. Values were reported as the mean \pm SEM. Differences in means were considered significant at $p < 0.05$.

Results

Murine Th-modified IL-5 DNA vaccines can bypass B cell tolerance

A DNA vaccine encoding murine IL-5 modified to contain a promiscuous foreign Th epitope, P30 from the tetanus toxoid (22) (Th-modified mIL-5.2), was designed and tested for its ability to induce a polyclonal Ab response that was cross-reactive with non-modified murine IL-5 (mIL-5.wt; see Fig. 1). Before vaccination, the constructs were tested in COS cell transient transfections to ensure that the encoded proteins were appropriately expressed. The

nonmodified wild-type murine IL-5 (mIL-5.wt) and the murine Th-modified IL-5 (mIL-5.2) constructs were both capable of being transiently expressed, as detected by Western blotting (data not shown).

The Th-modified IL-5 construct capable of expressing protein in COS cell transient transfection was further tested for immunogenicity by DNA vaccinations in mice. C3H mice were initially immunized intradermally with 100 μ g of DNA in 0.9% saline and subsequently boosted (100 μ g) six times at 2-wk intervals. Although the mIL-5.wt construct was unable to induce an anti-mIL-5 humoral immune response (0 of 40 mice tested), the mIL-5.2 Th-modified construct bypassed B cell immune tolerance and induced polyclonal Abs that were cross-reactive with native murine IL-5 (30 of 30 mice tested; Fig. 2A). Anti-mIL-5 (and anti-P30) Abs could be detected after the third immunization, and 100% of the mice had seroconverted after the fourth mIL-5.2 DNA immunization (data not shown). Antisera from mice vaccinated with mIL-5.2 DNA were tested for its ability to compete for the binding of native murine IL-5 by using a competition ELISA (Fig. 2B). Antisera from vaccinated mice were preincubated with mIL-5 and were capable of competing with an anti-mIL-5 mAb (TRFK4) for the binding of mIL-5 protein. Thus, polyclonal Abs from mIL-5.2-vaccinated mice efficiently blocked the interaction of neutralizing anti-murine IL-5 mAbs TRFK4/TRFK5 with the native ligand. Together these data suggest that the polyclonal Abs induced via mIL-5.2 DNA vaccination recognize native murine IL-5.

Eosinophilia and pulmonary lymphocyte inflammation are inhibited in mice vaccinated with Th-modified IL-5 DNA

The mIL-5.2 DNA vaccine was further characterized in three separate mouse models of allergic airways inflammation that mimic key pathological events characteristic of asthma (15, 28). The first model was a simple intranasal OVA-allergen model (intranasal model) that induces eosinophilia in the lungs of mice. This model allowed us to rapidly assess and validate the concept that Th-modified mIL-5.2 DNA vaccination could induce Abs that cross-react with native mIL-5 and reduce eosinophilia in the BALF of a large number of allergic mice. An OVA aeroallergen sensitization model

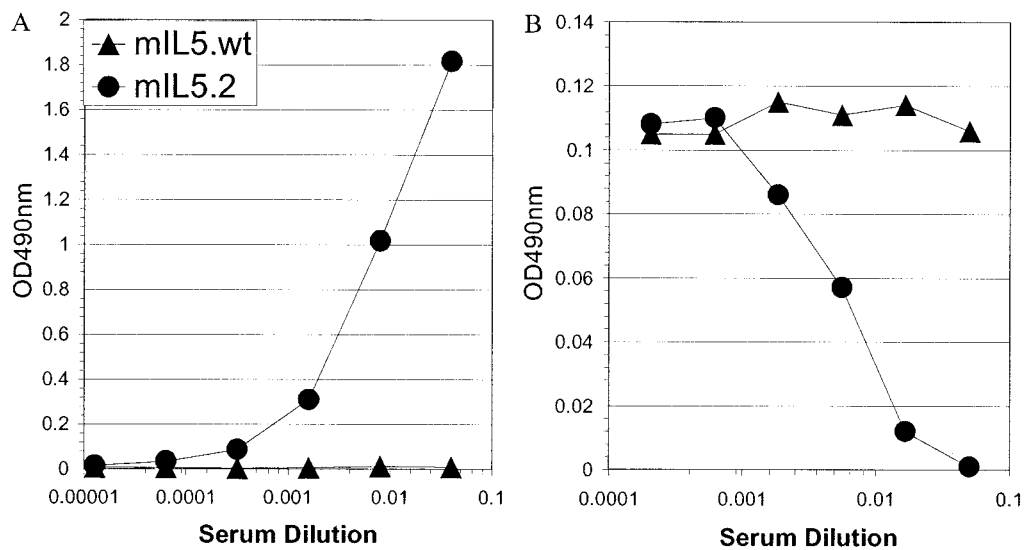


FIGURE 2. Th-modified IL-5 DNA vaccination induces polyclonal Abs that cross-react with native mIL-5. *A*, Anti-mIL-5 serum ELISA with pooled sera from mice vaccinated and boosted five times with 100 μ g of endotoxin-free mIL-5.wt (\blacktriangle) or mIL-5.2 Th-modified (\bullet) DNA in 0.9% sterile saline. Abs that cross-react with native murine IL-5 can be detected after the third DNA vaccination, and by the fourth DNA vaccination 100% of the mice have seroconverted. *B*, Competition ELISA of TRFK4/5 (R&D Systems) anti-mIL-5 mAbs and pooled sera from mIL-5.wt (\blacktriangle) and mIL-5.2 (\bullet) DNA vaccinated mice preincubated with mIL-5.

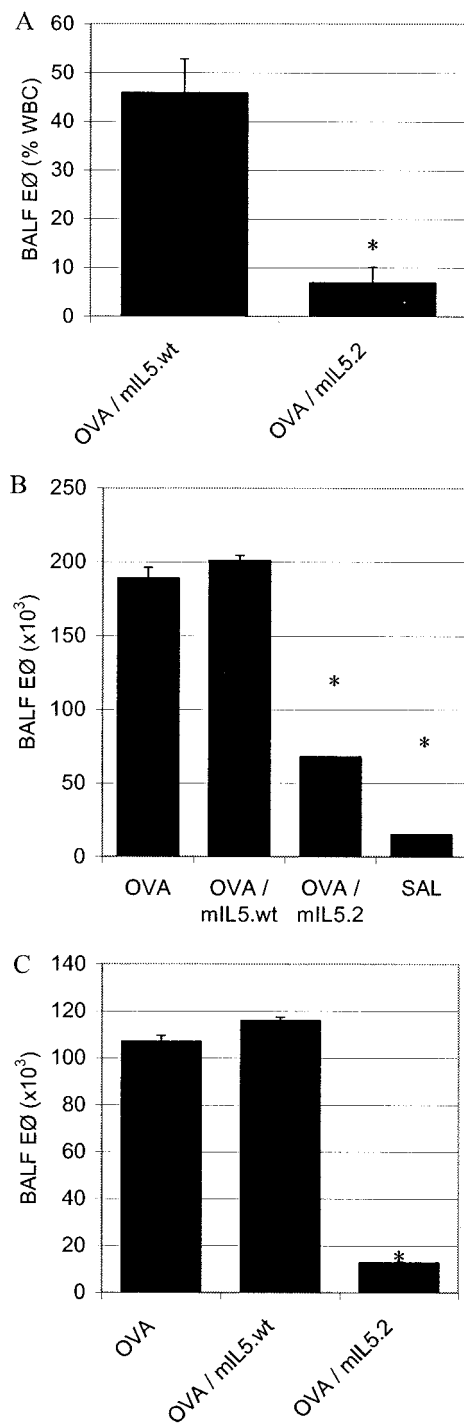


FIGURE 3. BALF eosinophilia is reduced in Th-modified IL-5 DNA vaccinated mice. *A*, OVA intranasal regimen; *B*, sensitization regimen; *C*, T cell transfer/OVA aerosol regimen. The frequency (*A*; mean \pm SEM of 19 (mIL-5.wt) or 30 (mIL-5.2) mice) and total number (*B* and *C*; mean \pm SEM of six mice) of eosinophils recovered from BALF of the airway lumen was determined 24 h after the last aeroallergen challenge. *, $p < 0.000001$ (*A*), $p < 0.05$ (*B*), and $p < 0.005$ (*C*) for OVA-sensitized mIL-5.wt-vaccinated mice compared with OVA-sensitized mIL-5.2-vaccinated mice.

(OVA sensitization model) was then later used to look at the AHR response in vaccinated mice. Thirdly, an adoptive transfer model (transfer model) of allergen-specific Th2 CD4⁺ T cells was used to address the ability of the vaccines to reduce disease symptoms in a model in which sensitization to the aeroallergen was performed in an environment free of the effects of the anti-mIL-5 immune

response. The number and frequency of eosinophils in BALF, blood, and lung tissue were assessed in all three models of OVA-induced allergic airways inflammation. In the intranasal model, mice were vaccinated six times with DNA and sensitized to OVA s.c. four times at weekly intervals. One week after the last sensitization, the mice were challenged with OVA intranasally three times over a 6-day period. One day later, BALF was collected for eosinophil counts. As shown in Fig. 3*A*, eosinophilia in BALF was dramatically reduced in mice vaccinated with Th-modified mIL-5.2 ($n = 30$ mice) compared with mIL-5.wt ($n = 19$ mice). Next, the effect of mIL-5.2 DNA vaccination on eosinophilia and the subsequent induction of AHR was determined in the OVA sensitization and transfer models of experimental asthma that employed OVA sensitization or adoptive transfer of Ag-specific CD4⁺ T cells (that secrete Th2 cytokines such as IL-5 and IL-4) to naive mice before allergen provocation of the lung. In the CD4⁺ T cell adoptive transfer model, the Th2 T cells are sensitized to allergen (OVA) in an environment free of neutralizing mIL-5 Abs, thus ruling out a failure to induce the model due to the vaccinations. Eosinophil recruitment into the BALF was inhibited in both aeroallergen models in response to inhaled allergen after mIL-5.2 DNA vaccination (Fig. 3, *B* and *C*, and Table I). Notably, blood eosinophilia was completely attenuated after mIL-5.2 DNA vaccination (Fig. 4, *A* and *B*). Moreover, active DNA vaccination with mIL-5.2 in the transfer model ameliorated tissue eosinophilia in the lungs of all mice (Fig. 4*C*). The inability of mIL-5.2 DNA-vaccinated mice to mount blood eosinophilia directly correlated with the inability of the bone marrow pool of eosinophils to expand in response to allergen provocation of the lung (results not shown). Lymphocyte numbers recruited to the lungs in the OVA sensitization model were also reduced in mIL-5.2 DNA-vaccinated mice. On day 19 after aerosol challenge, there were significantly reduced lymphocyte numbers in the BALF of mIL-5.2 DNA-vaccinated mice compared with wild-type mIL-5 DNA-vaccinated or nonvaccinated controls (Table I). Thus, neutralizing IL-5 via DNA vaccination effectively inhibits the recruitment of inflammatory cells to the sites of allergic inflammation by acting systemically.

Th-modified IL-5 DNA vaccination restores normal airways reactivity in OVA-sensitized mice

To determine the ability of mIL-5.2 DNA vaccination to reduce AHR to cholinergic stimuli, lung reactivity to β -methacholine was measured in the OVA sensitization and T cell transfer models 24 h after the last aeroallergen challenge (Fig. 5). Mice vaccinated

Table I. Reduced numbers of inflammatory T cells and eosinophils in the BALF of allergen-challenged mice after Th-modified IL-5 DNA vaccination

	Day 19 BALF ^a	
	T lymphocytes (cells/ml $\times 10^3$)	Eosinophils (cells/ml $\times 10^3$)
Saline	19 \pm 12 ^b	15 \pm 6
OVA	221 \pm 43	189 \pm 27
OVA/mIL5.wt	230 \pm 37	201 \pm 35
OVA/mIL5.2	110 \pm 26*	68 \pm 19**

^a BALF was collected on day 19, one day after the final OVA-aeroallergen challenge as described in *Materials and Methods*, and infiltrating cells were identified morphologically.

^b Mean cell numbers are shown (\pm SEM).

*, T lymphocytes $p < 0.005$ and **, eosinophils $p < 0.05$ for OVA-sensitized mIL-5.2-vaccinated mice when compared to OVA-sensitized mIL-5.wt-vaccinated mice.

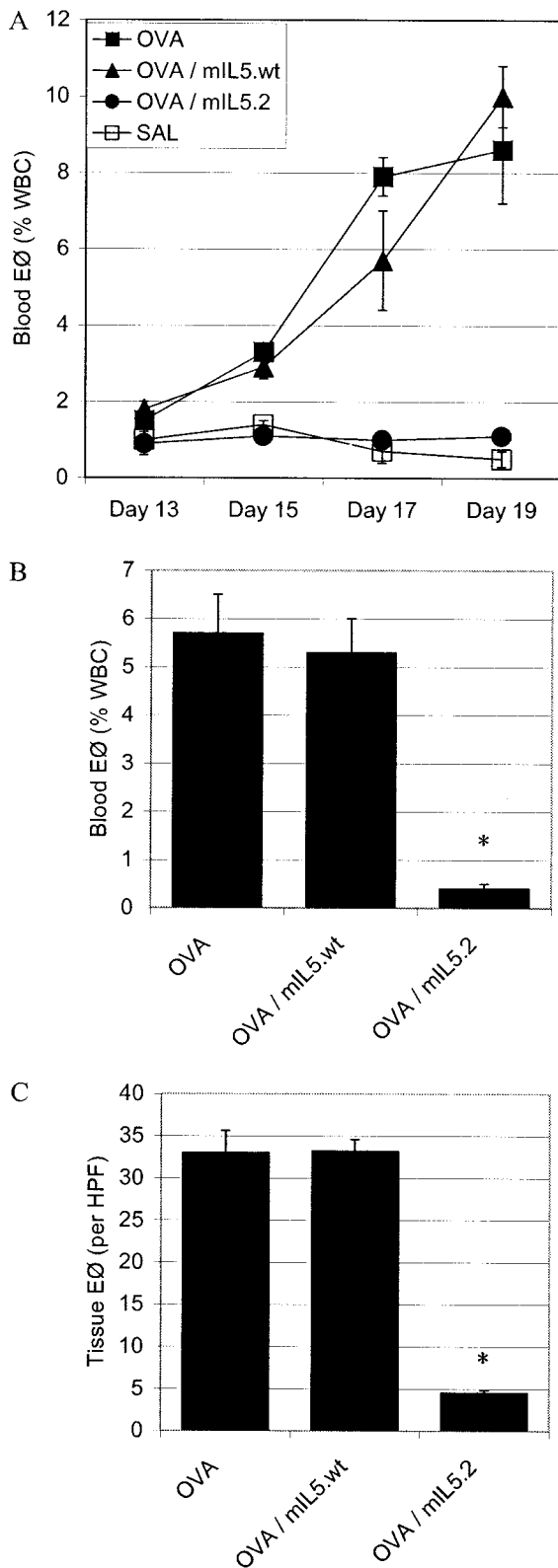


FIGURE 4. Blood and lung tissue eosinophilia are reduced in Th-modified IL-5 DNA-vaccinated mice. *A*, The percentages of eosinophils in the peripheral blood on various days using the sensitization regimen. *B*, The percentages of eosinophils in the peripheral blood 24 h after the last aeroallergen challenge in the T cell transfer/OVA aerosol regimen. *C*, The mean number of lung peribronchial/perivascular eosinophils per 10 similar high-powered fields (HPF; $\times 1000$ magnification) for each group 24 h after the last aeroallergen challenge in the Th2 cells transfer/OVA aerosol regimen. *, $p < 0.005$ for OVA-sensitized mL5.wt vaccinated mice compared with OVA-sensitized mL5.2-vaccinated mice (mean \pm SEM of six mice).

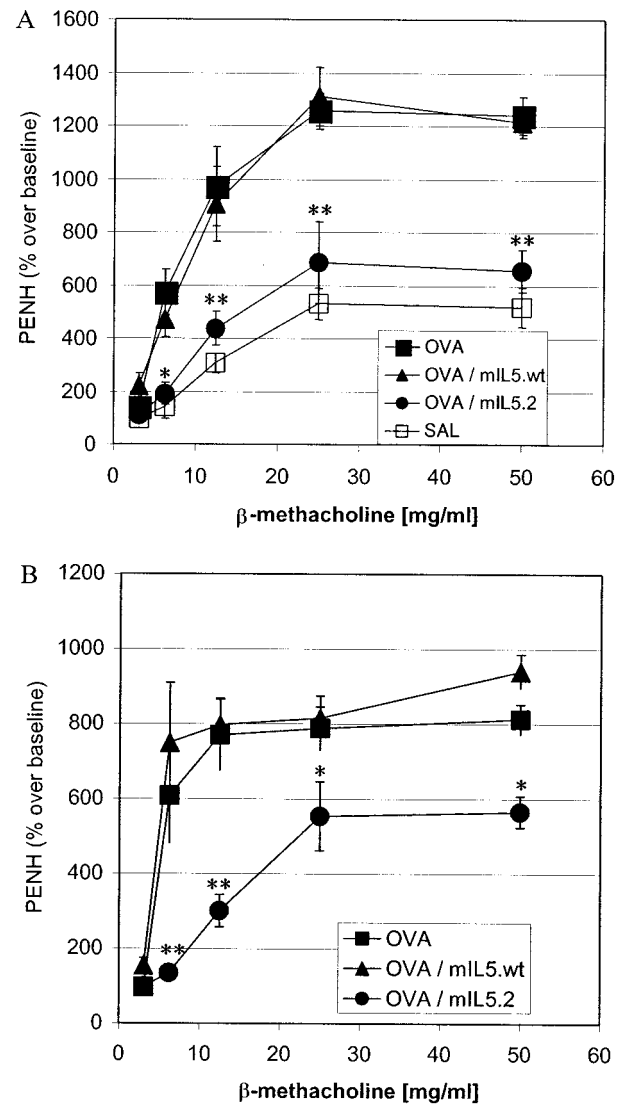


FIGURE 5. Reduced AHR to cholinergic stimuli in Th-modified IL-5 DNA-vaccinated mice. *A*, Sensitization regimen; *B*, CD4⁺ T cell transfer/OVA aerosol regimen. Reactivity to β -methacholine was measured by barometric plethysmography, and the data (mean of seven mice \pm SEM) represent the percent increase in Penh over baseline reactivity in the absence of cholinergic stimuli. Heightened reactivity was seen at all concentrations of methacholine by OVA-sensitized mL5.wt-vaccinated mice compared with that in OVA-sensitized mL5.2-vaccinated mice (*, $p < 0.05$; **, $p < 0.005$).

with mL5.wt DNA developed AHR similar to nonvaccinated OVA-sensitized controls (Fig. 5A) and naive mice that received CD4⁺ T cells (Fig. 5B) after aeroallergen challenge. By contrast, in the OVA sensitization model AHR in mice vaccinated with the mL5.2 DNA was reduced to a level similar to that in nonsensitized saline-treated control mice (Fig. 5A). Importantly, we also demonstrated that mL5.2 DNA was effective at inhibiting T cell-regulated AHR. The level of airways reactivity to β -methacholine in the T cell transfer model after mL5.2 DNA vaccination (Fig. 5B) was similar to that observed in saline-treated controls (Fig. 5A). Although there is conflicting evidence regarding the role of IL-5 in the development of AHR (15, 29), we show here that mL5.2 DNA vaccination can inhibit AHR in both OVA sensitization and T cell transfer models of experimental asthma.

Production of Th2 cytokines is reduced after DNA vaccination with Th-modified IL-5

To further characterize the mechanism of the mIL-5.2 DNA vaccine-mediated inhibition of eosinophilia and AHR, we measured the production of various cytokines in the lungs and by OVA-stimulated peribronchial lymph node (PBLN) cells. No significant differences between naive nontreated controls and mIL-5.wt DNA-vaccinated mice were seen in the production of IL-4, IL-5, IL-10, or IL-13 at the level of protein or message production. By contrast, mIL-5.2 DNA-vaccinated mice had significant reductions in IL-5 protein (40–60%) and message levels (Tables II and III). Surprisingly, IL-4 and IL-10 production were also reduced at the level of protein and message after mIL-5.2 DNA vaccination. No significant reductions were seen in the production of OVA-specific IgE levels or IL-13 (Tables II and III and data not shown). These data suggest that active vaccination against murine IL-5 can reduce not only IL-5 levels, but also other key cytokines involved in the pathogenesis of asthma, presumably by reducing the numbers of inflammatory cells (lymphocytes and eosinophils; see Table I) recruited to the lung that are producing Th2 cytokines.

Discussion

In this investigation, we show that active vaccination against IL-5 is a novel therapeutic approach for the treatment of asthma and potentially other eosinophilic disorders. Asthma as well as many other chronic diseases are associated with the aberrant expression of self-proteins. The expression of IL-5 in the lung is inversely correlated with pulmonary dysfunction in asthmatics, and the level of expression is directly correlated with the number of eosinophils detected in asthmatic airways (8, 10, 11). Animal studies have indicated that neutralizing IL-5 can profoundly attenuate eosinophilia and the subsequent damage caused by these leukocytes in the allergic lung (17). Reducing IL-5 levels can also reduce AHR independently of its role in eosinophilia (30), probably via the effects of IL-5 on airways smooth muscle (31, 32). The Th-modified IL-5 vaccine approach demonstrates the therapeutic potential for immunologically based vaccines directed against pathogenic self-proteins. By incorporating a promiscuous foreign Th epitope into self-proteins, thus providing sufficient T cell help, immunological tolerance to self-proteins can be bypassed (18–20). We show here that a murine Th-modified IL-5 DNA vaccine can induce an immune response that produces polyclonal Abs that are cross-reactive with native murine IL-5. Moreover, this immune

Table III. Reduced production of Th2 cytokines in PBLN cells of Th-modified IL-5 DNA-vaccinated OVA-sensitized mice^a

	OVA ^b		
	IL-4 (ng/ml)	IL-5 (ng/ml)	IL-13 (ng/ml)
Saline	ND	ND	ND
OVA	0.77 ± 0.05 ^c	61 ± 3.3	15.5 ± 2.5
OVA/mIL5.wt	0.71 ± 0.06	54 ± 9.1	16 ± 3
OVA/mIL5.2	0.31 ± 0.03*	26 ± 4.3*	13 ± 0.1

^a Cells from the PBLN were recovered from mice after exposure to an aerosol of OVA.

^b Cells were stimulated with OVA in MLC medium for 72 h, and cytokine ELISAs were performed on supernatants.

^c Mean cytokine production is shown (±SEM; *n* = 4 mice per group). Assays were repeated twice independently in duplicate.

* IL-5 and IL-4, *p* < 0.05 for OVA-sensitized mIL-5.wt-vaccinated mice when compared with OVA-sensitized mIL-5.2-vaccinated mice.

response reduces airways inflammation, AHR and the production of key pathogenic Th2 cytokines in the pulmonary compartments of mice exposed to allergen-induced models of experimental asthma.

Immune responses against self-proteins such as IL-5 are usually not generated due to immunological tolerance. B cell Ag receptors to self-proteins are normally removed from the repertoire to induce tolerance and avoid autoimmunity (33, 34). However, immature B cells undergoing the induction of tolerance are exquisitely sensitive to T cell help, which, if provided, can rescue B cells from the induction of tolerance and promote B cell development (35). Importantly, we demonstrate that vaccination with Th-modified IL-5 DNA provides abundant T cell help that is capable of inducing a B cell response that elicits cross-reactive immunity with native IL-5. Our studies suggest that a portion of B cell tolerance is maintained by the careful regulation of T cell help. Indirectly, tolerance of the CD4 T cell compartment regulates B cell tolerance. Vaccination with DNA encoding Th epitope-modified self-proteins (but not DNA encoding unmodified self-proteins; see Fig. 2) that can provide T cell help in the appropriate context bypasses B cell tolerance.

Analysis of cytokine production in draining lymph nodes revealed a general reduction in Th2-type cytokines. Reduced levels of IL-5, IL-4, and IL-10 were measured in mIL-5.2-vaccinated

Table II. Reduced production and expression of Th2 cytokines in PBLN cells and lungs of Th-modified IL-5 DNA-vaccinated mice that received CD4⁺ T cells^a

	OVA ^b				
	IL-4 (ng/ml)	IL-5 (ng/ml)	IL-10 (ng/ml)	IL-13 (ng/ml)	IFN-γ (ng/ml)
OVA	0.57 ± 0.03 ^c	39 ± 3.0	27 ± 3.0	14 ± 1.7	ND
OVA/mIL5.wt	0.63 ± 0.04	41.2 ± 6.8	25 ± 0.2	13 ± 2.0	ND
OVA/mIL5.2	0.24 ± 0.03*	23.7 ± 2.0*	17 ± 2.0*	11 ± 0.3	ND
	(transcript U/HPRT)	(transcript U/HPRT)	(transcript U/HPRT)	(transcript U/HPRT)	(transcript U/HPRT)
OVA	0.72 ± 0.03 ^d	0.59 ± 0.03	0.56 ± 0.03	0.71 ± 0.03	0.15 ± 0.01
OVA/mIL5.wt	0.74 ± 0.02	0.65 ± 0.01	0.59 ± 0.03	0.78 ± 0.03	0.15 ± 0.01
OVA/mIL5.2	0.22 ± 0.19**	0.27 ± 0.02**	0.37 ± 0.02**	0.75 ± 0.04	0.13 ± 0.02

^a Cells from the PBLN were recovered from mice after transfer of T cells and exposure to an aerosol of OVA.

^b Cells were stimulated with OVA in MLC medium for 72 h, and cytokine ELISAs were performed on supernatants.

^c Mean cytokine production is shown (±SEM; *n* = 4 mice per group). Assays were repeated twice independently in duplicate.

^d Total RNA was isolated from lungs and RT-PCR was performed to determine relative quantities of mRNA for various cytokines. Values were derived by dividing the mean of the triplicate values measured for the transcript of interest by the mean of triplicate HPRT values for the sample (*n* = 4 mice per group).

* IL-5, *p* < 0.05; IL-4, *p* < 0.005; IL-10, *p* < 0.05 for OVA-sensitized mIL-5.wt-vaccinated mice when compared with OVA-sensitized mIL-5.2-vaccinated mice.

** IL-5 and IL-4, *p* < 0.005 for OVA-sensitized mIL-5.wt-vaccinated mice when compared with OVA-sensitized mIL-5.2-vaccinated mice.

mice. Previous studies have shown that administering short non-specific immunostimulatory DNA sequences could inhibit eosinophilia, IL-5, and AHR in similar models of allergen-induced lung disease (36, 37). The proposed mechanisms in those studies suggest that both an immediate production of IL-12 and IFN- γ by the innate immune system and a general shift from a Th2 to a Th1 immune response by the adaptive immune system inhibit the activation of bone marrow-derived eosinophils and the subsequent generation of AHR. Although we cannot rule out a similar mechanism playing some role in the present studies, neither the mIL-5.wt nor the mIL-5.2 DNA vaccine induced a detectable Th1 immune response. IFN- γ levels were consistently low in all study groups (Table II). Our data suggest that our vaccine approach is highly specific for targeting IL-5 and that the OVA-allergen induces a Th2 immune response as seen in the cytokine profile (see Tables II and III) and anti-OVA Ab isotypes (data not shown). DNA encoding wild-type murine IL-5 (or vector-only controls) showed no effect on the general Th2 environment and did induce Th1 cytokine production (Table II) or skew Ab isotypes. In addition, it is unlikely that a general Th1 environment could skew the cytokine production by the adoptively transferred OVA-specific Th2 CD4⁺ T cells in such a short time frame. A more plausible explanation of the reduction in Th2 cytokine levels is that by neutralizing IL-5 and thus inhibiting T cell and eosinophil infiltrations into the lung, we have reduced the total number of cells capable of producing these Th2 cytokines (see Table I). The broad effects obtained by reducing IL-5 levels suggest that IL-5 production is central to a cascade of events that eventually results in the production of other Th2 cytokines and airways inflammation leading to AHR.

In summary, the Th-modified vaccine approach is capable of circumventing many of the problems associated with previous vaccine techniques, namely, bypassing immune tolerance and generating a polyclonal immune response. This approach also allows for longitudinal immunotherapy and potential resolution from chronic disease. Numerous studies have validated IL-5 as a therapeutic target for the treatment of asthma (8, 10, 11, 15, 38, 39), and promising results have been seen with anti-IL-5 mAb treatment in various animal models (16, 17, 40–46). Several clinical trials are currently evaluating the efficacy of anti-IL-5 mAb therapy in man, but have shown limited success to date in allergic asthmatics (47). Additional studies are required to determine the clinical efficacy of IL-5 neutralization in asthma as well as other eosinophilic disorders. By contrast to systemic humanized anti-IL-5 mAb administration, it is conceivable that in our vaccination approach, by using autologous B cells to deliver the therapeutic Abs at high concentration at the disease sites, we access microenvironments that are critical for disease expression and neutralize the pathogenic properties of IL-5. Although our DNA vaccine did not appear to drive a Th1 immune response, the ability of DNA vaccination to deviate immune responses toward a Th1 profile could be beneficial in treating allergy and could be exploited with our vaccine by adjusting the dose, route, or frequency of the DNA vaccine administration. Preliminary safety studies monitoring organ weights and general histology of selected tissues, including trachea and airways smooth muscle, show no differences between naive untreated and Th-modified IL-5 DNA-vaccinated mice (data not shown). Preliminary observations show that by 45–50 days after the last DNA vaccination Ab levels are declining (data not shown), as expected from previous vaccination studies with other Th-modified vaccines (A. Neisig, M. Hertz, and I. Dalum, unpublished observations). In conclusion, Th-modified IL-5 DNA provides a cost-effective therapeutic vaccine method for inhibiting pulmonary inflammation and AHR in response to allergen provocation of the airways. Active

vaccination against IL-5 represents a novel therapeutic approach for the treatment of asthma and other allergic conditions.

Acknowledgments

We thank Dana Leach for critical reading of this manuscript, the technical staff at Pharmexa, and Peter Birk and Martin Roland Jensen for directing the molecular constructions. We are especially thankful to Susanne Clausen (Arbejdsmiljøinstituttet, Denmark) for guidance establishing the animal models at Pharmexa.

References

- De Monchy, J. G., H. F. Kauffman, P. Venge, G. H. Koeter, H. M. Jansen, H. J. Sluiter, and K. De Vries. 1985. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am. Rev. Respir. Dis.* 131:373.
- Beasley, R., W. R. Roche, J. A. Roberts, and S. T. Holgate. 1989. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am. Rev. Respir. Dis.* 139:806.
- Azzawi, M., B. Bradley, P. K. Jeffery, A. J. Frew, A. J. Wardlaw, G. Knowles, B. Assoufi, J. V. Collins, S. Durham, and A. B. Kay. 1990. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am. Rev. Respir. Dis.* 142:1407.
- Ohashi, Y., S. Motojima, T. Fukuda, and S. Makino. 1992. Airway hyperresponsiveness, increased intracellular spaces of bronchial epithelium, and increased infiltration of eosinophils and lymphocytes in bronchial mucosa in asthma. *Am. Rev. Respir. Dis.* 145:1469.
- Nakajima, H., I. Iwamoto, S. Tomoe, R. Matsumura, H. Tomioka, K. Takatsu, and S. Yoshida. 1992. CD4⁺ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am. Rev. Respir. Dis.* 146:374.
- Broide, D. H., G. J. Gleich, A. J. Cuomo, D. A. Coburn, E. C. Federman, L. B. Schwartz, and S. I. Wasserman. 1991. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J. Allergy Clin. Immunol.* 88:637.
- Wardlaw, A. J., S. Dunnette, G. J. Gleich, J. V. Collins, and A. B. Kay. 1988. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma: relationship to bronchial hyperreactivity. *Am. Rev. Respir. Dis.* 137:62.
- Robinson, D. S., S. Ying, A. M. Bentley, Q. Meng, J. North, S. R. Durham, A. B. Kay, and Q. Hamid. 1993. Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. *J. Allergy Clin. Immunol.* 92:397.
- Walker, C., M. K. Kaegi, P. Braun, and K. Blaser. 1991. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J. Allergy Clin. Immunol.* 88:935.
- Robinson, D. S., Q. Hamid, S. Ying, A. Tscopoulos, J. Barkans, A. M. Bentley, C. Corrigan, S. R. Durham, and A. B. Kay. 1992. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N. Engl. J. Med.* 326:298.
- Robinson, D., Q. Hamid, A. Bentley, S. Ying, A. B. Kay, and S. R. Durham. 1993. Activation of CD4⁺ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J. Allergy Clin. Immunol.* 92:313.
- Walker, C., E. Bode, L. Boer, T. T. Hansel, K. Blaser, and J. C. Virchow, Jr. 1992. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am. Rev. Respir. Dis.* 146:109.
- Drazen, J. M., J. P. Arm, and K. F. Austen. 1996. Sorting out the cytokines of asthma. *J. Exp. Med.* 183:1.
- Bousquet, J., P. Chanez, J. Y. Lacoste, G. Barneon, N. Ghavanian, I. Enander, P. Venge, S. Ahlstedt, J. Simony-Lafontaine, P. Godard, et al. 1990. Eosinophilic inflammation in asthma. *N. Engl. J. Med.* 323:1033.
- Foster, P. S., S. P. Hogan, A. J. Ramsay, K. I. Matthaei, and I. G. Young. 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J. Exp. Med.* 183:195.
- Mausner, P. J., A. M. Pitman, X. Fernandez, S. K. Foran, G. K. Adams III, W. Kreutner, R. W. Egan, and R. W. Chapman. 1995. Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am. J. Respir. Crit. Care Med.* 152:467.
- Mausner, P. J., A. Pitman, A. Witt, X. Fernandez, J. Zurcher, T. Kung, H. Jones, A. S. Watnick, R. W. Egan, W. Kreutner, et al. 1993. Inhibitory effect of the TRFK-5 anti-IL-5 antibody in a guinea pig model of asthma. *Am. Rev. Respir. Dis.* 148:1623.
- Dalum, I., D. M. Butler, M. R. Jensen, P. Hindersson, L. Steinaa, A. M. Waterston, S. N. Grell, M. Feldmann, H. I. Elsner, and S. Mouritsen. 1999. Therapeutic antibodies elicited by immunization against TNF- α . *Nat. Biotechnol.* 17:666.
- Dalum, I., M. R. Jensen, P. Hindersson, H. I. Elsner, and S. Mouritsen. 1996. Breaking of B cell tolerance toward a highly conserved self protein. *J. Immunol.* 157:4796.
- Dalum, I., M. R. Jensen, K. Gregorius, C. M. Thomasen, H. I. Elsner, and S. Mouritsen. 1997. Induction of cross-reactive antibodies against a self protein by immunization with a modified self protein containing a foreign T helper epitope. *Mol. Immunol.* 34:1113.
- Kozak, M. 1987. An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 15:8125.

22. Panina-Bordignon, P., A. Tan, A. Termijtelen, S. Demotz, G. Corradin, and A. Lanzavecchia. 1989. Universally immunogenic T cell epitopes: promiscuous binding to human MHC class II and promiscuous recognition by T cells. *Eur. J. Immunol.* 19:2237.
23. Hogan, S. P., K. I. Matthaesi, J. M. Young, A. Koskinen, I. G. Young, and P. S. Foster. 1998. A novel T cell-regulated mechanism modulating allergen-induced airways hyperreactivity in BALB/c mice independently of IL-4 and IL-5. *J. Immunol.* 161:1501.
24. Hamelmann, E., J. Schwarze, K. Takeda, A. Oshiba, G. L. Larsen, C. G. Irvin, and E. W. Gelfand. 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* 156:766.
25. Webb, D. C., A. N. McKenzie, A. M. Koskinen, M. Yang, J. Mattes, and P. S. Foster. 2000. Integrated signals between IL-13, IL-4, and IL-5 regulate airways hyperreactivity. *J. Immunol.* 165:108.
26. Wynn, T. A., I. Eltoum, A. W. Cheever, F. A. Lewis, W. C. Gause, and A. Sher. 1993. Analysis of cytokine mRNA expression during primary granuloma formation induced by eggs of *Schistosoma mansoni*. *J. Immunol.* 151:1430.
27. Stamm, L. M., A. Raisanen-Sokolowski, M. Okano, M. E. Russell, J. R. David, and A. R. Satoskar. 1998. Mice with STAT6-targeted gene disruption develop a Th1 response and control cutaneous leishmaniasis. *J. Immunol.* 161:6180.
28. Hogan, S. P., A. Koskinen, K. I. Matthaesi, I. G. Young, and P. S. Foster. 1998. Interleukin-5-producing CD4⁺ T cells play a pivotal role in aeroallergen-induced eosinophilia, bronchial hyperreactivity, and lung damage in mice. *Am. J. Respir. Crit. Care Med.* 157:210.
29. Corry, D. B., H. G. Folkesson, M. L. Warnock, D. J. Erle, M. A. Matthay, J. P. Wiener-Kronish, and R. M. Locksley. 1996. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J. Exp. Med.* 183:109.
30. Tournoy, K. G., J. C. Kips, and R. A. Pauwels. 2001. The allergen-induced airway hyperresponsiveness in a human-mouse chimera model of asthma is T cell and IL-4 and IL-5 dependent. *J. Immunol.* 166:6982.
31. Hakonarson, H., N. Maskeri, C. Carter, and M. M. Grunstein. 1999. Regulation of TH1- and TH2-type cytokine expression and action in atopic asthmatic sensitized airway smooth muscle. *J. Clin. Invest.* 103:1077.
32. Hakonarson, H., N. Maskeri, C. Carter, S. Chuang, and M. M. Grunstein. 1999. Autocrine interaction between IL-5 and IL-1 β mediates altered responsiveness of atopic asthmatic sensitized airway smooth muscle. *J. Clin. Invest.* 104:657.
33. Hertz, M., and D. Nemazee. 1998. Receptor editing and commitment in B lymphocytes. *Curr. Opin. Immunol.* 10:208.
34. Goodnow, C. C. 1996. Balancing immunity and tolerance: deleting and tuning lymphocyte repertoires. *Proc. Natl. Acad. Sci. USA* 93:2264.
35. Buhl, A. M., D. Nemazee, J. C. Cambier, R. Rickert, and M. Hertz. 2000. B-cell antigen receptor competence regulates B-lymphocyte selection and survival. *Immunol. Rev.* 176:141.
36. Broide, D., J. Schwarze, H. Tighe, T. Gifford, M. D. Nguyen, S. Malek, J. Van Uden, E. Martin-Orozco, E. W. Gelfand, and E. Raz. 1998. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J. Immunol.* 161:7054.
37. Kline, J. N., T. J. Waldschmidt, T. R. Businga, J. E. Lemish, J. V. Weinstock, P. S. Thorne, and A. M. Krieg. 1998. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J. Immunol.* 160:2555.
38. Sur, S., G. J. Gleich, K. P. Offord, M. C. Swanson, T. Ohnishi, L. B. Martin, J. M. Wagner, D. A. Weiler, and L. W. Hunt. 1995. Allergen challenge in asthma: association of eosinophils and lymphocytes with interleukin-5. *Allergy* 50:891.
39. Ohnishi, T., H. Kita, D. Weiler, S. Sur, J. B. Sedgwick, W. J. Calhoun, W. W. Busse, J. S. Abrams, and G. J. Gleich. 1993. IL-5 is the predominant eosinophil-active cytokine in the antigen-induced pulmonary late-phase reaction. *Am. Rev. Respir. Dis.* 147:901.
40. Egan, R. W., D. Athwahl, C. C. Chou, S. Emtage, C. H. Jehn, T. T. Kung, P. J. Mauser, N. J. Murgolo, and M. W. Bodmer. 1995. Inhibition of pulmonary eosinophilia and hyperreactivity by antibodies to interleukin-5. *Int. Arch. Allergy Immunol.* 107:321.
41. Van Oosterhout, A. J., A. R. Ladenius, H. F. Savelkoul, I. Van Ark, K. C. Delsman, and F. P. Nijkamp. 1993. Effect of anti-IL-5 and IL-5 on airway hyperreactivity and eosinophils in guinea pigs. *Am. Rev. Respir. Dis.* 147:548.
42. Akutsu, I., T. Kojima, A. Kariyone, T. Fukuda, S. Makino, and K. Takatsu. 1995. Antibody against interleukin-5 prevents antigen-induced eosinophil infiltration and bronchial hyperreactivity in the guinea pig airways. *Immunol. Lett.* 45:109.
43. Gulbenkian, A. R., R. W. Egan, X. Fernandez, H. Jones, W. Kreutner, T. Kung, F. Payvandi, L. Sullivan, J. A. Zurcher, and A. S. Watnick. 1992. Interleukin-5 modulates eosinophil accumulation in allergic guinea pig lung. *Am. Rev. Respir. Dis.* 146:263.
44. Kung, T. T., D. M. Stelts, J. A. Zurcher, G. K. Adams III, R. W. Egan, W. Kreutner, A. S. Watnick, H. Jones, and R. W. Chapman. 1995. Involvement of IL-5 in a murine model of allergic pulmonary inflammation: prophylactic and therapeutic effect of an anti-IL-5 antibody. *Am. J. Respir. Cell Mol. Biol.* 13:360.
45. Shardonofsky, F. R., J. Venzor III, R. Barrios, K. P. Leong, and D. P. Huston. 1999. Therapeutic efficacy of an anti-IL-5 monoclonal antibody delivered into the respiratory tract in a murine model of asthma. *J. Allergy Clin. Immunol.* 104:215.
46. Nagai, H., S. Yamaguchi, N. Inagaki, N. Tsuruoka, Y. Hitoshi, and K. Takatsu. 1993. Effect of anti-IL-5 monoclonal antibody on allergic bronchial eosinophilia and airway hyperresponsiveness in mice. *Life Sci.* 53:L243.
47. Leckie, M. J., A. ten Brinke, J. Khan, Z. Diamant, B. J. O'Connor, C. M. Walls, A. K. Mathur, H. C. Cowley, K. F. Chung, R. Djukanovic, et al. 2000. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 356:2144.