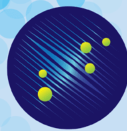
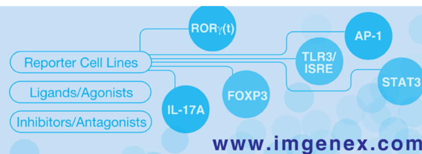

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J Immunol 2003; 171:2080-2089; ;
http://www.jimmunol.org/content/171/4/2080

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Print ISSN: 0022-1767 Online ISSN: 1550-6606.

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Airway IgG Counteracts Specific and Bystander Allergen-Triggered Pulmonary Inflammation by a Mechanism Dependent on FcγR and IFN-γ[1]

Sarita Sehra,* Gwenda Pynaert,* Kurt Tournoy,† Anuschka Haegeman,* Patrick Matthys,‡ Yohichi Tagawa,§ Romain Pauwels,† and Johan Grooten[2]*

Besides IgE, the Ab isotype that gives rise to sensitization and allergic asthma, the immune response to common inhalant allergens also includes IgG. Increased serum titers of allergen-specific IgG, induced spontaneously or by allergen vaccination, have been implicated in protection against asthma. To verify the interference of topical IgG with the allergen-triggered eosinophilic airway inflammation that underlies asthma, sensitized mice were treated by intranasal instillation of specific IgG, followed by allergen challenge. This treatment strongly reduced eosinophilic inflammation and goblet cell metaplasia, and increased Th1 reactivity and IFN-γ levels in bronchoalveolar lavage fluid. In contrast, inflammatory responses were unaffected in IFN-γ-deficient mice or when applying $F(ab')_2$. Although dependent on specific allergen-IgG interaction, inflammation triggered by bystander allergens was similarly repressed. Perseverance of inflammation repression, apparent after secondary allergen challenge, and increased allergen capture by alveolar macrophages further characterized the consequences of topical IgG application. These results assign a novel protective function to anti-allergen IgG namely at the local level interference with the inflammatory cascade, resulting in repression of allergic inflammation through an FcγR- and IFN-γ-dependent mechanism. Furthermore, these results provide a basis for topical immunotherapy of asthma by direct delivery of anti-allergen IgG to the airways. *The Journal of Immunology,* **2003, 171: 2080–2089.**

Mucosal surfaces lining body cavities, such as the gastrointestinal tract and pulmonary airways, are continuously exposed to attack by exogenous pathogens and, through food ingestion and Ag inhalation, to innocuous environmental Ags. Mucosal tolerance, preventing unwanted inflammation to innocent soluble protein Ags, has evolved along with specific and nonspecific defenses against colonization and invasion of mucosal surfaces by microorganisms. Mucus secretion by goblet cells, proteases such as lysozyme and lactoferrin, defensins, and active exit from the airways of particulate Ags via the mucociliary escalator constitute nonspecific defenses against invading pathogens. Specific immune recognition in contrast relies on secretory IgA, a particular class of Abs essential for mucosal immunity (1). From experimental and clinical data, immune exclusion appears to be the main mechanism by which secretory IgA confers resistance to infection (2–5). IgA in secretions is the product of local synthesis at mucosal surfaces by B cells, matured into polymeric IgA-producing cells under the influence of the Th2-type cytokines IL-4, IL-5, IL-6, and IL-10 (6). In line with the reliance of the respiratory tract on a secretory IgA-mediated humoral form of immunity, Th2-type differentiation is preferentially promoted after pulmonary or nasal immunization with Ags that, when administered by s.c. or i.p. route, prime for cell-mediated forms of immunity (7–9). The mucosal surfaces of the airways therefore may be considered as sites that favor the priming of Th2-type effector T cells.

Prototype immunopathologic outings of a pro-Th2 lung environment are respiratory allergies and asthma. Thus, subsequent to nasal immunization to airborne allergens and the generation of allergen-specific IgE Ab responses, re-exposure to the allergen may elicit allergic asthma in susceptible individuals (10, 11). Allergic asthma, a complex and chronic disease, is characterized by inflammation of the bronchial mucosa, involving activated eosinophils, mast cells, and $CD4^+$ T cells, as well as airway hyperresponsiveness, reversible bronchoconstriction, and elevated titers of circulating IgE. Observations from mouse models of allergic respiratory inflammation identified pulmonary cytokines characteristic of the Th2 subset of $CD4^+$ T cells, mainly IL-4, IL-5, IL-9, and IL-13, as crucial actors in the etiology of the human disease (12). Not surprising, the development of therapies targeted at neutralizing specific cytokines has recently attracted considerable attention. Yet these approaches are hampered by intercytokine functional redundancy, thus limiting the effectiveness of the treatment (13–15). Anti-IgE therapy exerts its action by reducing the amount of free IgE available to bind to effector cells (16, 17). This form of therapy has demonstrated limited clinical efficacy. The limitations in efficacy of the anti-IgE therapy might be due either to the fact that circulating IgE is not completely reduced or to the requirement for disease-modifying approaches, modulating upstream processes, such as the preferential Th2 skewing in responses to inhaled allergen.

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Received for publication January 29, 2003. Accepted for publication June 2, 2003.

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[1] This work was supported in part by the Regional Government of Flanders (Concerted Research Actions program) and by the Belgian Federal Government (Interuniversity Network for Fundamental Research and Interuniversity Attraction Poles). P.M. is a postdoctoral fellow with the Fund for Scientific Research-Flanders.

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Besides IgA and IgE, the Ab isotype that gives rise to sensitization in humans, the immune response to common inhalant allergens also includes IgG (18–20). Recent studies by Platts-Mills and coworkers (21, 22), investigating the relation between exposure levels to cat and dog allergens and development of asthma, demonstrated a progressive increase of specific serum IgG titers with extended exposure and a prevalence of the Th2 cytokine-dependent IgG4 isotype. An IgG response without IgE in exposed children, indicative of a modified Th2 response, coincided with absence of allergic disease and asthma. Also in allergic patients, immunotherapy by allergen vaccination is accompanied by an increment of allergen-specific IgG titers and especially IgG4 titers (23, 24), thus suggesting a protective effect of IgG against allergy and asthma. Protection may derive from blocking serum IgE-facilitated allergen presentation, thus raising the allergen threshold levels required for T cell activation (25). However, due to the systemic nature of the mechanism, this observation omits the eventuality of topical IgG effects occurring in the airways during allergen exposure and interfering with the allergen-triggered eosinophilic airway inflammation that causes asthma. Similar to IgA in secretions, airway IgG may neutralize inhaled allergens. In addition, through interaction with FcγR, IgG may promote activation of accessory cells and enable FcγR-mediated endocytosis of allergen-IgG complexes, thereby promoting allergen capture and presentation by APCs, such as alveolar macrophages and/or lung dendritic cells (26, 27). To verify the interference of these IgG effector functions with allergic airway inflammation, we directly administered IgG to the airways of sensitized BALB/c mice and monitored its effects on airway inflammation induced by a subsequent allergen challenge.

Materials and Methods

Mice

Specific pathogen-free female BALB/c mice were obtained from the Broekman Institute (Someren, The Netherlands). The generation and basic characteristics of IFN-γ-deficient mice of the 129 $\times$ BALB/c strain have been described previously (28). These mice were backcrossed for eight generations to the parental BALB/c strain. Mice were maintained under specific pathogen-free conditions; they were housed in sterile microisolator cages and were given sterile water and food. All mice were used between 8 and 12 wk of age.

Abs and reagents

Alum ($Al(OH)_3/Mg(OH)_2$), grade V OVA (both from Sigma-Aldrich, St. Louis, MO), and human catalase (h-cat;[3] Calbiochem-Novabiochem, San Diego, CA) were used for sensitization and airway exposure. Mouse Abs for immunophenotyping and intracellular cytokine staining were obtained from BD PharMingen (San Diego, CA). Purified anti-CD3 mAb (clone 145-2c11) was kindly provided by G. Leclercq (University Hospital, Ghent, Belgium). Mouse mAbs to OVA and h-cat were generated as described previously (29). Anti-OVA IgG1 (clone G13), IgG2a (clone 89.46.9), IgG2b (clone F2.66), and anti-h-cat IgG2a (clone 2VIG2), used for intranasal instillation, were purified from culture fluid by protein G affinity chromatography. Ab concentration was determined by ELISA using isotype-matched reference Abs as standards (Sigma-Aldrich). $F(ab')_2$ was derived from anti-OVA IgG2a by pepsin proteolytic cleavage, as described previously (30), using agarose-bound pepsin (Pierce Chemicals, Rockford, IL). The concentration of functional $F(ab')_2$ was determined by OVA-specific ELISA using a goat anti-mouse κ for detection (Southern Biotechnology Associates, Birmingham, AL) and a sample of whole Ab as reference. All mAbs and reagents for in vivo application contained <5 ng/ml endotoxin and were applied in endotoxin-free PBS.

Experimental protocols

Mice were sensitized by three i.p. injections (0.5 ml) at 1-wk intervals of OVA/alum (10 μg/1 mg) or h-cat/alum (100 μg/2 mg) on days 0, 7, and 14 of the protocol. Dual-sensitized mice received both OVA and h-cat complexed with 2 mg alum. Starting from day 21, mice were challenged for 2 consecutive days by intratracheal instillation (80 μl) of 10 μg OVA or 50 μg h-cat (short exposure protocol). Mice were first anesthetized with a mixture of ketamine and xylazine (100 and 10 mg/kg, respectively). Alternatively, mice were challenged from days 21 to 27 by daily, 20-min inhalations in a closed aerosol chamber of an aerosol generated by nebulization (Acorn II jet nebulizer; Vital Signs, Totowa, NJ; 2.5 bars operating pressure) of a 1% OVA solution (prolonged exposure protocol). For secondary challenge, the short exposure protocol was followed by a 5-day rest period and mice were rechallenged on days 28 and 29 by intratracheal instillation. Control mice received i.p. injections of PBS and/or challenges of PBS alone. IgG (50 μg) was administered by intranasal instillation (30 μl), 2 h before challenges on days 21 and 22 (short exposure protocol) or 2 h before the first and fourth inhalation from the prolonged exposure protocol. Preliminary experiments indicated that intranasal IgG instillation generated maximal intra-airway delivery of intact Ab, as opposed to nebulization that caused a significant loss of Ab reactivity (result not shown). Control mice were placebo treated by intranasal instillation of PBS.

Bronchoalveolar lavage (BAL) and differential cell counts

BAL was performed 48 h after challenge, unless otherwise indicated. Mice were sacrificed by i.p. injection (0.5 ml) of avertin (2.5% w/v in PBS). BAL was performed with 3 $\times$ 1 ml of Ca^{2+}- and Mg^{2+}-free HBSS (Life Technologies, Rockville, MD), supplemented with 0.05 mm EDTA. The cells recovered in BAL fluid were counted with a hemocytometer. Differential cell counts were determined on cytospin preparations stained with May-Grunwald-Giemsa (Sigma-Aldrich) by classification of 200 cells on standard morphology. $CD4^+$ and $CD8^+$ T cell subsets were quantified by flow cytometry.

Histological analysis

After BAL, lungs were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (2.5 μm thick) from all lobes were stained either with Congo Red (demonstrating eosinophils) or with periodic acid-Schiff (PAS; demonstrating goblet cells). Slides were coded, and the peribronchial (and perivascular) inflammation was graded in a blinded fashion using a reproducible scoring system described previously (31). Briefly, a value from 0 to 3 was adjudged to each tissue section scored. A value of 0 was adjudged when no inflammation was detectable, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 when most bronchi were surrounded by a thin layer of inflammatory cells (1–5), and a value of 3 when most bronchi were surrounded by a thick layer of inflammatory cells (>5). As 5–7 tissue sections per mouse were scored, inflammation scores could be expressed as a mean value per animal and could be compared between groups. To estimate the presence of mucus-producing cells, we counted the number of airways per section and adjudged a score of 0, 1, 2, or 3 to each airway when no, very few, $<50\%$, or $>50\%$ of the airway epithelial cells were PAS positive. In that way, each mouse and group was characterized by a distribution of scores that could be compared statistically.

Analysis of OVA uptake by tissue and BAL cells

OVA was covalently labeled with the green fluorescent dye Fluor X using a FluoroLink-Ab Fluor X labeling kit (Amersham Pharmacia Biotech, Rainham, U.K.). Sensitized mice received anti-OVA IgG2a (50 μg) or PBS by intranasal instillation (30 μl), followed 2 h later by intratracheal instillation (80 μl) of 10 μg OVA-Fluor X. After an additional 2 h, mice were sacrificed, BAL was performed, and lungs were excised. Single cell suspensions of lung tissue were prepared by digestion of minced tissue with RPMI 1640 medium supplemented with 2.4 mg/ml collagenase (Sigma-Aldrich) and 1 mg/ml DNase I (Roche Molecular Biochemicals, Basel, Switzerland) for 30 min at 37°C. The digest was filtered through a 70-μm cell strainer, and erythrocytes were lysed with NH_4Cl-Tris buffer. Before analysis of cell-bound OVA-Fluor X, BAL and lung single cell suspensions were incubated with unlabeled blocking anti-FcγR II/III mAb, followed by staining with biotinylated anti-CD11c, biotinylated anti-CD11b, and/or PE-conjugated anti-I-A^d. Samples stained with biotinylated Abs were additionally incubated with streptavidin-CyChrome. Cell staining was analyzed by three-color flow cytometry.

Cytokine measurements

Intracellular cytokine staining was performed as described previously (32). Briefly, cells recovered from BAL fluid were pooled, and triplicate cultures (10^6 cells/ml) were stimulated with anti-CD3 (5 μg/ml) and anti-CD28 (5 μg/ml). Two-hour cultures were supplemented with brefeldin A (10 μg/ml; Sigma-Aldrich) for an additional 4-h period. Intracellular cytokine staining

[3] Abbreviations used in this paper: h-cat, human catalase; BAL, bronchoalveolar lavage; PAS, periodic acid-Schiff; wt, wild type.

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was performed using blocking anti-FcγR II/III, FITC-conjugated anti-CD4, and PE-conjugated anti-IL-4 or anti-IFN-γ Abs. Duplicate samples were preincubated with excess unlabeled anti-cytokine Abs, followed by staining with corresponding fluorochrome-labeled Abs to quantitate nonspecific binding. The number of positive cells was counted using a FACSCalibur flow cytometer (BD Biosciences, Mountain View, CA) and analyzed using the CellQuest program. IL-4, IL-5, and IFN-γ levels in BAL fluid were determined by cytokine-specific ELISA, according to the manufacturer's instructions (Endogen, Woburn, MA). All ELISAs showed R-square values of >0.99.

Results

Airway IgG represses allergen-induced eosinophilic airway inflammation

Unlike secretory IgA, which is protected from enzymatic degradation by its linkage to the secretory piece (33), IgG in the bronchial lumen is prone to attack by proteases abundantly present on mucosal surfaces. Determination of Ab titers in the BAL fluid of naive BALB/c mice after intranasal instillation of anti-OVA IgG indeed revealed a gradual loss of anti-OVA reactivity; whereas 6 h after instillation over 70% of instilled Ab was detected, at 48 h this rapidly dropped to a few percent (Fig. 1). The same Ab administered by i.v. route maintained significant serum titers up to several months (data not shown). The rapid clearance from the airways of instilled IgG defines an approximate 24-h window in which IgG effector functions may be triggered by interaction with specific Ag. Accordingly, we opted as basic scheme for studying the immune modulatory activities of airway IgG on allergen-induced pulmonary inflammation for intranasal instillation of IgG, followed after 2 h by allergen exposure.

Injection of OVA emulsified in alum rendered BALB/c mice sensitive to a subsequent provocation by intratracheal instillation or inhalation of OVA. Using BAL for isolation of cells lining the airways, a marked increase of cells in BAL fluid recovered from sensitized mice was observed as compared with placebo-sensitized mice (PBS) or mice sensitized with OVA and challenged with PBS (Fig. 2*A*). Assessment of the cell composition by staining of cytospin slides revealed a major influx of eosinophils and mononuclear cells after OVA challenge (Fig. 2*B*). This OVA-induced eosinophilic airway inflammation, characteristic of allergic asthma, was observed using both short and prolonged allergen exposure protocols, although the extent of the inflammatory cell influx increased with the number of exposures (Fig. 2, *B* and *C*). We next verified the consequences of a prior treatment with anti-OVA IgG on the magnitude and composition of cell infiltration. Anti-OVA

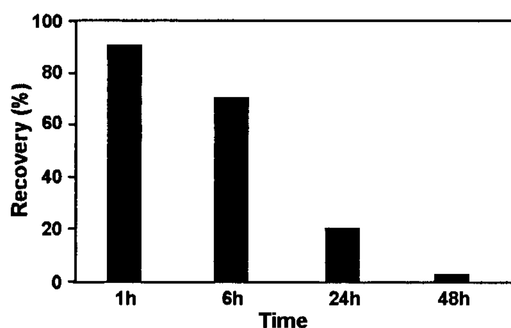

FIGURE 1. Clearance from the airways of instilled IgG. Anti-OVA IgG2a mAb was administered directly to the airways by intranasal instillation. Ag-specific IgG titers in the BAL fluid were determined by Ag- and isotype-specific ELISA. The recovery percentage was calculated from the mean titers ($n = 3$) using the original IgG preparation as standard. SEM on individual titers was below 10%, except at 24 and 48 h, in which the interindividual variation increased.

IgG2a was administered by intranasal instillation, thus creating a topical presence of allergen-specific IgG in the lumen of the airways. With both exposure protocols, the number of cells in BAL fluid recovered from mice treated in that way was consistently reduced by 50% or more (Fig. 2, *B* and *C*). This reduction was mainly attributable to a lesser presence of eosinophils. Numbers of inflammatory effectors associated with cell-mediated forms of immunity, macrophages and neutrophils, remained unchanged or were slightly reduced in the respective groups. Treatment with an irrelevant isotype control, namely anti-h-cat-IgG2a, had no effect on the size or composition of the cell infiltrate. In an independent experiment, comparing anti-OVA mAbs belonging to different IgG subclasses (IgG1, IgG2b), near-identical reductions of cell infiltration were observed, compared with the formerly used IgG2a mAb (Fig. 2*D*).

Dependence on the IgG Fc domain

Binding of IgG to Ag promotes stable interaction of the bound Ab with FcγRs. Because FcγRs specifically interact with the IgG Fc domain, removal of this domain generates a $F(ab')_2$ that is no longer recognized by FcγRs, but still retains the cognate activity of the whole Ab. To investigate whether interactions with FcγR are part of the repressive mechanism of anti-OVA IgG, equimolar concentrations of $F(ab')_2$ were administered to OVA-sensitized mice. Total cell numbers and eosinophil content in BAL fluid harvested from whole IgG-treated mice showed the characteristic, over 50% reduction as compared with placebo. In contrast, intranasal instillation of the Ab-derived $F(ab')_2$ failed to exert repressive activity on inflammatory cell influx, producing BAL cell counts comparable to placebo (Fig. 3).

To further characterize in the different groups the inflammatory responses ongoing within the lung tissue, lung sections were stained with Congo Red (demonstrating eosinophils) and scored for magnitude of peribronchial and perivascular cell infiltration. Histological analysis of the lungs from the placebo-treated mice exposed to the nebulized allergen showed infiltration of the airways as well as the perivascular areas with eosinophils and mononuclear cells (Fig. 4, *upper panels*). Alveolar septa were not infiltrated with inflammatory cells. Treatment of these allergen-exposed mice with anti-OVA $F(ab')_2$ did not alter the histology of the airways. In contrast, treatment with anti-OVA IgG2a before and during the allergen challenge reduced the airway inflammation significantly as compared with placebo- or $F(ab')_2$-treated mice (Fig. 4, *upper panels*). This difference was also seen in the perivascular areas, although no statistical significance was reached (data not shown). In placebo-treated animals, specific staining for mucus-producing cells (PAS) revealed that 32 ± 8% of the airways was free of PAS-positive cells (Fig. 4, *lower panels*). This figure is similar to that of mice treated with $F(ab')_2$, namely 29 ± 4% and $p > 0.05$. However, many more airways were free of PAS-positive cells in mice treated with anti-OVA IgG2a: 49 ± 4%; $p < 0.05$. Although no significant differences were found for the airways with few or moderate numbers of mucus cells (data not shown), there were significant differences in the presence of airways of which >50% of the epithelial cells were PAS positive: 53 ± 6% for placebo-treated animals vs only 32 ± 4% for anti-OVA IgG2a-treated animals ($p < 0.05$). From these results, an anti-inflammatory activity can be assigned to the presence of IgG in the lumen of the airways, requiring Fc domain-mediated effector functions and featuring a reduced eosinophil influx in the airways and lung perivascular areas, along with a reduction of goblet cell metaplasia.

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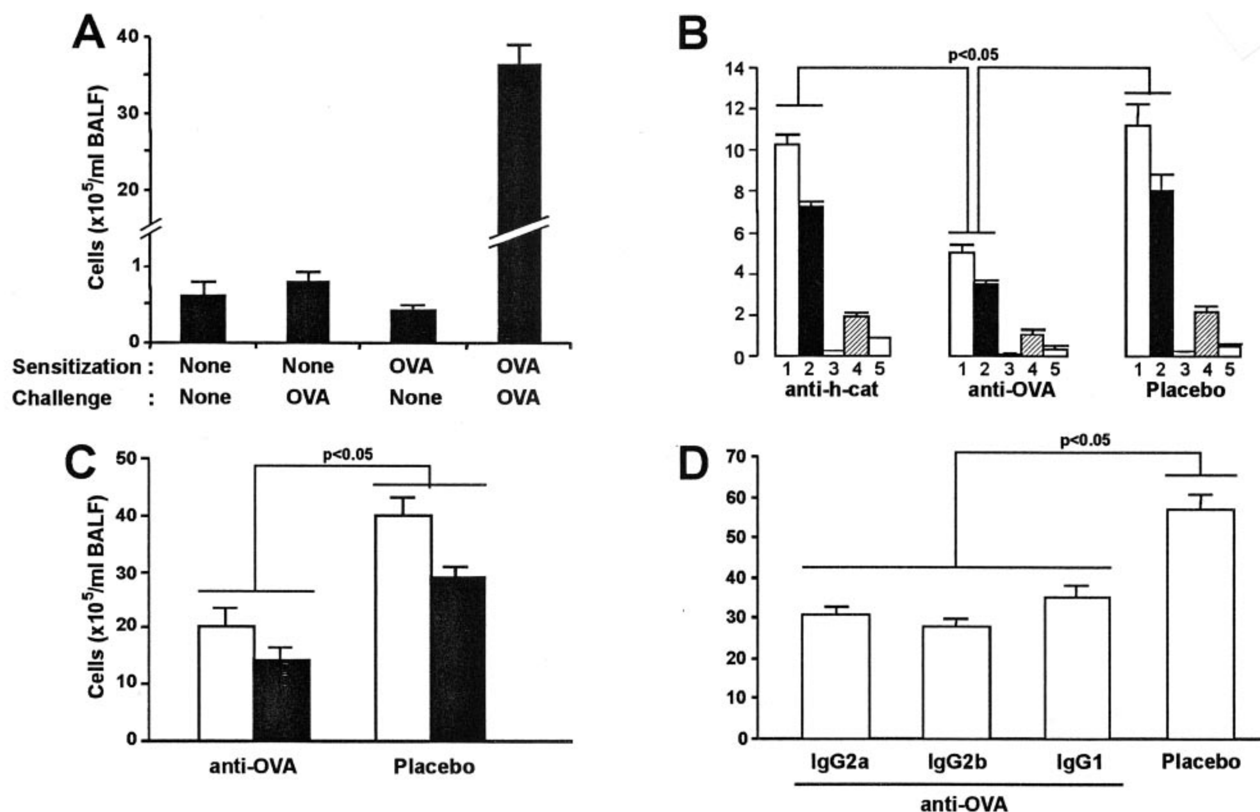

FIGURE 2. Intranasal instillation of anti-allergen IgG counteracts development of allergic airway inflammation. *A*, Total cell counts in BAL fluid from naive or OVA-sensitized mice, challenged by exposure on a daily basis to a series of seven OVA aerosols. *B*, OVA-sensitized mice were treated for 2 consecutive days with anti-OVA IgG2a. Anti-h-cat IgG2a and PBS were used as isotype and placebo controls. Two hours after each treatment, OVA was administered intratracheally to all mice (short exposure protocol). Forty-eight hours after the second allergen exposure, BAL was performed. Total nucleated cell counts and differential cell counts are shown. Frequencies of $CD4^+$ T cells were determined by flow cytometry. Data are expressed as means ± SEM (n = 5–7). For naive mice, macrophages represented the majority of the cells (not shown). Bar 1, total BAL cells; bar 2, eosinophils; bar 3, neutrophils; bar 4, macrophages; bar 5, $CD4^+$ T cells. *C*, OVA-sensitized mice were treated with anti-OVA IgG2a or PBS (placebo) on the first and fourth day of a 7-day OVA-aerosol exposure (prolonged exposure protocol). Shown are means ± SEM of total cell counts and eosinophil counts from BAL fluid collected 24 h after the last aerosol exposure (n = 5). *D*, The ability of airway IgG to repress allergic airway inflammation is not restricted to a single mAb or IgG subclass, but represents a function characteristic of anti-allergen IgG. Using the prolonged exposure regimen described in *C*, OVA-sensitized mice were treated with OVA-specific mAbs belonging to different IgG subclasses. Mean numbers ± SEM of nucleated cells present in the BAL fluid are shown (n = 5).

OVA uptake by tissue and BAL cells

FcγRs are expressed on the surfaces of lymphoid cells and accessory cells, such as macrophages, neutrophils, granulocytes, tissue dendritic cells, and others. As a means of monitoring what type of cell is engaged in capturing free OVA as opposed to OVA in the presence of IgG, we verified by flow cytometry the extent of cell-associated OVA in the airways. Administration of OVA covalently labeled with the green fluorescent dye Fluor X produced a distinctive fraction of green fluorescent cells in BAL and lung tissue cell suspensions isolated 2 h after OVA-Fluor X intratracheal instillation (Fig. 5, *upper panels*). However, treatment with anti-OVA IgG, 2 h before OVA-Fluor X instillation, doubled the fraction of OVA-positive cells present in the BAL fluid. In contrast, in cell suspensions from lung tissue only a modest increment was observed, which would indicate that the range of action of the instilled IgG is confined to the airway lumen and cells lining the airways. Immunofluorescent staining identified the OVA-positive cells in BAL and lung tissue samples from placebo-treated as well as IgG-treated mice as $CD11c^+$ (Fig. 5, *middle panels*), a marker for dendritic cells that is also expressed on macrophages, especially alveolar macrophages. A significant fraction of $CD11b^+$ macrophages was also detected in both types of samples, yet this population scored homogenously negative for OVA-Fluor X. When gated on OVA-Fluor X-positive cells, over 90% of the events showed increased autofluorescence (Fig. 5, *bottom panels*). Combined with MHC class II (I-A^d) expression levels below detection (result not shown), this result identifies alveolar macrophages as the main population responsible for OVA capture, the direct effect of IgG treatment being an increment of this cell fraction.

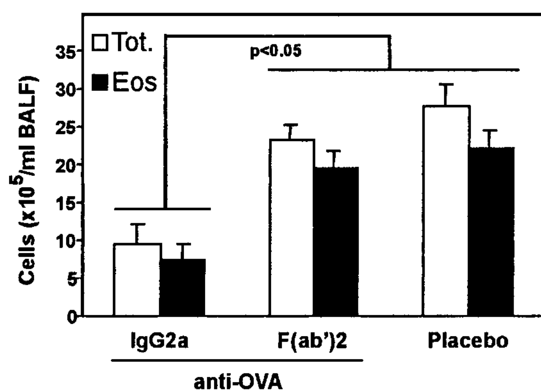

FIGURE 3. Repression of inflammation by IgG treatment requires the Fc domain. Using the prolonged exposure protocol, mice were treated with anti-OVA IgG2a and equimolar concentrations of derived $F(ab')_2$. Placebo-treated mice received PBS. BAL was performed 24 h later; total and differential cell counts were determined on BAL fluid cells. Results are expressed as means ± SEM (n = 5).

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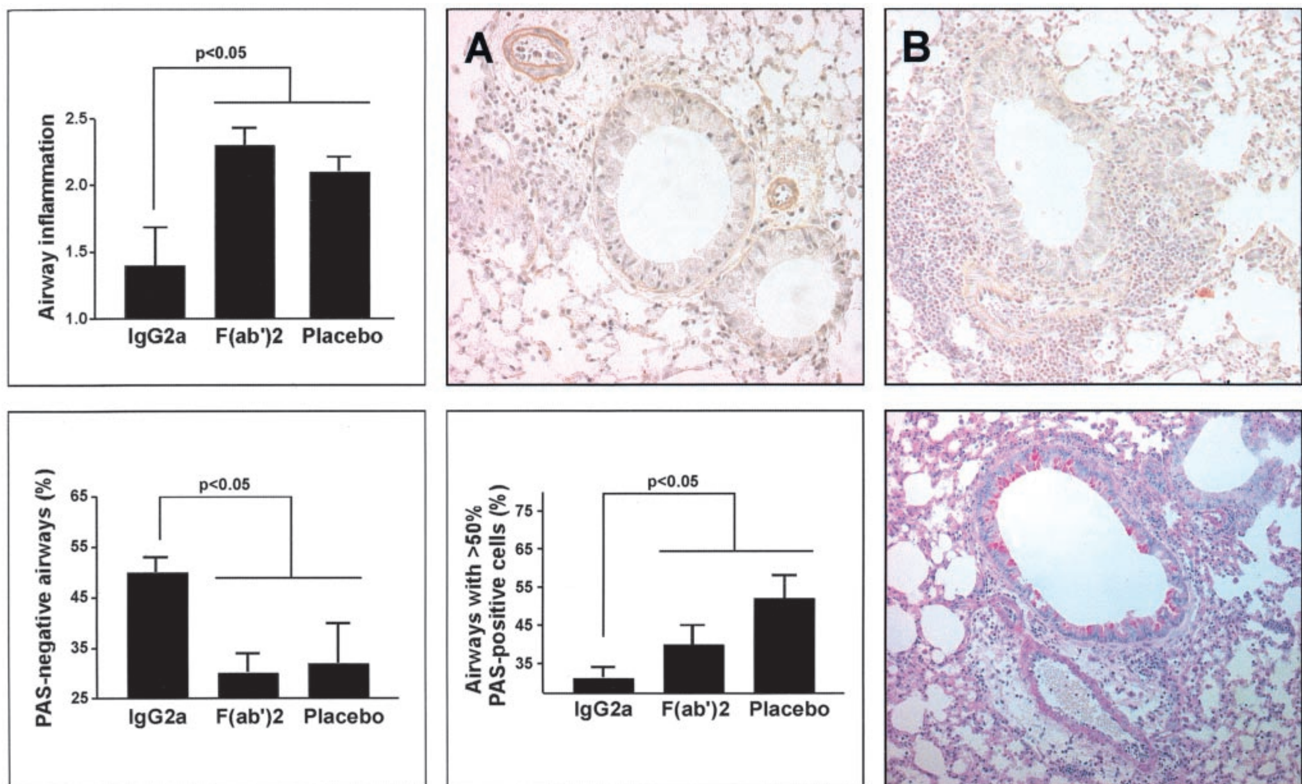

FIGURE 4. Histological lung analysis. Instillation of anti-OVA IgG, but not of $F(ab')_2$ or placebo, decreases the allergen-induced eosinophilic airway inflammation (*upper panels*). *A*, Representative of mice treated with IgG2a; *B*, representative of mice treated with $F(ab')_2$ or placebo. *Lower panels* represent the number of airways free of mucus-producing cells or containing >50% PAS-positive cells. The lower image illustrates a typical PAS staining (mucus-producing cells stain purple).

Th1/Th2 cytokine pattern in IgG-treated and OVA-challenged airways

Analysis of cytokine levels in the BAL fluid from mice exposed to inhaled OVA showed an increment of IFN-γ levels from near background in samples from placebo-treated mice to significant levels in BAL fluid from IgG-treated mice (Fig. 6, *upper panel*). In contrast, levels of the Th2-related cytokines IL-4 and IL-5 remained unchanged (Fig. 6, *lower panels*). To verify whether the IFN-γ increment in the BAL fluid reflects an increased proportion of Th1 cells, T cells from the BAL fluid were stimulated with anti-CD3 in the presence of anti-CD28, and stained for CD4 and intracellular cytokines. As shown in Fig. 7 (*upper panels*), a 2-fold increase in IFN-γ-secreting $CD4^+$ T cells was observed in the IgG-treated group, along with a slight reduction in the proportion of IL-4-secreting T cells. Although of low abundance, cytokine-positive events were abolished by preincubation with excess unlabeled blocking Abs, thus confirming the specificity of cytokine staining (Fig. 7, *bottom panels*).

Additional characteristics of inflammation repression: repression of bystander allergens, persistence of repression, and dependence on IFN-γ

To verify whether airway inflammation repression by IgG treatment arises from a specific induction of regulatory cytokines, exerting bystander activity on immune responses concomitantly triggered by unrelated Ags, we verified the airway inflammatory response to a second allergen. Mice, simultaneously sensitized to OVA and h-cat, were treated with either anti-OVA or anti-h-cat IgG2a and exposed to both model allergens, administered jointly. Five days after this single IgG treatment/dual Ag challenge, mice were re-exposed to either the specific or bystander allergen with respect to the specificity of the mAb instilled before. Thus, mice treated with anti-OVA IgG2a in conjunction with h-cat and OVA exposure were re-exposed to either OVA (specific allergen) or h-cat (bystander allergen) (Fig. 8*A*). Inversely, mice treated with anti-h-cat and exposed to both allergens were re-exposed to either specific or bystander allergen, viz h-cat or OVA, respectively (Fig. 8*B*). In both experimental setups, airway inflammation triggered by the secondary challenge with specific allergen (filled bars) showed a 60–80% reduction of airway eosinophilia. Strikingly, in both instances, a secondary challenge with the bystander allergen (striped bars) showed near-identical repression of airway eosinophilia compared with its specific counterpart. Thus, bystander repression along with perseverance of repression after clearance of IgG, 5 days after IgG instillation, represent additional attributes of the IgG-induced mechanism of allergic inflammation repression.

Having shown a lasting modification of the allergen-induced airway response, we next verified the role of IFN-γ, a cytokine up-regulated by IgG treatment. Using a similar first and second challenge experimental setup, but now applying a matched combination of IgG and allergen, viz treatment with anti-OVA IgG2a and challenges with OVA, total cell counts and eosinophil cell counts in BAL fluid from sensitized wild-type (wt) and IFN-γ knockout mice were verified. Whereas wt mice responded to IgG treatment by a pronounced reduction in total cell and eosinophil recruitment, such responses were absent in IFN-γ knockout mice (Fig. 8*C*). This result identifies IFN-γ as an important constituent of the repressive mechanism promoted by topical IgG.

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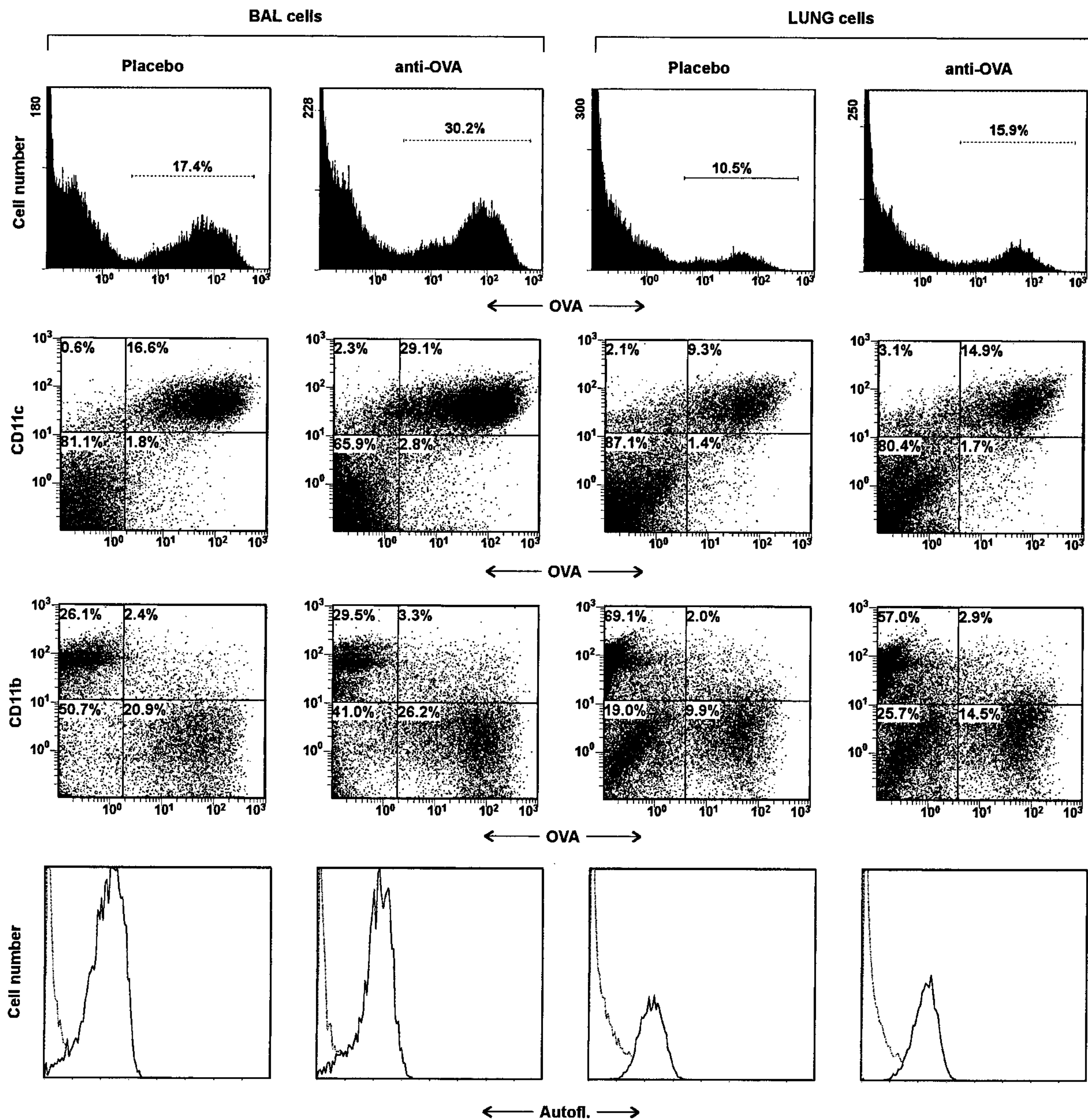

FIGURE 5. Topical IgG promotes OVA capture by CD11c^{+} alveolar macrophages. Placebo-treated mice or mice treated with anti-OVA IgG2a were analyzed for OVA-Fluor X-positive cells in the BAL fluid and lung tissue cell suspensions by flow cytometry. Mice ($n = 5$) were sacrificed 2 h after OVA-Fluor X instillation. Individual samples were pooled, and 120,000 events were analyzed. Large granular cells were gated based on forward and side scatter properties. Values inside the dot plots and histograms represent the percentage of cells exhibiting a particular fluorescence profile. Lower overlay histograms were in addition gated on OVA-Fluor X-positive cells (bold line). Data are representative of three separate experiments.

Discussion

To date, the treatment of chronic airway inflammation triggered in asthmatics by airborne allergens mainly depends on anti-inflammatory drugs, primarily corticosteroids, administered orally or by inhalation. Recently, a steroid-sparing treatment has been achieved in patients with moderate to severe allergic asthma by i.v. administration of a humanized anti-IgE Ab (34). IgE depletion by anti-IgE mAb effectively decreases the airway inflammation in allergen-provoked patients (35, 36). Although these treatments are adequate in temporarily relieving the symptoms, they apparently do not affect the underlying disease process. In an attempt to modify the disease process by interfering with the afferent phase of the inflammatory cascade, we topically administered anti-allergen IgG2a, hereby altering the immune properties of subsequently inhaled allergen. The resulting reduction in eosinophils present in BAL fluid and diminished influx of inflammatory cells in the peribronchial areas of the lungs, each constituting well-established parameters for allergic airway inflammation, assign an anti-inflammatory function to the intratracheally instilled anti-allergen IgG. This anti-inflammatory function was generated independently of the IgG isotype applied as indicated by the similar effects obtained also with mAbs from the IgG1 or IgG2b isotype. Repression of allergic airway inflammation was further confirmed by the reduced numbers of PAS-positive cells in lung tissue sections, thus indicating suppression of goblet cell metaplasia.

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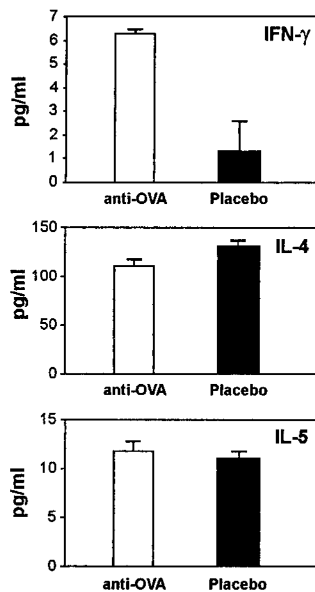

FIGURE 6. Increased IFN-γ levels in BAL fluid of IgG-treated mice. Mice were subjected to the prolonged exposure protocol, combined with IgG or placebo treatment. Twenty-four hours after the last OVA-aerosol exposure, BAL was performed, and levels of IFN-γ, IL-4, and IL-5 present in the fluid were determined by ELISA. Data represent means ± SEM ($n = 5$).

Reduced eosinophil influx was also reported for allergen-specific IgA (37). Immune exclusion by preventing access of the allergen to the lung immune system was proposed as mechanism for the action of anti-IgA on airway inflammation. Also, serum IgG generated by specific allergy vaccination, currently the only allergy treatment that not only has a symptomatic effect, but also influences the course of the allergic disease itself (38), promotes a blocking of serum IgE-facilitated allergen presentation, resulting in higher allergen threshold levels to obtain T cell activation (25). In contrast to these exclusively cognate modes of action, topical IgG antagonizes allergic airway inflammation by an alternative and active process as substantiated among others by the requirement for the IgG Fc domain and the increment of airway IFN-γ levels and Th1 reactivity. Although in mucosal secretions secretory IgA is the main isotype and IgG is underrepresented, a consequence of the barrier function of the lung epithelial layers and sensitivity of IgG to degradation by proteases abundantly present on mucosal surfaces (33), several lines of evidence support a contribution of IgG to mucosal immunity in adults. Thus, in the human lung, mucosal infection by respiratory syncytial virus was prevented by systemic administration of IgG (39). Inversely, humans deficient in IgG exhibit an increased incidence of infections caused by microorganisms that invade the respiratory tract. Mucosal secretions obtained from adult humans showed a distinct IgG specificity pattern when compared with serum of the same individuals (40). FcRn, the MHC class I-related FcR for IgG, mediates bidirectional transcytosis of IgG in polarized epithelial cells and was recently shown to be functionally expressed on bronchial epithelial cells of the adult human, thus providing a mechanism by which IgG may cross epithelial barriers to function in mucosal secretions (41–43). Our results therefore indicate that serum IgG, induced by extended exposure to common inhalant allergens in nonallergic individuals or during vaccination therapy, may contribute to the nonallergic response by acting also at the topical level, directly interfering with the processes that in the airways give rise to allergic inflammation.

In addressing the nature of the anti-inflammatory mechanism promoted by airway IgG, direct IgG-mediated functions were verified as well as their downstream consequences for the allergen-triggered airway response. The failure of isotype-matched anti-OVA or anti-h-cat Abs to counteract airway inflammation triggered by the opposite allergen in single allergen challenge experiments (Fig. 2*B*, and data not shown) demonstrates the requirement for a specific Ag-IgG interaction in addition to the requirement for the IgG Fc domain. Ag- and Fc-dependent cross-linking of FcγR mediates activation of inflammatory effectors and endocytosis of Ag by phagocytes (27, 44). Under normal conditions, low levels of IgG are present in the airway lumen and submucosa (45), rendering APCs dependent on pinocytosis for Ag acquisition or on endocytosis by alternative membrane-bound receptors, such as FcϵR, macrophage mannose receptor, and DEC-205 (46, 47). Pinocytosis is permissive to Ag presentation by MHC class II molecules in dendritic cells (47), but not in macrophages (48). By

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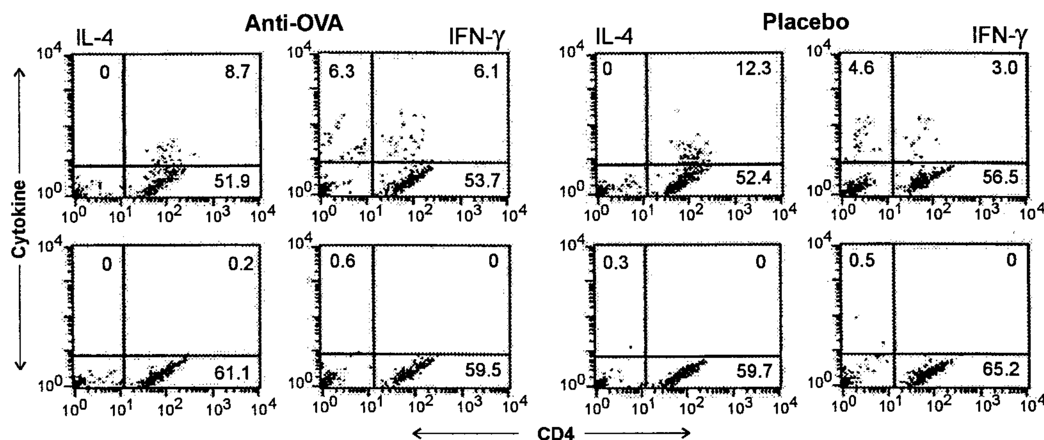

FIGURE 7. Shift in Th1/Th2 ratios by IgG treatment. Intracellular cytokine staining for CD4$^+$ T cells producing Th1- or Th2-related cytokines reveals a shift from a Th2-skewed response in placebo-treated airways (Th2/Th1 ratio of 4:1) to a more balanced response in the airways of IgG-treated mice (Th2/Th1 ratio of 1.4:1). Mice ($n = 4$) were treated as described in Fig. 6. Cells in BAL fluid from each treated group were pooled, stimulated in culture with anti-CD3 and anti-CD28, and stained for surface CD4 and intracellular IL-4 or IFN-γ. Quadstat analyses of two-parameter dot plots are shown. Forward and side scatter were used to gate lymphocytes. Five thousand events were analyzed. *Upper panels*, Show cells stained with the respective anti-cytokine Abs in the absence of blocking Abs. *Lower panels* show nonspecific cytokine staining, determined by preincubation with excess unlabeled anti-cytokine Abs.

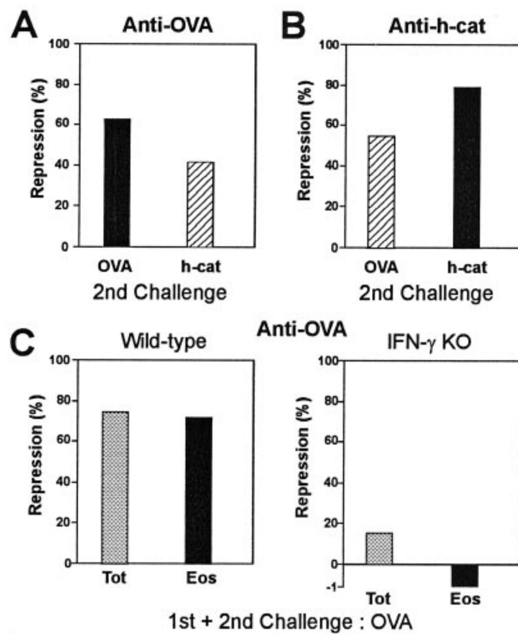

FIGURE 8. Repression of bystander allergen-triggered airway eosinophilia and dependence on IFN-γ. Allergic inflammation repression by topical IgG prevails after clearance of instilled IgG and affects both specific and unrelated bystander allergen-triggered responses. OVA and h-cat dual-sensitized mice were treated with either anti-OVA (*A*) or anti-h-cat (*B*), followed by exposure to both allergens (short exposure regimen). Five days after this single IgG treatment/dual Ag exposure, mice were exposed to a secondary challenge with either h-cat or OVA, followed 48 h later by BAL ($n = 4$). Shown is the repression percentage of eosinophil numbers present in the BAL fluid from IgG-treated mice. Filled bars represent matched IgG and allergen combinations; striped bars show mismatched combinations being model for bystander allergen activity. The repression percentage was calculated as (1 − (IgG treated − min)/(placebo treated − min)) × 100. Minimum values (min) represent mice not exposed to a secondary allergen challenge and amounted to 4 (±1) × 10^5 for all treated groups. Eosinophil counts for placebo-treated mice exposed to a secondary challenge amounted to 18 (±2) × 10^5 for OVA and h-cat. *C*, IFN-γ deficiency restores eosinophilic airway inflammation in IgG-treated mice. Wt and IFN-γ knockout mice were assayed according to a similar scheme of primary and secondary allergen challenges, however using solely a matched IgG and allergen combination, viz anti-OVA IgG2a and OVA. Gray bars show the repression percentage of total cell numbers present in the BAL fluid from IgG-treated mice; filled bars represent the repression percentage of eosinophil numbers ($n = 5$). Minimum values from mice not exposed to a secondary challenge were for wt mice, 0.8 (±0.2) × 10^5 total cells and 0.02 (±0.01) × 10^5 eosinophils; for IFN-γ knockout mice, 0.4 (±0.1) × 10^5 total cells and 0.03 (±0.01) × 10^5 eosinophils. Cell counts for placebo-treated mice exposed to a secondary challenge were for wt mice, 19.8 (±4.6) × 10^5 total cells and 13.4 (±3.1) × 10^5 eosinophils; for IFN-γ knockout mice, 9.4 (±2.6) × 10^5 total cells and 4.9 (±1.6) × 10^5 eosinophils.

enabling FcγR-mediated endocytosis of specific Ag, IgG instilled in the airways may facilitate Ag capture by cells lining the airways, either alveolar macrophages or dendritic cells. Pulmonary dendritic cells are identified using three simultaneous immunofluorescent criteria: low autofluorescence, CD11c positivity, and MHC class II positivity. Macrophages are high autofluorescence, CD11c positive, but dim or negative for MHC class II. Based on these criteria, our results using fluorescent-labeled OVA demonstrate an enhanced acquisition of OVA by alveolar macrophages, and not or only marginally by macrophages or dendritic cells isolated from lung tissue. Besides indicating a range of action of the instilled IgG limited to the lumen of the airways, this result raises the issue of how the binding of specific IgG to BAL cells influences the allergic inflammation occurring mainly peribronchially. Possibly, migration of macrophages across the epithelium is a dynamic bidirectional process involving egression, but also a reverse migration back into the stromal compartment. Although requiring further investigation, our observation that intratracheal instillation of macrophages similarly repressed the development of allergic airway inflammation (G. Pynaert and J. Grooten, unpublished results) confirms that macrophages primarily residing in the lumen of the airways are indeed capable of interfering directly or indirectly with inflammatory processes occurring in the peribronchial areas of the lungs. Also, to exert this anti-inflammatory activity, the instilled macrophages required ex vivo pulsing with OVA. Therefore, a likely consequence of the IgG-facilitated acquisition of OVA by alveolar macrophages is to enhance the Ag-presenting ability of the cells and hereby their capacity to specifically stimulate effector $CD4^+$ T cell subsets. Especially $CD4^+$ T cells of the Th1 lineage are probable targets, as indicated by the increment of secreted IFN-γ following IgG treatment along with the shift from a Th2-skewed response to a more balanced Th1/Th2 response, and in agreement with several studies showing that macrophage APC activity is associated with priming for Th1 cells (49–51).

In allergy, the Th1-promoting APC activity of lung macrophages has been reported to counteract airway eosinophilia by an IFN-γ-dependent mechanism (52). Also, our results using IFN-γ knockout mice showed a dependence on IFN-γ of the IgG-induced repression of allergic airway inflammation. The diminished capacity of IFN-γ-deficient mice to clear eosinophilic inflammation (53) may explain their unresponsiveness to IgG treatment. However, arguing against such mechanism, we found that in IFN-γ knockout mice eosinophilic inflammation 5 days after allergen challenge was cleared to levels comparable to those in wt mice (legend to Fig. 8*C*). Therefore, the observed dependence on IFN-γ most likely reflects a direct, although not necessarily exclusive, involvement of the cytokine in the inflammation repression/prevention by topical IgG. The combined action of IFN-γ and FcγR signaling may promote the differentiation of alveolar macrophages into functional APCs. Additionally, IFN-γ may act directly on inflammatory effector cells such as eosinophils (54–56). Also, metaplastic goblet cells are direct targets of IFN-γ (55, 57), in line with our observation of reduced goblet cell metaplasia in IgG-treated airways. Furthermore, inhibition by IFN-γ of inflammatory functions exerted by Th2-related cytokines, especially IL-4 and IL-13 (58, 59), provides for counterregulation of Th2-dependent inflammatory responses, as illustrated by the repressive effect on allergic inflammation of inhaled IFN-γ (60, 61). Correspondingly, the augmented Th1 cytokine profile and the IFN-γ-dependent repression of Th2-dependent inflammation observed in IgG-treated and allergen-challenged mice are indicative of counterregulation. Yet, in similar mouse models for allergic airway inflammation, transfer experiments with Th1 cells failed to counterbalance allergic inflammation and, to the contrary, aggravated pulmonary disease (62, 63). Ag-specific Th1 cells therefore do not protect against Th2-mediated allergic disease and, to the contrary, rather initiate an additional Th1-driven acute lung pathology (64). The contrasting outcome of IgG treatment, apparent from the absence of neutrophil recruitment, points to a more complex mechanism of repression involving additional regulatory cytokines such as IL-10 and/or TGF-β. Although induced by Ag-specific cognitive mechanisms, these regulatory cytokines exert bystander activity, repressing concomitant immune responses triggered by unrelated Ags. T regulatory cells, promoting immune tolerance through the production of IL-10 and/or TGF-β, are a prominent example of a repressive mechanism that, although activated in an Ag-specific way, extends its repressive activity to bystander responses (65, 66). Importantly, using

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dual-sensitized mice challenged with both allergens, but treated with a single mAb, we similarly observed repression of allergic inflammation triggered by the bystander allergen. Thus, contrarily to the requirement for a specific allergen-IgG interaction at the time of treatment, the repressive mechanism extends to bystander inflammatory responses. This bystander repression may be of relevance also in view of the broadening of airway sensitivity to supplementary allergens frequently observed in patients suffering from respiratory allergies or asthma. Whereas the increasingly Th2 skewing of allergic airways may promote Th2 reactivity to jointly inhaled unrelated Ags, our results indicate that topical IgG counters this broadening of airway sensitivity.

Taken together, the observed key immune actions of topical IgG, namely protection against allergic inflammation, prevalence of protection after clearance of IgG, and cross repression of airway inflammation triggered by secondary allergens, assign an important regulatory role to anti-allergen IgG responses in exposed, allergic and nonallergic individuals. Furthermore, these functional attributes render a topical application of anti-allergen IgG by direct administration to the airways an attractive approach to immunotherapy of allergic asthma.

References

1. Russell, M. W., M. Kilian, and M. E. Lamm. 1999. Biological activities of IgA. In *Mucosal Immunology*. P. L. Ogra, ed. Academic Press, San Diego, p. 225.
2. Williams, R. C., and R. J. Gibbons. 1972. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science 177:697.*
3. Kraehenbuhl, J. P., and M. R. Neutra. 1992. Molecular and cellular basis of immune protection of mucosal surfaces. *Physiol. Rev. 72:853.*
4. Brandtzaeg, P. 1996. History of oral tolerance and mucosal immunity. *Ann. NY Acad. Sci. 778:1.*
5. Phalipon, A., A. Cardona, J. P. Kraehenbuhl, L. Edelman, P. J. Sansonetti, and B. Corthésy. 2002. Secretory component: a new role in secretory IgA-mediated immune exclusion in vivo. *Immunity 17:107.*
6. Lycke, N. 1998. T cell and cytokine regulation of the IgA response. *Chem. Immunol. 71:209.*
7. Constant, S. L., K. S. Lee, and K. Bottomly. 2000. Site of antigen delivery can influence T cell priming: pulmonary environment promotes preferential Th2-type differentiation. *Eur. J. Immunol. 30:840.*
8. Hodge, L. M., M. Marinaro, H. P. Jones, J. R. McGhee, H. Kiyono, and J. W. Simecka. 2001. Immunoglobulin A (IgA) responses and IgE-associated inflammation along the respiratory tract after mucosal but not systemic immunization. *Infect. Immun. 69:2328.*
9. Jones, H. P., L. M. Hodge, K. Fujihashi, H. Kiyono, J. R. McGhee, and J. W. Simecka. 2001. The pulmonary environment promotes Th2 cell responses after nasal-pulmonary immunization with antigen alone, but Th1 responses are induced during instances of intense immune stimulation. *J. Immunol. 167:4518.*
10. Simecka, J. W., R. J. Jackson, H. Kiyono, and J. R. McGhee. 2000. Mucosally induced immunoglobulin E-associated inflammation in the respiratory tract. *Infect. Immun. 68:672.*
11. Corry, D. B. 2002. Emerging immune targets for the therapy of allergic asthma. *Nat. Rev. Drug Discov. 1:55.*
12. Yssel, H., and H. Groux. 2000. Characterization of T cell subpopulations involved in the pathogenesis of asthma and allergic diseases. *Int. Arch. Allergy Immunol. 121:10.*
13. Borish, L. C., H. S. Nelson, M. J. Lanz, L. Claussen, J. B. Whitmore, J. M. Agosti, and L. Garrison. 1999. Interleukin-4 receptor in moderate atopic asthma: a phase I/II randomized, placebo-controlled trial. *Am. J. Respir. Crit. Care Med. 160:1816.*
14. 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.*
15. Riffo-Vasquez, Y., and D. Spina. 2002. Role of cytokines and chemokines in bronchial hyperresponsiveness and airway inflammation. *Pharmacol. Ther. 94:185.*
16. Barnes, P. J. 2000. Anti-IgE therapy in asthma: rationale and therapeutic potential. *Int. Arch. Allergy Immunol. 123:196.*
17. Busse, W., J. Corren, B. Q. Lanier, M. McAlary, A. Fowler-Taylor, G. D. Cioppa, A. van As, and N. Gupta. 2001. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J. Allergy Clin. Immunol. 108:184.*
18. Ishizaka, K., T. Ishizaka, and M. M. Hornbrook. 1967. Allergen-binding activity of γ-E, γ-G and γ-A antibodies in sera from atopic patients: in vitro measurements of reaginic antibody. *J. Immunol. 98:490.*
19. Platts-Mills, T. A., M. J. Snajdr, K. Ishizaka, and A. W. Frankland. 1978. Measurement of IgE antibody by an antigen-binding assay: correlation with PK activity and IgG and IgA antibodies to allergens. *J. Immunol. 120:1201.*
20. Vailes, L. D., M. S. Perzanowski, L. M. Wheatley, T. A. Platts-Mills, and M. D. Chapman. 2001. IgE and IgG antibody responses to recombinant Alt a 1 as a marker of sensitization to Alternaria in asthma and atopic dermatitis. *Clin. Exp. Allergy 31:1891.*
21. Platts-Mills, T., J. Vaughan, S. Squillace, J. Woodfolk, and R. Sporik. 2001. Sensitization, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet 357:752.*
22. Perzanowski, M. S., E. Rönmark, T. A. Platts-Mills, and B. Lundbäck. 2002. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am. J. Respir. Crit. Care Med. 166:696.*
23. Aalberse, R. C., R. van der Gaag, and J. van Leeuwen. 1983. Serologic aspects of IgG4 antibodies. I. Prolonged immunization results in an IgG4-restricted response. *J. Immunol. 130:722.*
24. Muller, U., C. A. Akdis, M. Fricker, M. Akdis, T. Blesken, F. Bettens, and K. Blaser. 1998. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A_2 induces specific T-cell anergy in patients allergic to bee venom. *J. Allergy Clin. Immunol. 101:747.*
25. Van Neerven, R. J. J., T. Wikborg, G. Lund, B. Jacobsen, Å. Brinch-Nielsen, J. Arnved, and H. Ipsen. 1999. Blocking antibodies induced by specific allergy vaccination prevent the activation of $CD4^+$ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J. Immunol. 163:2944.*
26. Amigorena, S., J. Salamero, J. Davoust, W. H. Fridman, and C. Bonnerot. 1992. Tyrosine-containing motif that transduces cell activation signals also determines internalization and antigen presentation via type III receptors for IgG. *Nature 358:337.*
27. Watts, C. 1997. Capture and processing of exogenous antigens for presentation on MHC molecules. *Annu. Rev. Immunol. 15:821.*
28. Tagawa, Y., K. Sekikawa, and Y. Iwakura. 1997. Suppression of concanavalin A-induced hepatitis in IFN-$\gamma^{-/-}$ mice, but not in TNF-$\alpha^{-/-}$ mice: role for IFN-γ in activating apoptosis of hepatocytes. *J. Immunol. 159:1418.*
29. Gefter, M. L., D. H. Margulies, and M. D. Scharff. 1977. A simple method for polyethylene glycol-promoted hybridization of mouse myeloma cells. *Somatic Cell Genet. 3:231.*
30. Lamoyi, E. 1986. Preparation of $F(ab')_2$ fragments from mouse IgG of various subclasses. *Methods Enzymol. 121:652.*
31. Tournoy, K. G., J. C. Kips, and R. A. Pauwels. 2000. Endogenous interleukin-10 suppresses allergen-induced airway inflammation and nonspecific airway responsiveness. *Clin. Exp. Allergy 30:775.*
32. Winterrowd, G. E., and J. E. Chin. 1999. Flow cytometric detection of antigen-specific cytokine responses in lung T cells in a murine model of pulmonary inflammation. *J. Immunol. Methods 226:105.*
33. Lindh, E. 1975. Increased resistance of immunoglobulin A dimers to proteolytic degradation after binding of secretory component. *J. Immunol. 114:284.*
34. Milgrom, H., R. B. Fick, Jr., J. Q. Su, J. D. Reimann, R. K. Bush, M. L. Watrous, and W. J. Metzger. 1999. Treatment of allergic asthma with monoclonal anti-IgE antibody. *N. Engl. J. Med. 341:1966.*
35. Boulet, L. P., K. R. Chapman, J. Cote, S. Kalra, R. Bhagat, V. A. Swystun, M. Laviolette, L. D. Cleland, F. Deschesnes, J. Q. Su, et al. 1997. Inhibitory effects of an anti-IgE antibody E25 on allergen-induced early asthmatic response. *Am. J. Respir. Crit. Care Med. 155:1835.*
36. Fahy, J. V., H. E. Fleming, H. H. Wong, J. T. Liu, J. Q. Su, J. Reimann, R. B. Fick, Jr., and H. A. Boushey. 1997. The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am. J. Respir. Crit. Care Med. 155:1828.*
37. Schwarze, J., G. Cieslewicz, A. Joetham, L. K. Sun, W. N. Sun, T. W. Chang, E. Hamelmann, and E. W. Gelfand. 1998. Antigen-specific immunoglobulin-A prevents increased airway responsiveness and lung eosinophilia after airway challenge in sensitized mice. *Am. J. Respir. Crit. Care Med. 158:519.*
38. Bousquet, J., R. Lockey, and H. J. Malling. 1998. Allergen immunotherapy: therapeutic vaccines for allergic diseases: a WHO position paper. *J. Allergy Clin. Immunol. 102:558.*
39. Groothuis, J. R., E. A. Simoes, M. J. Levin, C. B. Hall, C. E. Long, W. J. Rodriguez, J. Arrobio, H. C. Meissner, D. R. Fulton, and R. C. Welliver. 1993. Prophylactic administration of respiratory syncytial virus immune globulin to high-risk infants and young children: the Respiratory Syncytial Virus Immune Globulin Study Group. *N. Engl. J. Med. 329:1524.*
40. Berneman, A., L. Belec, V. A. Fischetti, and J. P. Bouvet. 1998. The specificity patterns of human immunoglobulin G antibodies in serum differ from those in autologous secretions. *Infect. Immun. 66:4163.*
41. Dickinson, B. L., K. Badizadegan, Z. Wu, J. C. Ahouse, X. Zhu, N. E. Simister, R. S. Blumberg, and W. I. Lencer. 1999. Bidirectional FcRn-dependent IgG transport in a polarized human intestinal epithelial cell line. *J. Clin. Invest. 104:903.*
42. McCarthy, K. M., Y. Yoong, and N. E. Simister. 2000. Bidirectional transcytosis of IgG by the rat neonatal Fc receptor expressed in a rat kidney cell line: a system to study protein transport across epithelia. *J. Cell Sci. 113:1277.*
43. Spiekermann, G. M., P. W. Finn, E. S. Ward, J. Dumont, B. L. Dickinson, R. S. Blumberg, and W. I. Lencer. 2002. Receptor-mediated immunoglobulin G transport across mucosal barriers in adult life: functional expression of FcRn in the mammalian lung. *J. Exp. Med. 196:303.*
44. Ravetch, J. V., and S. Bolland. 2001. IgG Fc receptors. *Annu. Rev. Immunol. 19:275.*
45. Peebles, R. S., R. G. Hamilton, L. M. Lichtenstein, M. Schlosberg, M. C. Liu, D. Proud, and A. Togias. 2001. Antigen-specific IgE and IgA antibodies in bronchoalveolar lavage fluid are associated with stronger antigen-induced late phase reactions. *Clin. Exp. Allergy 31:239.*

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46. Jiang, W., W. J. Swiggard, C. Heufler, M. Peng, A. Mirza, R. M. Steinman, and M. C. Nussenzweig. 1995. The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature 375:151.*
47. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: down-regulation by cytokines and bacterial products. *J. Exp. Med. 182:389.*
48. Peppelenbosch, M. P., M. Desmedt, G. Pynaert, S. J. H. van Deventer, and J. Grooten. 2000. Macrophages present pinocytosed exogenous antigen via MHC class I whereas antigen ingested by receptor-mediated endocytosis is presented via MHC class II. *J. Immunol. 165:1984.*
49. Hsieh, C. S., S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy. 1993. Development of TH1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science 260:547.*
50. Chattergoon, M. A., T. M. Robinson, J. D. Boyer, and D. B. Weiner. 1998. Specific immune induction following DNA-based immunization through in vivo transfection and activation of macrophages/antigen-presenting cells. *J. Immunol. 160:5707.*
51. Desmedt, M., P. Rottiers, H. Dooms, W. Fiers, and J. Grooten. 1998. Macrophages induce cellular immunity by activating Th1 cell responses and suppressing Th2 cell responses. *J. Immunol. 160:5300.*
52. Tang, C., M. D. Inman, N. van Rooijen, P. Yang, H. Shen, K. Matsumoto, and P. M. O'Byrne. 2001. Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN-γ-dependent mechanism. *J. Immunol. 166:1471.*
53. Coyle, A. J., S. Tsuyuki, C. Bertrand, S. Huang, M. Aguet, S. S. Alkan, and G. P. Anderson. 1996. Mice lacking the IFN-γ receptor have impaired ability to resolve a lung eosinophilic inflammatory response associated with a prolonged capacity of T cells to exhibit a Th2 cytokine profile. *J. Immunol. 156:2680.*
54. Li, X. M., R. K. Chopra, T. Y. Chou, B. H. Schofield, M. Wills-Karp, and S. K. Huang. 1996. Mucosal IFN-γ gene transfer inhibits pulmonary allergic responses in mice. *J. Immunol. 157:3216.*
55. Cohn, L., R. J. Homer, N. Niu, and K. Bottomly. 1999. T helper 1 cells and interferon γ regulate allergic airway inflammation and mucus production. *J. Exp. Med. 190:1309.*
56. Huang, T. J., P. A. MacAry, P. Eynott, A. Moussavi, K. C. Daniel, P. W. Askenase, D. M. Kemeny, and K. F. Chung. 2001. Allergen-specific Th1 cells counteract efferent Th2 cell-dependent bronchial hyperresponsiveness and eosinophilic inflammation partly via IFN-γ. *J. Immunol. 166:207.*
57. Shi, Z. O., M. J. Fischer, G. T. De Sanctis, M. R. Schuyler, and Y. Tesfaigzi. 2002. IFN-γ, but not Fas, mediates reduction of allergen-induced mucous cell metaplasia by inducing apoptosis. *J. Immunol. 168:4764.*
58. Dickensheets, H. L., C. Venkataraman, U. Schindler, and R. P. Donnelly. 1999. Interferons inhibit activation of STAT6 by interleukin 4 in human monocytes by inducing SOCS-1 gene expression. *Proc. Natl. Acad. Sci. USA 96:10800.*
59. So, E. Y., H. H. Park, and C. E. Lee. 2000. IFN-γ and IFN-α posttranscriptionally down-regulate the IL-4-induced IL-4 receptor gene expression. *J. Immunol. 165: 5472.*
60. Lack, G., H. Renz, J. Saloga, K. L. Bradley, J. Loader, D. Y. Leung, G. Larsen, and E. W. Gelfand. 1994. Nebulized but not parenteral IFN-γ decreases IgE production and normalizes airways function in a murine model of allergen sensitization. *J. Immunol. 152:2546.*
61. Lack, G., K. L. Bradley, E. Hamelmann, H. Renz, J. Loader, D. Y. Leung, G. Larsen, and E. W. Gelfand. 1996. Nebulized IFN-γ inhibits the development of secondary allergic responses in mice. *J. Immunol. 157:1432.*
62. Hansen, G., G. Berry, R. H. DeKruyff, and D. T. Umetsu. 1999. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. *J. Clin. Invest. 103:175.*
63. Randolph, D. A., R. Stephens, C. J. Carruthers, and D. D. Chaplin. 1999. Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation. *J. Clin. Invest. 104:1021.*
64. Tournoy, K. G., J. C. Kips, and R. A. Pauwels. 2002. Is Th1 the solution for Th2 in asthma? *Clin. Exp. Allergy 32:17.*
65. Cottrez, F., S. D. Hurst, R. L. Coffman, and H. Groux. 2000. T regulatory cells 1 inhibit a Th2-specific response in vivo. *J. Immunol. 165:4848.*
66. Weiner, H. L. 2001. Induction and mechanism of action of transforming growth factor-β-secreting Th3 regulatory cells. *Immunol. Rev. 182:207.*

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