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Resolution of Der p1-Induced Allergic Airway Inflammation Is Dependent on CD4⁺CD25⁺Foxp3⁺ Regulatory Cells

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Allergic asthma, which is reaching epidemic levels in the Western world (1), is triggered by inhalation of environmental allergens like cat dander, pollen, and house dust mite proteins (2). One of the major dust mites in the United Kingdom is Dermatophagoides pteronyssinus (2), which secretes the allergenic cysteine protease Der p1 in feces (3). Inhalation of allergens in sensitive individuals leads to a cascade of immune responses characterized by allergen-specific IgE, mastocytosis, goblet cell hyperplasia and eosinophilia, contributing to airway hyperreactivity and symptomatic disease (1–4).

There are difficulties in the study of asthma in patients with established disease, and murine models have been extensively used to examine the role of the acquired immune response in induction of allergic airway inflammation (AAI)⁵ in sensitized animals (3, 5–11). Most of these studies examine events within the lungs 3–4 days after challenge, but there are fewer studies on the kinetics of resolution of disease markers after cessation of allergen exposure (12). The most widely used model system is OVA-induced allergy. However, although OVA is a gut allergen in humans (13), which may induce respiratory symptoms in a minority of patients when ingested (13), it is not a natural inducer of asthma. Using such models, the essential role of T cells in induction of AAI through Th2 development and the secretion of the cytokines IL-4, IL-5, IL-9, and IL-13 is well documented (14, 15). However, how T cells control the local airway immune response and their role in resolution of lung inflammation is still under characterized. It is widely accepted that allergic disease and asthma may be linked to a dysregulation in immune balance and in recent years, regulatory T cells (Tregs) have been demonstrated to play an essential role control of both innate and adaptive immune responses during Th2-mediated AAI (11, 16–18). Other studies have used murine models of OVA-induced AAI and TCR transgenic animals to determine the role of Ag-specific Tregs in induction and resolution of allergen-induced inflammation (11, 19, 20). Adoptive transfer of Ag-specific Tregs was reported to prevent induction of OVA-induced airway hyperreactivity, lung eosinophilia, and Th2 cytokine production in the lung (11). In a study using whole house dust mite extract as allergen, Lewkowich et al. (18) showed that depleting anti-CD25 Ab given to C3H mice before sensitization enhanced the allergic airway response but had no effect on the response in A/J mice and also increased the ability of lung DCs from C3H but not A/J mice to present the Ag.

However, there is still little information on the normal role of Tregs in resolution of airway inflammation or on whether transgenic Ag-specific Tregs act differently from naturally occurring or endogenously generated Tregs in sensitized wild-type animals. Naturally occurring Tregs constitutively express both CD25 and Foxp3 and constitute up to 10% of the peripheral T cell pool in naive animals (21). CD4⁺CD25⁺Foxp3⁺ T cells regulate immune...
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FIGURE 1. Experimental model. C57BL/6 mice were sensitized i.p. ×2 with 10 μg of Der p1 in alum and challenged i.t. ×2 with either PBS (mock challenged) or 10 μg of Der p1 in PBS (allergen challenged). To investigate resolution of inflammation, mice were culled at various time points from 2 to 21 days after challenge (protocol A). To investigate the role of IL-10 and CD25+ Tregs in this system, CD25+CD4+ T cells, and anti-IL-10R (1B1.2) or isotype control Ab were administered 1 day before i.t. challenge (protocol A) or anti-CD25 (PC61), or isotype control mAb was administered 1 wk before i.t. challenge (protocol B).

responses through the secretion of immunosuppressive cytokines such as IL-10 and TGFβ (21–23). Previous work showed that induction of nasal tolerance to Der p1 inhibits lung inflammation and

FIGURE 2. Histological analysis of lung tissue. A. Schematic representation of the lung compartments assessed for inflammation. B–D. Sections from lungs 4 days after second i.t. challenge with Der p1: peribronchiolar infiltrate stained with anti-eosinophil Ab (B); H&E stain (C); periodic acid-Schiff stain (PAS) for Goblet cells (D). E and F. Sections from lungs 4 days after second i.t. challenge with PBS: H&E stain (E); periodic acid-Schiff stain (PAS) for Goblet cells (F). Black bars, 100 μm.

FIGURE 3. Der p1 airway challenge induces acute lung inflammation, eosinophilia, and goblet cell hyperplasia. Interstitial lung inflammation was scored in perivascular (A), bronchial (B), peribronchial (C), and alveolar wall compartments (D). E. Percentage of eosinophils in interstitial infiltrate; numbers of eosinophils recovered in BAL (F); percentage of goblet cells in airway epithelium (G). Data accumulated from three experiments (3–4 mice/group/experiment). (D, day post challenge). ○, Individual mice; bars, median value per group; PBS, pooled results experiments (3–4 mice/group/experiment). * Significant difference (Mann-Whitney) from mock-challenged mice; †, Significant difference (Mann-Whitney) from mock-challenged mice (p < 0.001).
did restore the ability of dMLN cells to secrete Th2 cytokines suggesting that Treg control of AAI and effector T cell expansion in lymph nodes operate through different mechanisms. Overall, this study identifies a clear role for CD25+ cells in control and in resolution of AAI.

Materials and Methods

Animals

Female C57BL/6j mice (6–8 wk old) (Harlan-Olac) were housed in conventional specific and opportunistic pathogen-free facilities. All experiments were performed in accordance with the U.K. Home Office Scientific Procedures Act (1986) and local ethical approval.

Sensitization, airway challenge, and in vivo Ab treatment

As shown in Fig. 1, mice were sensitized by two i.p. injections of 10 μg of Der p1 absorbed on 2.25 mg of aluminum hydroxide (Imject ALUM; Pierce) in 100 μl. AAI was induced by two intratracheal (i.t.) instillations of 10 μg of Der p1 in 50 μl of PBS in anesthetized animals (0.1 mg/kg Avertin; 2,2,2-tribromoethanol). Sensitized control mice were mock challenged 1 day before i.t. challenge. Control and in resolution of AAI.

FIGURE 4. Systemic immune responses to Der p1 airway challenge. See Results and Materials and Methods for further details. A–D, In vitro responses of dMLN cells from individual mice 2, 4, 6, 14, and 21 days after Der p1 (D) or PBS challenge (P) in vivo. Representative data from four experiments (n = 3–5/group/experiment); bars = median group value. A, [3H]Thymidine incorporation at 72 h; B–D, cytokine secretion at 48 h. Cells cultured in medium alone secreted <100 pg/ml of any cytokine. E–H, Representative experiment (n = 4) showing responses of mononuclear cells isolated from Der p1-challenged lungs at 4 days post challenge. Lung mononuclear cells (MNCs) also proliferate in response to Der p1 (Dp1) but not medium only (m) and produce IL-5, IL-13, and IL-10. F–H also show that BAL fluid from the same animals contained detectable levels of IL-5, IL-13, and IL-10. Representative data from four experiments (n = 3–5/group/experiment) showing serum Ab responses on day 2 (J and K) and day 21 (J and L) post challenge for Der p1-specific IgG1 (J and K) and IgE (K and L). A, absorbance. Differences were considered significant (Mann-Whitney) when p < 0.05 (∗).
Lymph node and lung lymphocyte restimulation

Single cell suspensions of dMLN and PBS perfused whole lungs were made by passing tissues through 40-μm sterile sieves. Lung cells were then spun over Lympholyte-M (VH-Bio) to isolate mononuclear leukocytes.

Analysis of Tregs

Single cell suspensions from dMLN or lungs were resuspended at 5 × 10^6 cells/ml and stained with extracellular CD4 and CD25 (BD Pharmingen) and intracellular Foxp3 as recommended by the manufacturer (eBioscience). CD25 depletion was confirmed by analysis with anti-CD25 Abs (clones 7D4 and 3C7) and flow cytometry on a BD FACS Calibur using CELLQuest software.

Der p1-specific IgG1, IgG2a, and IgE

Microtiter wells were coated with 0.25 μg of Der p1 in 50 μl of 0.05 M carbonate bicarbonate buffer (pH 9.6). Nonspecific binding was blocked with 3% BSA in PBS. For IgG1 and IgG2a analysis, sera were double diluted from a 1/120 dilution in PBS-T (50 μl of 0.05 M carbonate bicarbonate buffer (pH 9.6) containing 0.05% Tween 20). Bound Ab was detected using biotinylated rat anti-mouse IgG1 antibody (clone LO-MG1-2; Serotec) or biotinylated mouse anti-mouse IgG2a antibody (clone 5.7; BD Pharmingen). For IgE measurement, sera were depleted of IgG using protein G-coupled beads (25) and double diluted from a 1/120 dilution in PBS-T. Bound Ab was detected using biotinylated rat anti-mouse IgE antibody (clone R35-118; BD Pharmingen). Binding was visualized using streptavidin-HRP (R&D Systems) and tetramethylbenzidine (R&D Systems).

Statistical analysis

Mann-Whitney tests were used to determine statistical differences between groups, where a value of p < 0.05 was considered to be significant.

Results

Acute Der p1-induced AAI resolves within 21 days

To establish a time course of AAI resolution, a highly reproducible, minimal sensitization and challenge model was established in C57BL/6J mice. Mice were sensitized i.p. twice with 10 μg of Der p1 in alum adjuvant and challenged i.t. twice with Der p1 in PBS. Control sensitized mice were mock challenged with PBS (Fig. 1). Animals that received no sensitizing injection, only one i.t. challenge did not develop AAI (data not shown). The time between last sensitization and first challenge could vary between 14 and 28 days without effect on AAI (data not shown). After the second challenge, mononuclear cells and eosinophils infiltrated into perivascular, peribronchial and bronchiolar lung tissue and into alveolar walls (Fig. 2). By day 2 post challenge, mice given Der p1 had moderate to severe lung inflammation in all compartments (Fig. 3, A–D), which resolved by...
day 21. The maximal eosinophil influx in lung tissue and BAL coincided with the peak in inflammation and gradually declined over time reaching control levels by day 21 (Figs. 2C and 2D). Goblet cell hyperplasia (Figs. 2E and 2F) peaked on day 4 post challenge but was not completely resolved by day 21. PBS-challenged mice were included at each time point but never developed AAI (Fig. 2F). The number of effector cells in the lung response resolved, the numbers of effector cells in dMLN decreased. Despite showing equivalent proliferative responses to Der p1, dMLN cells from mock-challenged mice secreted much less IL-5 and IL-13 and essentially no IL-10 when stimulated with Der p1 (Fig. 4, B–D). This indicated that although Der p1-reactive memory was similar in lymphoid tissue of both groups, the challenged mice had expanded allergic effector cell populations. IFN-γ was always undetectable in cells from mock or Der p1-challenged mice.

A total of $<10^5$ mononuclear cells could be isolated from lungs of mock-challenged mice so it was not possible to determine their responses at each time point. It was only possible to isolate sufficient numbers of cells for in vitro restimulation from Der p1-challenged lungs at the peak of inflammation on day 4 after challenge. Results from a representative experiment are shown in Fig. 4.
In BAL at the same time point were low but detectable, and IFN-IL-13, and IL-10 were secreted. Levels of IL-5, IL-13, and IL-10 cells in lung tissue were similar to those in dMLN and that IL-5, CD4 and lung there was a consistent, detectable population of significantly increased at day 4 and declined over time. In both dMLN found in both dMLN and lungs of mock-challenged mice. After investigate whether Tregs were involved in resolution of Der p1-in suppression of responses to foreign Ag, thus we aimed to inves-}

Percentage of proliferating cells at 72 h (CFSE incorporation); n

Der p1 had similar levels of specific IgG1 (Fig. 4).

However, by day 21, Der p1-challenged mice had enhanced serum IgG1 (Fig. 4). In contrast, Der p1-specific IgG titers were enhanced at day 2 post challenge with Derp1 (Fig. 4K), but by day 21 this declined almost to the level of mock-challenged mice (Fig. 4L). Der p1-specific IgG2α was undetectable at all time points (not shown).

**CD4** CD25** Foxp3** Tregs increase in the lung and dMLN upon Ag challenge

Endogenous CD4** CD25** Foxp3** Tregs have been implicated in suppression of responses to foreign Ag, thus we aimed to investi-}

Serum Dilution 1:

**IgE**

A

B

C

D

E

F

G

H

**FIGURE 8.** Depletion of CD25** Foxp3** Tregs enhances, whereas adoptive transfer reduces systemic immune responses to airway challenge. Mice were sensitized, challenged, and treated as in Fig. 1. On day 6 post Der p1 challenge, dMLN cells were cultured in medium alone (M) or with Der p1 (D). A. Percentage of proliferating cells at 72 h (CFSE incorporation); B–D, 48 h cytokine secretion; shown for individual mice. Bars, median group value. Representative results from three experiments (n = 4–5/group/experiment). Significant differences to medium only (*) or relevant control group ($) (p < 0.05, Mann-Whitney). E–H, Sera from Der p1 sensitized mice, treated with anti-CD25 or isotype control (E and G) or CD4** CD25** or CD4** CD25** cells (F and H) before challenge, analyzed for Der p1-specific IgG1 (G and H) and IgE (E and F) 6 days post challenge.

4, E and F, and show that responses of Der p1-reactive effector cells in lung tissue were similar to those in dMLN and that IL-5, IL-13, and IL-10 were secreted. Levels of IL-5, IL-13, and IL-10 in BAL at the same time point were low but detectable, and IFN-γ and IL-4 were undetectable. BAL collected at all other time points or from mock-challenged mice at any time point contained no detectable cytokines (not shown).

Sera from sensitized mice 2 days after challenge with PBS or Der p1 had similar levels of specific IgG1 (Fig. 4F). However, by day 21, Der p1-challenged mice had enhanced serum IgG1 (Fig. 4F). In contrast, Der p1-specific IgG titers were enhanced at day 2 post challenge with Derp1 (Fig. 4K), but by day 21 this declined almost to the level of mock-challenged mice (Fig. 4L). Der p1-specific IgG2α was undetectable at all time points (not shown).

**CD4** CD25** Foxp3** Tregs can modulate allergic pulmonary eosinophilia and lung inflammation

Having shown that Tregs accumulated in lungs and dMLN after allergen challenge we wished to know whether or not they were involved in resolution of AAI. Fig. 5 shows that low numbers of Treg cells were found in both dMLN and lungs of mock-challenged mice. After allergen challenge the numbers of CD4** Foxp3** CD25** Tregs sig-}

In cell transfer experiments, mice were given 5 × 10⁵ naïve CD4** CD25** or 5 × 10⁵ CD4** CD25** T cells i.v. 24 h before first airway challenge (Fig. 1). All CD25** CD4** T cells isolated were also Foxp3** by flow cytometry. To track migration of transferred cells, 5 × 10⁵ naïve CFSE-labeled Tregs were transferred as above. Four days after Der p1 challenge (9 days after transfer), when endogenous Treg numbers peaked in lungs and dMLN (Fig. 5), lungs, dMLN and spleens were removed and labeled cells detected by flow cytometry. Although numbers were very low, transferred cells were only found in the dMLN suggesting that they were recruited to and retained in the activated lymphoid tissue (Fig. 6G).

To assess Treg function in AAI, tissues were analyzed at day 6 post challenge when inflammation was well established, but starting to resolve (Figs. 2–4). In depletion experiments, isotype Ab i.p. and PBS i.p. were both used as controls for PC61 administration. Because these control treatments gave equivalent results in three separate experiments (p > 0.05), they are expressed as a total control group. Anti-CD25 Ab had no effect on overall lung inflammation (Fig. 7, A–D) indicating that mice were still capable of mounting a response to allergen challenge, i.e., depletion had not removed memory cells. However, there was increased eosinophilia in the pulmonary tissue (Fig. 7E) and BAL (Fig. 7F). In contrast, transfer of CD4** CD25** Foxp3** T cells from naïve mice before airway challenge reduced disease as lung inflammation and eosino-}

Transfer and depletion of CD25** Foxp3** T cells before airway challenge had profound effects on lung pathology. Whether transfer or depletion also reduced or exacerbated the adaptive
allergic responses was investigated by determining the dMLN T cell response and serum Ab levels. Depletion of CD25\(^+\)Foxp3\(^+\) T cells enhanced proliferation of dMLN cells to recall Ag (Fig. 8A). This coincided with elevated levels of the Th2 cytokines, IL-5 (Fig. 8B), and IL-13 (Fig. 8C) but there was no effect on IL-10 secretion (Fig. 8D). In contrast, adoptive transfer of CD25\(^+\)Foxp3\(^+\) T cells abrogated proliferation and decreased secretion of IL-5 and IL-13 (Fig. 8A–C), but enhanced levels of IL-10 compared with CD25\(^+\) cell recipients or control mice (Fig. 8D). Depletion of CD25\(^+\) T cells increased IgG1 and IgE whereas transfer inhibited Ab titers compared with control mice (Fig. 8, E–H).

**Suppression of lung inflammation by CD25\(^+\)Foxp3\(^+\) Tregs is independent of IL-10R function**

CD25\(^+\)Foxp3\(^+\) transfer increased production of IL-10 by dMLN cells from Der p1-challenged mice. To resolve whether IL-10 was important in prevention or early resolution of pulmonary inflammation, anti-IL-10R-blocking Ab was administered at the time of cell transfer. Blockade of IL-10R at the time of cell transfer had no effect on the inhibition of lung inflammation (Fig. 9, A–D) or eosinophilia (Fig. 9, E and F).

**IL-10R function is essential for control of allergen-induced draining lymph node cell proliferation and cytokine secretion by transferred CD25\(^+\)Foxp3\(^+\) CD4\(^+\) T cells**

Fig. 10, A–D, shows that, as above, adoptive transfer of CD25\(^+\)Foxp3\(^+\) CD4\(^+\) T cells decreased dMLN cell proliferation and this coincided with decreased IL-5 and IL-13 and increased IL-10 secretion. Administration of anti-IL-10R Ab at the time of transfer prevented the decreased proliferation and cytokine secretion.

**Treg influence on systemic Ab response is independent of IL-10R function**

As above, transfer of CD25\(^+\)Foxp3\(^+\) cells before airway challenge prevented secretion of Der p1-specific IgG1 and IgE Abs. Blockade of IL-10R binding at the time of transfer did not reverse this response in cell recipients (Fig. 10, E and G). Levels of IgG2a\(^b\) were low in all groups of mice (Fig. 10F).

**Discussion**

This paper reports that endogenous Tregs are important in resolution of Th2-mediated AAI induced by the native form of a natural human airway allergen, Der p1 in mice with an intact immune system. We show that endogenous Tregs from Ag naive syngeneic

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**FIGURE 9.** Blockade of IL-10R and transfer of CD25\(^+\)Foxp3\(^+\) Tregs prevents lung inflammation and eosinophilia. Mice sensitized, treated (PBS or isotype control Ab; or CD25\(^+\)Foxp3\(^+\) cells only; or isotype control and CD25\(^+\)Foxp3\(^+\) cells; or anti-IL-10R Ab and CD25\(^+\)Foxp3\(^+\) cells) and challenged with Der p1. On day 6 post challenge, lung inflammation was assessed in perivascular (A), bronchiorlar (B), peribronchiolar (C), and alveolar wall (D) compartments. Pulmonary (E) and BAL eosinophils (F) were also assessed. Data representative of three comparable experiments. Bars, median group value. Significant difference (+) to control (p < 0.05, Mann-Whitney).

**FIGURE 10.** IL-10R blockade dysregulates cytokine production and isotype class switching. Mice sensitized with Der p1, treated (CD25\(^+\)Foxp3\(^+\) cells only, isotype control and CD25\(^+\)Foxp3\(^+\) cells or anti-IL-10R Ab and CD25\(^+\)Foxp3\(^+\) cells), and challenged with Der p1. On day 6 post challenge, dMLN cells were cultured with medium alone (M) or with 50 \(\mu\)g ml\(^{-1}\) Der p1 (D). A, Percentage of cells proliferating after 72 h was assessed by CFSE incorporation. B–D, Concentration of cytokine secreted at 48 h. Bars, median. Serum was analyzed for Der p1-specific IgG1 (E), IgG2a (F), and IgE (G). Representative results (n = 4–5/group/experiment). Significant differences to control group (+) or medium alone ($) (p < 0.05, Mann-Whitney).
mice down-regulate eosinophil recruitment into lungs and Th2 effector cell function in draining lymph nodes. We also demonstrate that Treg function is IL-10R independent in the lung, but IL-10R dependent in the dMLN.

Previous studies reported on induction and modulation of AAI in murine models (15, 26) and TCR transgenic OVA-specific Tregs have been shown to down-regulate AAI in an IL-10-dependent fashion (11). However, this study did not determine whether endogenous Tregs were involved in induction and resolution of AAI, nor whether Tregs must be allergen-specific to control AAI. Using whole house dust mite extract as allergen, Lewkowich et al. (18) showed that depletion of natural CD25+CD4+ T cells before sensitization increased disease in C3H but not A/J mice and that this was due to alterations in lung dendritic cells. This study demonstrated that natural Tregs are important in determining whether in allergy results from allergen encounter but did not determine whether disease can be modulated by endogenous Tregs in already sensitized animals.

To determine whether Tregs were involved in AAI resolution and associated immune responses, we first established the time course of lung inflammation and systemic immunity in Der p1-sensitized and -challenged mice. Lung inflammation and eosinophilia peaked between 2 and 6 days after challenge and had largely returned to baseline levels by 21 days (Figs. 2 and 3). Serum Der p1-specific IgE levels were transiently boosted by i.t. challenge but decreased by 21 days whereas serum IgG1 levels were boosted by challenge and continued to increase 21 days later (Fig. 4). Recall responses in dMLN cells showed that effector cells capable of secreting IL-5, IL-13, and IL-10 were present in draining lymph nodes from 2 days after challenge and decreased by 21 days (Fig. 4).

We further demonstrated that CD25+CD4+Foxp3+ T cells are recruited into both lungs and draining lymph nodes of sensitized mice, peaking at 4 days after challenge when resolution starts (Fig. 5). To study whether these cells were functionally involved in resolution and/or disease induction we took a “two sided” approach of in vivo endogenous Treg depletion and adoptive transfer. When using depleting anti-CD25 Ab in sensitized animals it was a concern that we may simply remove effector T cells so sufficient time had to be left after sensitization to allow activated allergen-specific CD25+ effector cells to revert to “memory” i.e., CD25− status. That depletion before lung challenge did not appear to alter the overall lung inflammation (Fig. 7) and enhanced serum Ab responses (Fig. 8) indicated that treatment did not prevent generation of effector cells from pre-existing memory cells. However, Treg depletion in elevated eosinophilia (Fig. 7) and enhanced Der p1-specific serum IgG1 and IgE responses (Fig. 8). This indicates that Tregs normally “dampen down” AAI but cannot prevent eosinophilia and allergic Ab production induced by lung challenge. The results are consistent with previous reports that have suggested that Tregs are important in control of Th2 immune responses and lung eosinophilia (11, 20).

Other studies reported that Ag-specific Treg from DO11.10 transgenic mice can suppress lung eosinophilia. To establish whether or not endogenous Treg from allergen naive, wild-type mice would also be functional in AAI modulation, we transferred 5 × 105 CD4+CD25+Foxp3+ T cells from naive mice into sensitized animals 1 day before allergen challenge. These cells were recruited to and retained in dMLN after Ag challenge (Fig. 6). Treg transfer had the opposite effect of depletion and greatly reduced lung eosinophilia (Fig. 7) and goblet cell differentiation (data not shown) as well as inhibiting production of Der p1-specific serum IgE and IgG1 (Fig. 8).

Treg depletion enhanced levels of the Th2 cytokines, IL-5 and IL-13 secreted by dMLN cells while transfer of additional Tregs from allergen naive mice had opposite effects (Fig. 8). In contrast, IL-10 secreted by dMLN cells was unaffected by depletion but enhanced by transfer. The question arose from this whether IL-10 was necessary for Treg function in control of Der p1-induced AAI. To answer this, we treated mice with IL-10R blocking Ab at the time of Treg transfer. Unlike results reported for TCR transgenic OVA-specific Tregs (11), control of AAI in our model is independent of IL-10R signaling as administration of anti-IL-10R Ab at the time of Treg transfer had no effect (Fig. 10). In contrast, in the dMLN response, blockade of IL-10 signaling at the time of transfer prevented the inhibition of IL-5 and IL-13 seen when Tregs alone were given (Fig. 10). This indicates that IL-10 is necessary for Treg control of dMLN IL-5 and IL-13 secretion. Levels of IL-10 secretion by dMLN cells were unaffected by receptor blockade (Fig. 10).

Overall these results show that endogenous Tregs control resolution of airway inflammation and eosinophilia in the lung in an IL-10-independent manner while draining lymph node allergic Th2 cytokine responses are controlled by the same cell population in an IL-10-dependent manner. We believe that our results showing that natural/endogenous Tregs are important for resolution of AAI and that their function can be boosted by transferring cells from Ag naive donors add important information on the biology of AAI and credence to the idea that endogenous Tregs may be a therapeutic target.

Disclosures
The authors have no financial conflict of interest.

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