Alveolar Macrophages Contribute to Respiratory Tolerance by Inducing FoxP3 Expression in Naive T Cells

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Abstract

Alveolar macrophages (AMs) from mice and humans have long been known to contribute to maintaining tolerance in the lung. Studies have shown that AMs can induce anergy in CD4+ T cells. Nitric oxide, prostaglandins, and leukotrienes have been implicated in AM-mediated tolerance. However, it remains unclear what effect, if any, AMs exert on FoxP3 induction in CD4+ T cells from mice and humans, and whether or not other immunomodulators might play a role. AMs were isolated from bronchoalveolar lavage (BAL) fluid from either mice or humans, and cocultured with enriched naive CD4+FoxP3− T cells. We show here for the first time that AMs and AM-conditioned media (AM-CM) from mice and humans induced FoxP3 expression in naive CD4+ T cells in vitro, an outcome that was reversed in part either by inhibiting retinoic acid (RA) binding to its receptor (RAR), or by blocking transforming growth factor (TGF)–β1 signaling. A nasal administration of the RAR antagonist reduced the frequencies of CD4+FoxP3+ T cells in the lungs of mice after aerosol challenge with Bordetella pertussis. In addition, we found that the intranasal vaccination of mice with ovalbumin (OVA) protein in conjunction with an RAR inhibitor led to a significant increase in OVA-specific serum IgE. Our findings suggest that AMs can mediate tolerance in the lungs of mice and humans via RA and TGF-β1. These data may have implications in the development of nasal vaccines in the future.

KEYWORDS: alveolar macrophages, FoxP3, TGF-β1, retinoic acid

T cell–mediated immunity is important in the lung to protect the host from inhaled pathogens and environmental antigens (1–3). However, respiratory T-cell immunity must be tightly regulated to protect against tissue damage. Furthermore, most of the environmental antigens encountered by the lung are innocuous, so it is crucial for the resident immune cells to distinguish such inert antigens from those derived from pathogens, and respond appropriately.

Immunoregulatory mechanisms used by the lung include the components of the surfactant lining (4) and products secreted by alveolar Type II epithelial cells (5) as well as alveolar macrophages (AMs) (6). Rodent and human AMs have long been known for their active suppression of T-cell proliferation (7–13).

Immunoregulators associated with AMs include prostaglandins, leukotrienes (8, 14), IL-1 (15), and nitric oxide (3). The suppression of T-cell responses in tissues such as the lung is thought to contribute to peripheral tolerance to exogenous antigens and the control of autoreactive lymphocytes (16).

AMs comprise the most numerous (> 90%) type of immune cell in the normal lung (17). In the rat, AMs induce anergy in T cells, and this anergy is reversible upon the removal of AMs from the culture (6). In addition, T cells isolated from the lungs of rodents express less IL-2 receptor (CD25) than do splenocytes and bear the hallmarks of quiescence (18), whereas T cells isolated from human lungs produce less IL-2 than do autologous blood-derived T cells (19).

The majority of studies investigating lung tolerance and T cells were performed before the discovery of the transcription factor FoxP3. The importance of FoxP3 as the master regulator of regulatory T cells (Tregs) was first described in 1995 by Sakaguchi and colleagues (20). Treg cells can be broadly divided into two categories, namely, thymically derived natural (n) Treg (20) and inducible (i) Treg cells (21, 22). Depletions or defects in nTreg cells can lead to the emergence of a wide variety of autoimmune diseases and tissue destruction, as exemplified in *Scurfy* mutant mice (23), and the immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome in humans (24). A mutation in the gene encoding the FoxP3 transcription factor underlies both phenotypes. FoxP3 expression is currently regarded as the definitive marker for cells with a regulatory function in mice and humans (25, 26). We previously reported on the existence of $CD4^+FoxP3^+$ T cells that lack CD25 expression in murine lungs, and that might represent an inducible Treg cell population (27). Therefore, we wanted to investigate whether AMs from either mice or humans could induce FoxP3 expression in naive $CD4^+$ T cells *in vitro*.

AMs, obtained from the bronchoalveolar lavage (BAL) of mice or humans, enhanced FoxP3 expression in $CD4^+FoxP3^-$ T cells *in vitro*. This enhancement was in part mediated by retinoic acid (RA) and transforming growth factor (TGF)–β_1. RA is a biologically active metabolite of vitamin A, and is known to act as an immune modulator by enhancing the TGF-β_1–mediated induction of $FoxP3^+$ iTreg cells, and inhibiting the IL-17–polarizing effects of TGF-β_1 (28, 29). The activation of naive T cells in the presence of either AMs or AM-conditioned media (AM-CM) prevented T-cell proliferation, an effect that was reversed by

inhibiting the RA receptor as well as by TGF-β_1 binding. Inhibiting the RA receptor *in vivo* also reduced the frequencies of $CD4^+FoxP3^+$ T cells in the lungs of mice after *Bordetella pertussis* aerosol challenge. Our findings suggest that resident RA-secreting and TGF-β_1–secreting AMs are at least partly responsible for the induction of $CD4^+FoxP3^+$ T cells from infiltrating $CD4^+$ T cells *in vitro* and *in vivo*, in both mice and humans. Furthermore, an intranasal vaccination of mice with ovalbumin (OVA) protein and an RA receptor inhibitor significantly enhanced OVA-specific serum IgE. We suggest that the manipulation of RA signaling to prevent tolerance in the respiratory tract may help enhance the immunogenicity of nasal vaccines.

MATERIALS AND METHODS

Animals

BALB/c mice were purchased from Harlan (Oxon, UK). Animal experiments were approved by the Ethics Committee of Trinity College and by the Irish Department of Health.

Cell Sorting

T cells were isolated from murine spleens or human peripheral blood mononuclear cells (PBMC). Murine cells were stained with rat anti-mouse CD4-PE, CD45RB-FITC, and CD25-PE-Cy5 (eBioscience, Hatfield, UK). Naive $CD4^+CD25^-CD45RB^{high}$ T cells were sorted using a MoFlo cell sorter (DakoCytomation, Ely, UK). Human naive $CD4^+CD25^-CD45RA^+CD45RO^-$ and memory $CD4^+CD25^-CD45RA^-CD45RO^+$ human T cells were isolated using an EasySep naive T-cell isolation kit (StemCell, Grenoble, France).

Isolation of AMs

Murine AMs were obtained by bronchoalveolar lavage (BAL), and resuspended at 5×10^5 AMs/ml in serum-free RPMI.

Human AMs were obtained during bronchoscopy, under a protocol approved by the Ethics Board of St. James's Hospital. Cells were resuspended at 5×10^5 AMs/ml in RPMI supplemented with 10% type AB human serum (Sigma Chemical Co., St. Louis, MO), 50 μg/ml Fungizone, and 50 μg/ml cefotaxime.

After 3 hours of incubation, nonadherent cells were removed. The media were replenished, and adherent cells (predominantly AMs) were incubated overnight before each experiment.

Flow Cytometry

Cocultured cells were stained with antibodies directed against a number of surface and intracellular antigens (eBioscience). Labeled cells were acquired using a CyAn flow cytometer (Beckman Coulter, Lismeehan, Ireland).

Aldefluor Assay

Aldehyde dehydrogenase (ALDH) activity in individual cells was estimated using an Aldefluor assay (StemCell) according to the manufacturer's protocol, and was measured by flow cytometry and fluorescence microscopy.

T-Cell Coculture with AMs and Inhibitors

Murine $CD4^+CD25^-CD45RB^{high}$ or human $CD4^+CD25^-CD45RO^-$ naive T cells were activated with 2 μg/ml soluble anti-CD3 and cultured for 24 hours with AMs or AM-CM (harvested from an overnight AM culture). Murine T cells were activated with AM-CM in the presence of 50 μg/ml anti–TGF-β_1 (a gift from Dr. L. Boon, Bioceros, Utrecht, The Netherlands) or 5 μM retinoic acid receptor inhibitor (RARi; Enzo Life Sciences, Exeter, UK). Human T cells were activated with AM-CM in the presence of 1 μg/ml anti–TGF-β_1 (Source

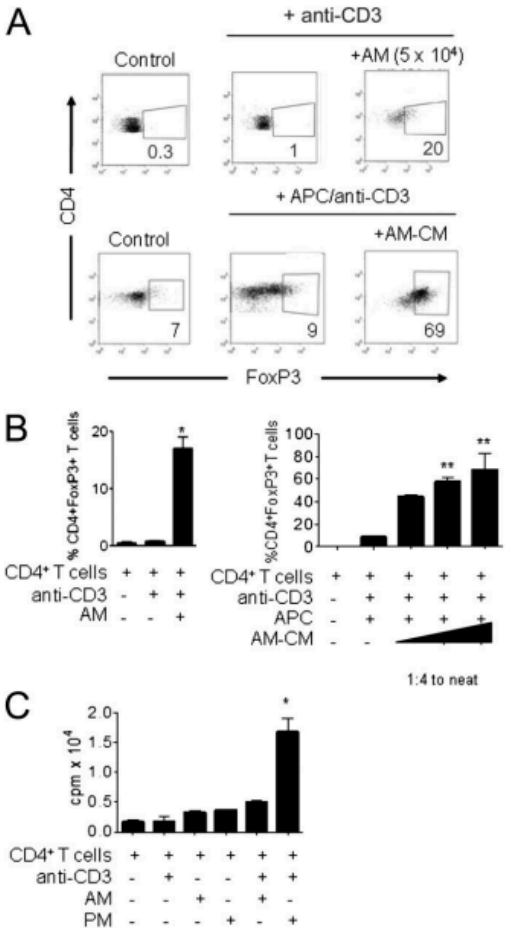

Figure 1. Murine alveolar macrophages (AMs) induce FoxP3 expression in naive $CD4^+$ T cells *in vitro*. AMs were isolated from bronchoalveolar lavage (BAL) samples taken from naive mice by adhering overnight at 37°C. AM-conditioned media (AM-CM) were generated by incubating freshly isolated AMs overnight at 37°C. $CD4^+CD25^-CD45RB^{hi}$ T cells were sorted from the spleens of naive mice, activated with 2 μg/ml soluble anti-CD3, and incubated in the presence of adhered AMs (5×10^4/ml) or AM-CM plus antigen-presenting cells (APCs; irradiated splenocytes). After 24 hours of culture, the frequency of $CD4^+FoxP3^+$ cells was determined after gating on the viable $CD4^+$ lymphocytes. (*A*) Representative dot plots depict the percentage of $CD4^+FoxP3^+$ T cells (gated on a viable $CD3^+CD4^+$ T cell gate) before (*left*) and 24 hours after (*right*) coincubation with either AMs or AM-CM. (*B*) Results represent the mean values of three experiments (main $P < 0.05$; *post hoc* test, $^*P < 0.05$ and $^{**}P < 0.01$, compared with $CD4^+$ cells activated in the presence of APCs). (*C*) Freshly sorted $CD4^+CD25^-CD45RB^{hi}$ cells (6×10^4/ml) were cocultured with either AMs (5×10^5/ml) from BAL or peritoneal macrophages (PMs) from murine peritoneal exudate and activated with immobilized anti-CD3 (2 μg/ml). Proliferation was determined by ^{3}H-thymidine incorporation 72 hours later (main $P < 0.05$; *post hoc* test, $^*P < 0.05$, compared with $CD4^+$ cells activated in the presence of PMs. The results represent median values for three experiments.

Bioscience, Cambridge, UK) or 20 μM RARi. The supernatant was retained for ELISA. Cells were analyzed by flow cytometry, or cocultures were replenished with medium containing ^{3}H-thymidine and incubated for a further 24 hours, and subsequently harvested using a Tomtec Harvester 96 (Receptor Technologies, Warwick, UK). Radioactive counts were determined using a WALLAC β counter (PerkinElmer, Waltham, MA).

Bordetella pertussis Challenge Model

Mice were exposed to live *B. pertussis* aerosol. Lung colony-forming units (CFUs) were determined at intervals after infection, as described elsewhere (22).

Intranasal Vaccination with RARi

Mice received either 75 or 150 μg RARi or PBS/ethanol vehicle intranasally every second day after *B. pertussis* exposure.

In a separate experiment, mice were immunized twice intranasally, 21 days apart, with 25 μg ovalbumin (OVA) protein (Hyglos GmbH, Bernried am Starnberger See, Germany), alone or with 5 μg cytosine-phosphate-guanidine (CpG) adjuvant. Both combinations were administered with or without 100 μg RARi. Control mice received OVA and CpG subcutaneously, or intranasal ethanol vehicle. Seven days after the second vaccination, mice were killed, and concentrations of OVA-specific serum IgE were determined by ELISA.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism software. Functionality was compared between study groups using nonparametric tests, namely, the Mann-Whitney test for comparing two groups, and the Kruskal-Wallis test followed by the Dunn test for multiple comparisons involving three or more groups.

RESULTS

AMs and AM-CM Can Induce FoxP3 in Naive Murine and Human CD4$^+$FoxP3$^-$ T Cells *In Vitro*

We previously reported that CD4$^+$FoxP3$^+$CD25$^-$ T cells with a regulatory function are present in the lungs of naive mice, and although they possess a high activation threshold (compared with nTreg cells), they can suppress effector T-cell proliferation *in vitro* via IL-10 (27). It remained unclear whether these Treg cells were of thymic origin or were induced in the lung. Because the suppressive function of thymically derived nTreg cells has been shown to be cell contact–dependent *in vitro* (30), we proposed that these cells might be induced in the lung (27).

AMs induce anergy in CD4$^+$ T cells, which is associated with the down-regulation of surface CD25 expression (6). However, the mechanism behind this induction of anergy has not been determined, and it could only be elucidated if AMs were added to T-cell culture within 24 hours of T-cell activation (6). We investigated whether AMs from murine BAL samples could induce FoxP3 expression in naive CD4$^+$ T cells *in vitro*. AMs comprise approximately 90% of all immune cells in the BAL fluid of naive animals, and therefore AMs were cocultured with naive T cells at a ratio of 9:1. The activation of naive T cells with soluble anti-CD3 in the presence of AMs caused a significant increase in the percentage of cells expressing FoxP3 ($P < 0.05$) after 24 hours (Figures 1A and 1B). Furthermore, AM-CM were used in the place of AMs to induce significant FoxP3 expression in activated naive CD4$^+$ T cells, in a dose-dependent manner (Figures 1A and 1B).

We next investigated whether AMs isolated from the lungs of naive mice by BAL could inhibit a proliferation of anti-CD3–activated naive CD4$^+$ T cells. Peritoneal macrophages (PMs), isolated by peritoneal lavage (see the online supplement for detailed methods), were used as a positive control. Macrophages

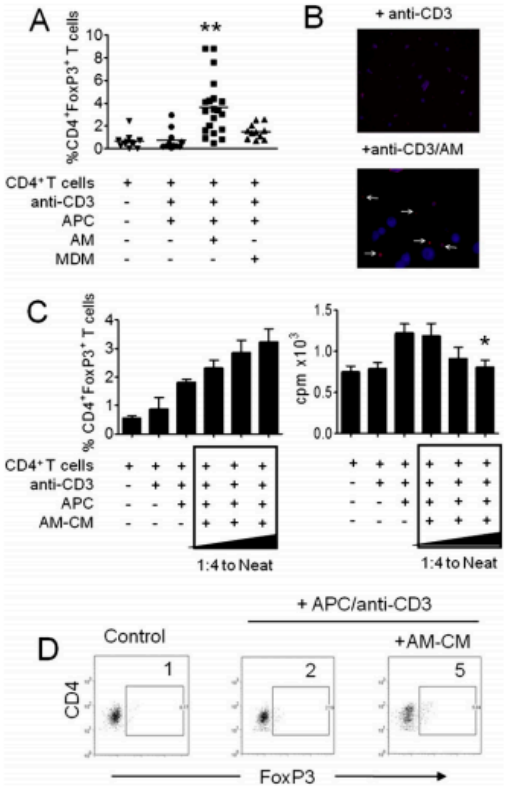

Figure 2. Human alveolar macrophages induce FoxP3 expression in naive CD4$^+$ T cells *in vitro*. AMs were isolated from the BAL fluid of outpatient volunteers who were undergoing clinically indicated bronchoscopies, but who did not demonstrate diagnosable lung disease. AMs were cultured overnight at a concentration of 5×10^5/ml. Alternately, monocyte-derived macrophages (MDMs) were generated from the peripheral blood mononuclear cells (PBMC) of healthy donors and cultured *in vitro* for 7–10 days before coculture with T cells. (*A*) Freshly isolated naive human CD4$^+$ CD25$^-$CD45RO$^-$ T cells (6×10^3) were activated with 2 μg/ml soluble anti-CD3 and cocultured (1:9) in the presence or absence of AMs or MDMs for a further 24 hours (main $P < 0.01$; *post hoc* test, $^{**}P < 0.01$, compared with CD4$^+$ cells activated in the presence of MDMs). Results represent the median values for three experiments. (*B*) Freshly isolated naive human CD4$^+$ CD25$^-$CD45RO$^-$ T cells (6×10^3) were activated as already described, and were cultured in the presence or absence of AMs at a ratio of 9:1 AMs/T cells. After 24 hours, cells were stained with anti-human FoxP3-PE (*red*) and Hoechst 3358 nuclear stain (*blue*), and the extent of FoxP3$^+$ T-cell induction was examined by fluorescence microscopy, using a Leica photomicroscope (×100 magnification; Medical Supply Company, Dublin, Ireland). Representative images show activated T cells in the absence of AMs (*top*) and T cells activated in the presence of AMs (*bottom*). *Arrows* indicate FoxP3$^+$ T cells. (*C*) Naive human CD4$^+$CD25$^-$CD45RO$^-$ T cells (6×10^3) were activated in the presence of AM-CM, harvested from allogeneic human AMs. FoxP3 expression was examined after 24 hours, whereas cell proliferation was determined by the uptake of ^{3}H-thymidine 48 hours later (main $P < 0.05$; *post hoc* test, $^*P < 0.05$, compared with CD4$^+$ cells activated in the presence of allogeneic APCs). Representative results from three separate experiments are shown. (*D*) Representative dot plots are based on the viable CD3$^+$CD4$^+$ T cell gate, depicting intracellular FoxP3 expression within T cells, 24 hours after activation in the absence or presence of neat AM-CM.

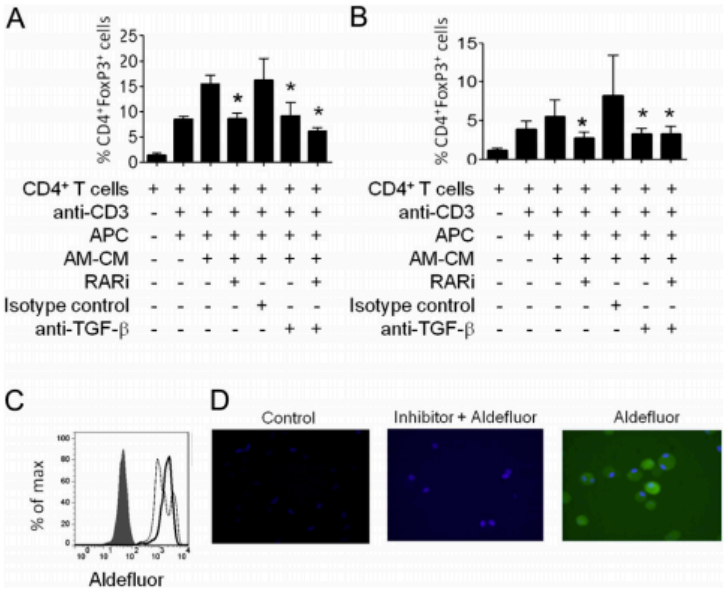

Figure 4. The inhibition of retinoic acid (RA) and TGF-β_1 reverses AM-induced FoxP3 expression in murine and human $CD4^+$ T cells *in vitro*. (*A*) $CD4^+CD25^-CD45RB^{hi}$ T cells were sorted from the spleens of naive mice and activated with 2 μg/ml soluble anti-CD3 plus irradiated spleen cells as APCs, with and without AM-CM in the presence or absence of an isotype control antibody, 50 μg/ml anti–TGF-β_1, 5 μM retinoic acid receptor inhibitor (RARi) alone, or RARi plus anti–TGF-β_1 (*$P < 0.05$, versus activated $CD4^+$ T cells cocultured with AM-CM alone). (*B*) Freshly isolated human naive $CD4^+CD25^-CD45RO^-$ T cells (6×10^3) were activated with 2 μg/ml soluble anti-CD3 and cocultured in the presence of AM-CM supplemented with 20 μM RARi, 1 μg/ml of neutralizing anti–TGF-β_1, or RARi and anti–TGF-β_1 for a further 24 hours (main $P < 0.05$; *post hoc* test, *$P < 0.5$, compared with $CD4^+$ cells activated in the presence of AM-CM alone). The results shown are representative of three experiments. (*C*) Representative histogram depicts aldehyde dehydrogenase–A1 (ALDHA1, Aldefluor; StemCell) expression as an indirect measurement of RA expression in viable, unstained control cells (*solid histogram*), peritoneal exudate cells (*thin line*), and BAL cells (*thick line*) isolated from a naive mouse. (*D*) Representative immunofluorescence images show ALDHA1 expression in human AMs. (*F*) Diethylaminobenzaldehyde (DEAB) was used as a competitive inhibitor and negative control for ALDH1A. DEAB specifically inhibits ALDH1. These results are representative of three separate experiments.

We subsequently examined ALDH1A expression as an indirect measurement of RA production in AM-CM. Using this indirect assay, we found that both murine (Figure 4E) and human (Figure 4F) AMs express ALDH1A (Figure 4E).

RARi Increases the Frequency of Lung $CD4^+FoxP3^+$ T Cells in *B. pertussis*–Infected Mice

We next examined whether the inhibition of RA could alter the course of respiratory infection *in vivo*. BALB/c mice were infected with *B. pertussis* and received either 75 μg or 150 μg RARi intranasally. Treatment with either dose of RARi exerted no effect on CFU values in the lungs (data not shown). Nonetheless, at 7 days after the challenge, the mice that received the higher dose of RARi demonstrated significantly reduced ($P < 0.001$) frequencies of lung $CD4^+FoxP3^+$ T cells, compared with vehicle-treated control cells (Figure 5A). The lower dose of RARi was seen to exert a significant ($P < 0.05$) effect on the frequency of $CD4^+FoxP3^+$ T cells on Day 14 after the challenge (Figure 5B), and both doses of RARi reduced the frequencies of these cells in lung tissue when the experiment was terminated on Day 21 after the challenge (Figure 5C).

Intranasal Immunization with Antigen and Adjuvant in the Presence of RARi Enhances Serum IgE in Mice

The mucosal administration of soluble antigen results in immunological nonresponsiveness (mucosal tolerance) (33). The mucosal delivery of antigen before systemic sensitization with the same antigen was reported to result in long-lasting immunosuppression rather than priming (34–36). We investigated whether the induction of $FoxP3^+$ T cells by RA-producing AMs was responsible for tolerance in the respiratory mucosa, and whether this tolerance could be prevented using RARi.

BALB/c mice were immunized intranasally with 25 μg OVA, alone or with CpG as adjuvant, with or without RARi. OVA-specific IgE was not detected in mice that received intranasal OVA alone, with RARi, or with CpG (Figure 5D). However, significant ($P < 0.001$) OVA-specific IgE was present in the serum of mice that were vaccinated with OVA + CpG in the presence of RARi, at concentrations similar to those in control mice that received OVA + CpG subcutaneously (Figure 5D).

DISCUSSION

We show for the first time that AMs induce intracellular FoxP3 expression in $CD4^+$ T cells, and promote IL-10 while suppressing IFN-γ production by the same T cells *in vitro*. AM-derived RA and TGF-β_1 play a significant role in FoxP3 induction in murine and human naive $CD4^+$ T cells. Furthermore, the inhibition of RA signaling significantly enhanced OVA-specific serum IgE concentrations in OVA-immunized mice, thereby partly preventing nasal tolerance.

APCs derived from the lung are poor activators of T cells *in vitro* (37, 38). We previously reported that lung APCs demonstrate a significantly reduced capacity to activate *B. pertussis*–specific T cells, compared with splenic APCs *in vitro* (39). Furthermore, the removal of AMs from lung mononuclear cell preparations restored the capacity of lung T cells to respond to polyclonal activation (39). Here we have shown that AMs released a soluble factor that promoted the peripheral conversion of naive and memory T cells into $FoxP3^+$ T cells. FoxP3 expression was significantly reversed by the addition of RARi. In the gut, RA in conjunction with TGF-β_1 and tolerogenic $CD103^+$ DCs plays a major role in the induction of $FoxP3^+$ Treg cells (31, 32, 40, 41). Similarly, RA has been shown to protect against graft rejection (42) by inducing Treg cells *in vivo*. We found that

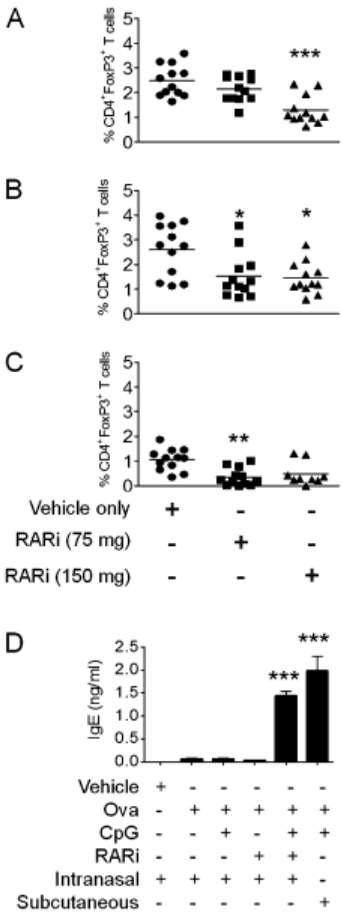

Figure 5. Intranasal treatment and vaccination of mice with RARi. Naive BALB/c mice were exposed to an aerosol containing a fixed amount of live *Bordetella pertussis* for 15 minutes. Infected mice received either 75 μg or 150 μg RARi intranasally every second day until Day 19 after the challenge, whereas control infected mice received vehicle (ethanol) only. Lungs were homogenized, and the frequencies of $CD4^+FoxP3^+$ T cells (based on the viable $CD3^+CD4^+$ T-cell gate) were examined by flow cytometry (*A*) 7 days, (*B*) 14 days, and (*C*) 21 days after challenge. *P* values were calculated based on the difference in $CD4^+FoxP3^+$ cell frequencies between mice that received RARi and mice that received vehicle (main $P < 0.05$; *post hoc* test, $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$). (*D*) Naive BALB/c mice were immunized twice intranasally, 21 days apart, with 25 μg ovalbumin (Ova) protein, alone or in the presence of 5 μg cytosine-phosphate-guanidine (CpG) as adjuvant. Both vaccine combinations were administered in the presence or absence of 100 μg RARi. Control mice were vaccinated subcutaneously with ovalbumin and CpG, or intranasally with vaccine vehicle (ethanol). Seven days after the second vaccination, the mice were killed and examined for the presence of serum ovalbumin–specific IgE. *P* values were calculated on the difference in IgE concentrations between mice that received ovalbumin versus mice that received vehicle. $^{***}P < 0.001$.

$TGF\text{-}\beta_1$ production by murine and human AMs played a similar role to that of RA in FoxP3 induction. Thus, like the DC-mediated induction of Treg cells in the gut, the AM-induced FoxP3 induction in $CD4^+$ T cells in the lung appears to require $TGF\text{-}\beta_1$ and RA.

Our previous findings demonstrated that $CD4^+FoxP3^+CD25^-$ T cells with regulatory function comprise the dominant $FoxP3^+$ cell in the lungs of mice (27). They may act as an ancillary regulatory arm in the lung, working with IL-10 to maintain immune tolerance and control immunity to infection in the lungs (27). Further study is required to show that T cells cocultured with AMs or AM-CM exercise a regulatory function *in vitro*.

The nasal administration of RARi to mice infected with *B. pertussis* resulted in a significant decrease in the frequency of $CD4^+FoxP3^+$ T cells in the lungs. We previously reported on the existence of two populations of Treg cells in the lung (27), and we found that both $CD4^+FoxP3^+CD25^-$ and $CD4^+CD25^+FoxP3^+$ populations played redundant roles in the control of immunity to infection (27). The inhibition of RA-induced inducible Treg cells by RARi alone is likely not enough to affect the bacterial burden. nTreg cells might well compensate for the absence of inducible Treg cells *in vivo*.

Effective intranasal vaccines are already in use clinically, and have been shown to work in experimental systems using pathogens (27, 43–45). Our findings suggest that RARi could be used to enhance the efficacy of nasal vaccinations against soluble antigens. The tolerance induced by the nasal or oral route is mediated by $CD4^+$ T cells, and results in decreased T-cell proliferation, which in turn prevents isotype switching to IgE (34, 35). The intranasal administration of OVA along with CpG and RARi prevented the induction of nasal tolerance and resulted in systemic immunity to OVA, as demonstrated by the presence of OVA-specific IgE in serum. Of course, intranasal treatment can modify multiple aspects of the immune response, and not just the respiratory tract. Studies in which plasmid DNA–lipid complexes were administered intranasally disseminated throughout the respiratory and gastrointestinal tracts, draining the lymph nodes and spleen (46).

Th2 cytokines promote IgG_1 and IgE production (33), and this production can be inhibited by IFN-γ (47). The adoptive transfer of $CD4^+$ T cells from nasally tolerized mice can suppress IgE production in primed recipients (33, 34), but blocking IFN-γ before adoptive transfer can facilitate IgE production (33). Other lung cells have been reported to produce RA, such as bronchial epithelial cells (48), and therefore AMs may not comprise the only cells exerting a regulatory effect via this molecule. Nonetheless, a specific role for AMs in regulating this system is supported by a study in which the elimination of AMs *in vivo* resulted in increased IgE production in the lungs of presensitized rats, when subsequently exposed to antigen aerosols (49). Our data confirm that nasal tolerance inhibits IgE immune responses in mice. The data also provide evidence that AMs and the $FoxP3^+$ T cells they induce may be responsible for promoting nasal tolerance, at least in mice. RARi may have a significant role to play in the development nasal vaccination strategies in humans.

Author disclosures are available with the text of this article at www.atsjournals.org.

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