Induction of Prolonged Asthma Tolerance by IL-10 – Differentiated Dendritic Cells: Differential Impact on Airway Hyperresponsiveness and the Th2 Immunoinflammatory Response

Aarti Nayyar, Wojciech Dawicki, Hui Huang, Meiping Lu, Xiaobei Zhang and John R. Gordon

*J Immunol* published online 25 May 2012
http://www.jimmunol.org/content/early/2012/05/25/jimmunol.1103286

**Supplementary Material**
http://www.jimmunol.org/content/suppl/2012/05/25/jimmunol.1103286.6.DC1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Induction of Prolonged Asthma Tolerance by IL-10–Differentiated Dendritic Cells: Differential Impact on Airway Hyperresponsiveness and the Th2 Immunoinflammatory Response

Aarti Nayyar,* 1 Wojciech Dawicki,† 1 Hui Huang,* Meiping Lu,* Xiaobei Zhang,* and John R. Gordon†

IL-10–differentiated dendritic cells (DC10s) can prevent allergen sensitization and reverse the asthma phenotype in mice with established disease. However, little is known about the time-frames over which this tolerance is effective. We report that at 2 wk after i.p. or transtracheal delivery of 1 × 10^6 OVA-, but not house dust mite-, presenting DC10s to OVA-asthmatic mice, significant diminution of airway hyperresponsiveness (AHR) was first apparent, whereas AHR was abrogated between 3 and 10 wk posttreatment. At 13 wk, AHR returned to pretreatment levels but could again be reversed by DC10 retreatment. The impact of a single DC10 treatment on airway eosinophil and Th2 cytokine responses to recall OVA challenge, and on OVA-specific IgE/IgG1 responses, was substantial at 3 wk posttreatment, but progressively increased thereafter, such that at 8 mo, airway eosinophil and Th2 responses to recall allergen challenge remained ∼85–95% suppressed relative to saline-treated asthmatic mice. Four biweekly DC10 treatments, whether transtracheal or i.p., reduced all asthma parameters to near background by 8 wk, whereas s.c. DC10 treatments did not affect AHR but did reduce the airway Th2 responses (i.e., DC10 had no discernible effects). Repeated challenge of the DC10-treated mice with aerosolized OVA (100 µg/ml) did not reverse tolerance, but treatment with the indoleamine-2,3-dioxygenase antagonist 1-methyltryptophan or neutralizing anti–IL-10R from days 12 to 21 after DC10 therapy partially reversed tolerance (Th2 cytokine responses, but not AHR). These findings indicate that DC10-induced Th2 tolerance in asthmatic animals is long lived, but that DC10s employ distinct mechanisms to affect AHR versus Th2 immunoinflammatory parameters. The Journal of Immunology, 2012, 189: 000–000.

Asthma is characterized by intermittent and reversible airway obstruction, airway hyperresponsiveness (AHR), and eosinophilic airway inflammation, driven by underlying Th2 immune responses (1). Conventional treatments have not addressed the immunologic basis of this disease but rather are primarily symptomatic in nature, targeting either the AHR (e.g., bronchodilators) or inflammatory responses (e.g., steroids) (2). Numerous reports have clearly indicated that immunologic tolerance can be induced in multiple disease models through use of regulatory dendritic cells (DCs) (3–7) that are either generated by differential culture conditions or genetically modified. The abilities of such tolerogenic DCs to prevent experimental Ag sensitization have been documented in model systems ranging from cancer (8) to allergic diseases (9). TGF-β–differentiated DCs have been reported to ∼50% reduce the localization of allergenic disease to the airways in animals that are sensitized systemically to an allergen, but this was not an allergen-specific effect (10), and IL-10–differentiated DCs (DC10s) are similarly effective in blocking asthma sensitization (11). Quiescent allergen-presenting, splenic CD8α+ DCs can ∼50% reverse established asthmatic responses in a mouse model of asthma, including AHR, eosinophilias and Th2 responses, but systemic allergen-specific IgE/IgG1 responses are relatively resistant to tolerization with such DCs (12). In contrast, we have reported that specific allergen-presenting DC10s are highly effective in reversing AHR and airway Th2 recall responses in a mouse model of asthma, including AHR, eosinophilias and Th2 responses, but systemic allergen-specific IgE/IgG1 responses are relatively resistant to tolerization with such DCs (12).
the life span of that tolerance or its durability under allergen rechallenge conditions.

In our study, we explored these issues using OVA-presenting DC10s in a mouse model of OVA-asthma. Specifically, we followed the clinical course of tolerance over ~8 mo in DC10-treated animals, assessed the impact of the anatomic route by which DC10s are given, as well as the impact of multiple DC10 treatments on the asthma phenotype. We show that DC10s can provoke a prolonged and highly effective allergen-specific tolerization of the asthmatic Th2 immunoinflammatory responses in the treated animals, although targeting of AHR is only transiently effective.

Materials and Methods

Animals, reagents, and materials

Female BALB/c mice were obtained from our institutional Animal Resource Centre; all animal treatments were in accord with the guidelines of the Canadian Council on Animal Care. The reagents/materials used and their sources have largely been reported (7, 12, 13). Additional reagents and their sources include the following: 1-methyltryptophan (1-MT) and placebo slow-release polymer pellets (release rate, 10 mg/day; Innovative Research of America, Sarasota, FL); neutralizing anti–IL–10R Abs (obtained through H. Tabel (University of Saskatchewan, Saskatoon, SK, Canada) (15)): mAbs, MIP-1α (CCL3) and MIP-3β (CCL20) (R & D Systems, Minneapolis, MN); FITC-labeled anti-mouse CD11b (clone M1/70), CD14 (clone mouse rC5-3), CD16/CD32 (clone 2.4G2), CD19 (clone 1D3), and CD45RB (clone 16Aa); and FITC-labeled isotype control rat IgG2b, IgG2a, and IgG1, hamster IgG1, IgG2b, and IgM, mouse IgG2a, and IgG2b Abs (Pharmingen Canada, Mississauga, ON, Canada); FITC-labeled anti-CD205 (clone MCA949F) and F4/80 anti-macrophage/monocyte Abs (clone CL:A3-1) (Serotec, Oxford, U.K.).

Generation of DCs

BM-derived DCs were generated largely as noted (16). Briefly, bone marrow cells were seeded in RPMI 1640 supplemented with 1% antibiotics/antimycotics, 50 μM 2-ME, and 10% heat-inactivated FCS (complete medium) containing 20 ng/ml GM-CSF. The culture medium was replaced on days 6 and 8 with fresh GM-CSF–containing medium, and on day 10 the nonadherent DCs were resuspended in complete medium supplemented with 7.5 ng/ml GM-CSF alone (to generate immature cells) or with 50 ng/ml of either IL-10 (to generate tolerogenic cells; DC10s) or, for some experiments, TNF (to generate immunostimulatory DC-TNFs). As noted previously (16), the removal of the adherent cells at day 10 and transfer of the nonadherent cells into low-dose GM-CSF substantially increases the purity of DCs in our cultures (90–95% DCs). On day 13, a portion of the cells from each culture were pulsed for 2 h at 37°C with 50 μg/ml OVA (OVA-DC10) or, as a negative control for Ag specificity (7), house dust mite allergen (HDM-DC10); then the cells were washed.

DC characterization

The cells were assessed by FACS for expression of the noted markers using a FACSScan (Becton Dickinson, Mountain View, CA), with isotype-matched control Ab for all populations. To assess phagocytosis, cells were incubated for 30 min at either 4°C or 37°C with FITC-dextran (100 μg/ml), then washed with ice-cold PBS, fixed in 0.5% paraformaldehyde, and analyzed by FACS (17). CCR5 and CCR7 expression was determined using microchemotaxis assays with MIP-1α and MIP-3β, respectively, as the specific ligands, as noted (18). Our DC10s express modestly to markedly lower levels of CD40, CD54, CD80, and CD86, and MHCII relative to mature DC-TNF (7); avidly phagocytosed FITC-dextran and were responsive to MIP-1α, but not to MIP-3β, and expressed substantially higher levels of TGF-β than did immature DCs (Supplemental Fig. 1). They were not appreciably contaminated with monocytes (CD14), neutrophils (CD16/32), macrophages (F4/80), B cells (CD19) or T cells (CD3), as determined by FACS (i.e., isotype control-level staining only).

ELISA

Our ELISA protocols have been reported in detail previously (5, 7, 12, 19). Cell culture supernatants and bronchoalveolar lavage (BAL) fluids were not diluted, but plasma samples were diluted 1:10 in PBS with Tween 20. The detection limits for our cytokine ELISAs are routinely 5–10 pg/ml.

Statistics

All data were expressed as the mean ± SEM. Group statistics were assessed by one-way ANOVA, with Tukey’s multiple comparison post
hoc testing (Sigma Stat Version 2.0, SPSS, Chicago, IL). Data not normally distributed were log transformed for analysis. Significance was assigned when $p$ values were $\leq 0.05$.

**Results**

**DC10 treatment induces a robust, but transient, reversal of AHR in asthmatic animals**

Tolerogenic DCs can induce a seemingly robust blockade of AHR, whether the DCs are used prophylactically (10, 11) or therapeutically (7, 12), but none of these studies have examined the durability of that tolerance over prolonged time-frames. In our study, we first delivered $1 \times 10^7$ OVA-presenting immature DCs (OVA-imm DC), DC10s (OVA-DC10), DC-TNFs (OVA-DC-TNF), or saline (asthma) (protocol, Fig. 1A) administered i.t. into asthmatic mice and then assessed their AHR over time. None of the treatments significantly affected AHR at 1 wk, but by week 2 the OVA-DC10 group began to show signs of improvement ($p = 0.03$, versus saline-treated asthmatic mice), and by day 21 their AHR was fully normalized ($p \leq 0.01$; Fig. 1B). The airways of the OVA-asthmatic animals that were treated with OVA-pulsed immature DCs or DC-TNFs remained fully hyperresponsive (each, $p < 0.05$, versus saline-treated asthmatic mice). Delivery of OVA-DC10s i.p. reversed AHR with identical kinetics, whereas s.c. injection of DC10s had no impact on AHR (Supplemental Fig. 2). The airways of the OVA-DC10-treated mice remained fully normalized at 8 wk, but, interestingly, at 10 wk the effects of the DC10s on AHR began to wane and by week 13 the airways of the DC10-treated mice were fully hyperresponsive again (Fig. 1B). A second treatment with $1 \times 10^7$ OVA-DC10s at week 14 again fully abolished AHR within 3 wk (data not shown).

**Allergen-specific asthmatic Th2 tolerance induced by DC10 treatments is prolonged and progressive**

OVA-DC10 treatment also affected the pulmonary eosinophil and Th2 responses of the asthmatic mice, as determined at 3 wk after treatment, although not as much as it had affected AHR. The animals were treated as above, or, as a control for allergen specificity of DC10, they were treated with HDM-presenting DC10s. All animals were challenged with OVA aerosol 1 d before sacrifice to reactivate their pulmonary Th2 responses (protocol, Fig. 2A).

The airways of OVA-immune DC- or saline-treated asthmatic animals were rich in eosinophil infiltrates, whereas OVA-DC10 treatment reduced eosinophilia by $59.8 \pm 3.6\%$ ($p < 0.01$, versus asthmatic mice); the irrelevant allergen control HDM-DC10 treatments had no significant effect on the eosinophil response ($p > 0.05$, versus asthmatic mice; Fig. 2B, left panel). The circulating levels of OVA-specific IgE were also significantly reduced in the OVA-DC10- ($p \leq 0.05$, versus asthmatic mice), but not in the OVA-immune DC- or HDM-DC10–treated mice (for both, $p > 0.05$ versus asthmatic mice; Fig. 2B, middle panel). IgG1, but not IgG2a, levels were also significantly reduced by the OVA-DC10 treatment (Supplemental Fig. 3A), as were BAL fluid levels of Th2 cytokines (Fig. 2B, right panel). Thus, IL-4, IL-5, IL-9, and IL-13 levels were each reduced ($p \leq 0.01$ or 0.05, versus asthmatic animals). The airway levels of IL-12 and IFN-γ were also reduced by the OVA-DC10 treatment (IL-12: asthmatic, $279 \pm 117$ pg/ml; DC10-treated, $94 \pm 23$ pg/ml; IFN: asthmatic, $873 \pm 385$ pg/ml; DC10-treated, $124 \pm 51$ pg/ml), indicating that we had not simply induced Th2 $\rightarrow$ Th1 immune deviation. HDM-DC10s did not alter the airway Th2 cytokine responses of the OVA-asthmatic animals (data not shown), confirming that tolerance induced by DC10 is allergen specific (7).

Our observation that DC10 treatment ablated AHR for only $\sim$3 mo led us to question whether it would have similarly short-term effects on asthmatic Th2 immunoinflammatory disease. Thus, we treated OVA-asthmatic mice with saline (asthmatic) or OVA-presenting DC10s or immature DCs, as above, and we followed the treatment effects for 8 mo, using the animals’ serum IgE levels as a noninvasive measure of tolerance (protocol, Fig. 2A). One day before sacrifice, we challenged the mice with concentrated (10 mg/ml) aerosols of OVA to invoke airway recall responses. As might be expected for animals that had not been exposed to allergens for a prolonged period of time, the circulating levels of OVA-specific IgE in the saline-treated asthmatic mice waned across the 8 mo of the experiment ($p \leq 0.01$), but nevertheless remained elevated relative to normal control mice or DC10-treated asthmatic animals. IgE levels of the DC10-treated mice were markedly lower than those of the saline-treated asthmatic mice at all times, approaching background within 4 mo of treatment (99% suppressed) and remaining suppressed at 8 mo ($p \leq 0.001$, $p \leq 0.001$, $p \leq 0.001$).
versus levels in either the saline- or the immature DC-treated mice; Fig 2C, left panel). The airway Th2 recall responses were also markedly affected by the DC10 treatment; the BAL fluid levels of IL-4, IL-5, IL-9, and IL-13 were 86–96% lower (each, p \leq 0.001) than those of immature DC- or saline-treated asthmatic animals (Fig. 2C, middle panel). This trend was mirrored also by the in vitro Th2 cytokine responses of the animals’ lung tissue mononuclear cells to the recall allergen challenge (Fig. 2C, right panel). The responses of the cells from the saline-treated asthmatic animals were significantly lower than those of the OVA-presenting immature DC-treated mice (for each cytokine, p \leq 0.001), whereas the lung T cell IL-4, IL-5, IL-9, and IL-13 responses of the DC10-treated asthmatic animals were reduced by 91–99% relative to the immature DC-treated mice (for each cytokine, p \leq 0.05). A single DC10 treatment also protected the animals repeatedly given DC10s, whether t.t. or i.p. (Fig. 3C, left panel) and i.v. delivery routes (protocol, Fig. 3A). We assessed AHR at 8 wk, prior to challenging the mice with OVA aerosols, and sacrificed them 1 d later, as above. The bronchial responsiveness of the t.t. and i.p. DC10 recipients was fully normalized at 8 wk, but the AHR of the asthmatic s.c. (Fig. 3B) and i.v. (data not shown) DC10 recipients was not discernibly affected by four DC10 treatments.

Repeated t.t. DC10 treatments reduced the IgE (Fig. 3C, left panel) and IgG1 responses by 90–93% at 8 wk (Supplemental Fig. 4), whereas the OVA-specific IgG2a and IgA responses were reduced by 87% and >98%, respectively (data not shown). The i.p. delivery of DC10s similarly reduced the OVA-specific IgE (Fig. 3C, left panel), IgG1, and IgG2a responses by 84%, 94%, and 87%, respectively, DC10 given by the s.c. route did not significantly impact the IgE response (Fig. 3C; p > 0.05). Again, i.v. delivery of DC10 was completely ineffective (data not shown).

The airway Th2 cytokine responses were markedly reduced in the animals repeatedly given DC10s, whether t.t. or i.p. (p \leq 0.01 or 0.001, respectively; Fig. 3C, right panel). Of interest, s.c., but not i.v., delivery of DC did significantly reduce airway Th2 cytokine responses (p \leq 0.01; Fig. 3B). Intriguingly, the levels of IL-10 and TGF-\(\beta\) in the airways of the t.t. and i.p. DC10 recipient mice were upregulated, and substantially so (>5-fold) for TGF-\(\beta\) (Supplemental Fig. 4), whereas this did not occur in the s.c. DC treatment group. For example, BAL IL-10 levels in the saline- and i.p. DC10-treated asthmatic mice were 141 and 197 pg/ml,
We had previously noted that, in vitro, IDO plays a role in CD8+ T cell tolerance (12), and others have reported that IL-10 expression by DC10s (11) is important to their tolerogenic functions. We therefore assessed whether these molecules also have roles in the effector phases of DC10-induced tolerance, administering either neutralizing anti–IL-10R Abs or the IDO antagonist 1-MT (via s.c. slow-release pellets) to groups of DC10-treated asthmatic animals from day 12, just before the effects of DC10 treatment were first discernible, to the time of sacrifice (day 21) (Fig. 5). The anti–IL-10R Ab treatments had no significant impact on bronchial responsiveness (dose of methacholine provoking a 20% fall in pulmonary airflow: anti–IL-10R, 1.57 mg/ml; isotype control Ab, 1.58 mg/ml) or the OV A-specific IgE response (Fig. 5A, upper left panel), their airway Th2 cytokine, IFN-γ (upper right panel), or eosinophil (lower left panel) responses, or the ex vivo expression of Th2 cytokines or IFN-γ by lung T cells (lower right panel)—in each case relative to saline-challenged DC10-treated asthmatic animals. The experiments depicted were repeated three times (n ≥ 5 animals per group).

A critical factor related to therapeutic induction of tolerance is that the treated individual be able to resist spurious allergen challenges without reverting to the asthma phenotype. To assess this, we induced tolerance in OVA-asthmatic mice with DC10s given three times over 4 wk; then 2 wk later, we began a series of physiologically relevant allergen challenges (protocol, Fig. 4A). Thus, on days 42, 49, and 53, we exposed the mice for 10 min to nebulized aerosols of 100 μg/ml OV A, and each time we assessed the effects of DC10 treatment were first discernible, to the time of sacrifice (day 21) (Fig. 5). The anti–IL-10R Ab treatments had no significant impact on bronchial responsiveness (dose of methacholine provoking a 20% fall in pulmonary airflow: anti–IL-10R, 1.57 mg/ml; isotype control Ab, 1.58 mg/ml) or the OV A-specific IgE response (Fig. 5A, upper left panel), their airway Th2 cytokine, IFN-γ (upper right panel), or eosinophil (lower left panel) responses, or the ex vivo expression of Th2 cytokines or IFN-γ by lung T cells (lower right panel)—in each case relative to saline-challenged DC10-treated asthmatic animals. The experiments depicted were repeated three times (n ≥ 5 animals per group).

We had previously noted that, in vitro, IDO plays a role in CD8α+ DC-induced asthmatic T cell tolerance (12), and others have reported that IL-10 expression by DC10s (11) is important to their respective TGF-β levels were 95 and 338 pg/ml, respectively.

A critical factor related to therapeutic induction of tolerance is that the treated individual be able to resist spurious allergen challenges without reverting to the asthma phenotype. To assess this, we induced tolerance in OVA-asthmatic mice with DC10s given three times over 4 wk; then 2 wk later, we began a series of physiologically relevant allergen challenges (protocol, Fig. 4A). Thus, on days 42, 49, and 53, we exposed the mice for 10 min to nebulized aerosols of 100 μg/ml OV A, and each time we assessed the animals’ AHR 3 d later. On day 57, we sacrificed the mice and analyzed their asthma phenotype, as above. We observed no discernible impact of repeated allergen exposure on DC10-induced normalization of AHR and no significant increases in airway eosinophilia or levels of Th2 or Th1 cytokines (Fig. 4B). We also assessed in vitro expression of these cytokines in the lung-draining mediastinal lymph node cells, and found no reversal of tolerance at that level either (Fig. 4, lower right panel). These data suggested that DC10-induced tolerance is not highly sensitive to reversal by spurious environmental allergen exposures.

Roles for IL-10 and IDO in DC10-mediated allergen tolerance

We had previously noted that, in vitro, IDO plays a role in CD8α+ DC-induced asthmatic T cell tolerance (12), and others have reported that IL-10 expression by DC10s (11) is important to their respective TGF-β levels were 95 and 338 pg/ml, respectively.
have shown that TGF-β (10) or IL-10– (10) differentiated DCs can blunt development of the asthma phenotype in systemically sensitized animals and that DC10s can substantially reverse the allergen challenge but did not significantly affect AHR (p > 0.05), IL-9 levels (p = 0.056), or the IL-4 response (data not shown), relative to isotype control Ab-treated mice. (A) Antagonizing IDO had no significant impact on AHR, but did significantly affect the IL-5, IL-9, and IL-13 responses to recall allergen challenge, and also affected the circulating OVA-specific IgG1, but not IgE, levels. The experiments depicted were repeated three times (n ≥ 5 animals per group). *p ≤ 0.05, **p ≤ 0.01 versus their relevant saline-treated asthmatic control group animals.

Discussion

Our data demonstrate that delivery of specific allergen-presenting DC10s administered either directly into the airways or i.p. can be highly effective in reducing the Th2 asthma phenotype in experimental animals for up to 8 mo, but that AHR is reduced to background for only ~3 mo. On the contrary, we had reported previously that airway delivery of quiescent OVA-presenting splenic CD8α⁺ DCs modestly (~50%) reverses AHR, eosinophilia, and pulmonary Th2 responses, but is somewhat less effective in reducing the allergen-specific IgE and IgG1 responses (12). Others have shown that TGF-β– (10) or IL-10– (10) differentiated DCs can blunt development of the asthma phenotype in systemically sensitized animals and that DC10s can substantially reverse the asthma phenotype, as determined 4 wk posttreatment in a model of established house dust mite asthma (7, 11). Tolerance associated with TGF-β–differentiated DCs (10), or with CD8α⁺ DCs (12), is not fully allergen specific, whereas DC10-induced tolerance is driven by cognate processes (7, 11). We found that i.v. delivery of DC10 was completely ineffective in inducing tolerance in asthmatic mice, and this agrees with other findings (26–28), although unlike cells delivered via the tail vein, IL-10 transgene-expressing DCs do prolong graft survival if injected into the portal vein (28).

AHR, or hyperirritability of bronchial smooth muscle, is the hallmark of asthma (29), such that its amelioration would be an ideal outcome of asthma therapeutics, and this was one of the more remarkable findings in DC10-induced tolerance. We found that the OVA-DC10 treatments were effective in reducing AHR, essentially abrogating it within 3 wk, although this normalization lasted only 3 mo. We are not aware of any reports of transient corrections of AHR with regulatory DCs, and at this time we have no direct experimental evidence relating to the mechanisms mediating this effect. Evidence from other reports indicates that AHR can be related functionally to multiple distinct mechanisms. One is IgE/mast cell dependent (30, 31), and another is eosinophil/IL-5 dependent and mast cell independent (32), and we have reported that AHR can develop in the absence of any evidence of IgE or Th2 responses when animals are sensitized with very low doses of allergen (19). This latter model (19) may reflect some of the processes that regulate “intrinsic” versus extrinsic asthma etiology and pathogenesis (33). In the current study, reversal of AHR began prior to observable effects on IgE levels, or IL-5 and eosinophil responses, and this clearly indicates that a dampening of smooth muscle responses can be implemented even in the face of substantial classical Th2 allergen sensitivity. It has been shown that expression of an IL-10 transgene within the airways similarly suppresses AHR (34) and also that IL-10 can block smooth muscle cell activation by inflammatory stimuli in vitro or in vivo, at least in part by directly inhibiting 1-kb degradation and NF-kB nuclear translocation (35). We have found in our laboratory that DC10s, delivered i.p. rapidly migrate to the airways (M. Lu, H. Huang, and J.R. Gordon, unpublished observations), and we reported in this article and elsewhere that human and mouse DC10s secrete elevated levels of IL-10 relative to control DCs (5, 7, 13). Thus, IL-10 released locally in the lungs by the treatment DC10s could potentially have been in part responsible for amelioration of AHR. We (H. Huang, W. Dawicki, and J.R. Gordon, unpublished observations) and others (3, 11) have found that knockdown or knockout of IL-10 expression by DC10 eliminates their abilities to affect AHR, and that is consistent with the argument that the DC10’s IL-10 secretion may initiate normalization of AHR. Our DC10 tracking data also indicate that the numbers of treatment DC10s decline in the lung at the same time as these cells begin to appear in the lung-draining lymph nodes (M. Lu, H. Huang, and J.R. Gordon, unpublished observations), and this suggests that either an alternate factor must regulate AHR at this time or that an alternate source of IL-10 must be or become positioned in the lungs, at least temporarily. Others have reported that chronic aeroallergen exposure upregulates regulatory T cell function and thereby dampens AHR in a rat model of asthma, and that this suppression of bronchial responsiveness requires continual allergen challenge; interrupting the chronic challenge process leads to loss of tolerance and a return of AHR (36). However, in our model AHR returns to the animals despite a continuation of Th2 tolerance, and we have reported that DC10 treatments drive the conversion of Th2 effector T cells in the lungs into CD4⁺CD25⁺Foxp3⁺ regulatory T cells that employ IL-10 as a primary tolerogenic mechanism (13). In concert with this, we have observed conversion of the tolerant animals’ endogenous lung DCs into regulatory cells that also secrete IL-10.
metric relationship exists between the numbers of Ag-presenting cells and the ensuing immune response (41). Our DC10s were CCR7low, whereas at 4–8 mo this effect had increased to ~80%. Similarly, at 3 wk the eosinophil responses to allergen challenge were ~60% below those at the time of treatment, and at 8 mo posttreatment this had increased to ~90% suppression, and the suppression of the Th2 cytokine response was similarly progressive. As noted above, we have shown that DC10s activate regulatory T cells in the lungs of asthmatic mice, with regulatory T cells recovered at 3 wk after DC10 treatment having significantly more activity than those recovered at earlier times (13). We do know, however, that these regulatory T cells subsequently convert the animals’ endogenous lung DCs to a regulatory phenotype (C. Li, W. Dawicki, H. Huang, and J.R. Gordon, unpublished observations), and this feed-forward cascade of events might be anticipated to progressively improve the asthma phenotype. Others have reported, of course, that regulatory T cells have a significant impact on DCs, inducing them to take on a regulatory phenotype (36–39). The time-frames required for a single DC10 treatment to achieve full effect on the asthma phenotype were still quite prolonged relative to what might otherwise be desired, although allergen-specific immunotherapy protocols can require significantly more time yet to reduce allergic symptomatology by ~75% (e.g., 2 y) (40). As such, the observation that multiple DC10 treatments much more rapidly reduced disease severity was important—in our hands four biweekly DC10 treatments brought allergen sensitivity to near background within 8 wk. It is interesting then that DC10s that were delivered i.v. had no discernible impact on asthma, as had been reported previously (26). Intravenous delivery of “tolerogenic” DCs in a mouse model of multiple sclerosis is also ineffective in reversing disease, whereas s.c.-delivered cells were effective in that model (27).

It is intriguing that the cells delivered s.c. in our model were only partially effective in reversing the asthma phenotype. It has been reported that CCR7+ bone marrow-derived DCs that are injected s.c. (i.e., in the footpad) efficiently migrate to the draining lymph nodes of mice and there trigger immune responses, whereas CCR7− DCs do not do so efficiently (41). It was noted that a geometric relationship exists between the numbers of Ag-presenting DCs and the resulting T cell response, wherein decreasing by half the numbers of DCs in the draining nodes reduces by 4-fold the ensuing immune response (41). Our DC10s were CCR7−, such that it could have been they were not able to readily traffic from the skin to the draining lymph nodes and therefore were not as efficient as DC10s that do efficiently reach the lung-draining lymph nodes (i.e., t.i. and i.p. DC10s), as noted above. Certainly, the s.c.-delivered DC10s had little impact on AHR, and that would be consistent with a need for these cells, or the regulatory T cells they induce (13), to reside within the lungs to affect bronchial reactivity. One could speculate that regulatory T cells induced in one compartment (e.g., skin-draining lymph nodes) may not be as efficiently traffic to alternate compartments (e.g., the lungs), such that they may not have been present in sufficient numbers to impact AHR. Thus, for example, CD103+ TGF-β and retinoic acid-expressing DCs from the intestine induce regulatory T cell responses to innocuous gut symbionts, and these induced regulatory T cells express CCR9 and α4β7 integrin, such that they recirculate back to the gut but not other compartments (42). Whether other types of regulatory DCs induce regulatory T cells that recirculate to yet other compartments is an interesting question, the answer to which could have ramifications for compartment-specific applications (e.g., the CNS in multiple sclerosis) of DC immunotherapies.

Our data suggest that both IL-10 and IDO play a role in DC10-dependent reversal of the asthmatic Th2 phenotype, but not in reversal of AHR, at least during the early effector phases of tolerance. Others have reported that IL-10 expression by IL-10–differentiated DCs is required to prevent development of the asthma phenotype in systemically sensitized mice (11) and that IL-10 expression by host cells, presumed to be regulatory T cells, is required for induction of tolerance in asthmatic mice that have been treated with IL-10–lentivirus-transfected DCs (14); the latter authors did not assess tolerization of AHR (14). In addition, we have reported that neutralization of IL-10 prevents DC10-induced regulatory T cells from suppressing effector T cell responses in vitro in our OVA-asthma system (13). It is important to keep in mind that other cells that express Th2 cytokines (e.g., mast cells) can also be regulated by IL-10 (43). Indeed, mast cell-derived IL-10 may well play regulatory roles in asthma and tolerance (44). Although we have no experimental insights into why the anti–IL-10R treatments reduced airway IL-5 and -9 levels and eosinophil responses to allergen recall challenge in our model, but not the IL-4 or IgE responses or AHR, others have also reported that administration of anti–IL-10 during the resolution phase of the asthmatic response attenuates the natural resolution of airway eosinophil responses to recall allergen challenge without affecting AHR (45). Given the linkage between IL-4 and B cell IgE production (46), it would not be unexpected that these two parameters might run in parallel, as we had observed.

In our hands, i.p. DC10 treatments led to a state of tolerance sufficiently robust that three allergen challenges across 11 d did not lead to a return of the asthma phenotype, as determined by assessments of AHR, airway eosinophilia and Th2 cytokine responses, and lung-draining lymph node Th2 responses. Others had demonstrated previously that tolerance induced by treatment of asthmatic mice with DCs transfected with an IL-10–lentivirus construct, such that the cells expressed very high levels of IL-10 relative to our DC10, is also robust (14), although retrovirus-transfected DCs would likely not be employed clinically. The concentration of OVA with which we challenged our mice (100 µg/ml) was higher than levels reported previously to induce immediate and late-phase bronchoconstriction responses in allergen-challenged asthmatic individuals (i.e., 15–20 ng HDM or 5 µg/ml ragweed) (21, 22). Moreover, as noted, our preliminary titrations had indicated that aerosol challenge with 100 µg/ml of nebulized OVA induces ~80% maximal airway eosinophil responses in asthmatic mice.

In conclusion, we have found that IL-10–differentiated DCs differentially impact the AHR and Th2 immunoinflammatory components of the asthma phenotype. As has been reported multiple times, such regulatory cells induce a normalization—in our hands, abrogation—of AHR within 3 wk of treatment; however, although this effect is relatively prolonged, it is nevertheless transient. Within 10–12 wk of the DC10 treatment, AHR returns to pretreatment levels, although it can again be reduced to baseline by retreatment of the asthmatic animals. In contrast, amelioration of the Th2 immunoinflammatory response is progressive over at least 8 mo, when the Th2 phenotype has largely waned. Repeating the...