Transgenic expression of granulocyte-macrophage colony-stimulating factor induces the differentiation and activation of a novel dendritic cell population in the lung

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The role of granulocyte-macrophage colony-stimulating factor (GM-CSF) in the differentiation of dendritic cells (DCs) during pulmonary viral infection was investigated by using a mouse model of GM-CSF transgene expression established with an adenoviral vector (AdGM-CSF). GM-CSF gene transfer resulted in increased levels of GM-CSF in the lung, which peaked at day 4 and remained increased up to day 19. A striking cellular response composed predominantly of macrophage-like cells was observed in the lung receiving AdGM-CSF but not control vector. By FACS analysis, the majority of these cells were identified at an early time point as macrophages and later as mature/activated myeloid DCs characterized by CD11b<sup>bright</sup>, CD11c<sup>bright</sup>, MHC class I<sup>bright</sup>, and B7<sub>1</sub><sup>bright</sup>. In contrast, GM-CSF had a weak effect on a small DC population that was found present in normal lung and was characterized by CD11c<sup>bright</sup> and CD11b<sup>low</sup>. By immunohistochemistry staining for MHC II, the majority of activated antigen-presenting cells were localized to the airway epithelium and peribronchial/perivascular areas in the lung. A concurrently enhanced Th1 immune response was observed under these conditions. The number of CD4 and CD8 T cells was markedly increased in the lung expressing GM-CSF, accompanied by increased release of interferon (IFN)γ in the lung. Furthermore, lymphocytes isolated from either lung parenchyma or local draining lymph nodes of these mice but not the control mice released large amounts of IFNγ on adenosine antigen stimulation in vitro. These findings reveal that GM-CSF promotes the differentiation and activation of a myeloid DC population primarily by acting on macrophages during pulmonary immune responses.

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Introduction

The lung is constantly exposed to the external environment. Thus, the generation of pulmonary immune responses against invading pathogens, including bacteria and viruses, is critical for host defense. Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) essential for the development of primary immune responses against microbial pathogens. Pulmonary DCs are normally distributed within the airway mucosa, alveolar septa, and perivascular parenchyma. Like DCs from other nonlymphoid tissues, pulmonary DCs are originated from either “myeloid” or “lymphoid” precursors and are believed to be functionally immature APCs in the tissue where they exert a sentinel function. On encounter with antigens, DCs move to the T-dependent areas of secondary lymphoid organs where they become mature DCs to activate antigen-specific lymphocytes. Granulocyte-macrophage colony-stimulating factor (GM-CSF), a well-known hematopoietic growth factor, is indispensable for DC differentiation and maturation from bone marrow progenitors or peripheral blood monocytes in a number of in vitro systems. It also has regulatory effects on purified pulmonary DCs. However, relatively little is known about whether GM-CSF has such effects on DCs in vivo. GM-CSF-transduced tumor cells were shown to induce the most potent antitumor immunity compared with tumor cells transduced with other cytokines, and GM-CSF transgenic mice were found to have increased DCs in the peritoneal cavity and lymphoid organs. Furthermore, GM-CSF gene-deficient mice were more susceptible to lung infections by a range of opportunistic microorganisms. Indeed, GM-CSF has been found in increased amounts in a number of pulmonary immune and inflammatory conditions.

Although these lines of evidence suggest a regulatory role of GM-CSF in host immune-inflammatory responses in the lung, the precise role of GM-CSF in the differentiation/activation of DCs in the lung during immune responses has remained to be elucidated. Because GM-CSF is induced primarily under immune-inflammatory conditions in the lung, its effect on APCs in the lung may be best studied in a transgenic model in which GM-CSF is expressed concurrently with an ongoing immune-inflammatory response.

In this study, we utilized a GM-CSF transgene model established by local lung delivery of a replication-deficient adenoviral GM-CSF gene transfer vector to examine the effect of GM-CSF on the differentiation and activation of pulmonary APCs, particularly DCs. This viral-mediated transgene approach targets GM-CSF transgene to the airway epithelial cells and gives rise to a prolonged...
raised GM-CSF level in the lung and thus allows us to study the effect of GM-CSF on APCs in the lung during host immune responses to viral infection.

### Material and methods

#### Gene transfer vectors and antibodies

Construction and characterization of a replication-deficient adenoviral gene vector expressing murine GM-CSF (AdGM-CSF) have been previously described. An adenoviral vector Addl70-3 without transgene was used as a control. UV-inactivated wild-type adenovirus was used as adenoviral antigens for an in vitro antigen stimulation assay. Anti-mouse monoclonal antibodies, including FITC-labeled anti-CD8a (clone 53-6.7), PE-labeled anti-CD11c (clone HL3) and anti-CD4 (clone GK1.5), and biotinylated anti-CD3 (clone 145-2C11), anti-B7.1 (clone BB1), and anti-natural killer (NK) cell (clone DX5) antibodies, were purchased from PharMingen (Mississauga, ON, Canada). FITC-labeled anti-CD11b (anti-Mac1, clone M1/70.15) was produced, purified on protein G, and labeled with biotin. The mAb 2.4G2 (anti-FcR IIb/III) was produced as ascites and purified on protein G.

Binding of biotinylated antibodies was identified by streptavidin-conjugated peridinin chlorophyll protein (Becton Dickinson, San Jose, CA).

#### Mice and GM-CSF gene transfer in the lung

Female Balb/c and C3H mice of 10- to 14-week age were purchased from Harlan Laboratories (Indianapolis, IN) and housed under specific pathogen-free conditions before use at McMaster University Central Animal Facility. Mice were anesthetized and AdGM-CSF or Addl70-3 was intranasally (i.n.) delivered to mouse lung by a standardized procedure that we have previously described. Briefly, a dose of 0.6 × 10⁹ plaque-forming units (pfu) of viral vector was diluted with phosphate-buffered saline (PBS) to a total volume of 30 µL and delivered into mouse lungs with a fine pipette tip in 2 aliquots (15 µL each). Mice were killed at days 2, 4, 7, 12, or 19 postgene transfer. We have previously shown that, following i.n. vector delivery, transgene is expressed primarily by bronchial epithelial cells and, to a certain extent, by alveolar macrophages.

#### Bronchoalveolar lavage (BAL) and cytologic analysis

At each time point, BAL was performed as previously described. A total of 450 µL of PBS was used to lavage the lung, and usually 350 µL of BAL fluids were retrieved. BAL fluids were then spun in a microcentrifuge at 5000 rpm for 5 minutes, and supernatants were stored at −20°C until cytokine measurements. Cell pellets were resuspended in PBS and total cell numbers were counted on a hemocytometer. Cytospins were prepared by cytocentrifugation (Shandon Inc, Pittsburgh, PA). Differential cell counts were determined on Diff-Quik-stained (Baxter, McGaw Park, IL) cytospins by randomly counting 400-500 cells per slide.

#### Flow cytometric analysis of T-cell and NK-cell subsets in BAL and APCs in the lung

To phenotype immune cell subsets in the lung, BAL-derived cells were pooled from three AdGM-CSF-treated mice or five Addl70-3-treated mice 7 days after infection. About 0.3 × 10⁶ cells were blocked by an anti-Fc receptor antibody 2.4G2 for 15 minutes and then labeled with a combination of mAbs of biotinylated anti-CD3, PE-anti-CD4 and FITC-anti-CD8, or FITC-anti-CD3 and biotinylated anti-NK DX5. The basic staining procedure was carried out as previously described. A FACScan instrument was used (Becton Dickinson, Sunnyvale, CA) to collect list mode data (10,000 total events) for analysis. Analysis was carried out using Lysys II software (B-D) by first setting a forward- and side-scatter gate that included lymphocytes but excluded dead cells and debris.

In separate experiments, mice were killed 5 or 12 days after delivery of AdGM-CSF, Addl70-3, or PBS. The lungs were removed from the chest with the heart and a portion of the trachea intact. Pulmonary vasculature was perfused with 5 mL of warm calcium and magnesium-free 1 X HBSS containing 5% fetal calf serum (FCS), 100 U/mL penicillin, and 100 g/mL of streptomycin via the right ventricle of the heart. Total lung mononuclear cells were isolated by collagenase digestion, followed by discontinuing gradient centrifugation as previously described. Approximately 0.3 × 10⁶ cells were labeled with mAbs in a combination of FITC-CD11b, PE-CD11c, and biotinylated-antibody to a surface molecule of interest (MHC class II or B7.1) or biotinylated-isotype control antibody rat immunoglobulin (Ig)G2a. List mode data (20,000 total events) were collected on FACScan and analysis was performed on cells gated in high forward and scatter region (R1 region), distinct from regions defined for lymphocytes and debris (see Figure 1A). More than 90% of CD11c-positive cells were found within R1 region by back-gating analysis. The cells from R1 region were further divided into three subpopulations (R2-R4) on the basis of CD11b and CD11c expression (Figure 1B). The unstained cells gated on R1 region were used as negative controls, and the background was approximately 2% and 1.2% for PE-CD11c and FITC-CD11b, respectively. The absolute numbers of cell subsets based on CD11c and CD11b were calculated on the basis of the total number of cells recovered per mouse, multiplied by the fraction of cells in the high scatter region, and then multiplied by the fraction of cells with a given CD11c, CD11b phenotype.

#### T-cell enrichment from lung and draining lymph nodes and in vitro stimulation with adenoviral antigen

Mice were killed 12 days after delivery of AdGM-CSF or Addl70-3. Total lung mononuclear cells were isolated as above and were enriched for T cells by using a mouse T-cell-enrichment column (R&D System, Minneapolis, MN) according to manufacturer’s instructions. The resultant

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population contained more than 90% T lymphocytes with 5% to 10% antigen-presenting cells. Cell suspensions were cultured in RPMI-10 medium (RPMI 1640, supplemented with 10% FCS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM L-Glutamine). From the same mice, mediastinal lymph nodes were collected and kept in HBSS, then gently ground between two sintered glass slides. The resultant lymphocyte suspension was filtered through one layer of nylon membrane (55 µm) and centrifuged at 1200 rpm for 10 minutes at 4°C. The cell pellets were washed once with PBS and resuspended in RPMI-10 medium.

Approximately 0.2 × 10^6 lung-derived or 0.3 × 10^6 lymph node-derived cells were seeded into 96-well plates and cultured with or without 1 × 10^6 puw/Well of UV-inactivated adenovirus at 37°C for 72 hours. Other wells received 8 µL/mL of an irrelevant mycobacterial antigen (PPD). The supernatants were taken at 72 hours and stored at −20°C until cytokine measurement.

### Immunohistochemical staining for MHC II expression

Lung tissues were obtained at 12 days after delivery of AdGM-CSF or Addl70-3 and fixed in 30 mL of 4% formaldehyde in 75 mmol/L phosphate buffer (pH 7.4) for 24 hours. Paraffin sections were deparaffinized in xylene followed by 100% ethanol and then placed in freshly prepared methanol H_2O_solution for 30 minutes (200 mL methanol, 10 mL hydrogen peroxide, and 0.5 mL concentrated HCl) to block endogenous peroxidase activity. After rehydration, the slides were subjected to hot Hewlett’s Epitope Recovery Buffer (10 mmol/L citrate/phosphate buffer, pH 6.85) for 10 minutes and then allowed to cool down to room temperature. The slides were blocked with 1% BSA/PBS for 15 minutes, followed by incubation with 1:100 rat anti-mouse biotinylated MHC II M5 mAb in 1% BSA/PBS overnight at room temperature. Following the incubation with 1:300 secondary biotinylated rabbit anti-rat antibody at room temperature for 1 hour and 1:1200 streptavidin/peroxidase conjugate for 90 minutes, the slides were developed by a conventional substrate/chromogen solution and counter-stained with 50% hematoxylin for 2 minutes.

### Cytokine measurements

The level of cytokines in the BAL and culture supernatants was determined by using mouse specific enzyme-linked immunosorbent assay (ELISA) kits. Interferon (IFN)γ and GM-CSF ELISA kits were purchased from R&D Systems. The sensitivity of detection for these ELISA kits was 2 pg/mL, MIP-1 and MCP-1 ELISAs were developed as previously described.

### Results

**GM-CSF transgene protein levels and cellular responses in the lung post-GM-CSF gene transfer**

To determine the level of GM-CSF transgene protein in the lung, BAL fluids collected at various time points following lung GM-CSF gene transfer were measured for murine GM-CSF by ELISA. The level of GM-CSF markedly increased by day 2, peaked by day 4, still remained high at day 7, and significantly decreased by day 12 in the lung of mice after GM-CSF gene transfer (Table 1). In contrast, little GM-CSF was detected in the BAL from mice receiving the control vector Addl70-3. To evaluate cellular responses to GM-CSF in the lung, total and differential cell counts in the BAL were determined at various time points. The control vector induced only minimal cellular responses throughout the entire experiment (Table 2). In contrast, GM-CSF induced an increase in total cell numbers that peaked at day 12, being 8 times as many as in mice receiving Addl70-3 (Table 2). Macrophages/monocytes represented a major cell type among increased leukocytes. The number of lymphocytes also markedly increased. For instance, at day 12, there were 50.4 × 10^4, 24 × 10^4, and 13.2 × 10^4 of macrophages/monocytes, lymphocytes, and neutrophils, respectively, in BAL from the lung of mice expressing GM-CSF versus 10.8 × 10^5, 0.64 × 10^5, and 0.17 × 10^4, respectively, in the lung of mice receiving Addl70-3 (Table 2). By day 19, although the cellular response almost completely resolved in the lung of control mice, the number of macrophages/monocytes in the lung of mice receiving AdGM-CSF still remained elevated. In addition to macrophages, lymphocytes, and neutrophils, there was also a small, but significant, increase in the number of eosinophils. The delayed peak cellular response, as compared with the earlier peak time of GM-CSF transgene product in the lung, suggests that such increased cell responses are not just the effect of GM-CSF on cell influx and that it is more likely a result of the enhancement by GM-CSF of cellular immune responses to viral infection that normally takes 7 to 10 days to peak. Consistent with the cellular profiles observed in the BAL, total mononuclear cells isolated from the lung tissue of mice expressing GM-CSF at day 12 were 10 times as high as that in control groups (data not shown).

**Phenotypes of APCs induced by GM-CSF transgene expression in the lung**

Having demonstrated that GM-CSF induced a marked cellular response of primarily monocyteic nature in the lung, we analyzed the phenotype of this macrophage/monocyte population by FACS. To this end, total mononuclear cells were isolated from mouse lung tissue receiving AdGM-CSF, Addl70-3, or PBS at day 12 and stained with different combinations of mAbs to various leukocyte
surface markers, including CD11b (Mac-1), CD11c, MHC II, and B7.1. A cell population (Figure 1A, R1 region) showing higher forward- and side-scatter properties was gated and subdivided into three populations, based on their relative CD11c and CD11b expression. These populations were cells expressing CD11bbright and CD11cbrill (R2 region), CD11bbright and CD11cbrill (R3 region), or CD11bbright and CD11clow (R4 region), respectively (Figure 1B). CD11blow/CD11cbrill cells were lung residential DCs, phenotypically similar to some DCs found in lymphoid organs.11,25-27 CD11bbright/CD11cbrill cells represented a myeloid DC phenotype also found in lymphoid organs11,26 and were a novel DC population induced by GM-CSF that we now identified in the lung. CD11bbright/ CD11clow cells were macrophages.25 In PBS- or Addl70-3-treated animals, about 60%-80% of CD11c+ cells were CD11bbright/CD11clow. In contrast, GM-CSF expression resulted in a striking increase in the percentage of CD11bbright/CD11clow double-positive cells, which accounted for about 90% of CD11c+ cells (Figure 1B). Thus, GM-CF induced an approximately 44-fold increase in the number of CD11bbright/CD11clow DCs over that found in PBS- or Addl70-3-treated groups (Figure 1C). GM-CSF also induced a fourfold and threefold increase in the number of CD11bbright CD11clow cells and CD11clow/CD11cbrill cells, respectively (Figure 1C). These results indicate that GM-CSF plays a major inductive role in the differentiation of the CD11bbright/CD11clow double-positive myeloid DC population.

Activation of DCs and macrophages by GM-CSF transgene expression in the lung

To examine the activating effect of GM-CSF on each APC population during viral infection, we also examined expression of phenotypic activation markers MHC class II and costimulatory molecule B7.1 that are expressed in high intensity by activated phenotypic activation markers MHC class II and costimulatory population during viral infection, we also examined expression of GM-CSF expression on CD11bbright/CD11clow macrophages in R4 region (Figure 1B). The absolute cell numbers for double positive for CD11c and MHC II or B7.1 derived from single mouse in different groups.

**Figure 2.** Effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on activation of R2 region CD11bbright/CD11clow population during pulmonary adenoviral infection. Following the procedure described in Figure 1, (A) MHC II and B7.1 expression were examined on R2 region for each group. Open histograms represent the background staining with the isotype control antibody, whereas solid histograms indicate staining with relevant monoclonal antibody against the indicated surface molecule. M1 markers represent the limit defining the expression at high levels of the corresponding marker. In the case of B7.1 expression, the majority of cells from R2 region in control groups expressed at dull level that labeled as M8. (B) The absolute cell numbers for double positive for CD11c and MHC II or B7.1 derived from single mouse in different groups.

**Figure 3.** Histograms showing staining with relevant monoclonal antibody against the indicated surface molecule. M1 markers represent the limit defining the expression at high levels of the corresponding marker. In the case of B7.1 expression, the majority of cells from R2 region in control groups expressed at dull level that labeled as M8.
CD11c\textsuperscript{bright}, MHC class II\textsuperscript{bright}, and B7.1\textsuperscript{bright} in the lung, we investigated whether this DC population could have derived from a macrophage population expanded by GM-CSF at an earlier time. To this end, a group of mice were killed 5 days post-AdGM-CSF gene transfer. Mononuclear cells were isolated, immunostained, and analyzed in the way we did at day 12. A comparison was made between 5 days and 12 days post-GM-CSF gene transfer. GM-CSF markedly induced a CD11b\textsuperscript{bright}/CD11c\textsuperscript{low} macrophage population at day 5 after transgene expression (Figure 5). These cells accounted for 46.3\% of total analyzed cells as opposed to 16.3\% found in PBS controls (Figure 1B) and had increased MHC II expression (data not shown). In comparison, only 9\% of analyzed cells were CD11b\textsuperscript{bright}/CD11c\textsuperscript{bright}. At day 12, however, while macrophage population decreased dramatically to only 15.4\% of total cells, 57.6\% of cells were CD11b\textsuperscript{bright}/CD11c\textsuperscript{bright}. Of note, the percentage of CD11b\textsuperscript{low}/CD11c\textsuperscript{bright} cells remained similar to that found at day 5 (11.8\% vs 9\%). Thus, the phenotypic shift from macrophages to myeloid DCs during the course of GM-CSF transgene expression strongly suggests that CD11b\textsuperscript{bright}/CD11c\textsuperscript{bright} DCs induced by GM-CSF were primarily derived from a macrophage population activated earlier by GM-CSF.

Localization of APC in the lung by immunohistochemistry

We next examined the localization of APC in the lung by immunohistochemistry with an anti-MHC II mAb. Few MHC II-positive cells were found in the lung of mice receiving AddI70-3 (Figure 6A). In contrast, many MHC II-bearing cells were localized

![Figure 3. Effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on activation of R3 region CD11b\textsuperscript{bright}/CD11c\textsuperscript{bright} population during pulmonary adenoviral infection.](image)

![Figure 4. Effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on activation of R4 region CD11b\textsuperscript{bright}/CD11c\textsuperscript{low} population during pulmonary adenoviral infection.](image)
to peribronchial and perivascular areas, many of which could represent activated GM-CSF-induced DCs although morphologically they appear to be macrophages (Figure 6B). In addition, some MHC II-positive cells were also localized to the airway epithelium, and because these cells possessed dendrites or projections, they were most likely intraepithelial dendritic cells (Figure 6B).

Enhanced immune responses in the lung by GM-CSF transgene expression

The primary function of DCs is to activate antigen-specific T cells, enhancing their proliferation and cytokine responses during primary immune responses.1,4,28,30 Having demonstrated that GM-CSF transgene expression markedly promoted the differentiation and activation of myeloid APC populations in the lung during immune responses to adenoviral infection, we further examined and compared the level of immune responses with pulmonary viral infection in the lung of mice receiving Ad70-3 or AdGM-CSF. To this end, we first examined by FACS analysis the number of immune subsets including NK, CD4, and CD8 T cells in the lymphocytic population present in BAL fluids recovered at day 7 postgene transfer. In our previous studies, we have found that the cellular profiles of lymphocytes, macrophages, and granulocytes in BAL fluids always mirror those seen at the histopathologic level.18-22 We have previously shown that T-cell responses are an important aspect of host anti-adenoviral immune response in the lung.31 The number of NK cells was found similar between control and GM-CSF groups (Figure 7). However, there were approximately 34-fold and 16-fold increases in the number of CD4 and CD8 T cells, respectively, in the lung of the GM-CSF group (Figure 7).

Further, we examined the level of an antiviral Th1 cytokine IFNγ in the lung. The level of this cytokine was only marginally increased in the lung of mice receiving the control viral vector. In sharp contrast, there was a significant induction of IFNγ in the lung of the GM-CSF group, which peaked at day 7 and remained high at day 12 and markedly declined by day 19 (Figure 8). In addition, we also observed increased levels of chemokines MIP-1α and MCP-1 in the BAL in the AdGM-CSF group, particularly at early time points (data not shown).

Furthermore, we isolated lymphocytes from both lung tissue and mediastinal lymph nodes 12 days after administration of AdGM-CSF or Ad70-3 and investigated the level of Th1-type lymphocyte response to adenoviral antigen stimulation in vitro by measuring the release of IFNγ. As shown in Figure 9, although there was an antigen-specific recall IFNγ response by lymphocytes isolated from the lung or mediastinal lymph nodes of mice infected with the control virus Ad70-3, such a response was enhanced many times in lung- or mediastinal lymph node-derived lymphocytes from mice infected with AdGM-CSF. These findings suggest that GM-CSF enhances a Th1-type host immune response to viral infection.

Discussion

In this study, we have used a unique mouse model of adenoviral-mediated GM-CSF transgene expression to investigate the role of GM-CSF in pulmonary DC differentiation and activation during an immune response to viral infection in the lung. We have shown an increased cellular response composed predominantly by macrophage/microcyte-like cells following GM-CSF gene transfer in the lung. By FACS analysis, two DC populations, CD11blow/CD11c bright and CD11b bright/CD11c bright, were identified with the

![Figure 5. Major effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) on macrophages at earlier times in the lung.](image-url)

![Figure 6. Distribution of MHC II positive dendritic cells (DCs) and macrophages in the lung.](image-url)
GM-CSF is released by airway epithelial and endothelial cells, fibroblasts, macrophages, and carcinoma cells in response to a number of stimuli in vitro17,32-36 and has been found increased in the lungs during a number of pulmonary immune conditions.17 However, the precise role of GM-CSF in the pathogenesis of these pulmonary conditions has remained poorly understood. Results from our current study have not only demonstrated a potent effect of GM-CSF on the differentiation and activation of DCs and macrophages but also revealed the nature of a novel DC phenotype driven by GM-CSF during an immune response to pulmonary viral infection. Our results suggest that GM-CSF has a weak effect on the percentage of each subtype.

CD11b<sup>low</sup> and CD11c<sup>high</sup>. However, GM-CSF dramatically induces the emergence of a DC population characterized by CD11b<sup>high</sup>, CD11c<sup>high</sup>, MHC II<sup>high</sup>, and B7.1<sup>high</sup>, in addition to its activating effect on macrophages characterized by CD11b<sup>high</sup>, CD11c<sup>low</sup>, MHC II<sup>high</sup>, and B7.1<sup>high</sup>. Recently, Suda et al have reported that the number of DC precursors present in the pulmonary vascular compartment is 76% greater than that in the vena cava, and these precursors, on exposure to GM-CSF in vitro, have a strong ability to activate alloreactive T cells. It is possible that some of the GM-CSF-expanded DCs observed in our study derived from such precursors. However, Palucka et al have demonstrated that human macrophages could convert into DC in vitro in the presence of GM-CSF. Our current study has provided the first in vivo evidence to support such conversion. We found that macrophage population was induced 5 days after GM-CSF transgene delivery and dramatically decreased at the time when a myeloid DC population emerged at day 12. Thus, these findings, together with the fact that GM-CSF is a well-known stimulator of macrophage proliferation,17,18,39 strongly suggest that GM-CSF-induced DCs derived primarily from expanded macrophages. In further support of our findings, such CD11b-expressing myeloid DCs have recently been identified in lymphoid organs.11,25 Compared with lymphoid-derived DCs, myeloid-derived DCs are believed to play a differential immune-stimulatory role in host defense.30,40-42 This selective effect on myeloid DCs by GM-CSF suggests that GM-CSF is a proimmune cytokine in the lung. Indeed, we observed a markedly enhanced immune response at both cellular and cytokine levels during adenoviral infection in the lung. Our recent demonstration that airway allergic sensitization to repeated airway ovalbumin challenges only occurred in mice that expressed GM-
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