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Plasmacytoid Dendritic Cells Limit Viral Replication, Pulmonary Inflammation, and Airway Hyperresponsiveness in Respiratory Syncytial Virus Infection

Hongwei Wang, Nina Peters, and Jürgen Schwarze

Plasmacytoid dendritic cells (pDC), as major producers of IFN-α, are thought not only to be pivotal in antiviral immunity, but also to limit allergic inflammation. In this study, we delineate the role of pDC in a mouse model of respiratory syncytial virus (RSV)-induced airway inflammation. Bone marrow-derived pDC generated high levels of IFN-α upon RSV infection, and the percentage of pDC expressing MHC class II and maturation-associated costimulatory molecules was increased. However, their weak Ag-presenting capacity was not enhanced. Furthermore, pDC induced marked levels of IL-10 in T cell cultures irrespective of infection. In vivo, numbers of pDC in the lung increased early after RSV infection and remained elevated throughout the inflammatory phase and the resolution phase of infection. Depletion of pDC resulted in increases in peak RSV titers, pulmonary inflammation, and airway hyperresponsiveness. In contrast, adoptive transfer of activated pDC to the airways reduced RSV copy numbers. In conclusion, RSV infection induces activation of murine pDC with robust IFN-α production, limiting replication and accelerating elimination of RSV. In addition to this innate response, pDC also may play an immune regulatory role in reducing pulmonary inflammation and inhibiting the development of airway hyperresponsiveness. The Journal of Immunology, 2006, 177: 6263–6270.

Respiratory syncytial virus (RSV), infections, which occur worldwide and can result in bronchiolitis in infants and in severe lower respiratory tract infection and pneumonia in the elderly, constitute a major health and economic burden (1, 2). In addition, RSV bronchiolitis is a risk factor for asthma development in childhood (3, 4), and RSV, like other respiratory viruses, is an important trigger of asthma exacerbations in children and adults (5, 6). Severe RSV disease is associated with excessive pulmonary inflammation, and animal models indicate that this is due primarily to host immune responses, rather than the direct cytopathic effects of the virus. T cells in particular, including type 2 cytokine-producing CD4+ and CD8+ T cells, are central for RSV-induced airway inflammation (7) and the development of airway hyperresponsiveness (8, 9).

Primary T cell responses are initiated by professional APC such as dendritic cells (DC), which also determine the quality of T cell responses and contribute to the establishment of T cell memory (10). Two major subsets of DC have been recognized: 1) myeloid DC, the principal APC; and 2) plasmacytoid DC (pDC), which are thought to be pivotal cells in antiviral immunity. pDC also have been described in a murine model of allergic airway inflammation, where they seem to play a suppressive role and may induce regulatory T cells (11). Depletion of pDC in this model enhanced the development of allergic airway inflammation. The antiviral effects and possibly antiallergic properties of pDC, which also have been called natural IFN-producing cells, may be due to their ability to produce high levels of IFN-α upon activation by viral and bacterial stimuli. Interestingly, many viruses, including RSV, have developed strategies to reduce type 1 IFN production in host cells or to evade their effects (12). Nonstructural proteins 1 and 2 convey some protection for RSV from antiviral effects of type 1 IFN by inhibiting the activation of IFN regulatory factors (13–15), and RSV has been found to be a poor inducer of type 1 IFN production in many cell types, including epithelial cells, macrophages, and myeloid DC (16, 17). In contrast, human pDC produce high levels of IFN-α in response to RSV infection (17, 18).

In this study, we delineate the role of lung pDC for antiviral responses and for pulmonary inflammation and lung function changes in RSV infection. We demonstrate that murine pDC are permissive for RSV infection, and that infection induces robust production of IFN-α. In addition, infection results in phenotypic maturation of pDC but does not increase their ability to induce T cell proliferation. Importantly, using depletion and adoptive transfer of pDC in a murine model of RSV infection, we demonstrate central roles for activated pDC in the lung, inhibiting replication and enhancing elimination of the virus, as well as limiting RSV-induced pulmonary inflammation and airway hyperresponsiveness.

Materials and Methods

Virus

Human RSV (type A (A2 strain), free of chlamydia or mycoplasma contamination; LGC Promochem) was plaque purified and grown in HEP-2 cell (LGC Promochem) culture in RPMI 1640 medium supplemented with 2% FBS, 2 mM l-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen Life Technologies). Virus was titered by immunoplaque assay using biotin-conjugated goat anti-RSV Ab (Biogenex) as described previously (19). Resulting stock solution contained 1 × 10⁷ PFU/ml RSV.

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For controls, RSV was inactivated by UV irradiation (UV-RSV) for 15 min at 1200 mJ/cm² on ice using the UV Stratalinker 2400 (Stratagene).

**Mice**

Female BALB/c AnNCr mice, 8–12 wk of age, free of specific pathogens, were obtained from Charles River Laboratories and kept under specific pathogen-free conditions. All experimental animals were used under a protocol approved by the Home Office (London, U.K.).

**Isolation of lung and bone marrow cells**

Lungs were harvested from naive mice or on days 2, 6, 9, 14, and 21 after RSV infection. Before harvest, lungs were gently perfused via the right ventricle with 5–10 ml PBS containing 0.6 mM EDTA to remove blood cells from the pulmonary circulation. Lungs were then cut into small pieces and incubated with collagenase type IA-S (0.7 mg/ml PBS; Sigma-Aldrich) and type IV bovine pancreatic DNase (30 mg/ml PBS; Sigma-Aldrich) for 30 min at 37°C. After digestion, lung cells were dispersed by shearing through a 20-gauge needle, followed by filtration through a nylon screen cell strainer (70 mm). Single-cell suspensions were washed with PBS, recontaining erythrocytes were lysed using ACK lysis buffer (0.15M NH₄Cl, 1 mM KHCO₃, and 0.1 mM EDTA), and viable cells were counted by trypan blue exclusion.

Bone marrow cells were flushed from femurs with PBS supplemented with 2% heat-inactivated FBS (Invitrogen Life Technologies) and resuspended in Tris-ammonium chloride at room temperature for 3 min to lyse RBC. They were then used for culture of pDC.

**Generation of murine pDC**

Murine bone marrow-derived pDC were generated as described previously (20). Briefly, bone marrow cells were cultured for 9 days at 37°C 5% CO₂ in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 0.3 mg/ml L-glutamine (PSG), and 10% FBS, all from Invitrogen Life Technologies, and 200 ng/ml recombinant human FMS-like tyrosine kinase 3-ligand (Flt3-L; a gift from Amgen, Thousand Oaks, CA), rhC3d (Peprotech, Rocky Hill, NJ), and 200 ng/ml recombinant human CCL-3 (SDF-1, 200 U/ml), 10 ng/ml IL-7, and 10 ng/ml IL-15.

For one experiment, bone marrow-derived myeloid DC were generated in culture with GM-CSF as described previously (22).

**Flow cytometry**

Following FcR blockade with anti-mouse CD16/CD32 (24G2; BD Biosciences), cells were stained with the following anti-mouse Abs (all from BD Biosciences, except for DO.11.10 TCR-specific mAb (KJ1-26) for OVA323–339-specific CD8 T cells) and FITC-conjugated anti-RSV Ab (DakoCytomation).

**Cytokine detection**

IFN-α was detected in supernatants of pDC infected with RSV, mock-infected pDC, or cultured in the presence of CpG 1826 using ELISA as described previously (23). Anti-IFN-α multisubset Ab (F18 Hycult; Hycult Biotechnology) was used as capture Ab and rabbit polyclonal anti-IFN-α (PBL Biomedical Laboratories) as detection Ab. Murine IL-10, IFN-γ, and IL-4 levels were measured by ELISA using Ab pairs from BD Bioscience. The assays were performed according to the manufacturer’s instructions. The detection limits were as follows: 15 pg/ml IL-4, 30 pg/ml IL-10, and 60 pg/ml IFN-γ.

**Ag-specific T cell proliferation**

OVA323–339-specific DO11.10 T cells were sorted and labeled with 5 nM CFSE as described previously (24). For proliferation assays, OVA323–339 peptide-pulsed or mock-pulsed, lung-derived myeloid DC (5 × 10⁴ per well) were cultured with CFSE-labeled CD4⁺ DO11.10 T cells (2.5 × 10⁵ per well) in 200 μl of complete medium in 96-well flat-bottom plates at 37°C in 5% CO₂. Assays were performed in triplicate. Cells were harvested after 4 days of culture, and CFSE fluorescence intensity was determined by flow cytometry.

**Experimental protocols in vivo**

Mice were infected under light anesthesia with isoflurane intranasally with 1 × 10⁴ FFU of RSV in 100 μl of UV-RSV as control. RSV infection was confirmed by ex vivo plaque assay (25). Briefly, on day 4 postinfection, mice were killed, and lungs were harvested and homogenized in 1 ml of RPMI 1640 medium without FCS. The homogenate was clarified by centrifugation, and HEp-2 cells were then incubated with serial dilutions of lung supernatant for immunoplaque assays (19). RSV titers were determined as plaque-forming unit per lung.

To deplete pDC, some groups of mice received four i.p. injections of 100 μg of 120G8 Ab (a gift from Schering-Plough, Kenilworth, NJ) in 100 μl of PBS or isotype control on days –1 to 2 after RSV infection (11, 26). On day 4 postinfection, depletion of pDC was assessed by flow cytometry in total lung cells, and RSV titers were determined by immunoplaque assay. On day 9, lung function and airway inflammation were assessed. In separate experiments, mice received activated pDC or PBS before infection. CD11b⁺ CD11c⁺ B220⁺ pDC (21) were isolated as described above and treated with 10 μg/ml CpG1826 (5′-TCCATGACGT TCCGACGT-3′) (Sigma-Genosys) for 24 h were adoptively transfered by intratracheal inoculation 6 h before RSV infection. RSV titers were determined by quantitative PCR on day 9 postinfection.

**Lung function**

Lung function was assessed using a whole-body plethysmograph (Buxco Europe) as described previously (27). In the plethysmograph, mice were exposed to nebulized PBS and, subsequently, to increasing concentrations of aerosolized methacholine (Sigma-Aldrich) in PBS for 3 min using an Aerogen aerosol delivery system (Buxco Europe). During each aerosolization and for 4 min afterward, enhanced pause (Penh) values were recorded, averaged, and expressed as absolute values.

**Bronchoalveolar lavage (BAL) and lung histology**

BAL was performed on day 9 after infection. Mice were sacrificed in deep anesthesia with pentobarbital sodium. Following cannulation of the trachea, lungs were lavaged through a polyethylene tube with 3 × 1.0 ml of PBS. Cell viability was assessed by trypan blue exclusion, and total cell numbers in BAL fluid were counted by hemocytometer. One hundred-microliter aliquots of BAL fluid were centrifuged onto Cytospin slides (using Model 2 Cytospin, Shandon Scientific), and 500 BAL cells, stained with Diff-Quick (Reagen), were differentiated by light microscopy at a magnification of ×1000 (oil immersion).

Following BAL, lungs were inflated with 1 ml of formalin solution (10% formalin in PBS) and fixed in formalin solution for histology. Tissue sections (2–3 μm) were cut, stained with H&E or periodic acid-Schiff, and assessed by light microscopy for peribronchial, perivascular, and parenchymal inflammation.

**Statistical analysis**

Groups were compared using GraphPad Prism 4.02 (GraphPad). The Mann-Whitney U test was used for the comparison of two groups, and the general linear models test with post hoc analysis was used for comparisons of more than two groups. Values of p < 0.05 were considered statistically significant. Values for all measurements are expressed as the mean ± SEM unless stated otherwise.
Results

Murine pDC are susceptible to RSV infection

Mouse DC were generated from bone marrow cells cultured in the presence of FMS-like tyrosine kinase 3 ligand (Flt3-L), resulting in two major populations, CD11b<sup>+</sup>CD11c<sup>+</sup>B220<sup>−</sup> myeloid DC and lineage-negative, CD11b<sup>−</sup>CD11c<sup>−</sup>B220<sup>−</sup>Ly6c<sup>−</sup> pDC. The latter are shown in Fig. 1A. From these cultured DC, pDC were isolated for additional experiments by FACS sorting, and a final purity of 99% was achieved.

To determine the susceptibility of murine pDC to RSV infection, these cells were cocultured for 1 h with live RSV or UV-RSV and then washed. RSV replication was assessed at different time points by quantitative PCR. RSV L-gene copies were detectable by 2 h after infection and peaked at 24 h, with 16,770 ± 5,268 copies per 2 μg of RNA, indicating viral replication. Following UV-RSV exposure, only minimal expression of RSV L-gene (1,068 ± 439.7 copies per 2 μg of RNA) was detected (Fig. 1B). Already at 36 h, copy numbers of RSV L-gene decreased again. In addition to RSV replication, we also detected intracellular production of RSV protein in pDC (Fig. 1C). These results indicate that murine pDC are permissive for RSV entry and limited replication.

RSV infection of pDC stimulates secretion of IFN-α

Next, we assessed production of IFN-α in pDC infected with RSV. Supernatants were collected 24 h after infection, and IFN-α concentrations were assessed by ELISA. Live virus, but not UV-RSV, induced high levels of IFN-α production in pDC, reaching similar concentrations as seen in influenza virus-infected controls (Fig. 2). Naive, PBS-treated pDC produced only minimal amounts of IFN-α. Thus, RSV infection is a strong stimulus for IFN-α production in pDC.

RSV infection induces maturation of pDC but does not enhance their ability to induce T cell proliferation

To assess the effects of RSV infection on the phenotype of pDC, we monitored expression of MHC class II (MHC-II) and costimulatory molecules by flow cytometry. In the absence of RSV infection, 45.1 ± 3.8% of pDC expressed MHC-II at intermediate levels. RSV infection increased the frequency of MHC-II expressing pDC to 73.3 ± 4.9% (p < 0.05, n = 3), but the intensity of MHC-II expression remained unchanged (Fig. 3). Monitoring the expression of CD40, CD80, and CD86 in noninfected pDC, we found these costimulatory molecules on only a minority of cells. Twenty-four hours after RSV infection, CD40 expression did not change significantly, while the percentage of pDC expressing CD80 and CD86 increased significantly from 17.76 ± 1.19% and 24.28 ± 2.51%, respectively, to 34.52 ± 5.27% and 64.22 ± 1.98% (p < 0.05, n = 3) after infection. In contrast, ICOS ligand (B7RP1) was expressed on 55.6 ± 5.5% of naive pDC, and the percentage of pDC expressing this marker seemed to decrease after...

**FIGURE 1.** RSV infection of murine pDC. A, Murine pDC were generated from bone marrow cells cultured with Flt3-L. Resulting CD11b<sup>−</sup> cells were analyzed by flow cytometry for expression of lineage markers, CD11c, B220, and Ly6C. Representative dot plots are shown, and percentages of Lin<sup>−</sup>CD11C<sup>+</sup> and B220<sup>−</sup>Ly6C<sup>−</sup> cells are indicated. B, At several time points (2, 24, and 36 h) after exposure to live RSV or UV-RSV, copy numbers of RSV L-gene were determined using quantitative RT-PCR in isolated pDC (purity 99%) containing <1% of myeloid DC (n, p < 0.05, n = 8; significant differences between the two groups at each time point). C, RSV protein was detected in pDC by flow cytometry 24 h after RSV infection or exposure to UV-RSV. FLt3-L-cultured bone marrow cells that did not express CD11b were stained for CD11c and intracellularly for RSV F and N protein using a mixture of mAbs. Representative dot plots are shown, and percentages of CD11C<sup>−</sup>RSV<sup>+</sup> cells are indicated.
RSV infection to 29.8 ± 2.2% (nonsignificant). Similarly, programmed death ligand 1 (PDL-1) and 2 (PDL-2), two inhibitory costimulatory molecules, were found on the majority of naive pDC. RSV infection resulted in a significant decrease in the percentage of PDL-2 expressing pDC, from 53.65 ± 8.96% to 26.62 ± 1.62% (p < 0.05, n = 3), while the percentage of PDL-1 expressing pDC did not change significantly. In addition, OX40 Ligand, a costimulatory molecule implicated in the polarization of T cell responses, was expressed only in <10% of pDC irrespective of RSV infection. Taken together, these results indicate that pDC mature to some extent following RSV infection. In addition, RSV infection did not change the expression of CD11c and B220 and did not induce CD11b (data not shown).

The changes in expression of costimulatory molecules suggested that pDC might display enhanced Ag-presenting capacity following RSV infection. We therefore assessed their ability to induce Ag-specific T cell proliferation and compared pDC with myeloid DC. T cell proliferation was assessed as reduction in

![FIGURE 3](image)

**FIGURE 3.** RSV-induced pDC maturation. Twenty-four hours after RSV infection (thick black curve, R) or after mock infection (shaded curve, C), pDC were analyzed by flow cytometry for expression of a panel of surface markers related to their maturation status. In addition to MHC-II, the following costimulatory molecules were analyzed: CD40, CD80, CD86, B7RP1, PDL1, PDL2, and OX40L. Positive staining (R1) was defined based on isotype controls. Representative histograms from three independent experiments are shown, and percentages of cells positive for each marker are indicated.

![FIGURE 4](image)

**FIGURE 4.** RSV-infected pDC induced weak T cell proliferation. RSV-infected pDC, sham-infected pDC, or myeloid DC generated in GM-CSF culture from bone marrow cells were cocultured with naive, CFSE-labeled, CD4⁺, OVA-specific T cells from DO 11.10 mice. As a control, the T cells were cultured alone. After 4 days, proliferation was determined by reduction in CFSE intensity in T cell populations using flow cytometry. CD4⁺ T cells were gated, and CFSE staining intensity is shown using histograms. The results presented are representative of three independent experiments.

![FIGURE 5](image)

**FIGURE 5.** Pulmonary pDC following RSV infection. Mice were infected with RSV or sham infected, lungs were harvested, and cells isolated at different time points after infection. *A,* Freshly isolated lung cells were stained for lineage markers, CD11c and B220 and analyzed by flow cytometry. Representative dot plots show Lin⁻CD11c⁺B220⁻ lung cells which were regarded as pDC. *B,* The graph represents numbers of lung pDC in naive mice and on days 2, 6, 9, 14, and 21 after RSV infection (++, p < 0.01; +, p < 0.05, n = 12; significant differences vs naive mice from three independent experiments).
CFSE intensity by flow cytometry in cocultures of OVA peptide-pulsed DC with OVA-specific, TCR-transgenic T cells. Noninfected, myeloid DC induced proliferation of all T cells, resulting in five populations with decreased CFSE intensity, each representing a round of proliferation. In contrast, naive pDC only induced partial and weak T cell proliferation (one round), and this was not changed by RSV infection (Fig. 4). Control T cells without DC did not proliferate at all. To exclude that cell death of pDC following RSV infection prevented increases in Ag-presenting capacity, viability and apoptosis were monitored in pDC. Trypan blue exclusion did not show differences in percentages of dead cells between naive and RSV-infected pDC. In addition, flow cytometric analysis of propidium iodide and annexin V staining did not reveal increased necrosis or apoptosis in pDC following RSV infection (data not shown).

Analyzing cytokine levels in these cocultures, we found that both naive and RSV-infected pDC induced vigorous production of IL-10 (naive pDC/T cells, 528 ± 41 pg/ml; RSV pDC/T cells, 489 ± 49 pg/ml, n = 4), but not of IFN-γ or IL-4 (data not shown). This suggests that pDC may play a regulatory role that is maintained after RSV infection.

**pDC are recruited to the lung in RSV infection**

To investigate the role of lung pDC in respiratory viral infection in vivo, we initially monitored their numbers during the course of infection. Lung cells were isolated by collagenase digestion on days 2–21 after RSV or sham infection and assessed by flow cytometry. pDC in lung cell isolates were defined as lineage-negative CD11c+B220+ cells (Fig. 5A). Numbers of lung pDC in sham-infected controls, inoculated with UV-RSV, did not differ significantly from those in naive mice (data not shown). After RSV infection, numbers of pDC increased significantly already on day 2 postinfection. A maximal 10-fold increase from 2.95 ± 0.24 × 10^4 pDC per naive lung to 31.93 ± 1.81 × 10^4 pDC per lung was reached on day 6 postinfection (Fig. 5B). Thereafter, pDC numbers declined slowly but stayed significantly elevated until day 21, when still almost twice as many lung pDC were found, compared with controls. Parallel to absolute numbers, percentages of pulmonary pDC also were increased significantly. As early as day 2 after RSV infection, 1.23 ± 0.21% of lung cells were pDC, compared with 0.30 ± 0.03% in sham-infected controls. On day 6 postinfection, a maximal percentage of 1.59 ± 0.36% pDC of lung cells was reached. By day 21, their percentage decreased to 0.57 ± 0.04% of lung cells.

**Lung pDC control RSV replication and enhance virus elimination**

To define the contribution of pulmonary pDC to antiviral immunity in RSV infection, we used two approaches: depletion and adoptive transfer of pDC. RSV-induced recruitment of pulmonary pDC was efficiently inhibited by administration of the pDC-specific Ab 120G8 (26). Repeated i.p. injection of this Ab resulted in a 55–90% reduction of lung pDC on day 4 after RSV infection (Fig. 6A). Numbers of myeloid DC were not affected (data not shown). Depletion of lung pDC was associated with significant increases in RSV titers on day 4 postinfection as determined by immunoplaque assay (Fig. 6B). This suggests that pDC recruited to the lung early in infection play an important role in limiting viral replication. Next, we asked whether an excess of lung pDC enhances virus eradication. We adoptively transferred 1 × 10^6 bone marrow-derived pDC, activated with CpG oligonucleotide. Intratracheal administration of these cells, but not of PBS, as a control resulted in almost complete eradication of RSV from the lung by day 9 postinfection, with <10 RSV L-gene copies per 2 μg of RNA on average. Without pDC transfer, >100 copies per μg of RNA of RSV L-gene were detected 9 days after infection (Fig. 6C). Taken together, these findings indicate that lung pDC play a major role in limiting RSV replication and in elimination of the virus.
pDC limit airway inflammation and airway hyperresponsiveness in RSV infection

To determine whether lung pDC also influence the consequences of RSV infection, we assessed lung function and pulmonary inflammation in RSV infected mice with and without pDC depletion. Using barometric whole-body plethysmography, we observed airway hyperresponsiveness on day 9 after RSV infection in mice depleted of pDC, but not in isotype Ab-treated controls (Fig. 6D). A significant increase in baseline Penh values after RSV infection was not affected by pDC depletion.

Airway and parenchymal lung inflammation was monitored in BAL cells and in lung sections harvested on day 9 postinfection. Analysis of BAL cells revealed increased numbers of neutrophils (7250 ± 1797 per ml, n = 8; p < 0.05) in RSV infection in the absence of pDC, compared with RSV-infected controls (1125 ± 236 per ml). Numbers of macrophages and lymphocytes were not affected by pDC depletion. In addition, scoring of histological sections stained with H&E revealed that airway and parenchymal inflammation following RSV infection was increased in the absence of pDC with marked peribronchial, perivascular, and alveolar infiltrates (Fig. 7). Further, goblet cell hyperplasia was observed in medium-sized to large airways following RSV infection only if pDC were depleted (data not shown). Thus, a reduction in pDC numbers in the lung during RSV infection aggravates both pulmonary inflammation and lung function changes.

Discussion

This study aimed to delineate the role of lung pDC in antiviral responses, pulmonary inflammation, and airway hyperresponsiveness in RSV infection using a mouse model. Initially, we studied responses of murine bone marrow-derived pDC to RSV infection in vitro. Confirming recent findings in human pDC (17, 18), we found that murine pDC can be infected with RSV, and that infection with this virus induces robust IFN-α production in a replication-dependent manner, similar to previous findings in influenza virus-infected murine pDC (28). The robust induction of IFN-α in pDC by A2 strain of RSV observed in this study and by Guerrero-Plata et al. (17) in human pDC seems to differ from recent findings by Schlender et al. (12), who reported a 5-fold reduction in IFN-α production by pDC infected with A2 strain of RSV, compared with pDC infected with Long strain. In contrast with Long strain, A2-RSV retains normal nonstructural proteins 1 and 2, which have been shown to reduce the production of type 1 IFN by interfering with the activation of IFN regulatory factors (13, 29). Our data and that of Guerrero-Plata et al. indicate that, although RSV nonstructural genes may reduce IFN-α production by pDC, they do not abrogate it.

Further, we found increases in the percentage of pDC expressing MHC-II and costimulatory molecules CD80 and CD86 after RSV infection and a marked reduction in the inhibitory molecule PD-L2, suggesting maturation. In the absence of enhanced Ag-presenting capacity in pDC, and without induction of CD11b or

FIGURE 7. pDC depletion enhances RSV-induced pulmonary inflammation. Mice were infected with RSV (1 × 10⁵ PFU per mouse) following depletion of pDC or after isotype Ab treatment. Controls were sham infected with UV-RSV or noninfected. Lungs were harvested on day 9 postinfection, fixed, and histological sections were stained with H&E. A, Representative microphotographs from two experiments are shown at ×200 magnification. B, Inflammation of small/medium-sized airways and of alveolar parenchyma was scored separately. Inflammation scores are illustrated (**, p < 0.01, n = 8; 120G8/RSV vs RSV).
changes in CD11c and B220 expression, it is clear that pDC did not develop into myeloid DC after RSV infection as has been reported for lymphocytic choriomeningitis virus infection (30). Although this difference in outcomes could be due to differences between these two viruses, it is more likely to be a consequence of different experimental approaches. In our study, pDC were isolated after differentiation of bone marrow cells in Flt3-L culture and then infected with RSV in vitro. In contrast, Zuniga et al. (30) infected mice with lymphocytic choriomeningitis virus and then isolated pDC from the bone marrow, which were subsequently cultured in the presence of Flt3L. This difference and the inability of splenic pDC to change to myeloid DC indicate that differentiated, peripheral pDC do not develop into myeloid DC upon viral infection, whereas viral infection may induce subpopulations of pDC in the bone marrow, which can differentiate to myeloid DC.

Maturation, as judged by increased expression of MHC-II and costimulatory molecules without enhanced ability to induce T cell proliferation, has also been described in human myeloid DC. In these cells, Ag-presenting capacity was reduced even after RSV infection (31, 32). This phenomenon seems to occur only with some respiratory viruses. Decreased ability to induce T cell proliferation also has been reported after infection of DC with adenovirus (33), but it was not observed after infection with human metapneumovirus (32) or influenza virus (34). The mechanisms involved in this dissociation of phenotypic and functional maturation remain to be determined. Taken together, the in vitro responses of murine and human pDC to RSV infection are similar, suggesting that the mouse is a valid model to study the role of pDC in RSV infection.

In vivo, pDC are rare cells, accounting for 0.2–0.8% of mononuclear cells in peripheral blood and lymphoid organs in both humans and mice (35). In naive mouse lungs, pDC numbers also were low and accounted for ~0.3% of lung cells. Following RSV infection, pDC numbers in the lung increased quickly and were markedly elevated on day 2 postinfection. After a maximal 10-fold increase by day 6 postinfection, pDC numbers declined already during the phase of marked airway inflammation and further during subsequent resolution of disease. The majority of pDC were cleared from the lung after ~14 days, the life span of pDC (36). The kinetics of RSV-induced pDC recruitment to the lung differed markedly from myeloid pulmonary DC. The latter expanded in numbers in the lung comparatively late in infection, only from day 6 postinfection, with the advent of severe pulmonary inflammation (25). Interestingly, both DC subsets remain significantly elevated beyond resolution of disease. The early influx of pDC to the lungs suggests that they may play an important role in innate, antiviral responses. Indeed, in this study, we demonstrate that depletion of ~90% of pDC from the lung resulted in significantly increased peak RSV titers on day 4 postinfection. In addition, adoptive transfer of activated pDC to the airways reduced RSV copy numbers during the resolution of infection. The early effects of pDC on viral replication are likely to be due to their innate ability to produce large amounts of IFN-α, which likely enhances type 1 IFN production in non-pDC and exerts direct antiviral effects, including up-regulation of MxA in infected cells (37). In addition, activated pDC are known to activate NK cells and NK T cells (38), which probably contribute significantly to viral clearance in RSV infection.

In addition to IFN-α, both naive and RSV-infected pDC induced robust levels of IL-10 in coculture with T cells. IL-10 has been linked to the development of regulatory T cells, which can be induced by pDC (39). An induction of regulatory T cells by pDC also would explain the poor T cell proliferation induced by these cells in vitro. In models of allergic sensitization, pDC inhibit the development of allergic airway inflammation by induction of regulatory T cells (11). Hence, we asked whether lung pDC also play a regulatory role in RSV-induced pulmonary inflammation. In the mouse model, RSV infection leads to inflammatory infiltrates of both airways and alveolar parenchyma, and this is associated with a reduction in baseline lung function and airway hyperresponsiveness to methacholine provocation (40). Depletion of pDC during RSV infection resulted in increased parenchymal inflammation, more pronounced peribronchial and perivascular infiltrates, and mucus cell hyperplasia in larger airways. In addition, RSV-infected mice depleted of pDC, but not controls, displayed significantly increased airway hyperresponsiveness on day 9 postinfection, which is usually only observed from days 4–6 postinfection. Using a similar mouse model of pDC depletion during RSV infection, Smit et al. (41) reported that depletion of pDC enhanced RSV-induced cytokine production by T cells from mediastinal lymph nodes in addition to inducing enhanced airway hyperresponsiveness. These findings indicate that the presence of normal numbers of lung pDC limits the severity of RSV-induced pulmonary inflammation and the development of airway hyperresponsiveness. Although these effects of lung pDC could be secondary to their antiviral properties, they also may reflect the induction of immune regulation in RSV infection. Smit et al. (41) addressed this question by treating pDC-depleted and RSV-infected mice with IFN-α. This significantly reduced viral load but did not affect the enhanced T cell cytokine responses or the enhanced airway hyperresponsiveness after pDC depletion (41), indicating that pDC do indeed play an immune regulatory role in RSV infection.

In conclusion, we provide direct evidence that pulmonary pDC in RSV infection play important roles in limiting viral replication, enhancing viral clearance, and reducing airway and parenchymal inflammation and associated airway hyperresponsiveness. In addition to their antiviral properties, pDC may have an immune regulatory role in RSV disease.

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Disclosures

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