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IL-4Rα on dendritic cells in neonates and Th2 immunopathology in respiratory syncytial virus infection

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RECEIVED DECEMBER 26, 2016; REVISED FEBRUARY 24, 2017; ACCEPTED MARCH 17, 2017. DOI: 10.1189/jlb.AA1216536R

ABSTRACT
Respiratory syncytial virus (RSV) is one of the leading causes of bronchiolitis in children, and severe RSV infection early in life has been associated with asthma development. Using a neonatal mouse model, we have shown that down-regulation of IL-4 receptor α (IL-4Rα) with antisense oligonucleotides in the lung during neonatal infection protected from RSV immunopathophysiology. Significant down-regulation of IL-4Rα was observed on pulmonary CD11b+ myeloid dendritic cells (mDCs) suggesting a role for IL-4Rα on mDCs in the immunopathogenesis of neonatal RSV infection. Here, we demonstrated that neonatal CD11b+ mDCs expressed higher levels of IL-4Rα than their adult counterparts. Because CD11b+ mDCs mainly present antigens to CD4+ T cells, we hypothesized that increased expression of IL-4Rα on neonatal CD11b+ mDCs was responsible for Th2-biased RSV immunopathophysiology. Indeed, when IL-4Rα was selectively deleted from CD11b+ mDCs, the immunopathophysiology typically observed following RSV reinfection was ablated, including Th2 inflammation, airway-mucus hyperproduction, and pulmonary dysfunction. Further, overexpression of IL-4Rα on adult CD11b+ DCs and their adoptive transfer into adult mice was able to recapitulate the Th2-biased RSV immunopathology typically observed only in neonates infected with RSV. IL-4Rα levels on CD11c+ cells were inversely correlated with maturation status of CD11b+ mDCs upon RSV infection. Our data demonstrate that developmentally regulated IL-4Rα expression is critical for the maturity of pulmonary CD11b+ mDCs and the Th2-biased immunopathogenesis of neonatal RSV infection. J. Leukoc. Biol. 102: 153-161; 2017.

Introduction
RSV causes acute lower respiratory tract infections in infants, and it is the leading cause of bronchiolitis in children under one year of age [1, 2]. RSV infection severe enough to require hospitalization during infancy is associated with increased risk of wheezing and development of asthma [3, 4]. Currently, no effective vaccines or therapeutics for RSV exist. The development of such products is partially hindered by our lack of knowledge of the infant immune system and its response to RSV infection.

RSV infection in infants induces a Th2-biased immune response, which is partly responsible for RSV pathogenesis [5, 6]. A study comparing cytokine levels in the serum of RSV- vs. influenza-infected infants found higher concentrations of Th2 cytokines (IL-4 and IL-5) in RSV-infected children, suggesting that the Th2-biased response is not simply an age-specific response but also a virus-specific response [7]. Animal studies have reported similar findings [8, 9]. Age at initial RSV infection is important in determining the Th2 bias [10]; infection of neonatal mice and reinfection upon adulthood induces a Th2-biased immune response leading to Th2 cytokines (IL-4, IL-5, and IL-13), airway remodeling, mucus hyperproduction, and airway hyperreactivity [9-12]. Further, polymorphisms in IL-4Rα have been associated with RSV disease severity [13]. We have previously shown that down-regulation of IL-4Rα with antisense oligonucleotide during primary RSV infection in neonatal mice mitigated Th2-biased immunopathophysiology upon reinfection [12], which raises the
intriguing possibility that IL-4Ra has a role in the immunopathogenesis of neonatal RSV infection. DCs are APCs that initiate and dictate the ensuing adaptive immune response after exposure to a pathogen. DCs are functionally divided into two main classes: mDCs and pDCs. mDCs present Ags to T cells and help T cells to differentiate [14], whereas pDCs produce type I IFNs to combat viral infection [15]. mDCs are further classified into CD11b+ mDCs, which mainly present Ags to CD4+ T cells [16, 17], and CD103+ mDCs, which mainly cross-present Ags to CD8+ T cells [16]. Several functional and phenotypic differences have been shown between neonatal and adult DCs [18-20]. Additionally, RSV infection induces significantly less recruitment of pDCs and, thus, type I IFNs in the lung of neonatal vs. adult mice [21]; pDCs from cord blood express lower levels of type I IFNs compared with pDCs from adult peripheral blood in response to RSV stimulation ex vivo [22]. Neonatal mDCs express lower levels of costimulatory molecules (CD80 and CD86) and produce less IL-12 (a Th1 cytokine) in response to LPS compared with their adult counterparts [18, 19].

In the present study, we demonstrated an age-dependent expression of IL-4Ra on pulmonary DCs. Neonatal CD11b+ mDCs (CD11c+ MHCII+ CD11b+) and pDCs (CD11c+ PDCA-1+) expressed higher levels of IL-4Ra compared with their adult counterparts, whereas neonatal CD103+ mDCs (CD11c+ MHCII+ CD103+) expressed less IL-4Ra than they did in adults. Because the age at initial infection is important in determining the Th2-biased immune response to RSV infection [9, 10, 23] and CD11b+ mDCs are mainly responsible for Ag presentation and activation of CD4+ T cells [16, 17], we hypothesized that the elevated expression of IL-4Ra on neonatal mDCs is responsible for the Th2-biased immunopathogenesis of neonatal RSV infection. To test that hypothesis, we infected mice deficient in IL-4Ra CD11b+ mDCs [24] and evaluated the Th2-biased immune response to RSV in vitro and in vivo. Furthermore, we adoptively transferred mDCs that overexpressed IL-4Rα into adult mice to confirm the importance of IL-4Ra in the induction of Th2-biased immune responses to RSV. Our data demonstrate that high levels of IL-4Ra, as occurs in infancy, inhibit the maturation of CD11b+ mDCs, and, for the first time to our knowledge, our data establish a critical role for IL-4Ra on CD11b+ mDCs in initiating the Th2-biased immunopathogenesis of neonatal RSV infection.

MATERIALS AND METHODS

Mice

BALB/c mice were purchased from Envigo (Cambridgeshire, United Kingdom) and were housed in a specific pathogen-free animal facility at the University of Tennessee Health Science Center (Memphis, TN, USA). CD11c-specific IL-4Ra-/- mice (IL-4Ra-/-/CD11c-) or their littermates (IL-4Ra-/-/A5/dg) were generated by crossing IL-4Ra-/- mice with CD11c-/-/IL-4Ra-/- mice (gifts from Frank Brombacher, University of Cape Town, South Africa). All animals were treated with medium, sham = mice treated with medium.

Africa and were generously donated by Deborsha Herbert, University of California, San Francisco, CA, USA) [24]. All mice were on BALB/c background. Breeders were time mated, and age-matched pups were used for all experiments. All animal experiments were performed according to Guidelines of Care and Use of Laboratory Animals and approved by University of Tennessee Health Science Center Animal Care and Use Committee.

Experimental design and RSV infection

Neonatal (5 d old) or adult mice (6 wk old) were infected intranasally with human RSV strain A/2 (2 × 10^5 50% tissue culture infective dose (TCID50) /g body weight; Advanced Biotechnologies, Eldersburg, MD, USA) in 10 μl (neonates) or 50 μl (adults) of SFM (GE Healthcare Life Sciences, Little Chalfont, United Kingdom) or SFM alone (sham). A subset of the cohort was allowed to mature and was reinfected 4 wk later. At 6 d after reinfection, various endpoints were measured, including lung function and histopathology, BALF cytokines and cellularity, and T cell profile.

BMDCs transduction and transfer

Bone marrow cells were isolated from the thia and femur of adult mice (5-7 wk old) mice, as previously described [25] and then cultured in DC media (RPMI 1640; GE Healthcare Life Sciences) supplemented with 10% heat-inactivated FBS (Thermo Fisher Scientific, Waltham, MA, USA), 100 U/ml penicillin (GE Healthcare Life Sciences), 100 mg/ml streptomycin (GE Healthcare Life Sciences) human FLT3 200 ng/ml (Tonbo Biosciences, San Diego, CA, USA) for 6 d at a density of 1 × 10^6/ml in a 6-well plate [26]. BMDCs were then harvested and infected with third-generation lentiviral vectors (pLV-IL-4Ra-eGFP or control pLV-eGFP) (Cyagen Biosciences, Santa Clara, CA, USA) at a multiplicity of infection 20 in Opti-MEM media (Thermo Fisher Scientific) and 5 μg/ml of polybren (Cyagen Biosciences) for 5 h. These cells were cultured in DC medium for 4 d. Transduced cells were then infected with RSV at a multiplicity of infection 50 overnight, and CD11b+ mDCs that were positive for eGFP were sorted with a SH800 cell sorter (Sony Biotechnology, San Jose, CA, USA). These cells were adoptively transferred intranasally into the lungs of mice (1 × 10^7 cells in 50 μl of SFM media). Control mice received 50 μl of SFM media.

RSV gene expression in the lung

RSV gene expression in the lungs was quantified using real-time RT-PCR that correlates well with a plaque assay or TCID50 assay [9, 21, 27]. Lungs were isolated, and total RNA was extracted using the Total RNA extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. CDNA was synthesized using Oligo (dT) and SuperScript III first-strand synthesis system (Thermo Fisher Scientific). Real-time PCR was performed using Platinum SYBR Green qPCR Supermix (Thermo Fisher Scientific) with the following primers: NS1 forward primer (5’-CACAACTGGCAATGTGTTCAAA-3’) and NS1 reverse primer (5’-TTAGACCATTAGGGTAGAGCAATG-3’).

Cell staining and flow cytometry

To measure the expression of surface markers on pulmonary DCs, single-cell suspensions were prepared from mouse lungs using gentleMACS Octo Dissociator (Miltenyi Biotec, San Diego, CA, USA), as previously reported [21]. Single cells were then stained with fixable viability dyes, Abs to CD11c (N418), CD11b (M1/70; BioLegend, San Diego, CA, USA), CD103 (2E7), PDCA-1 (eBio 927; eBioscience, San Diego, CA, USA), and either IL-4Ra (mIL-4Ra; BD Biosciences, San Jose, CA, USA) or MHCII (MS/5114.15.2), CD80 (16-10A1), and CD86 (GL-1). All Abs and dyes were from eBioscience unless otherwise stated. The staining data were acquired on a FACScan Canto II (BD Biosciences) flow cytometer and analyzed with FlowJo software (version 10; Tree Star; Ashland, OR, USA.). Because the MFI of IL-4Ra FMO controls were different among the mice at different ages (i.e., 1 d, 5 d, and 6 wk), the IL-4Ra MFI was normalized by subtracting the MFI of the age-matched FMO controls from the actual values.
To measure the T cell profile in the lung, single cells from lungs were isolated as mentioned and stimulated for 5 h at 37°C in RPMI 1640, containing 10% heat-inactivated FBS (Thermo Fisher Scientific), 100 U/ml penicillin (GE Healthcare), 100 mg/ml streptomycin (GE Healthcare), 5 ng/ml PMA (Sigma-Aldrich; St. Louis, MO, USA), 500 ng/ml ionomycin (Sigma-Aldrich), and protein transport inhibitor (1 μl/10^6 cells; GolgiPlug, BD Biosciences). Cells were then stained with fixable viability dye, fixed with fixation buffer, permeabilized with permeabilization buffer, and labeled with Abs to CD3 (17A2), CD4 (RM4-5; BioLegend), IFN-γ (XMGI1.2), and IL-4 (BDV6-24G2). All Abs, dyes, and buffers were from eBioscience unless otherwise stated. Flow data were then acquired and analyzed as described. Cells were gated on lymphocytes based on forward and side scatter light properties, singlets, and live cells and then on the CD5 and CD4 double-positive population.

**BALF cellularity**

BALF was isolated with 1 ml of PBS and 0.5% BSA; 20,000 cells from BALF were centrifuged onto slides and stained with a Hema-3 staining kit (Thermo Fisher Scientific). Cells were then differentiated and counted by one unbiased observer using an EVOS microscope (Thermo Fisher Scientific).

**Pulmonary function test**

Airway resistance was assessed with the FlexiVent FX system (Scireq, Montreal, QC, Canada) to increasing doses of methacholine (Sigma-Aldrich). Mice were anesthetized with ketamine/xylazine (180/10 mg/kg) and mechanically ventilated at a tidal volume of 10 ml/kg and a frequency of 2.5 Hz, using a computer-controlled piston ventilator. Resistance was calculated by recording pressure and volume in the airways using the single-compartment model. All data were normalized to their individual baseline resistance values [(Value – baseline)/Baseline] and plotted as normalized resistance.

**Lung histopathology**

After euthanasia, hearts of mice were perfused with PBS, and lungs were removed and gravity infiltrated with zinc formalin (Thermo Fisher Scientific) and then fixed for 24 h. Lung tissues were then embedded in paraffin, sectioned, and stained with H&E or PAS. Stained lung sections were visualized, and images acquired with an EVOS microscope (Thermo Fisher Scientific).

**Cytokines in the lung**

Cytokine levels, including IL-4, IL-12 (p40), and IFN-γ, were measured in the BALF with a Milliplex kit (EMD Millipore, Billerica, MA, USA) and a Luminex system (Luminex, Austin, TX, USA), per the manufacturer's instruction. Each sample was analyzed in duplicate. IL-13 in the lung homogenates was quantitatively scored in a blinded fashion based on the following scale: 1, very low; 2, low; 3, moderate; and 4, severe. PAS staining was scored by a quantitative method [28].

**In vitro T cell activation assay**

Pulmonary CD11b+ mDCs (CD11c+MHCII+CD11b+) were isolated first with the CD11c Positive Selection Kit (StemCell Technologies, Vancouver, BC, Canada) and then with an SH800 cell sorter (Sony Biotechnology) from lungs of neonatal mice at 6 d after infection with RSV. CD4+ T cells were isolated from the spleen of naïve adult BALB/c mice with a CD4 positive selection kit (StemCell Technologies) and stained with 0.5 μM CFSE (Thermo Fisher Scientific). CFSE-stained CD4+ cells (1 × 10^6) were then cocultured with CD11b+ mDCs (2 × 10^5) in the presence of 1 μg/ml CD3 Ab (17A2; eBioscience) for 72 h. Cells were stimulated, stained, and analyzed by flow cytometry to determine the Th subpopulations. The purity of isolated CD11b+ mDCs or CD4+ cells was >90 percent.

**Statistical analyses**

Data were plotted as means ± SEM and analyzed using GraphPad Prism 6 (GraphPad, La Jolla, CA, USA). Student's t test or 2-way ANOVA with Bonferroni post hoc tests were used to compare the means among groups, where appropriate. Differences were considered statistically significant at P < 0.05.

**RESULTS**

**Expression of IL-4Rα on DCs was age dependent**

In our previous study [12], we observed that the most significant down-regulation of IL-4Rα after anti-sense oligonucleotide treatment occurred in pulmonary DCs, and that down-regulation correlated with decreased Th2-biased immunopathologies during RSV reinfection, suggesting a role for IL-4Rα on DCs in RSV immunopathogenesis [12]. To explore that possibility, we first quantified IL-4Rα expression on various types of pulmonary DCs from mice at different ages (gating strategy in Supplemental Fig. 1). Specifically, we measured expression of IL-4Rα on pulmonary CD11b+ mDCs (CD11c+MHCII+CD11b+), CD103+ mDCs (CD11c+MHCII+CD103+), and pDCs (CD11c1c; PDCA-1) from neonatal (1 or 5 d old) or adult mice (6 wk old) via flow cytometry (Fig. 1A). The expression of IL-4Rα on CD11b+ mDCs declined as age increased, with 1-d-old pups expressing the greatest amount (Fig. 1B). Interestingly, IL-4Rα expression on CD103+ mDCs increased with age, with adults expressing the greatest amount (Fig. 1C). Similar to CD11b+ mDCs, pDCs down-regulated IL-4Rα expression as age increased (Fig. 1D). These data suggest that the expression of IL-4Rα on pulmonary DCs is developmentally regulated and cell specific.

**Deletion of IL-4Rα on CD11c+ cells attenuated Th2-biased immune responses upon RSV reinfection**

Having confirmed that neonatal CD11b+ mDCs express elevated levels of IL-4Rα, we further examined the role of IL-4Rα on CD11b+ mDCs in polarizing the Th2-biased immune response to RSV. We used a mouse model in which IL-4Rα is specifically deleted on CD11c+ cells (IL-4Rα<sup>−/−</sup>) by crossing IL-4Rα<sup>lox/lox</sup> mice with CD11c<sup>Cre</sup>IL-4Rα<sup>−/−</sup> mice [24]. In IL-4Rα<sup>−/−</sup> mice, the expression of IL-4Rα is decreased on CD11b+ mDCs, CD103+ mDCs, and alveolar macrophages but not on T cells (Supplemental Fig. 2). The littermate controls (IL-4Rα<sup>−/loxDC</sup>) have 1 copy of intact IL-4Rα. IL-4Rα<sup>−/−</sup>DC and IL-4Rα<sup>−/loxDC</sup> neonatal mice were infected with RSV (IL-4Rα<sup>−/−</sup>DCRR and IL-4Rα<sup>−/loxDCRR</sup>) or medium (IL-4Rα<sup>−/−</sup>DCRR sham or IL-4Rα<sup>−/loxDCRR</sup> sham) at 5 d of age and reinjected with RSV 4 wk later. At 6 d after reinfection, we analyzed the CD4+ T cell responses from the lungs of those mice. As expected, the IL-4Rα<sup>−/loxDCRR</sup> mice that had one copy of intact IL-4Rα mounted a Th2-biased immune response upon RSV reinfection, although the magnitude of this Th2 bias was smaller than in BALB/c mice, as we previously published [12]. Importantly, we observed a significant decrease in the percentage of CD4+ IL-4- T cells in the IL-4Rα<sup>−/−</sup>DCRR mice compared with the IL-4Rα<sup>−/loxDCRR</sup> mice (Fig. 2A). There was also reduction in CD4+ IFN-γ+ IL-4- T cells in IL-4Rα<sup>−/−</sup>DCRR mice vs. IL-4Rα<sup>−/loxDCRR</sup> mice (Fig. 2A). This reduction in Th2 cells was accompanied by a decrease in IL-13 (Table 1) in
lung homogenates after RSV reinfection; in fact, IL-13 levels in lung homogenates were similar to uninfected groups (IL-4Rα2/2 DCsham or IL-4Rα2/loxDCsham). IL-4 was very low in all groups and below the limit of detection in the uninfected groups. Although no difference was observed in the percentage of CD4+ IFN-γ+ T cells between the RSV-infected groups, we did observe an elevation in IL-12p40 levels in the BALF of IL-4Rα2/2 DCRR mice vs. IL-4Rα2/loxDCRR (Table 1). These results were not due to a difference in relative viral gene expression of RSV (Fig. 2B) or baseline numbers of DCs during initial infection (Supplemental Fig. 3). These data indicate that IL-4Rα on CD11b+ mDCs has a role in the Th2-biased immune response to neonatal RSV infection in vivo.

Deletion of IL-4Rα on CD11c+ cells protected mice from immunopathophysiology upon RSV reinfection

In addition to the pulmonary CD4+ T cell responses, we also examined pathophysiologic endpoints, including BALF cellularity and pulmonary function and pathology in IL-4Rα−/−DCRR and IL-4Rα−/loxDCRR mice. We observed substantially reduced inflammatory responses in the airways of IL-4Rα−/−DCRR mice, as evidenced by reduced total cells, macrophages, and eosinophils in the BALF compared with IL-4Rα−/loxDCRR mice (Fig. 3A). As shown in Fig. 3B, RSV-induced airway hyperreactivity in IL-4Rα−/loxDCRR mice, whereas airway resistance in IL-4Rα−/−DCRR mice was no different than that of the sham controls. No significant differences in resistance were detected between groups of sham-infected mice. Peribronchiolar and perivascular inflammation was significantly reduced in IL-4Rα−/−DCRR vs. IL-4Rα−/loxDCRR mice (Fig. 3C). Strikingly, there was little-to-no mucus production in the airways of IL-4Rα−/−DCRR mice, whereas significant amounts of airway mucus were obvious in IL-4Rα−/loxDCRR mice (Fig. 3D). That observation is consistent with the fact that airway resistance in IL-4Rα−/−DCRR mice was no different from that of sham-infected mice. The decrease in airway inflammation, airway hyperreactivity, and mucus hyperproduction in IL-4Rα−/−DCRR mice suggests that elevated IL-4Rα expression on CD11b+ mDCs is partially responsible for the immunopathophysiology upon RSV reinfection.

Absence of IL-4Rα on CD11b+ mDCs dampened Th2 responses to RSV infection in vitro

Although the expression of IL-4Rα decreased in neonatal CD11b+ mDCs in the IL-4Rα−/−DC mice, it was also decreased in
CD103+ mDCs and alveolar macrophages (Supplemental Fig. 2). To examine the role of IL-4Rα specifically on CD11b+ mDCs to induce a Th2-biased immune response to RSV, we performed in vitro T cell–activation assays. Pulmonary CD11b+ mDCs were isolated from RSV-infected neonatal IL-4Rα2/2 DC or IL-4Rα2/loxDC mice. Those cells were subsequently cocultured for 3 d with splenic CD4+ T cells isolated from naïve adult BALB/c mice, and T cell phenotypes were then measured by flow cytometry. As expected, neonatal CD11b+ mDCs from IL-4Rα2/loxDC mice induced a substantial amount of Th2 cells (CD4+IL-4+), and mimicking our in vivo data, CD11b+ mDCs from IL-4Rα2/2 DC mice induced significantly less Th2 cells and a decreased ratio of Th2/Th1 (CD4+IL-4+/CD4+IFN-γ+) cells (Fig. 4). These data suggest that IL-4Rα expression on CD11b+ mDCs promotes Th2-biased immune responses to RSV.

Loss of IL-4Rα expression on CD11b+ mDCs enhanced their maturation response to RSV

To delineate the mechanism by which IL-4Rα on CD11b+ mDCs induced Th2-biased responses to RSV in neonates, we measured the expression of maturation markers (CD80 and CD86) on CD11b+ mDCs. Wild-type neonates and adults or IL-4Rα2/2 DC neonates (IL-4Rα2/loxDCNR) were infected with RSV, and the expression of CD80 and CD86 was measured on pulmonary CD11b+ mDCs at 14 d after infection by flow cytometry (Fig. 5). Consistent with previous studies [18, 19], neonatal CD11b+ mDCs were less mature (i.e., reduced expression of CD80 and CD86) compared with their adult counterparts during RSV infection. In the absence of IL-4Rα, the expression of CD80 and CD86 was significantly enhanced on CD11b+ mDCs (IL-4Rα2/loxDCNR compared with neonatal mice infected with RSV). These data suggest that elevated expression of IL-4Rα on...
neonatal CD11b+ mDCs suppresses the ability of those cells to mature in response to RSV infection, resulting in a Th2-biased, adaptive immune response.

Overexpression of IL-4Rα on adult CD11b+ mDCs exacerbated Th2 responses to RSV infection in adult mice

Expression of IL-4Rα on CD11b+ mDCs declines significantly with age. To further confirm the pathogenic role of IL-4Rα, we overexpressed IL-4Rα on adult mDCs. Specifically, we transduced adult BMDCs with IL-4Rα (pLV-IL-4Rα-eGFP) or eGFP (pLV-eGFP) expressing lentivirus. Successful transduction was verified by flow cytometry (Supplemental Fig. 4). CD11b+ mDCs that were positive for eGFP were then sorted and adoptively transferred to the lungs of naïve adult mice; 1 wk later, those mice were infected with RSV. At 6 d after infection, we analyzed pulmonary CD4+ T cell responses. Overexpression of IL-4Rα on adult CD11b+ mDCs (pLV-IL-4Rα-eGFP) resulted in a lower percentage but no significant change in the number of CD4+IFN-γ+ T cells (Fig. 6A). A higher percentage and number of CD4+IL-4+ T cells were observed when IL-4Rα was overexpressed on adult CD11b+ mDCs compared with the control groups (Fig. 6A and B).

There was no change in the percentage of CD4+IFN-γ+IL-4+ cells with overexpression of IL-4Rα on adult CD11b+ mDCs, but there was an increase in the number of those cells (Fig. 6B).

**DISCUSSION**

Infants are particularly vulnerable to severe RSV infection; both human and animal studies implicate a Th2-biased immune response in the pathogenesis of RSV in infants [5, 6, 9–11]. In particular, single-nucleotide polymorphisms in the IL-4Rα gene that increase the receptor response to its cytokines (IL-4 or IL-13) are associated with increased IgE production, hospitalization rates from RSV infection, and susceptibility to asthma [13, 29]. Pharmacologic down-regulation of IL-4Rα in the lung is protective against RSV disease in neonatal mice and is effective in preventing Th2-biased immunopathogenesis [12]. In the current study, we observed increased expression of IL-4Rα on pulmonary CD11b+ mDCs in neonatal, compared with adult, mice. Deletion of IL-4Rα on neonatal CD11c+ cells (including CD11b+ mDCs and other cells) resulted in a significant reduction in Th2 polarization, RSV-associated airway hyperreactivity, inflammation, and eosinophilia after RSV reinfection in vivo. This was
accompanied by an increase in IL-12 production. In vitro T cell–activation assays demonstrated the specificity of IL-4Rα on neonatal CD11b+ mDCs in the induction of Th2 responses to RSV. Because adult CD11b+ mDCs express significantly less IL-4Rα, complimentary experiments in which adult CD11b+ mDCs were made to overexpress IL-4Rα were also performed. Overexpression of IL-4Rα on adult CD11b+ mDCs with the transfer of those cells into the lungs of adult mice before RSV infection was able to recapitulate the Th2-biased immunopathogenesis observed in neonatal mice. Mechanistically, we demonstrated that pulmonary CD11b+ mDCs were less mature in neonates compared with adults in response to RSV infection and that deletion of IL-4Rα on CD11b+ mDCs enhanced their maturation, altering Th2/Th1 immune responses to RSV.

The maturation status of DCs is important to their function, and the costimulatory molecules CD80 and CD86 are thought to be essential as a second signal for T cell activation. Here, we demonstrated that the levels of CD80 and CD86 on CD11b+ mDCs were associated with the axis of CD4+ T cell differentiation (i.e., Th1 vs. Th2). Neonatal CD11b+ mDCs expressed less CD80 and CD86 than their adult counterparts did (Fig. 5) and induced fewer Th1 cells in response to RSV infection [9]. Interestingly, CD103+ mDCs, which mainly activate CD8+ T cells, have also been shown to be immature in neonates compared with adults, and the maturation status of CD103+ mDCs influences the epitope hierarchy of CD8+ T cells in response to RSV infection [30]. Thus, it is exciting to speculate that maturation status of CD11b+ mDCs may also dictate epitope hierarchy of CD4+ T cells in response to RSV infection—a concept that we are currently exploring. We further demonstrated that the expression of CD80 and CD86 on CD11b+ mDCs was partially regulated by the expression of IL-4Rα because deletion of IL-4Rα on CD11b+ mDCs restored some expression of CD80 and CD86 after RSV infection. The mechanism or mechanisms by which IL-4Rα is developmentally regulated and how IL-4Rα regulates the expression of CD80 and CD86 are currently unclear and warrant further investigation.

The ratio of Th2/Th1 cells decreased by 50% in the IL-4Rα−/−mDC mice compared with IL-4Rα−/loxDC mice both in vivo and in vitro (Fig. 2 and 4). Although the reduction was not massive, it was significant. The reduction in Th2/Th1 ratio was accompanied by improved lung function and significantly decreased lung pathologies. We observed this phenomenon in our previous publication [12]. A subtle reduction in IL-4Rα on Th cells and DCs significantly improved lung pathology and abolished lung dysfunction after RSV reinfection. Our data indicate that the pathophysiology is determined by the balance of Th1/Th2 immune responses and that a small shift from the Th2-biased immune response is all that is necessary to improve lung pathophysiology.

As with our previous studies [9, 12], we observed a CD4+ T cell population expressing both IFN-γ and IL-4 after RSV re-infection. Initially, those cells were thought to be pre-Th1/Th2 cells that were in the intermediate stage during the commitment process of Th1/Th2 cells [31, 32]. However, recent data
Figure 6. Overexpression of IL-4Rα on adult CD11b+ mDCs exacerbated Th2 responses to RSV infection in adult mice. BMDCs were isolated from adult mice, transduced with pLV-IL-4Rα-eGFP or pLV-eGFP control, and infected with RSV. CD11b+ mDCs that were positive for eGFP were sorted and adoptively transferred into the lungs of adult mice. Those mice were then infected with RSV, and 6 d later, pulmonary Th subsets were quantified. (A) Percentage of CD4+ T cells expressing IFN-γ or IL-4 or both IFN-γ and IL-4. (B) Number of CD4+ T cells expressing IFN-γ, IL-4, or both IFN-γ and IL-4. Data are representative of 2 independent experiments with 3–4 mice/group. *P < 0.05.

We have shown that IL-4Rα expression on CD11b+ mDCs is developmentally regulated, and greater expression of IL-4Rα is associated with a less-mature phenotype of the CD11b+ mDCs in response to RSV infection, resulting in Th2-biased immune responses. The deletion of IL-4Rα on CD11b+ mDCs significantly decreased the Th2-biased immunopathogenesis during RSV infection by allowing enhanced maturation of CD11b+ mDCs. Our data suggest that modulating IL-4Rα signaling on CD11b+ mDCs may be a strategy for developing RSV therapeutics and that vaccine strategies should investigate such signaling in determining efficacy.

**AUTHORSHIP**

S.A.C. was the principal investigator and designed the study, analyzed the data, and wrote the manuscript. B.S. designed and performed most of the experiments, analyzed the data, and wrote the manuscript draft. D.Y. designed the study, analyzed the data, and wrote the manuscript. J.S. assisted in the in vitro T cell activation assay and the Flexivent experiment. D.T.S. performed the IL-13 ELISA experiment. S.J., G.I.L. and A.A.S. helped in flow cytometry experiments. J.N.H. assisted in lentiviral transduction experiments.

**ACKNOWLEDGMENTS**

This work was supported by U.S. National Institutes of Health grants to S.A.C. from the National Institute of Allergy and Infectious Disease (R01 AI090059) and from the National Institute of Environmental Health Sciences (R01 ES015050, R01 AI090059).
REFERENCES


KEY WORDS:
CD11b+ mDCs – RSV – immunopathogenesis

MARKED PREFERENCES

DISCLOSURES
The authors have no conflicts of interest.