Differential Chemokine Expression following Respiratory Virus Infection Reflects Th1- or Th2-Biased Immunopathology

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Respiratory syncytial virus (RSV) is a major viral pathogen of infants that also reinfects adults. During RSV infection, inflammatory host cell recruitment to the lung plays a central role in determining disease outcome. Chemokines mediate cell recruitment to sites of inflammation and are influenced by, and influence, the production of cytokines. We therefore compared chemokine production in a mouse model of immunopathogenic RSV infection in which either Th1 or Th2 immunopathology is induced by prior sensitization to individual RSV proteins. Chemokine expression profiles were profoundly affected by the nature of the pulmonary immunopathology: “Th2” immunopathology in BALB/c mice was associated with increased and prolonged expression of CCL2 (MCP-1), CXCL10 (IP-10), and CCL11 (eotaxin) starting within 24 h of challenge. C57BL/6 mice with “Th2” pathology (enabled by a deficiency of CD8⁺ cells) also showed increased CCL2 production. No differences in chemokine receptor expression were detected. Chemokine blockers may therefore be of use for children with bronchiolitis.

Chemokines are chemoattractive mediators that recruit inflammatory cells to sites of infection. In many viral infections, excessive inflammation contributes substantially to disease, which might be modulated by antichemokine therapy. The role of viral immunopathology is especially evident in infants and young children with bronchiolitis caused by respiratory syncytial virus (RSV), in which an excessive inflammatory cell recruitment results in occlusion of the small airways and alveoli (26).

Chemokines selectively control the recruitment of T-cell subsets. For example, Th2 cells express CCR3 and CCR4, while Th1 cells express CXCR3 (29). Different T-cell subsets also make different chemokines; for example, CCL5 (RANTES) is preferentially expressed by Th1 cells (32). Conversely, CCL2 (MCP-1) secretion is stimulated by interleukin-4 (IL-4) (22) and directly mediates T-cell differentiation into the Th2 cytokine-secreting phenotype (9). A role for chemokines in allergic pulmonary inflammation has been demonstrated in mice primed to single antigens, such as ovalbumin (OVA). In particular, CCL2, CCL3 (MIP-1 α), CCL5, and CCL13 (MCP-4) are important mediators of inflammatory cell recruitment to the lung, and CCL11 (eotaxin) is the principal chemoattractant for eosinophils in this model (20).

Chemokines therefore may determine both the extent of pulmonary inflammation and the nature of the cellular infiltrate in RSV infection. The BALB/c mouse model allows us to

prime for alternative immunopathological outcomes of RSV infection, which can be biased toward a Th1 or Th2 phenotype through prior vaccination with single proteins of RSV (1). Importantly, in this model, the Th1 or Th2 immunopathology is induced with the same challenge virus and is dependent on the prior sensitization. In naïve mice, RSV infection recruits NK cells into the airways during the first 2 to 3 days, and thereafter CD4⁺ and CD8⁺ T cells are recruited, peaking at 7 to 10 days after infection; significant proportions of these T cells secrete gamma interferon (IFN- γ) (16). CD8⁺ T cells are the most prominent cell subset during resolution of infection. Mice preprimed to the fusion (F) protein of RSV develop an enhanced Th1 response upon subsequent challenge with virus. IFN- γ -secreting CD4⁺ and CD8⁺ T cells are recruited to the lung in equal proportions. Conversely, mice primed to the attachment glycoprotein (G) develop a Th2 cytokine response after challenge. Th2 T cells and eosinophils are recruited, and NK and CD8⁺ T-cell responses are reduced (14).

This simple switch between different cytokine responses to the same viral infection provides a robust model for examining the control of chemokine secretion. Therefore, we set out to examine the association of certain chemokines and their receptors with these divergent immunopathological responses. We show that mice primed with G protein (Th2) have enhanced levels of CCL2, CCL3, CCL11, and CXCL10, whereas mice primed with the F protein (Th1) have enhanced levels of CCL3 only. In a CD8-deficient C57BL/6 (Th2) model, we show enhanced levels of CCL2.

MATERIALS AND METHODS

Mice and virus stocks. Female BALB/c and C57BL/6 wild-type or CD8 α chain-deficient C57BL/6 mice (5) (Harlan Olac Ltd., Wigginton, United Kingdom) were maintained under specific-pathogen-free conditions according to institutional and United Kingdom Home Office guidelines and used when 6 to 10 weeks old. RSV (A2 strain) and a recombinant vaccinia virus expressing either the RSV fusion protein (rVV-F), the RSV attachment glycoprotein (rVV-G), or

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FIG. 1. Chemokine expression following RSV infection. Groups of four birth-cohorted BALB/c mice were challenged with 2×10^6 PFU RSV, and 24 h, 48 h, and 7 days later, levels of chemokine mRNA in whole lungs were assessed by an RNase protection assay. (Inset) Baseline expression in uninfected mice (0 h) and in mice that had been challenged with Hep-2 cell lysates (the cells in which RSV stocks were grown). Data are shown as n -fold increase over control data $\pm$ standard error. Data are representative of three similar studies.

a control construct (rVV- β gal) were grown in HEP-2 cells and assayed for infectivity as described previously (3). UV inactivation of RSV was performed in a UV Stratalinker (Stratagene) for 10 min. At least four mice were given identical treatments in each study and compared to identical birth cohort controls. Data shown are representative of repeated studies.

Mouse infection protocol. Mice were scarified on the rump with 3×10^6 PFU of recombinant vaccinia virus. Two to 3 weeks later, mice were challenged intranasally with 2×10^6 PFU of RSV. Mice were sacrificed by pentobarbitone injection and exsanguinated via the femoral vessels. Bronchoalveolar lavage (BAL) fluid was obtained by inflation of the lungs via the trachea six times with 1.5 ml of 12 mM lidocaine in Eagle's minimum essential medium (16). BAL eosinophilia was assessed after staining of cytospin preparations with hematoxylin and eosin (H&E), and the BAL cell-free supernatant was retained for enzyme-linked immunosorbent assays (ELISAs). Lung tissue was snap-frozen for RNA extraction.

RPA. Total lung RNA was extracted using RNA-Stat-60 (Tel-Test Inc., Friendswood, TX). Chemokine or chemokine receptor mRNA expression was determined using probe kits mCK5 and mCR5, respectively (PharMingen, San Diego, CA). Probes were transcribed using T7 RNA polymerase in the presence of α - 32 P-labeled UTP (Amersham Pharmacia Biotech, Uppsala, Sweden). RNase protection assays (RPAs) were carried out using the RPA III kit (Ambion Inc., Austin, TX), results were analyzed by polyacrylamide gel electrophoresis, and band intensity was assessed electronically (Storm PhosphorImager using ImageQuant, version 4.2a, software; Molecular Dynamics Instruments, Sunnyvale, CA). Results were normalized to values for two housekeeping genes (L32 and GAPDH). It is noteworthy that the BALB/c genome contains a deletion in the 3' untranslated region of the CXCL10 gene in a region to which the radio-labeled probe binds. Consequently, the protected probe is 162 residues long instead of 181 residues, as in other strains (11). Previous studies of this infection model using this technique did not take account of this error in the manufacturer's reagent, effectively swapping the protected bands for CXCL10 and CCL2 (10, 34). The mRNA results for these two chemokines have thus been exchanged in these studies.

Chemokine ELISAs. Concentrations of chemokines in BAL fluid were assessed by ELISAs using the following antibody pairs: for the CCL2 ELISA, an anti-mouse CCL2 monoclonal antibody (MAb) and a biotinylated anti-CCL2 MAb (2H5; PharMingen); for the CCL3 ELISA, anti-mouse CCL3 and peroxidase-conjugated anti-CCL3 (R&D Systems); for the CCL5 ELISA, anti-mouse CCL5 and biotinylated anti-mouse CCL5 polyclonal antibodies (R&D Systems); for the CCL11 ELISA, an anti-mouse CCL11 MAb and biotinylated anti-CCL11 polyclonal immunoglobulin G (R&D Systems); for the CCL24 ELISA, purified anti-mouse CCL24 polyclonal immunoglobulin G (R&D Systems) and a peroxidase-conjugated anti-goat F(ab')₂ fragment (Jackson Laboratories).

Statistical analysis. Values were tested for statistical significance by the Mann-Whitney test or analysis of variance using Minitab (version 12.22; Minitab Inc.), making allowance for multiple comparisons and correcting significances. P values of <0.05 were considered to be significant, as indicated on the figures and in the text.

RESULTS

Lung chemokine expression in primary RSV infection. The mRNA expression levels of a number of chemokines were analyzed in total-lung RNA from BALB/c mice after primary RSV infection. Figure 1 shows the induction of chemokine mRNA in RSV-infected mice 24 and 48 h and 7 days after intranasal inoculation, corresponding to the time of initial cell recruitment (24 to 48 h) and the time of the peak number of total lung cells (7 days). Values are shown as n -fold increases over levels after inoculation with a lysate of HEP-2 cells, the cell line in which RSV is grown.

In uninfected lungs (Fig. 1, inset), very low levels of several chemokine mRNA species were detectable; CCL5 was clearly expressed at the highest levels prior to infection. Intranasal inoculation with HEP-2 cell lysates upregulated none of the chemokines examined. After RSV infection, mRNA levels of all chemokines examined were significantly upregulated. CXCL10 mRNA levels were most noticeably increased (>100 -fold increase; $P < 0.05$ versus HEP-2), but levels were reduced after 48 to 72 h. They remained above background after 7 days. A similar pattern was seen in expression of CCL2, CCL3 (MIP-1 α), CCL4 (MIP-1 β), and CXCL2 (MIP-2). Seven days after infection, CCL5 was the most common mRNA species of those examined, relative to the housekeeping genes, although not when expressed as n -fold increase over controls, because of the high constitutive expression of mRNA for this chemokine. Taking into account the manufacturer's error in the probe set used (see Materials and Methods), our results agree with those of other studies of primary infection (10, 34).

Chemokine expression in Th1 or Th2 cytokine-biased RSV infection. Figure 2A shows CCL2 mRNA levels in RSV-challenged mice previously primed with rVV expressing different RSV proteins. mRNA levels in whole lungs 24 h after RSV infection, the time of peak expression in all cases, are shown. CCL2 was undetectable in uninfected lungs and after instillation of a control HEP-2 lysate, in contrast to its rapid upregulation after infection with live RSV. While CCL2 mRNA production was marginally increased in rVV-F-primed mice (Th1

FIG. 2. CCL2 expression reflects the immunopathological response to RSV infection. (A) Levels of CCL2 mRNA were assessed by RNase protection assays in whole lungs of BALB/c or C57BL/6 mice 24 h following RSV infection. Each mouse was tested individually ($n = 4$ per group). Some mice had been primed 2 to 3 weeks earlier with either rVV-F, rVV-G, or the control rVV-βgal. Results are expressed as protected band intensity and are percentages of the intensity of the housekeeping genes L32 and GAPDH. (B) In similar experiments, CCL2 protein levels were assessed in cell-free BAL fluids by ELISAs at different time points following RSV infection. *, $P < 0.05$ for comparison of rVV-G- and rVV-F-primed BALB/c mice.

secondary response), there was significantly higher production after rVV-G priming (Th2 secondary response; 2.5-fold increase; $P < 0.02$ versus rVV-F).

We have previously shown that not all mouse strains develop Th2-driven pulmonary eosinophilia after RSV challenge (15). This, therefore, provides a model with which to test whether it is the priming to the G protein per se or the Th2 response to it that causes increased CCL2 expression. CCL2 mRNA expression in C57BL/6 mice, which develop a strong Th1 response following rVV-G priming, was not significantly different from that in rVV-F-primed BALB/c mice ($P > 0.05$), suggesting that the cytokines produced in Th2 priming are specifically associated with increased CCL2 mRNA levels.

Examination of CCL2 protein levels in BAL fluid confirmed the mRNA results (Fig. 2B). CCL2 protein was also produced over the first 24 h of infection; in the Th2 cytokine-associated secondary response, significantly higher concentrations were present 48 and 72 h after infection compared to rVV-F-primed mice ($P < 0.02$ and $P < 0.008$, respectively). CCL2 production in rVV-G-primed C57BL/6 mice, which develop a Th1 response after RSV challenge, was dramatically different from that in similarly treated BALB/c mice.

CCL3 mRNA expression was also induced by RSV infection,

FIG. 3. CCL3 expression in response to RSV infection. (A) Levels of CCL3 mRNA in whole lungs of BALB/c mice were assessed by RNase protection assays. Each mouse was tested individually ($n = 4$ per group). Some groups of mice had been primed 2 to 3 weeks earlier with either rVV-F, rVV-G, or rVV-βgal. Results are expressed as protected band intensity and are percentages of the intensity of the housekeeping genes L32 and GAPDH. Each data point is the mean for four animals. Error bars, standard errors. (B) CCL3 protein levels in cell-free BAL fluids from rVV-G- or rVV-F-primed and RSV-infected BALB/c mice were assessed by ELISA. The limit of detection is 15 pg/ml (dotted line).

and its expression was greatly enhanced in both rVV-F- and rVV-G-primed mice (Fig. 3A). In primary infection, significantly enhanced levels of CCL3 mRNA were seen only at 7 days after challenge. This was reflected at the protein level in BAL fluid (Fig. 3B). CCL3 is detectable in BAL fluid early in primary infection, but these levels also peak on day 7. Protein concentrations in BAL fluid are higher in both rVV-F- and rVV-G-primed mice than in primary challenge.

In many instances, CCL11 is a critical chemokine for the recruitment and retention of eosinophils in inflamed tissue (2). Low levels of CCL11 mRNA were detectable prior to RSV infection and were not altered by control inoculations, HEp-2 lysate, or UV-inactivated RSV (Fig. 4A). Over the first 24 h, there was a significant increase in the detectable mRNA levels in all groups, with CCL11 mRNA expression induced to a higher level and more rapidly in rVV-G-primed mice. This was short-lived, and by 48 h there was no significant difference between groups. At 7 days postinfection, the CCL11 level in rVV-G-primed mice had increased significantly compared to that in rVV-F-primed mice. The level of CCL11 protein de-

FIG. 4. CCL11 expression in response to RSV infection. (A) Levels of CCL11 mRNA in whole lungs of BALB/c mice following RSV infection were assessed by RNase protection assays. Some mice had been primed 2 to 3 weeks earlier with rVV-G, rVV-F, or rVV-βgal. Results are expressed as protected band intensity and are percentages of the intensity of the housekeeping genes L32 and GAPDH. *, $P < 0.05$ for comparison of rVV-G- and rVV-F-primed BALB/c mice. (B) CCL11 protein levels in cell-free BAL fluids from rVV-primed and RSV-infected BALB/c mice were assessed by ELISA. The limit of detection was 37.5 pg/ml (broken line).

tectable by ELISA in BAL fluid remained at or around background levels throughout RSV infection (Fig. 4B). There was intermittent low-level CCL11 protein detectable in BAL fluid from rVV-G-primed mice, peaking at 24 h postinfection, similarly to mRNA levels. CCL24 (eotaxin-2) protein expression was not seen in BAL fluid; values were below the assay sensitivity at all time points examined regardless of treatment (data not shown).

As with CCL2 mRNA expression, CXCL10 mRNA was induced in rVV-F-primed mice to a level similar to that detected in primary RSV infection. In rVV-G-primed mice, however, CXCL10 mRNA levels were threefold greater 12 and 24 h following infection ($P < 0.015$) (Fig. 5). Other chemokines, including XCL1, CCL4, CXCL12, and CX3CL1, were examined (data not shown); these chemokines were induced by RSV infection, but there was no difference between Th1- and Th2-primed animals.

Role of CD8⁺ lymphocytes in CCL2 production during RSV infection. We hypothesized that in rVV-G-primed BALB/c mice, enhanced CCL2 expression correlates with the absence of IFN-γ-producing CD8⁺ T cells. To test the role of CD8 T cells in controlling the production of CCL2, we primed either wild-type or CD8-deficient C57BL/6 mice with rVV-G followed by RSV challenge. This model was chosen because

FIG. 5. CXCL10 expression in response to RSV infection. Levels of CXCL10 mRNA in whole lungs of BALB/c mice following RSV infection were assessed by RNase protection assays. Some groups of mice ($n = 4$) had been primed 2 to 3 weeks earlier with rVV-G, rVV-F, or rVV-βgal. Results are expressed as protected band intensity and are percentages of the intensity of the housekeeping genes L32 and GAPDH. Each data point is the mean for four animals. Error bars, standard errors. *, $P < 0.05$ for comparison of rVV-G- and rVV-F-primed BALB/c mice.

C57BL/6 mice do not normally undergo eosinophilia following rVV-G priming due to the presence of IFN-γ-producing CD8⁺ T cells. In contrast, CD8⁺ T-cell-deficient rVV-G-primed C57BL/6 mice recruit similar numbers of cells to the lung as wild-type animals but undergo a Th2-type response and recruit eosinophils to BAL fluid, a response similar to that seen in rVV-G-primed BALB/c mice (15). CCL2 mRNA expression was increased in CD8-deficient mice, although expression followed a kinetics different from that for rVV-G-primed BALB/c mice (Fig. 6). This finding again associates CCL2 production with a Th2 response to RSV infection and suggests that CD8⁺ cells play a role in limiting the production of CCL2 during RSV infection.

Lung chemokine receptor expression. In total lungs of naïve mice, CCR2 mRNA was the most highly expressed chemokine receptor, although CCR1 and CCR5 mRNAs were also detectable (Fig. 7A). During the first 24 h after RSV infection, CCR2 underwent a rapid, transient increase in expression. Following this, increases in CCR1, -2, and -5 mRNAs began at 48 h and broadly paralleled cell infiltration (Fig. 7A, right axis). CCR2 was the most highly expressed chemokine receptor throughout. Chemokine receptor expression was not altered by prior exposure to RSV proteins (Fig. 7B). CC chemokine receptor expression followed the same pattern in primary RSV infection as in secondary infection, although slightly delayed, consistent with delayed cell recruitment (data not shown). There was no significant mRNA expression at the whole-lung level of CCR3 or CCR4 (not shown), archetypal *in vitro*-polarized Th2 chemokine receptors, even in mice undergoing Th2 cytokine-associated secondary inflammation.

DISCUSSION

The selective expression of chemokines or their receptors in association with Th1 or Th2 cytokine-biased pulmonary inflammation may play a central role in determining the outcome of disease. We have demonstrated that different chemokine profiles are associated with Th1- or Th2-biased immunopathology in response to RSV challenge. CCL2 expression was in-

FIG. 6. (A) CCL2 mRNA expression is increased in rVV-G-primed CD8-deficient mice. Levels of CCL2 mRNA were assessed by RNase protection assays in whole lungs of wild-type and CD8^{-/-} C57BL/6 mice primed with rVV-G and challenged 2 weeks later with RSV. Results are expressed as protected band intensity and are percentages of the intensity of the housekeeping genes L32 and GAPDH. Controls are mRNA levels after rVV scarification prior to RSV infection and 24 h after intranasal inoculation of a Hep-2 cell lysate. *, $P < 0.05$ for comparison of wild-type and CD8^{-/-} mice. (B) CCL2 protein levels in cell-free BAL fluids from wild-type and CD8^{-/-} RSV-infected C57BL/6 mice were assessed by ELISA. The limit of detection is 37.5 pg/ml. In both panels, each data point is the mean for four animals. Error bars, standard errors.

creased and prolonged during Th2 cytokine-mediated immunopathology both in BALB/c mice and in normal or CD8-deficient C57BL/6 mice. Expression levels of both CCL11 and CXL10 also increased in Th2-primed BALB/c mice.

A number of other chemokines examined, such as CCL3, showed no clear association with either inflammatory phenotype but were upregulated following priming with either RSV F (Th1) or RSV G (Th2) protein. Expression of a number of CC chemokine receptors in the lung was not significantly altered in cytokine-biased infection. There was a significant increase in CCR1, CCR2, and CCR5 expression but no detectable CCR3 or CCR4 expression even in Th2 cytokine-mediated immunopathology. We conclude that although early chemokine expression is likely to be an innate response to viral replication, prior priming to individual RSV proteins can alter cytokine expression even at very early time points after infection, indicating that specific primed/memory T cells that track to the lung from distant sites may have an influence on these early responses.

CCL2 expression was increased during Th2 immunopathology in both BALB/c and CD8-deficient C57BL/6 mice, indi-

FIG. 7. Chemokine receptor expression following RSV infection. Expression of chemokine receptor CCR1, CCR2, and CCR5 mRNAs in whole lungs of BALB/c mice was assessed by RNase protection assays. (A) Primary RSV infection. (B) Mice primed with rVV and challenged 2 weeks later with RSV. Results are expressed as percentages of the intensity of the housekeeping genes L32 and GAPDH. Each data point is the mean for four animals. Error bars, standard errors.

catting that the overexpression of CCL2 is a feature of the Th2 cytokine response. In other models of Th2-mediated pulmonary inflammation, CCL2 has been shown to be a key mediator of both cell recruitment and the augmentation of Th2 cytokine production by T cells (20). CCL2 promotes differentiation of CD4⁺ T cells to a Th2 phenotype, and mice deficient in CCL2 do not mount Th2 responses and produce low levels of Th2 cytokines (9, 17). CCL2 expressed at the site of inflammation can drain through the lymphatics to the lymph nodes, where it directly affects T-cell differentiation (27), and blocking CCL2 during the early stages of allergen challenge reduces T-cell and eosinophil recruitment in a model of OVA sensitization (7). CCL2 is induced by expression of IL-4 and inhibited by IFN- γ in vitro (13). Therefore, our data support a role for CCL2 in Th2-mediated immunopathology, even in virally induced eosinophilia. The CCL2 in our model may, but need not, come directly from T cells, as it is likely that CCL2 expression would also be induced from macrophages and from epithelium and surrounding parenchymal cells in response to IL-4 (4, 36, 37). CCL2 is detectable in nasal lavage fluid during active infection with RSV (25), although no association with Th2 cytokine-driven aspects of immunopathology has been previously documented. It is interesting to hypothesize that CCL2 may play a role in driving the differentiation of RSV-specific Th2 cells or that CCL2 can sustain and amplify an existing Th2 cell population in the lung. It cannot be ruled out, however, that the increase in CCL2 expression may be merely a consequence of increased Th2 cytokine expression and not part of the differentiation process. Experiments to block CCL2 function will be

needed to resolve whether CCL2 is a cause or a consequence of Th2 cell recruitment and differentiation in this model.

CCL3 has been shown to play a role in models of allergic inflammation: CCL3 is a chemoattractant for eosinophils, activated T cells, and monocytes, and neutralization *in vivo* partially reduces eosinophilia and airway hyperresponsiveness (7, 23). However, *in vitro*, CCL3 drives the development of Th1 cells (18). In our model, CCL3 production was elevated in mice primed for either Th1 or Th2 responses compared to primary infection. Since CCL3 recruits activated T cells, our data suggest that it may be an important factor for sustaining and amplifying an already primed T-cell response, rather than inducing the differentiation of a particular subset of T-helper cells.

CCL11 is a chemoattractant for eosinophils, basophils, mast cells, and Th2 cells which express its receptor, CCR3 (30). CCL24 has a similar function despite being structurally unrelated (37). In our model we saw a rapid early peak of CCL11 expression following the Th2 type rVV-G priming. This mRNA peak declined at 48 h but rose again at the 7-day time point. CCL11 protein levels increased following rVV-G priming at 24 h but not at 7 days. The CCL11 expression profile is similar to the one we see with CCL5 (RANTES) (F. J. Culley et al., submitted for publication), where there is a biphasic response, consisting of an early phase that we believe is caused by resident cells and a later phase, associated with an influx of lymphocytes. In the later phase mRNA is transcribed, but protein is not expressed; however, the protein can be expressed following RSV restimulation. In the rVV-F-primed mice, we did not see enhanced CCL11 levels. These data agree with our previous findings, where treatment with anti-CCL11 reduces disease in rVV-G-primed mice but not rVV-F-induced pathology (24).

The significantly higher expression of CXCL10 in rVV-G-primed mice following RSV infection was unexpected. Given that CXCL10 is induced by IFN- γ and indeed may be part of the IL-12 signaling pathway (28), it seems counterintuitive that expression is increased in Th2 cytokine-mediated immunopathology. However, our model contrasts with many other models of pulmonary inflammation in that we are using a replicating whole-virus infection to induce immunopathology. Even in rVV-G-primed mice, there is significant expression of IFN- γ (33). Since the preferential recruitment of Th1 cells by CXCL10 also depends on chemokine receptor and adhesion molecule expression on target cells, our results demonstrate that the expression of CXCL10 *per se* is insufficient to determine that the inflammation is of a Th1 cytokine phenotype.

There were no significant differences in chemokine receptor mRNA levels detected in our different models. The increases in receptor expression observed may be a rapid direct response to RSV infection, for instance, in macrophages or in epithelium, as seen in the initial peak of CCR2 expression at 12 h after RSV infection. Expression later in infection is likely to occur on recruited inflammatory cells, and indeed the increase in expression of CCR1, CCR2, and CCR5 follows increases in expression of their ligands CCL2, CCL4, and CCL5. Expression of CCR3 and CCR4 is seen on Th2-polarized cells *in vitro*, CCR3 is expressed on eosinophils, and both are seen in pulmonary allergic inflammation (6, 21, 31). However, even in Th2 cytokine-biased RSV infection, we did not detect CCR3

mRNA. It is possible that there is reduced transcription of CCR3 mRNA in eosinophils and lymphocytes once they are recruited to the lung. However, this may reflect the very low expression of the CCR3 ligands CCL11 and CCL24. It is a possibility, therefore, that the infiltrating cells in RSV-induced immunopathology do not express significant amounts of chemokine receptors that have previously been identified in Th2 cytokine inflammatory responses.

The mechanism by which rVV-G induces Th2 immunopathology in BALB/c mice following subsequent RSV challenge is not fully understood. Our studies demonstrate that differences in chemokine secretion patterns occur as early as 12 h following challenge. Since the mechanism by which rVV-G scarification induces the overall Th2 cytokine response to RSV is dependent on T cells (8), it is likely that rapidly responding T cells migrate from the site of primary vaccination to the lung and are responsible for this early response. These could be a population of circulating $\gamma\delta$ T cells that are recruited, having been primed in the skin, or an $\alpha\beta$ T-cell population that is able to respond quickly to infection. This mechanism is seen in *Leishmania major* infection of BALB/c mice, in which an ineffective, Th2 cytokine-dominated response to the parasite is seen (19). In this case, an NK1.1⁻ T-cell population with restricted $\alpha\beta$ T-cell receptor diversity ($V\alpha 8^+ V\beta 4^+$) responds to parasitic infection within 16 h and rapidly secretes very large amounts of IL-4, which are necessary and sufficient to sustain the subsequent Th2 cytokine response to infection (12). It is quite possible, therefore, that an analogous response could be established through the priming of G-specific $V\beta 14^+ CD4^+$ T cells, which dominate the T-cell response during rVV-G-primed Th2 responses in BALB/c mice (35). These cells may then influence the chemokine environment in the lung to augment and sustain Th2 immunopathology. It is also possible that T cells in the lung are remnants of the circulating pool of cells that are primed following cutaneous vaccination and that they have no specific lung homing ability. In considering these alternatives, it is relevant that the interval between priming and challenge makes little difference to the characteristics of the disease or cellular response if the priming antigen is G or F. For example, mice that are primed 3 months prior to challenge behave very similarly to mice that are primed 2 or 3 weeks earlier (unpublished data).

We have shown that an eosinophilic Th2-mediated response to RSV was associated with increased and prolonged production of CCL2, CCL3, CXCL10, and CCL11 during virus infection. This pattern of chemokine and receptor expression is not markedly different from that seen in particulate antigen-driven models of lung eosinophilia, such as that using OVA (7). Selective blockade of chemokines may elucidate the mechanism of immunopathology in RSV disease and lead to novel interventional therapeutics for children with bronchiolitis.

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