Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract disease in infants and young children and is associated with bronchiolitis (3, 4). Bronchiolitis is manifested by obstruction of the airways and is associated with the inflammatory response to infection (7–9, 17, 31). The viral and host factors contributing to the inflammatory response are not well understood but likely involve the production of cytokines and chemokines by immune and respiratory epithelial cells. For example, in mice, the enhanced disease that occurs following formalin-inactivated RSV vaccination is associated with a Th1 cytokine response (6, 10, 11, 30), while live RSV infection does not induce enhanced disease and is associated with a Th2 cytokine response (15, 27, 29). The link between cytokine production and enhanced disease is supported by abrogation of enhanced disease when interleukin 4 (IL-4) and IL-10 are neutralized with antibodies (6). Recent studies suggest that the RSV G glycoprotein is an important determinant of the cytokine response associated with enhanced disease (12, 18, 19). For example, G and/or SH glycoproteins alter Th1 cytokine, particularly gamma interferon (IFN-γ), expression as well as decrease polymorphonuclear leukocyte (PMN) and NK cell trafficking to the lung (27, 29). It has been proposed that lack of IFN-γ and CD8⁺ T-cell regulation of the CD4⁺ T-cell response to RSV infection may contribute to enhanced disease (14, 26).

Antigen nonspecific granular cells that produce chemokines govern the earliest stages of the inflammatory response. Chemokines promote an influx of immune cells to the site of infection, which in turn express chemokines that help refine the inflammatory response. Several groups have shown in vitro that epithelial cells respond to RSV infection by expressing IL-8, RANTES, MIP-1α, and MIP-1β (13, 22), suggesting that these chemokines are important during RSV infection; however, the characteristics of the chemokine response to in vivo RSV infection have not been well studied.

In this study, we examined the kinetics of chemokine mRNA expression by pulmonary leukocytes following primary infection of 4- to 6-week-old female BALB/c mice (Harlan Sprague Dawley Laboratories, Indianapolis, Ind.) with two strains of RSV, one which has the G and SH genes (B1) and one which lacks them (CP52), or with the JS strain of parainfluenza virus type 3 (PIV-3), all of which were propagated as described previously (28, 29). Mice were intranasally infected with 10⁴ PFU of B1, CP52, or PIV-3 diluted in phosphate-buffered saline (PBS) or with uninfected Vero cell-free lysate (VCL) (GIBCO, Grand Island, N.Y.). At various times postinfection (p.i.): bronchoalveolar lavage (BAL) cells from 4 to 6 mice/time point were collected by washing the lungs three times with 1 ml of PBS (GIBCO) containing 1% bovine serum albumin (Sigma, St. Louis, Mo.). Total cell numbers in the BAL cells of B1-infected mice ranged from 3.4 × 10⁵ to 9 × 10⁵ cells/ml, in CP52-infected mice they ranged from 5 × 10⁵ to 8.5 × 10⁵ cells/ml and in PIV-3-infected mice they ranged from 5 × 10⁵ to 12 × 10⁵ cells/ml. RNA isolation and multiprobe RNase protection analysis were performed according to the instructions of the probe manufacturer (PharMingen, San Diego, Calif.). BAL cells were used for RNA extraction. Total RNA was extracted using RNA STAT-50 LS (TEL-TEST Inc., Friendswood, Tex.) as described by the manufacturer. Chemokine mRNA was detected by RNase protection analysis using the RiboQuant Multi-Probe RNase Protection Assay System (PharMingen). 32P-labeled antisense RNA probes specific for eight chemokine mRNA sequences and two housekeeping mRNA sequences were used to detect CC chemokines (RANTES, Eotaxin, MIP-1α, MIP-1β, MIP-2, MCP-1, and TCA-3), CXC chemokine (IP-10), and the L32 and GAPDH housekeeping genes.

The mean results from experiments (n ≥ 3) examining chemokine mRNA expression at 0, 8, 16, 36, 72, 144, and 240 h p.i. with B1, CP52, and PIV-3 are shown in Fig. 1 and 2. Overall, there was low constitutive expression of the macrophage inflammatory proteins MIP-1β, MIP-1α, and MIP-2, as well as of MCP-1 and IP-10, followed by a marked early chemokine response to infection and a return to constitutive levels, followed by a small, second increase in chemokine expression later in the infection. At 8 h p.i., MIP, IP-10, and MCP-1 expression peaked for the two RSV strains, whereas at 18 h p.i. MIP and TCA-3 expression peaked for PIV-3 (Fig. 1 and 2). By 36 h p.i., except for that of RANTES, chemokine expression had returned to near constitutive levels (Fig. 1). The magnitude of RANTES mRNA expression varied following virus infection or VCL treatment, and no virus-specific increases were detected (Fig. 1). Overall, treatment of mice with uninfected VCL induced a chemokine expression pattern similar to that for B1 infection, but having a much lower magnitude.

There was consistently higher expression of MIP, MCP-1,
and IP-10 mRNAs following CP52 infection than there was following B1 infection (Fig. 1 and 2). To control for by-products in the virus inoculum that might affect chemokine induction, mice were infected with sucrose gradient-purified (5) B1 or CP52 virus (data not shown). Both purified viruses induced MIP, RANTES, IP-10, and MCP-1 mRNA expression between 8 h and 18 h p.i.; however, CP52 induced higher chemokine expression than B1 (e.g., 34% greater for MIP-1\(\alpha\), 37% greater for MIP-1\(\beta\), 22% greater for MIP-2, and 67% greater for IP-10). TCA-3 mRNA was also induced by purified CP52 but was not observed following infection with CP52-infected VCL; however, the magnitude of expression was significantly lower than that observed following PIV-3 infection. Since the titers of B1 and CP52 in the lung are comparable at day 3 p.i. (29), the differences in MIP, MCP-1, and IP-10 expression are likely due to the absence of G and/or SH proteins.

As observed previously (29), the absence of the G and SH genes (as in strain B1), whereas PIV-3-infected mice had intermediate levels of cells positive for these surface markers (data not shown).

The present study suggests that G and/or SH gene expression reduces MIP, MCP-1, and IP-10 mRNA expression. Chemokines interact with receptors expressed by Th1 cells (CCR5, CXCR3, and CXCR5) (23; P. Loetscher, M. Uguccioni, L. Bordoli, M. Baggiolini, B. Moser, C. Chizzolini, and J. M. Dayer, Letter, Nature 391:344–345, 1998) and with those preferentially expressed by Th2 cells (CCR3, CCR4, and CCR8) (16, 20, 21, 25, 32). MIPs interact with CCR1 and CCR5, MCP-1 interacts with the CCR2 receptor, and IP-10 interacts with the CXCR3 receptor on Th1 cells (1, 2, 24, 33). In earlier studies we showed decreased Th1 cytokine (IFN-\(\gamma\)) expression after infection with B1 compared to after infection with CB52, which lacks these genes (29). The decreased MIP, MCP-1, and IP-10 expression associated with G and/or SH glycoproteins likely impairs the Th1 response; thus, these chemokines may be important in RSV immunity or disease pathogenesis.

REFERENCES


