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IL-21 Promotes the Pathologic Immune Response to Pneumovirus Infection

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IL-21 is a cytokine with pleiotropic actions, promoting terminal differentiation of B cells, increased Ig production, and the development of Th17 and T follicular helper cells. IL-21 is also implicated in the development of autoimmune disease and has antitumor activity. In this study, we investigated the role of IL-21 in host defense to pneumonia virus of mice (PVM), which initiates an infection in mice resembling that of respiratory syncytial virus disease in humans. We found that PVM-infected mice expressed IL-21 in lung CD4+ T cells. Following infection, Il21r−/− mice exhibited less lung infiltration by neutrophils than did wild-type (WT) mice and correspondingly had lower levels of the chemokine CXCL1 in bronchoalveolar lavage fluid and lung parenchyma. CD8+, CD4+, and γδ T cell numbers were also lower in the lungs of PVM-infected Il21r−/− mice than in infected WT mice, with normal Th17 cytokines but diminished IL-6 production in PVM-infected Il21r−/− mice. Strikingly, Il21r−/− mice had enhanced survival following PVM infection, and moreover, treatment of WT mice with soluble IL-21R-Fc fusion protein enhanced their survival. These data reveal that IL-21 promotes the pathogenic inflammatory effect of PVM and indicate that manipulating IL-21 signaling may represent an immunomodulatory strategy for controlling PVM and potentially other respiratory virus infections. The Journal of Immunology, 2012, 188: 1924–1932.

Interleukin-21 is a pleiotropic four α-helical bundle type I cytokine that is produced primarily by CD4+ T cell populations, including T follicular helper (Tfh) cells, Th17 populations, and NK cells, and acts on a broad range of target cells including B cells, T cells, NK cells, dendritic cells (DCs), macrophages, and epithelial cells (1). The functional IL-21R is a heterodimer of an IL-21–specific protein, IL-21R, and the common cytokine receptor γ-chain, γc, which is shared by the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (2) and is encoded by the gene that is mutated in humans with X-linked SCID (3). IL-21 functions as a T cell comitogen and can also cooperate with IL-7 and IL-15 to drive expansion of CD8+ T cells (4). Within the B cell lineage, it is a critical regulator of Ig production, contributes to Ig class switch, and drives terminal B cell differentiation to plasma cells via its regulation of the PRDM1 gene, which encodes BLIMP1 (5, 6). Interestingly, IL-21 is also proapoptotic for B cells (5, 7, 8) and NK cells (9) and can suppress DC function (10). IL-21 plays a role in both CD8+ T cell and NK cell function related to anti-tumor responses, and IL-21 exhibits potent anticancer activity in a number of animal models (1). Although IL-21 exerts positive effects on differentiation and function, it also has inhibitory effects on immune responses based on its role in B cell apoptosis, its induction of IL-10 in T and B cells leading to immunosuppression (11), and its suppressive effects on DC function (10). IL-21 also promotes the development and expansion of the inflammatory Th17 subset of CD4+ T cells (12–14) as well as Tfh cells (15).

IL-21 can promote inflammation in a number of disease-related settings. For example, in the NOD mouse model of type 1 diabetes, IL-21 signaling promotes pancreatic islet inflammation and destruction, as shown by the lack of disease when mice are crossed to the Il21r−/− background (16, 17). Analogously, IL-21 also promotes autoimmune systemic lupus erythematosus related to its role in T cell activation, with diminished disease development in two different mouse models when IL-21 signaling is blocked (18, 19). Moreover, in the experimental autoimmune encephalitis mouse model of multiple sclerosis, IL-21 promotes the differentiation and expansion of IL-17–producing CD4+ T cells, and the absence of IL-21 signaling has been associated with reduced severity of disease (12, 13).

Despite the large body of information known as being related to the biology of IL-21, relatively limited information is available regarding its role following viral infection. In HIV-infected individuals, IL-21–expressing CD4+ T cells were present, and these numbers decreased as viral levels declined (20). Additionally, IL-21–producing HIV-specific CD8+ T cells were induced following HIV infection, and interestingly, the presence of these cells correlated better with HIV-1 control than did IL-21–producing CD4+ T cells (21). However, these findings were only correlative, and a full understanding of the role of IL-21 in host defense to HIV requires additional investigation. IL-21 is also elevated during lymphocytic choriomeningitis virus infection of mice, and experiments with Il21r−/− mice revealed a role for IL-21 in controlling CD8+ T cell function during this infection (22–24). However, the role of IL-21 in the pathogenesis of acute respiratory viruses has not yet been explored. In this study, we focus on...
pneumonia virus of mice (PVM), a negative-strand RNA virus that is in the same family (Paramyxoviridae) and genus (Pneumovirus) as human respiratory syncytial virus (RSV) (25, 26). PVM infection can result in disease in mice similar to the more severe forms of RSV infection, with viral replication in respiratory epithelial cells that is associated with the infiltration of neutrophils and lymphocytes and the production of IFN-γ and the chemokines CCL2, CCL3, CXCL1, and CXCL10. In this study, we examined the role of IL-21 in both the innate and adaptive responses to PVM infection and unexpectedly found that mice lacking Il21r−/− exhibited prolonged survival to PVM. Moreover, treatment of wild-type (WT) mice with a soluble IL-21R–Fc fusion protein was protective, indicating a role for IL-21 in promoting the pathogenic effects of this respiratory virus.

Materials and Methods

Mice

WT C57BL/6J mice were obtained from The Jackson Laboratory. Il21r−/− mice (6) were analyzed at 8–12 wk of age. Mice expressing the Il2r1mCherry transgenic mice have been described (27). Transgenic mice expressing the human IL-21 cDNA under the control of the H-2Kb promoter and IgM enhancer have been described (5). All experiments were performed under protocols approved by the National Heart, Lung, and Blood Institute and National Institute of Allergy and Infectious Diseases Animal Care and Use Committees and followed National Institutes of Health guidelines for use of animals in intramural research.

Virus inoculation

Virus stocks (PVM strain J3666) were prepared as previously described (28). Mice were anesthetized briefly via inhalation of 20% halothane and inoculated intranasally with 12–60 PFU PVM in 50–100 μl PBS. IL-21R/Fc fusion protein was obtained from R&D Systems. Human Fc γ control protein was obtained from BioXCell.

Flow cytometric analysis

Single-cell suspensions from BAL fluid or lung tissue were surface-stained in FACS buffer (PBS containing 0.5% BSA and 0.02% azide) using Abs from BD Biosciences. For intracellular staining of cytokines, cells were activated with PMA (10 ng/ml) and ionomycin (1 μM; Sigma-Aldrich) for 4 h in the presence of Golgi Plug (BD Biosciences). Cells were first surface-stained with anti-CD8, fixed, and permeabilized with CytoFix/Cytoperm (BD Biosciences). Data were acquired with either a BD FACS Canto II or LSR II (BD Biosciences) and analyzed with FlowJo software (Tree Star).

DC isolation

Splenic DCs were isolated by collagenase digestion followed by positive selection with pan-DC microbeads (Miltenyi Biotec). Splenic DCs were seeded at 2 × 10^6/sample in 24-well plates, rested at 37°C for 1 h, and stimulated with cytokines. Supernatants were collected, and IL-6 levels were measured by ELISA (BD Biosciences).

RNA preparation and real-time PCR

RNA was extracted from lung tissue by homogenization in TRIzol (Invitrogen) followed by RNA cleanup with the RNeasy kit (Qiagen). RNA was

Histology

Lungs were inflated before excision, fixed in 10% formalin, and embedded in parafin, 5-μm thick sections were cut, and slides were stained with H&E. Clinical scores for morphology, inflammation, and edema were evaluated.

Bronchoalveolar lavage fluid and lung cell preparation

Lungs were inflated intratracheally with 1 ml cold 0.1% BSA in PBS, and recovered fluid was used for ELISA and to prepare bronchoalveolar lavage (BAL) cells. Lung tissue was minced into small pieces using a razor blade and digested in a solution containing 0.5 mg/ml Liberase (Roche) and 0.5 mg/ml DNase I (Sigma-Aldrich) in serum-free RPMI 1640 for 30 min at 37°C. Digested tissue was then pushed through a cell strainer with a syringe. Cells were centrifuged, and RBCs were lysed with ACK, followed by two washes with complete RPMI 1640.

FIGURE 1. IL-21 is expressed by lung CD4+ T cells following PVM infection. C57BL/6J WT mice were inoculated with PVM, sacrificed at indicated time points, and lung RNA isolated from lung tissue, and RT-PCR analysis of the PVM SH (A) and Il21 (B) genes was performed. Shown are values ± SEM relative to Rpl7 expression (n = 5 for each time point). C–F, Il21r1mCherry transgenic reporter mice (TG) or WT littermates were infected with PVM, lung cells were isolated 6 d later, and IL-21 expression measured by flow cytometry after surface staining with anti-CD4 and anti-TCRβ. A representative experiment is shown in C–E; the experiment was performed three times with similar results. F, Summary of three experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
reverse-transcribed using Omniscript (Qiagen). PCR reactions to quantitatively detect cytokine and chemokine RNAs used probe sets from Applied Biosystems and the 7900HT Sequence Detection System. Relative levels of PVM mRNA expression (29) were determined by quantitative RT-PCR, with normalization to Rpl7 expression.

ELISAs

Lung tissue was homogenized in cold PBS, and tissue debris was removed by centrifugation (14,000 rpm for 10 min). Levels of CXCL1 in equal volumes of BAL fluid and equal amounts of protein from lung homogenates were measured using a kit (Antigenix).

Statistical analysis

Two-tailed paired t tests were performed using Prism 4.0 (GraphPad). In the figures, statistical significance is indicated by *p < 0.05, **p < 0.01, and ***p < 0.001.

Results

IL-21 expression is induced following infection with PVM

To determine whether IL-21 is expressed in the lung in response to PVM infection, WT mice were inoculated intranasally with PVM virions, and IL-21 mRNA expression was measured in the lung at time points during the 10 d following infection. Expression of the PVM SH gene as an indicator of virus burden (29) was greatest at day 6 after inoculation (Fig. 1A). As expected, infected mice developed pneumonia and began to succumb to infection starting at day 7, and SH gene expression declined, with it no longer being detected among survivors at day 10 (Fig. 1A). Il21 mRNA was detected as early as day 5, with a peak at day 6, and levels declined thereafter (Fig. 1B). Interestingly, at day 10, virus was no longer detected in lung tissue, but Il21 mRNA could be detected in the surviving mice.

To identify IL-21–producing cells, we used transgenic reporter mice in which the mCherry reporter is inserted at the translation start site of the Il21 gene in a bacterial artificial chromosome clone by recombineering technology. These mice were inoculated with PVM, and single-cell suspensions from lung tissue and lymphoid organs were examined ex vivo by flow cytometry. mCherry expression was detected in ~5% of CD4+ T cells in lung tissue of uninfected mice, with expression increasing to ~10–15% at day 6 after inoculation (Fig. 1C, 1D), which corresponded to peak IL-21 mRNA expression (Fig. 1B), whereas mCherry expression was not detected in NK1.1+ cells (NK cells or NKT cells), γδ T cells, or CD8+ T cells in the lung (Supplemental Fig. 1). In addition to this acute response in the lung, a systemic immune response also developed, as indicated by the increase in mCherry expression from

FIGURE 2. Reduced lung inflammatory pathology in PVM-infected Il21r−/− mice. Representative lung sections (stained with H&E) from either uninfected (Ctl) or PVM-infected WT and Il21r−/− mice. A, Compared to the Il21r−/− mice, WT lungs showing severe cell infiltration at days 5 and 6 after PVM inoculation. Scale bar, 100 μm for all panels. B, Higher-magnification views show the inflammatory cells include lymphocytes (arrows) and neutrophils (arrowheads). Scale bar, 20 μm.

FIGURE 3. Reduced cellular infiltration in lungs of Il21r−/− mice in response to PVM infection. Total number of cells in BAL fluid (A) or of cells isolated from one lobe of the lung (B) were measured at the indicated time points after PVM infection of WT or Il21r−/− mice. The total number of neutrophils was calculated after analyzing the percent Ly6G+CD11b+ cells in either the BAL fluid (C) or lung (D). E and F, RNA was isolated from the other lung lobe in these mice, and RT-PCR was used to evaluate the relative levels of matrix metalloprotease-8 or S100A8 mRNA in the lungs of these PVM-infected mice. Shown are the means ± SEM from one of three experiments with similar results (n = 5–8 mice/group). *p < 0.05, **p < 0.01, ***p < 0.001.
~1.9% of splenic CD4+ T cells in uninfected reporter mice (TG Ctrl) to ~5.9% at day 6 (TG PVM) (Fig. 1D). CD4+ T cells expressing IL-21 were enriched for expression of the ICOS/CXCR5 surface markers characteristic of Tfh cells (Fig. 1E, 1F) compared with CD4+ T cells that did not express IL-21, with a particularly high percentage of Tfh cells in mediastinal lymph node (MLN) IL-21 expressors.

Reduced lung inflammation in response to PVM infection in Il21r−/− mice

The increased numbers of IL-21–producing CD4+ T cells in the lung after PVM infection suggested that this cytokine might participate in antiviral host defense and/or viral pathogenesis. To investigate this possibility, WT and Il21r−/− mice were infected with PVM, and cellular inflammatory responses were evaluated. As observed previously (30, 31), lung tissue from PVM-infected WT mice exhibited progressive, severe inflammation diffusely through the lung at days 5 and 6, whereas lungs from Il21r−/− mice had only mild and focal perivascular inflammation at these time points (Fig. 2A). Infiltration of both neutrophils (arrowheads) and lymphocytes (arrows) were evident in the WT mice (Fig. 2B). We also examined BAL fluid from PVM-infected mice and found significantly fewer cells in the BAL fluid from the Il21r−/− mice than from WT mice on days 5, 6, and 9 postinoculation (Fig. 3). Granulocytic infiltration is a hallmark of the early inflammatory response to PVM (28), and there were fewer Ly6G+ CD11b+ neutrophils in the BAL fluid at days 5, 6, and 9 (Fig. 3C) and lung parenchyma (Fig. 3D) at day 6 in the Il21r−/− than WT mice inoculated with PVM. Consistent with this, at day 6 post-inoculation, Il21r−/− mice also had significantly decreased levels of mRNAs encoding matrix metalloproteinase-8 and S100A8 (Fig. 3E, 3F), two neutrophil-derived proteins involved in mediating the inflammatory/lung remodeling response (32, 33).

Because T cells were reported to be important for the resolution of sublethal PVM infection (34), we assessed the presence of CD4+ T cells, CD8+ T cells, γδ+ T cells, and NK cells in the lung during the course of PVM infection in WT and Il21r−/− mice (Fig. 4). CD4+ T cell infiltration was apparent by day 6 after inoculation, but to a greater degree in WT mice than in Il21r−/− mice, with elevated CD4+ T cell numbers persisting through day 9 postinfection (Fig. 4A). Increased numbers of CD8+ T cells were observed in the lungs of WT mice as early as 3 d after PVM inoculation but then declined, whereas fewer CD8+ T cells were observed in the lungs of infected Il21r−/− mice (Fig. 4B). Although γδ+ T cells infiltrated both WT and Il21r−/− lungs equivalently starting on day 5, fewer γδ+ T cells were seen in the Il21r−/− lungs at days 6 and 9 (Fig. 4C). NK cell numbers were also significantly lower in the lung parenchyma in the Il21r−/− mice (Fig. 4D). Thus, the recruitment of CD4+, CD8+, and γδ T cells, as well as NK cells, was diminished in the absence of IL-21 signaling.

CD8+ T cell function was previously reported to be suppressed in PVM-infected lungs (35). Interestingly, the percentage of lung CD8+ T cells producing IFN-γ increased during the course of infection in both WT and Il21r−/− mice (Fig. 4E), but because of the reduced number of CD8+ T cells in the lungs of Il21r−/− mice (Fig. 4B), the total number of IFN-γ–producing CD8+ T cells was significantly diminished (Fig. 4F). PVM peptides that allow the detection of viral-specific CD8+ T cells have not been identified; thus, these CD8+ T cells may include both PVM-specific cytotoxic
cells as well as bystander cells activated by the inflammatory environment.

Reduced levels of IL-6 in lungs of PVM-infected Il21r−/− mice

The inflammatory response to PVM in WT mice includes rapid infiltration of both neutrophils and lymphocytes into the lung, and, as noted above, these responses were reduced in Il21r−/− mice. Neutrophil infiltration in PVM infection is coordinated by multiple cytokines and proinflammatory chemokines (28, 30, 31). In other settings, IL-17 has been shown to promote neutrophil responses, leading to the induction of cytokines and chemokines that augment the levels of neutrophil progenitors and the subsequent expansion of peripheral neutrophil populations (36). Because IL-21 promotes the differentiation of IL-17–producing cells (12—14), we measured the levels of cytokines associated with Th17 and Tc17 responses after PVM infection. Levels of both Il17a (Fig. 5A) and Il22 (Fig. 5B) mRNAs in lung tissue were not significantly different in WT and Il21r−/− PVM-infected mice. Levels of both Il17a (Fig. 5C) and Ifng (Fig. 5D) mRNAs tended to be slightly lower in the Il21r−/− mice at day 6 after inoculation, but the differences were not statistically significant. Il1b mRNA was significantly decreased at days 6 and 9 (Fig. 5E), although differences in IL-1β protein were not observed (data not shown). However, Il6 mRNA was significantly decreased at days 5, 6, and 9 (Fig. 5F), with a corresponding decrease in IL-6 protein at day 6 in both BAL fluid and lung tissue (Fig. 5G, 5H). These results suggest that IL-21 signaling may directly or indirectly control the production of IL-6 during PVM infection.

Diminished levels of CXCL1 in PVM-infected Il21r−/− mice

Because the accumulation of neutrophils in the lungs was diminished in PVM-infected Il21r−/− mice (Fig. 3), we examined the expression of several chemokines known to promote neutrophil recruitment during PVM infection (37). Interestingly, Cxcl1 mRNA was less potently induced in lung tissue of Il21r−/− mice than in WT mice during PVM infection (Fig. 6A), whereas mRNA encoding CXCL10, a chemokine involved in lymphocyte recruitment, was induced with slightly more rapid kinetics in WT mice (Fig. 6B), but by day 6, the levels were not significantly different in WT versus Il21r−/− mice. We observed no significant difference in the expression of the chemokine CCL3 (also known as MIP-1α), which has been implicated in neutrophil recruitment and immunomodulatory protection in PVM infection (28) (Fig. 6C), and although lower Cxcl2 mRNA expression was observed in Il21r−/− mice at day 6 (Fig. 6D), there was no significant difference in CXCL2 protein expression in the lung (data not shown). Corresponding to the diminished levels of Cxcl1 mRNA (Fig. 6A), CXCL1 protein levels were significantly lower in BAL fluid (Fig. 6E) and lung homogenate (Fig. 6F) at days 5 and 6 after PVM infection of the Il21r−/− mice, correlating with the reduced neutrophil infiltration in the lungs of these mice.

Similar immune responses in lung draining lymph nodes from PVM-infected Il21r−/− mice

Because of the roles that IL-21 plays in the development of cellular immune responses, it was of interest to determine whether changes in cellularity, chemokines, and cytokines were specific to the lung response or whether they reflected an overall deficiency of the immune response in Il21r−/− mice. We thus examined lymphoid populations in the MLN at days 0 and 6 after PVM infection and found that both WT and Il21r−/− MLN undergo T cell expansion (Fig. 7A), including both CD4+ (Fig. 7B) and CD8+ (Fig. 7C) in response to infection, although this was reduced in the Il21r−/−

![FIGURE 5](https://www.jimmunol.org/) Levels of Th17 cytokines are similar, but IL-6 levels are reduced in lung tissue of PVM-infected Il21r−/− mice. Lung RNA was isolated from WT and Il21r−/− mice after PVM infection and relative levels of mRNA encoding IL-17A (A), IL-22 (B), TNF-α (C), IFN-γ (D), IL-1β (E), and IL-6 (F) were quantitated by RT-PCR. IL-6 protein levels in BAL fluid (G) or lung homogenate (H) were measured by ELISA. Shown are the means ± SEM from one of three experiments with similar results (n = 5–8 mice/group). *p < 0.05, **p < 0.01, ***p < 0.001.
MLN (Fig. 7A–C). Similar levels of Ifng, Tnfa, and Il6 mRNAs were measured in the MLN, although Il17a and Il1b mRNA levels were lower in the Il21r−/− MLN (Fig. 7D–H). These data indicate that although lymphoid expansion was lower in the Il21r−/− MLN, most inflammatory cytokine responses were similar, suggesting that the cellular immune response was not completely defective in the knockout mice.

**Constitutive expression of IL-21 leads to increased cellular infiltration to the lung and increased IL-6 production**

The production of IL-21 by CD4+ T cells in the normal lung suggested that IL-21 may play a role in normal lung homeostasis as well as in the development of the inflammatory response to PVM infection. To investigate this possibility, we examined lung cellularity in transgenic mice that constitutively express IL-21 in immune cells (TG21) (5). We observed increased cellularity in both the BAL fluid (Fig. 8A) and the lung (Fig. 8B), with an elevated percentage of neutrophils in both BAL and lung in the uninfected TG21 mice (Fig. 8C). Levels of Cxcl1 mRNA (Fig. 8D) and Il6 mRNA (Fig. 8E) were both increased in the TG21 lungs. Interestingly, however, levels of the neutrophil-expressed Mmp8 mRNA were similar in WT and TG21 lungs (Fig. 8F), suggesting that the enhanced recruitment of neutrophils to the lung occurred without an associated inflammatory response. To determine whether IL-21 could directly induce the production of IL-6, we purified DC populations and found that IL-21 significantly induced both IL-6 mRNA and protein (Fig. 8G, SH) in these cells.

**Prolonged survival of PVM-infected Il21r−/− mice**

Above, we observed an increase in IL-21 production in response to PVM infection as well as decreased neutrophil and lymphocyte accumulation and diminished production of IL-6 in Il21r−/− mice. It was unclear, however, whether IL-21 mediated the PVM pathogenic response or instead promoted host defense. To clarify the role of IL-21, we inoculated WT and Il21r−/− mice intranasally with a dose of PVM previously shown to be sufficient to kill WT mice and determined their survival (Fig. 9A). As anticipated, 60% of the WT mice died on day 7, and all were dead by day 10 after PVM inoculation. In contrast, 90% of the Il21r−/− mice were still alive at day 7, and 30% of the Il21r−/− mice survived beyond day 10, with 5% (1 out of 19 mice) surviving through day 21. Thus, the absence of IL-21 signaling conferred a survival advantage (p < 0.0001), even though there was no significant difference in PVM SH gene expression, as an indicator of virus copy number, in WT versus Il21r−/− lungs (Fig. 9B). These results suggest that IL-21 does not have a significant effect on viral replication or clearance but that it promotes the inflammatory response following infection with PVM, with earlier and augmented mortality in response to PVM infection in WT mice.

To determine whether the enhanced survival of the Il21r−/− mice was a direct result of the effects of IL-21 in the lung during the response to PVM infection, we next used an IL-21R/Fc fusion protein to block IL-21 activity. When WT mice were intratracheally treated with the IL-21R/Fc fusion protein 1 d prior and 2 d after PVM inoculation, there was significantly higher survival than in mice treated with an Fc control protein (Fig. 9C), even though no significant differences in virus replication, based on SH gene expression, were detected between these two groups at day 6 of infection (Fig. 9D). Moreover, when we inoculated mice with a lower dose of PVM, treatment with the IL-21R/Fc fusion protein conferred complete protection (Fig. 9E), and again there were no differences in viral burden (Fig. 9F). These data indicate that the survival advantage seen in Il21r−/− mice did not result from developmental differences but rather resulted from the lack of IL-21 signaling.
In this study, we have shown that IL-21 signaling plays a critical role in promoting the lung inflammatory response to acute pneumovirus infection. IL-21 expression in the lung in response to acute or chronic viral infections in general and to PVM in particular has not previously been reported. Our studies revealed that IL-21 mRNA levels are significantly elevated in lung tissue after PVM infection and that this induction parallels increased lung inflammation. Correspondingly, studies with Il21−/− mCherry reporter mice showed that enhanced IL-21 expression in the lung after PVM infection is specific for CD4+ T cells. Interestingly, uninfected lungs also contained a small but detectable population of CD4+ T cells expressing mCherry, suggesting a potential role for IL-21 in basal lung homeostatic responses to environmental Ags.

To determine if the production of IL-21 promoted or diminished PVM-induced disease, we examined PVM-inoculated Il21r−/− mice and found they had significantly reduced lung pathology, with decreased infiltration of both neutrophils and lymphocytes. Previous studies showed that CCL3/MIP-1α, a chemokine produced by lung epithelium in response to RSV infection (38), was critical for neutrophil accumulation in the lungs and survival after PVM infection (39). Interestingly, CCL3 levels in the Il21r−/− mice were at most modestly diminished, although neutrophil infiltration was significantly reduced. In contrast, the level of CXCL1, which also mediates neutrophil chemotaxis (40), was much less potently induced in the BAL fluid and lungs of Il21r−/− mice than in WT mice; thus, the diminished CXCL1 may at least in part explain the diminished neutrophil accumulation in Il21r−/− mice.

Our results reveal a potential role for IL-21 in normal lung homeostasis. Uninfected IL-21 transgenic mice had increased lung cellularity, including neutrophilia. Additionally, lungs from TG21 mice had increased levels of IL-6 and CXCL1, both involved in mediating inflammatory pathology. Interestingly, IL-21 could directly induce IL-6 expression in DCs, providing a direct link between IL-21 and the inflammatory process. Previously IL-6 was shown to induce IL-21 expression in CD4+ T cells (14, 41). Our results suggest that IL-21 can potentially amplify its own induction via the induction of IL-6.

T cell responses significantly contribute to the host response to sublethal PVM infection, with both CD4+ and CD8+ T cell subsets contributing to viral clearance (34). Although both CD4+ and CD8+ T cell numbers were decreased in the Il21r−/− mice, there was no apparent change in viral replication or clearance. Th17 cytokines are known to mediate inflammation, and IL-21 serves to promote Th17 differentiation (12–14); however, there was no apparent decrease in Th17 cells or of IL-17A or IL-22 production in the lungs of PVM-infected Il21r−/− mice. Thus, Th17 differentiation does not appear to play a substantial role in PVM infection.

It was previously reported that most PVM-specific CD8+ T cells produce diminished levels of cytokines upon antigenic stimulation (35). In our studies, however, there was no difference in the percentage of CD8+ T cells that could produce IFN-γ in WT and Il21r−/− mice. However, the total number of CD8+ T cells was greatly decreased, consistent with the known role of IL-21 for CD8+ T cell expansion and differentiation (4, 42), and thus the absolute number of IFN-γ+CD8+ T cells was also greatly reduced.

**FIGURE 7.** Cellular infiltration and cytokine production in lung-draining lymph nodes after PVM infection of WT and Il21r−/− mice. A, Total cellularity was measured in MLN either before or at day 6 after PVM infection. Flow cytometry was used to assess the total number of CD4+ (B) or CD8+ (C) T cells based on the percent of each population and the total cell number. D–H, MLN RNA was isolated, and relative levels of the indicated cytokines were measured by RT-PCR. Shown are pooled data from three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
in the Il21r−/− lungs. It is possible that although the absence of IL-21 did not affect viral clearance in the primary response to PVM, CD8+ T cells primed in the absence of IL-21 might have defective memory responses to PVM upon secondary challenge.

In summary, we have demonstrated that IL-21 plays a previously unappreciated role in the host response to PVM, promoting an unanticipated pathogenic inflammatory response to the virus that is associated with reduced host survival, which is in marked contrast to the protective role played by IL-21 in chronic lymphocytic choriomeningitis virus infections and its correlation with control of HIV. Importantly, we also found that pretreatment of WT animals with a soluble IL-21R-Fc fusion protein was protective, an ob-

FIGURE 8. IL-21 increases lung cellularity and induces IL-6 production. A–C, Total cellularity was measured in BAL fluid (A) and lungs (B) of WT and IL-21 transgenic mice (TG21). The percent of neutrophils was determined by flow cytometry of Ly6G+CD11b+ cells (C). Lung RNA was isolated, and relative levels of Cxcl1 (D), Il6 (E), and Mmp8 (F) mRNA were measured by RT-PCR. Splenic DCs were isolated and stimulated in vitro with IL-21 for 5 h, at which time Il6 mRNA was measured by RT-PCR (G), and IL-6 protein was measured at 16 h by ELISA (H). *p < 0.05, **p < 0.01, ***p < 0.001.

FIGURE 9. Prolonged survival after PVM infection in Il21r−/− mice or WT mice treated with an IL-21R/Fc fusion protein. A, WT and Il21r−/− mice were infected with PVM, and their survival was monitored daily over the next 3 wk. Infection of the long-term survivor was documented by confirming seroconversion to PVM Ags (SMART-M12, El Cerrito, CA; data not shown). Statistical significance was evaluated using Kaplan–Meier survival curve (Prism; GraphPad). n = 19 mice per group. B, RT-PCR evaluation of the PVM SH gene in total lung RNA. Shown are the means ± SEM from one of three experiments with similar results (n = 5–8 mice/group). C and E, WT mice received 50 μg of either IL-21R/Fc or control Fc intratracheally 1 d prior to and 2 d postinoculation with PVM, and their survival was monitored. Mice in C and E received 60 and 12 PFU, respectively, of PVM intranasally. In C, p = 0.001 and in E, p = 0.039, with 10 mice/group in each panel. D and F, PVM SH expression was determined by RT-PCR of total lung RNA at day 6 after PVM infection. D corresponds to the experiment in C, and F corresponds to the experiment in E. Shown are the means ± SEM from five mice in each group.
IL-21 PROMOTES THE INFLAMMATORY RESPONSE TO PVM


Supplemental Figure 1. IL-21 is not expressed by lung NK1.1⁺, γδ⁺ cells or CD8⁺ T cells following PVM infection. Il21-mCherry transgenic reporter mice (TG) or WT littermates were infected with PVM, lung cells were isolated 6 days later, and IL-21 expression measured by flow cytometry after surface staining with anti-NK1.1 (A), anti-γδ (GL3) (B), or anti-TCRβ plus anti-CD4 (C). A representative experiment is shown; the experiment was performed twice with similar results.