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*J Immunol* 2010; 185:2231-2239; Prepublished online 12 July 2010; doi: 10.4049/jimmunol.1000733

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Respiratory Virus-Induced TLR7 Activation Controls IL-17–Associated Increased Mucus via IL-23 Regulation

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The response to respiratory syncytial virus (RSV), negative strand ssRNA virus, depends upon the ability to recognize specific pathogen-associated targets. In the current study, the role of TLR7 that recognizes ssRNA was examined. Using TLR7−/− mice, we found that the response to RSV infection in the lung was more pathogenic as assessed by significant increases in inflammation and mucus production. Although there appeared to be no effect of TLR7 deficiency on type I IFN, the pathology was associated with an alteration in T cell responses with increases in mucogenic cytokines IL-4, IL-13, and IL-17. Examination of dendritic cells from TLR7−/− animals indicated a preferential activation of IL-23 (a Th17-promoting cytokine) and a decrease in IL-12 production. Neutralization of IL-17 in the TLR7−/− mice resulted in a significant decrease in the mucogenic response in the lungs of the RSV-infected mice. Thus, without TLR7-mediated responses, an altered immune environment ensued with a significant effect on airway epithelial cell remodeling and goblet cell hyper/metaplasia, leading to increased mucus production. The Journal of Immunology, 2010, 185: 2231–2239.

The role of innate immune responses for establishing the most appropriate and least pathologic responses for destruction and clearance of the infectious or deleterious agents is the first line of defense and is responsible for recognizing the vast array of pathogens with a limited set of fixed germline-encoded receptors. To deal with this challenge with infectious agents, innate immunity relies on the detection of patterns or conserved molecular motifs unique to various classes of pathogens (1–3). The heterogeneity of viral glycoproteins along with their ability to genetically drift from season to season creates even greater challenges for innate immune recognition of viruses. To circumvent these obstacles, the innate immune system has evolved mechanisms to detect characteristics of viral nucleic acids that are either distinct in structure (dsRNA) or subcellular location (ssRNA). Recognition of viral nucleic acids triggers the induction of type I IFNs that induce an antiviral state in virally infected cells (4–6). Activation of specific chemokines during RSV infection of epithelial cells can be upregulated via TLR3. Our laboratory identified that although CCL5 and CXCL10 can be upregulated via a TLR3-dependent pathway, CXCL8 is dependent upon a MyD88-dependent pathway (7). Thus, multiple TLR pathways likely contribute to the generation of an effective anti-RSV response. Interestingly, our findings suggest that RSV infection of TLR3−/− mice (a MyD88-independent mechanism) leads to altered immune environment and promotes increased IL-13 that is associated with increases in mucus production (13). Thus, alteration of the dsRNA recognition pathway leads to a pathogenic phenotype. Likewise, when MyD88 pathways are deleted in mice, an even more pathogenic environment is induced with increased eosinophilia, mucus overexpression, and an overall induction of a Th2 cytokine environment (14). In the current study, we have examined one of the important MyD88-dependent pathways associated with viral RNA detection, TLR7. Although the present perception is that plasmacytoid dendritic cells (pDCs) are the primary cell population that expresses TLR7 (15, 16), several other cell populations appear to be able to upregulate its expression, including myeloid DCs (mDCs), B cells, and macrophages (17–19). In the present studies, the deletion of TLR7 results in the generation of pathogenic responses during RSV infection and initiates an increase in a number of pathogenic cytokines, especially IL-17, which appears to be responsible for the increased mucus responses observed in TLR7−/− mice.

Materials and Methods

Mice

B6 wild-type (WT) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). TLR7−/− mice on a B6 background were originally provided by Dr. S. Akira (Osaka, Japan) and a breeding colony subsequently
established at the University of Michigan (Ann Arbor, MI). All animal work was performed in accordance with the University of Michigan Committee on Use and Care of Animals policy.

**Respiratory syncytial virus**

Our laboratory utilizes the antigenic subgroup A strain of RSV, referred to as Line 19. This isolate was obtained from a sick infant at the University of Michigan (20) and has been demonstrated in animal models to mimic human infection by stimulating mucus production using an inoculum of 1 × 105 PFU/mouse by intratracheal administration (21).

**Real-time TaqMan PCR**

The smallest lobe was removed and homogenized in 1 ml Trizol reagent (Invitrogen, Carlsbad, CA). RNA was isolated as described (Invitrogen), and 5 μg was reverse-transcribed to assess gene expression. Detection of cytokine mRNA in lung samples was determined using predeveloped primer/probe sets (Applied Biosystems, Foster City, CA) and analyzed using an ABI Prism 7500 Sequence Detection System (Applied Biosystems). Transcript levels of Muc5ac, Gob5, Il13, and Il4 were determined using custom primers, as previously described (22, 23). Gapdh was analyzed as an internal control and gene expression was normalized to Gapdh. Fold changes in gene expression levels were calculated by comparison with the gene expression in uninfected mice, which were assigned an arbitrary value of 1.

**Lung and lymph node leukocyte isolation**

Lung leukocytes were isolated from enzyme-dispersed lung tissue. Right lungs from each mouse were excised, washed in PBS, minced, and digested enzymatically for 45 min in 15 ml lung digestion buffer (RPMI, 5% FCS, 1 mg/ml collagenase [Roche Applied Science, Indianapolis, IN], and 30 μg/ml DNase [Sigma-Aldrich, St. Louis, MO]). Lung-associated lymph nodes (LALNs) were dispersed similar to lungs, except that only 5 ml digestion buffer was used. Following erythrocyte lysis using ammonium chloride (NH₄Cl) buffer, cells were washed and resuspended in media (RPMI, 5% FCS). Total lung leukocyte numbers were assessed in the presence of trypan blue using a hemocytometer; viability was >85%.

**Lymph node restimulation**

LALN cell suspensions were plated in duplicate at 1 × 10⁶ cells/well followed by restimulation with either media or RSV (multiplicity of infection [MOI] ~0.5). Cells were incubated at 37°C for 24 h and supernatants collected for analysis on the Bio-Rad Bioplex 200 system according to the manufacturer’s protocol (Bio-Rad, Hercules, CA). Kits (Bio-Rad) containing Ab beads to Th cytokines (IL-17, IFN-γ, IL-4, IL-5, IL-13) were used to assay for cytokine production in each of the samples.

**Lung protein analysis**

The levels of CCL5 and CXCL10 in lung homogenates were assessed using multiplex bead-based arrays (Invitrogen) and analyzed on the Bioplex 200 system (Bio-Rad).

**Generation of bone marrow-derived DCs**

Bone marrow was harvested from B6 or TLR7–/– mice and seeded in tissue-culture flasks in RPMI 1640-based complete media with 10 ng GM-CSF/ml (R&D Systems, Minneapolis, MN). Cells were fed every 3 d, and loosely adherent cells were collected after 10 d. The majority of the cells, >85%, were CD11c+ bone marrow-derived DCs (BMDCs) and subsequently were infected with RSV (MOI ~0.5) or stimulated with specific TLR ligands: CpG (HyHel Biotech, Uden, The Netherlands), LPS (Sigma-Aldrich), or 3M-011 (3M, St. Paul, MN). The cells were then assessed for gene and protein expression as well as used for flow cytometric analysis.

**Flow cytometric analysis**

Except TLR7, all Abs for flow cytometry (BD Pharmingen, San Diego, CA; Biologend, San Diego, CA; or eBioscience, San Diego, CA) were used to stain intact cells per the manufacturer’s instructions and analyzed on an LSR II cytometer (BD Biosciences, San Jose, CA). For TLR7 staining, cells were permeabilized using fixation/permeabilization solution (BD Biosciences) and stained with anti-mouse TLR7 Abs (Ingenex, San Diego, CA). Isotype control Abs were used to demonstrate specificity of our staining and to establish the criteria for our flow cytometry populations.

**Anti–IL-17A Abs**

Anti-mouse IL-17A was generated by immunization of rabbits with recombinant murine IL-17A. The polyclonal sera was tested for cross-reactivity to a panel of other cytokines, including other IL-17 family members, and no cross-reactivity was observed. Abs were isolated from total sera using Montage protein A affinity chromatography (Millipore, Bedford, MA). Mice were treated with ~3 mg anti–IL-17A Ig or control Ig every other day beginning at day 4 postinfection to allow our studies to address whether blocking IL-17 might have therapeutic potential.

**Statistics**

Data was analyzed using Prism software (GraphPad, San Diego, CA). Unless otherwise specified, data shown are representative of two or more experiments. Statistical significance in all experiments was determined by one-way ANOVA, followed by a Newman-Keuls post test. Significant differences were regarded as p < 0.05. Statistical comparisons of quantitative PCR (QPCR) data were determined from normalized cycle threshold values, preconversion to fold increases.

**Results**

**Increased expression of TLR7 during RSV infection**

The expression of TLR7 has been identified on multiple immune cell populations but is thought to be most closely associated with APCs, such as DCs and B cells. To determine if RSV infection upregulates TLR7 expression, infected animals were assessed for TLR7 expression by QPCR. The lungs (Fig. 1A) demonstrated a time-dependent increase in TLR7 expression that first appeared on day 4 and increased and plateaued on days 8 and 12, respectively. When cells from the RSV-infected mice (day 8 of infection) were assessed by intracellular staining for TLR7 by flow cytometry, the primary cells that expressed TLR7 were B cells, pDCs, and the strongest staining pattern for mDCs (conventional) based upon their median fluorescence intensity in the lung (Fig. 1B, 1C). Thus, it appears that numerous immune cells express TLR7, primarily in CD11b+CD11c+ conventional DCs, but there was no increase in TLR7 intensity on a per-cell basis in RSV compared with uninfected animals. Rather, the increase in mRNA expression levels is likely due to an increased recruitment of those cells in animals infected with RSV.

**Deletion of TLR7 enhances RSV-induced goblet cell hyperplasia**

The role of TLRs for the recognition of pathogen-associated molecular patterns, such as virus-associated ssRNA by TLR7, is thought to be critical in the recognition of an infectious process. TLR7 signaling is dependent upon MyD88 adaptor-mediated pathways, and our previous studies have established that deletion of MyD88 results in a skewed response with heightened levels of Th2 cytokines, eosinophilia, and increased mucus production. In the present studies, when TLR7–/– mice were infected with RSV, there was also an increase in pathologic responses similar to that observed in the MyD88–/– mice, especially the expression of mucus and increased numbers of goblet cells in the airways (Fig. 2A). Similarly, we observed that TLR7–/– mice had increased expression of mucus-associated genes Muc5ac and Gob5 compared with RSV-infected WT animals (Fig. 2B). Interestingly, there was not a remarkable increase in inflammatory cells in the TLR7–/– mice as evidenced in the lung sections or individual populations of cells including T cells and DC subsets in either the lung or draining lymph nodes (LALNs), as assessed by flow cytometry (Fig. 2C). When we examined viral clearance, we found no significant changes as assessed by viral gene expression within the lungs of infected mice as well as assessing viral titer at day 3 of infection by viral plaque assays (data not shown). Thus, in the absence of TLR7, there was primarily a pathogenic alteration of the RSV-induced responses that was primarily centered on airway pathology.

**Alteration in cytokine profiles in TLR7–/– mice with RSV-induced disease**

Several studies have identified that type I IFN is most often associated with an appropriate immune response and that TLR7 can mediate these effects (24–28). Given the above data indicating that...
mDCs appeared to have the highest expression of TLR7, we chose to focus on BMDCs as an in vitro model to assess the role of TLR7 in the innate immune response to RSV. When we examined type I IFN in DCs after RSV infection, there was no alteration of either IFNα or IFNβ in the TLR7−/− compared with WT DC populations (Fig. 3A). Furthermore, examining chemokines that are associated with type I IFN induction, CCL5 and CXCL10, demonstrated a similar induction with not only RSV but also a TLR3/polyinosinic:polycytidylic acid (poly I:C) stimulus (Fig. 3B). In addition, when we examined the type I IFN-dependent transcription factor IFN regulatory factor-1 in RSV-infected or poly I:C-stimulated DCs, it was similarly upregulated in WT and TLR7−/− DCs (Fig. 3C). Finally, several studies have demonstrated that DC maturation in general and specifically during RSV infection is dependent upon type I IFN. The TLR7−/− DCs were able to mature properly as demonstrated by examination of CD80 expression (Fig. 3D). These data suggest that a defect in type I IFN-associated responses are unlikely to be the proximate cause for the dysregulated response to RSV observed in TLR7−/− mice.

To begin to understand the changes in immune responses in the TLR7−/− animals that correspond to the pathologic alterations observed, lungs from RSV-infected mice were isolated and dispersed into single-cell suspensions. The single-cell suspensions were then given RSV (MOI 0.5) to initiate a recall response to RSV, and cytokine levels in supernatants were assessed using bioplex proteomics analysis after 24 h (Fig. 4A). The data indicate
that although there was no alteration in IFN-γ production in the TLR7−/− mice compared with WT, a very significant increase in IL-17A production could be observed, as well as significantly more Th2 cytokines IL-4 and IL-13 (Fig. 4A). Among these, only IL-17A was persistently elevated in lung homogenates at day 9 post-infection (27.24 ± 5.67 pg/ml versus 8.30 ± 1.98 pg/ml in controls; p < 0.05). Thus, the increased production of IL-17A, as well as Th2 cytokines IL-4 and IL-13, corresponded with associated increases in mucus gene expression and goblet cell hyperplasia.

To further examine if there was an alteration in APC function in the TLR7−/− mice that would alter the subsequent production of T cell-derived cytokines, we again used BMDCs from WT and TLR7−/− mice. BMDCs were infected with RSV (MOI ∼0.5) or other TLR agonists and assessed for expression of IL-6, IL-12p40 (the common chain), as well as the IL-12– and IL-23–specific chains p35 and p19, respectively. No significant increased differences in the mRNA expression of induction of IL-6 were found, but rather a trend toward a decrease (WT 15.0 ± 3.0-fold versus TLR7 8.7 ± 1.6-fold increase over uninfected). However, the data in Fig. 4B illustrate that although IL-12p40 expression was similar between the WT and TLR7−/− BMDCs, there was a significant decrease in IL-12p35 and concomitant increase in IL-23p19 in TLR7−/− BMDCs. These data indicate a skewing of the DC response to RSV, which could explain the increased level of IL-17 observed in the TLR7−/− mice following RSV infection. Based on evidence from the literature regarding the recognition of RSV via TLR4, we hypothesized that TLR4 may mediate the IL-23p19–promoting signal in BMDCs. To test this hypothesis, BMDCs were stimulated with various TLR agonists, and the induction of IL-23p19 was assessed via QPCR. Compared to other TLR agonists CpG (TLR9) and 3M-011 (TLR7), LPS was the most potent inducer of IL-23p19 (Fig. 4C). Taken together, these data suggest that IL-12/IL-23 signaling in response to RSV may be mediated by the opposing actions of TLR7 and TLR4, with TLR7 favoring IL-12 and TLR4 favoring IL-23.

To determine whether TLR7 deficiency would alter the balance of IL-12 versus IL-23 signaling in vivo, we assessed the induction of IL-12 and IL-23 in the lungs of RSV-infected mice. TLR7−/− mice and WT controls were infected with RSV, and the expression of IL-12 p35, IL-12/IL-23p40, and IL-23 p19 were assessed at day 3 postinfection via QPCR of lung RNA. No detectable induction of IL-12p40 was observed in either group (Fig. 4D), and the induction of IL-12p35 was similar between the two groups (Fig. 4D). Similar to the results obtained with BMDCs in vitro, a dramatic increase in IL-23p19 was observed in the lungs of TLR7−/− mice, relative to WT controls (Fig. 4D). In addition, when we examined other IL-17–associated cytokines in lungs of infected
mice, there were no significant changes in IL-6, a cytokine required for IL-17A induction (Fig. 4E). Importantly, no alteration in a closely related IL-17F and no changes in IL-17-inducible chemokines CXCL1 or CXCL2 were observed during RSV infection at day 3 post RSV infection (Fig. 4E). Together, these results in vitro and in vivo data support the conclusion that dysregulation in innate cytokine production in BMDCs, away from Th1 promoting IL-12 and in favor of Th17-promoting IL-23, results in dysregulated immune responses in the lungs of TLR7−/− mice, leading to increased pathology.

**IL-17 overexpression is a dominant pathogenic signal in TLR7−/− animals**

Our data indicated that the predominant cytokine that was upregulated in TLR7−/− animals was IL-17. Results have suggested that IL-17 may directly induce mucus production from epithelial cell populations in the lung (29, 30). To address the role played by IL-17 in the immunopathology observed in RSV-infected TLR7−/− mice, we generated anti–IL-17 Abs and treated TLR7−/− animals using passive immunization during RSV infection. Control or anti–IL-17 Abs were administered systemically (i.p.) beginning at day 4 post RSV infection, with predefined endpoint at 24 h post RSV infection. Anti–IL-17 Abs were administered in RSV-infected control B6 and TLR7−/− BMDCs (Fig. 3A). The induction of chemokines CCL5 and CXCL10 in control B6 and TLR7−/− BMDCs. BMDCs were cultured with media alone, poly I:C (20 μg/ml), 3M-011 (10 mM), or RSV, and the levels of chemokines in the supernatant were measured at 24 h via Bioplex multiplex assay. The expression of IFNβ (WT-RSV, 0.5 ± 0.5%; TLR7-RSV, 19.3 ± 6.8%; TLR7-RSV–anti–IL-17, 6.0 ± 3.7% PMNs of total BAL leukocytes). Thus, these data overall suggest that, in the absence of TLR7 signaling, inappropriate activation of the IL-17A pathway during RSV infection plays a prominent role in promoting increased mucus production and alters inflammation.

**Discussion**

The response to viral infections within the lung must proceed in a balanced and programmed manner to optimally clear the virus without damaging critical pulmonary function or promoting pathologic responses. This is particularly important in individuals with underlying disease, such as those with chronic obstructive pulmonary disease, asthma, or end-stage fibrotic illness. It is clear that the determination of the direction of the immune responses relies on innate immune cell activation and the mediators that they produce. In the current study, the innate response and subsequent T cell response to RSV, an ssRNA virus, depends upon the presence of ssRNA recognition through TLR7 to promote a relatively nonpathogenic response. Original studies described that TLR7 was necessary for recognition of ssRNA and elicited both IL-12 and type I IFNs necessary for Th1-type immune responses (31–33). The response of the TLR7−/− BMDCs in the present studies demonstrated that without TLR7, there was a change in the balance of innate cytokines with increases in Th1 inducing IL-23 and a decrease in Th1 inducing IL-12. Interestingly, in the absence of TLR7, there was a change in the balance of innate cytokines with increases in Th1 inducing IL-23 and a decrease in Th1 inducing IL-12. Interestingly, in the absence of TLR7, there was a change in the balance of innate cytokines with increases in Th1 inducing IL-23 and a decrease in Th1 inducing IL-12.
of TLR7, no alteration in RSV clearance could be detected. How-
however, the immune response and subsequent pulmonary phenotype
was greatly affected, with a significant increase in cytokines and
mucus production, demonstrating that a link between viral clear-
ance and immunopathology was not evident. Although several
immune cytokines that were upregulated, IL-4, IL-13, and IL-
17, have clearly been linked to the generation of pathogenic
responses, IL-17A was the most noteworthy. Numerous studies
have suggested that by targeting TLR activation in general and
TLR7 specifically, a skewing of the immune response away from
Th2 could be accomplished (27, 34–39). In addition, a recent
study demonstrated that type I IFN induced by TLR7 regulates
IL-17 production (40). It may be the absence of this pathway that
alters the response, perhaps through regulation of IL-23. Thus, our
findings support these previous findings using a clinically relevant
animal model of pulmonary viral infection.

Several studies have examined the role of TLRs in RSV and other
respiratory viral infections. TLR4 was first implicated in RSV
infections (6, 41), and although initially controversial (42), nu-
merous studies have identified a clear connection between TLR4
and RSV clearance (6, 41, 43, 44) as well as a link involving
TLR4 polymorphisms and severe RSV-induced disease in infants
(9, 45–47). The ability of RSV F protein to bind and induce acti-
vation of TLR4 suggests that even the initial binding of RSV
to the cell surface may provide an early innate activation signal
that is important to sensing the infection. Discrete amino acid
changes in F protein appear to regulate the induction of IL-13 and
mucus in different strains of RSV (48) and may be linked to
TLR4 activation. A most profound pathogenic effect
was found when MyD88
-/- mice were used with RSV infection
and included increased Th2 cytokines, enhanced mucus produc-
tion, and profound eosinophilia (14). This latter response was
likely due to the removal of multiple TLR pathways, including
TLR2, TLR4, and TLR7. The response in MyD88
-/- mice
appeared to be of a similar intensity as that described in
STAT1
-/- mice (30, 50), perhaps linking it to a type I IFN re-
sponse that may be important early in the antiviral immunity. In
addition, when the only MyD88-independent sensing TLR was
removed, TLR3, a pathogenic effect was also observed linked to
alteration of IL-13 production, leading to increased mucus pro-
duction (12). Recent studies have clearly established that TLR4
pathways lead to increased expression of IL-23 via AP-1 activa-
tion, which can be especially enhanced by coactivation with IFN-γ (51). Interestingly, IL-23 along with IL-1β induces
IL-17 from γ/δ T cells and can subsequently enhance CD4

**FIGURE 4.** A. Effect of TLR7 deficiency on early lung cytokine production during RSV infection. Control and TLR7
-/- mice were infected with RSV, and the lungs were harvested at day 3 postinfection. Single-cell suspensions were obtained via enzymatic
digests, and cells were restimulated with RSV (MOI ~0.5). Cytokine production was assessed via Bioplex multiplex assay. Similar
results were obtained in three independent experiments. B. Role of TLR7 in mediating IL-12 versus IL-23 signaling in vitro. BMDCs were isolated and cultured from WT and TLR7
-/- mice in the presence of media, the TLR7 agonist 3M-011, or RSV
(MOI ~0.5) for 24 h. The induction of IL-12p35, IL-12/23p40, and IL-23p19 were determined via QPCR. C. Effect of TLR
agonists on the induction of IL-23 p19 in DCs. BMDCs from control B6 mice were treated with media alone, CpG (1 μg/ml), LPS (1 μg/ml), or 3M-011 (10 mM) and the expression of IL-23 p19 was assessed at 4 h via QPCR. D. Effect of TLR7 defi-
cency on the induction of IL-12 and IL-23 in vivo. Control and TLR7
-/- mice were infected with RSV, and the expression of
IL-12p35, IL-12/IL-23p40, and IL-23p19 were determined at day 3 postinfection via QPCR of whole-lung RNA. E. Control and
TLR7
-/- mice were infected with RSV, and the expression of
IL-6, IL-17F, CXCL1, and CXCL2 were determined at day 3 postinfection via QPCR of whole-lung RNA. Data represent mean ± SEM from lungs of five
to six mice per group. p< 0.05.
T cell-derived IL-17 (52). The latter mechanism may be critical in driving IL-17 in the absence of TLR7 signaling by shifting the balance away from IL-12 and toward IL-23. Although the present studies did not observe an increase in IL-6 levels in TLR7 \(^{-/-}\) mice nor in isolated DCs, and because IL-6 and IL-17 may be critical and synergize to induce mucus gene expression (29), the pathologic phenotype in the TLR7 \(^{-/-}\) may be due to the increase in IL-17 in the presence of sufficient IL-6 production. Thus, it is clear that the pathogen-sensing system induced by multiple TLRs is critical at several levels for driving an appropriate antiviral response.

In the context of innate cell activation, TLR2 and TLR4 are found predominantly on the surface of innate cells and allow the early recognition of the virus, subsequently followed by TLR3 and TLR7 that are found primarily in the endosomal compartment and recognize ds- and ssRNA. These later TLRs appear to have a prominent impact on shaping the T cell immune response. In addition, because RSV infects predominantly via cytoplasmic entry, other nonendosomal sensing systems are also important, such as retinoic acid-inducible gene-I (53). A recent study that used mice deficient in the primary adapter molecule for retinoic acid-inducible gene-I, mitochondrial antiviral signaling protein, infected with RSV demonstrated a significant abrogation of early cytokine production, but pathogenesis later in disease was not investigated (54). Alteration of any of these pathogen-sensing pathways may lead to an alteration in either the clearance of the virus and/or the immunopathology of the response. Thus, there may be multiple associated mechanisms for induction of severe disease that may result from genetic or environmental alteration of pattern recognition receptor expression.

Establishing an altered immune environment of the lung early in development may have subsequent impact on later responses in specific susceptible individuals. Although it is still controversial whether severe RSV disease predisposes children to subsequent pulmonary disease, clear epidemiology studies have linked early RSV disease with persistent pulmonary problems (55–60). Interestingly, several studies have now linked TLR7 polymorphisms with altered responses to HIV and hepatitis C virus-induced responses, and, most interesting, the TLR7 polymorphisms have been associated with development of asthma (61–64). Thus, similar to TLR4 polymorphism studies, a link may exist between TLR7 polymorphisms and RSV disease and associated persistent pulmonary problems. An early and consistent observation related to IL-17 overproduction has been the induction of CXC chemokines that leads to increased neutrophil accumulation that can impact local tissue damage and increase mucus production via elastase release (65–67). Although we did not observe changes in CXC chemokines in the TLR7 \(^{-/-}\) animals, we were able to detect an increase in PMN accumulation in TLR7 \(^{-/-}\) that was reduced with neutralization of IL-17. The changes in the ability of particular or multiple TLR systems may lead to multiple disease phenotypes in the lung including asthma and chronic obstructive pulmonary

**FIGURE 5.** Neutralizing IL-17 beginning on day 4 of RSV infection of TLR7 \(^{-/-}\) mice attenuates mucus production. A, PAS staining of lung sections from control Ig or anti–IL-17–treated, RSV-infected TLR7 \(^{-/-}\) mice at day 8 postinfection (original magnification ×100). Expression of mucus-associated genes Muc5ac and Gob5 (B), IL-4, IL-13, and IL-17 (C) was assessed via qPCR of lung RNA in uninfected control (UC), control Ig RSV-infected mice, and in anti–IL-17–treated animals. Columns represent the mean fold increase over uninfected controls, and error bars represent SEM. *p < 0.05.
disease, diseases that most severely manifest themselves through viral exacerbation responses and altered inflammatory cell accumulation.

The overall response generated in a viral response relies upon multiple means to sense the pathogen with an emphasis on generating the most successful clearance response that is not pathogenic. In these studies, the absence of TLR7 does not alter viral clearance, but does allow a more pathogenic response that includes more mucus via an IL-17A-mediated mechanism. It is noteworthy that a similar alteration of IL-17F was not observed in the TLR7−/−, an observation that suggests clear differences in the regulation of the two family members. Thus, we can consider that a TLR7-mediated event allows for fine-tuning of the immune responses during respiratory viral infections limiting pathology.

Disclosures

The authors have no financial conflicts of interest.

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