

Respiratory syncytial virus infection in the absence of *STAT1* results in airway dysfunction, airway mucus, and augmented IL-17 levels

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Background: Respiratory syncytial virus (RSV) is the leading infectious cause of respiratory failure and wheezing in infants and young children. Prematurity is the greatest risk factor for severe RSV-induced disease, and recent studies suggest that premature children have lower levels of the type I IFNs (α/β), for which signal transducer and activator of transcription (STAT) 1 is a critical intracellular signaling molecule. **Objective:** We hypothesized that RSV infection in *STAT1* knockout (*STAT1* KO) mice would result in both increased airway resistance and airway hyperresponsiveness. **Methods:** Wild-type (WT) and *STAT1* KO mice on a BALB/c background were either RSV or mock infected. Phenotypic response to infection was assessed by means of plethysmography, immunohistochemistry, and lung cytokine measurement. **Results:** We found that *STAT1* KO mice infected with RSV (*STAT1* KO-RSV) had greater baseline lung resistance ($P = .05$) and airway responsiveness ($P < .001$) than mock-infected *STAT1* KO (*STAT1* KO-MOCK), RSV-infected wild type (WT-RSV), and mock-infected wild type (WT-MOCK) mice. In addition, the *STAT1* KO-RSV mice showed induction of mucus production and expression of gob-5 and Muc5ac, conditions not present in any of the other 3 groups. IL-17, a cytokine that regulates Muc5ac expression, was expressed in the lungs of the *STAT1* KO-RSV mice, whereas lung levels of IL-17 were undetectable in the remaining groups. Expression of the

IL-23-specific p19 subunit was also increased in the *STAT1* KO-RSV mice but not in the WT-RSV mice.

Conclusion: These results show that *STAT1* has an important regulatory role in RSV-induced alteration of airway function. (J Allergy Clin Immunol 2005;116:550-7.)

Key words: Viral, inflammation, rodent, cytokines, mucus, IL-17, IL-23

Respiratory syncytial virus (RSV) infection is an important cause of respiratory failure in infants¹ and is the most common infection leading to wheezing in young children.² Premature birth is the greatest risk factor for severe RSV-induced bronchiolitis that results in significant respiratory compromise.³ The reasons why prematurity increases the risk for development of more severe airway disease with RSV infection are not totally understood. First, epithelial sloughing and increased mucus production in the airways of infants with severe RSV infection clearly narrow the already small luminal diameter of their bronchi.^{3,4} Second, developmental and mechanical factors in the neuromuscular apparatus might influence airway function.⁵ In this article we address immune-mediated processes as a possible third major factor in the pathogenesis of severe RSV disease. In particular, we focus on the potential role of IFN signaling. Premature infants have significantly fewer IFN- α -producing cells among PBMCs than do term infants, and the frequency of IFN- α -producing cells in both groups is lower than in adults.^{6,7} Therefore deficient IFN- α activity might be a factor in the heightened RSV-induced illness in premature infants compared with that in older children.

We recently reported that the absence of signal transducer and activator of transcription (STAT) 1-dependent IFN signaling in mice fundamentally changes the immune response to RSV infection.⁸ In wild-type (WT) mice on a BALB/c background, RSV infection results in a predominant T_H1 immune response, with high levels of IFN- γ in the lung.⁹ However, RSV infection in *STAT1* KO mice of the same genetic background elicits a mixed T_H2/T_H1 response with high levels of IL-4, IL-5, and IL-13 and even higher levels of IFN- γ than seen in the

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Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
ECP: Eosinophil cationic protein
KO: Knockout
PAS: Periodic acid–Schiff
PFU: Plaque-forming units
RSV: Respiratory syncytial virus
STAT: Signal transducer and activator of transcription
WT: Wild-type

WT mouse.⁸ Given that T_H2 cytokine responses to RSV infection have been reported in the lower airway secretions of children with RSV-induced respiratory failure,^{10,11} we hypothesized that RSV infection would increase airway obstruction, airway hyperresponsiveness (AHR), and airway epithelial mucus production in *STAT1* KO mice. Such experimental findings would suggest that a defect in IFN- α/β – and IFN- γ –induced signaling is a potential explanation for the increased risk of severe RSV-induced respiratory symptoms in premature infants.

METHODS

Mice

Pathogen-free 8-week-old female BALB/c mice were purchased from Charles Rivers Laboratories (Wilmington, Mass). *STAT1* KO mice on a BALB/c background were maintained under specific pathogen-free conditions. In caring for animals, the investigators adhered to the “Guide for the Care and Use of Laboratory Animals” prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (revised 1996).

Maintenance of RSV stock

The A2 strain of RSV was provided by Dr Robert Chanock, National Institutes of Health. Master stocks and working stocks of RSV were prepared as previously described.⁹

Mouse infection

On day 0, mice were infected with RSV or given mock-infected culture media intranasally, as previously described.⁹ In some experiments, UV light–inactivated RSV made from the same stock of live RSV used in all experiments was administered intranasally. Briefly, the mice were anesthetized with intramuscular ketamine, 40 μ g/g, and xylazine, 6 μ g/g. When held upright with the neck fully extended, the mice readily inhaled a 100- μ L inoculum of undiluted stock virus (10⁵ plaque-forming units [PFU] of RSV) placed over their nostrils with a micropipette. Pulmonary infection was confirmed in a subset of mice by means of plaque assay in HEp-2 cells, as previously described.⁹

Quantitation of IFN- γ , IL-13, IL-17, and IL-23 p19 in lung tissues

Levels of IFN- γ , IL-13, and IL-17 in lung tissues of the 4 groups of mice were measured with commercially available ELISA kits (R&D Systems, Minneapolis, Minn), according to the manufacturer’s protocols and as previously described.⁸ An RT-PCR assay used to

detect both IL-23p19 mRNA and rRNA was performed, as previously described.¹²

Methacholine challenge

Mice were anesthetized with intraperitoneal injections of pentobarbital sodium (85 mg/kg), and a tracheostomy tube was placed to bypass the potential confounding factor of virally induced nasal congestion on lung resistance. The mice were then placed in a whole-body plethysmography chamber and mechanically ventilated, and methacholine dose-response curves were obtained, as previously described.¹³

Bronchoalveolar lavage

After the methacholine challenge was completed, the mice were killed, and 600 μ L of 5% BSA in sterile saline was instilled into the endotracheal tube. Approximately 400 μ L was harvested, on which cell counts and differentials were then performed, as previously described.¹⁴

Muc5ac Western blotting

One hundred micrograms of the protein in each sample was solubilized in electrophoresis sample buffer, and electrophoresis and Western blotting were performed, as previously described.¹⁵ The polyclonal antibody HO8 (antisynthetic peptide mouse gastric mucin) was generated in chickens by using mouse Muc5ac tandem repeat peptide conjugated with keyhole limpet hemacyanin as the immunogen.¹⁶

gob-5 Western blotting

For western blotting analysis, 100 μ g of protein was loaded per lane and separated, as previously described.¹³ The mouse anti-gob-5 antibody was generously provided by Takeda Chemical Industries (Tsukuba, Japan).

Protocol for examining lung sections

Mice were killed by means of cervical dislocation on day 14, and the lung block was removed. Fixation and staining were performed by using methods previously described. Expression of mucus by periodic acid–Schiff (PAS) stain, Muc5ac, and gob-5 was evaluated on the basis of distribution and amount. The amount of mucus identified by PAS stain and Muc5ac or gob-5 staining was evaluated in main, mid, and peripheral bronchi in both lungs and graded as follows: 0, none; 1+, minimal; 2+, moderate; and 3+, maximal. Distribution of mucus was noted. The immunohistochemical analysis for gob-5 and Muc5ac was performed, as previously described. Slides were examined by one observer in a blinded fashion.

Statistical analysis

Results are expressed as means $\pm$ SEM. Histopathologic changes of the lung in different experimental groups, measurements of IL-23 p19 by means of real-time PCR, and plaque assay data were compared with a Mann-Whitney *U* test for unpaired data. Measurements of cytokine proteins, weight loss, and dose-response curves to methacholine (analysis of repeated measures) were compared by means of ANOVA. Differences were considered to be significant at a *P* value of less than .05.

RESULTS

STAT1 KO mice have increased baseline lung resistance compared with WT mice with primary RSV infection

To determine whether RSV infection caused lower airway obstruction in *STAT1* KO mice, we measured

baseline lung resistance at day 14 in RSV- and mock-infected WT and *STAT1* KO mice on a BALB/c background. We found no difference in the baseline lung resistance of the WT-MOCK (1.30 ± 0.04 cm H₂O/mL/s), WT-RSV (1.36 ± 0.03 cm H₂O/mL/s), and *STAT1* KO-MOCK (1.32 ± 0.03 cm H₂O/mL/s) groups; however, the *STAT1* KO-RSV (1.46 ± 0.05 cm H₂O/mL/s) group had significantly greater baseline lung resistance compared with the WT-MOCK ($P = .007$), WT-RSV ($P = .05$), and *STAT1* KO-MOCK ($P = .01$) groups. Therefore in RSV-infected mice the inability to signal through *STAT1* causes a significant increase in baseline lung resistance.

***STAT1* KO mice have increased AHR when compared with WT mice with primary RSV infection**

Methacholine challenges were performed on days 7 (Fig 1, A) and 14 (Fig 1, B) to determine the effect of the inability to signal through *STAT1* on RSV-induced AHR. On day 7, the *STAT1* KO-RSV group had significantly greater airway reactivity compared with that of the WT-MOCK, WT-RSV, and *STAT1* KO-MOCK groups, as well as that of *STAT1* KO and WT mice challenged intranasally with UV light-inactivated RSV ($P < .001$ for all groups). There was no difference in the results of the methacholine challenges between the WT-MOCK, WT-RSV, WT-UV-RSV, *STAT1* KO-MOCK, and *STAT1* KO-UV-RSV groups. Similar results were obtained on day 14. These results indicate that RSV infection leads to increased AHR in *STAT1* KO mice, whereas RSV infection in WT mice does not.

Eosinophils are increased in the bronchoalveolar lavage fluid of *STAT1* KO mice with RSV infection

Bronchoalveolar lavage (BAL) was performed in all 4 groups of mice (Fig E1 in the Online Repository in the online version of this article at www.mosby.com/jaci) to determine whether inflammation was associated with the increase in AHR in the *STAT1* KO-RSV mice. Total cells were significantly increased in the *STAT1* KO-RSV mice ($11.8 \pm 1.5 \times 10^5$ /mL) compared with those in the *STAT1* KO-MOCK ($1.1 \pm 0.2 \times 10^5$ /mL), WT-RSV ($2.1 \pm 0.4 \times 10^5$ /mL), and WT-MOCK ($0.9 \pm 0.3 \times 10^5$ /mL) groups ($P < .001$ for all groups). Similarly, the number of macrophages was significantly greater in the BAL fluid of the *STAT1* KO-RSV mice ($4.9 \pm 0.7 \times 10^5$ /mL) compared with that in the *STAT1* KO-MOCK ($1.1 \pm 0.2 \times 10^5$ /mL), WT-RSV ($2.0 \pm 0.4 \times 10^5$ /mL), and WT-MOCK ($0.9 \pm 0.3 \times 10^5$ /mL) groups ($P < .001$). Additionally, the *STAT1* KO-RSV mice had $5.4 \pm 0.7 \times 10^5$ /mL eosinophils in the BAL fluid, whereas there were no eosinophils detected in the BAL fluid in any other group ($P < .001$). Finally, the *STAT1* KO-RSV group had significantly greater lymphocytes ($1.4 \pm 0.2 \times 10^5$ /mL) in the BAL fluid ($P < .001$), whereas the only other group in which lymphocytes were detected in the BAL fluid was the WT-RSV mice

($0.1 \pm 0.1 \times 10^5$ /mL). Therefore the increased AHR in the *STAT1* KO-RSV mice was accompanied by a greater level of inflammation than in the other groups and uniquely included significant eosinophilia.

RSV infection in *STAT1* KO mice induces mucus production

To determine the mechanism by which RSV leads to both increased lung resistance and AHR in *STAT1* KO mice, we evaluated the lung histopathology on day 14 in each group. We found, by means of PAS staining, that there was significant (2+) mucus production in the *STAT1* KO-RSV mice compared with the almost nonexistent mucus production in the WT-MOCK, WT-RSV, and *STAT1* KO-MOCK groups (Fig E2 in the Online Repository in the online version of this article at www.mosby.com/jaci). Therefore the increased mucus production in the *STAT1* KO-RSV mice is likely an important component of the increased lung resistance and AHR present on day 14 in this group.

RSV infection in *STAT1* KO mice upregulates gob-5 expression

Mouse gob-5 (mCLCA3) is a member of the Ca²⁺-dependent chloride channel (CLCA) family (originally termed the CaCC family)¹⁷ and has a selective expression in airway goblet cells of allergic sensitized mice.¹⁸ gob-5 is an important regulator of mucus production and has been reported to modulate Muc5ac expression.^{18,19} To determine whether gob-5 expression was increased in the airways of the *STAT1* KO-RSV mice, we performed histochemical and Western blot analysis for gob-5 on day 14. We found no gob-5 expression in the WT-RSV, WT-MOCK, and *STAT1* KO-MOCK groups, whereas there was distinct gob-5 staining in the *STAT1* KO-RSV group (Fig E3 in the Online Repository in the online version of this article at www.mosby.com/jaci). This gob-5 staining in the *STAT1* KO-RSV group was principally in the epithelium of the large airways and paralleled in intensity the staining seen with PAS. To confirm these histochemical findings, we also performed Western blots on lung homogenates by using the anti-gob-5 antibody. We found gob-5 protein in the lungs of the *STAT1* KO-RSV group by means of Western blotting, but there was no gob-5 protein in the lungs of the WT-RSV group.

RSV infection in *STAT1* KO mice upregulates Muc5ac expression

To determine the gene regulation of mucus expression in the *STAT1* KO-RSV mice, we performed histochemical and Western blot analysis for Muc5ac, a mucin gene product that regulates mucus production in the lung.^{20,21} on day 14. Muc5ac is the murine counterpart to the human gene, and the MUC5AC gene product is a major component of respiratory secretions.^{22,23} We found no Muc5ac expression in the WT-RSV, WT-MOCK, and *STAT1* KO-MOCK groups, whereas there was abundant Muc5ac staining in the *STAT1* KO-RSV groups (Fig E4 in the Online Repository in the online version of this article at

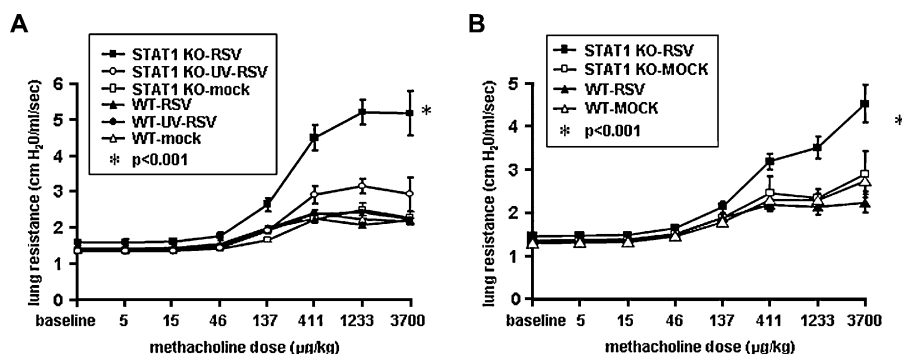


FIG 1. Airway responsiveness measured on day 7 (**A**; $n = 7$ in all groups) and day 14 (**B**; $n = 10$ in the RSV-infected groups and $n = 7$ in the mock-infected groups). * $P < .001$.

www.mosby.com/jaci). This Muc5ac staining in the *STAT1* KO-RSV group was principally in the epithelium of the large airways. To confirm these histochemical findings, we also performed Western blots on lung homogenates by using the anti-Muc5ac antibody. We found significant Muc5ac protein in the lungs of the *STAT1* KO-RSV group by means of Western blotting, but there was no Muc5ac protein in the lungs of the WT-RSV group.

Comparison of expression of lung cytokines in *STAT1* KO and WT mice

We have previously reported that IL-13 production was induced in *STAT1* KO mice with RSV infection and that the peak of expression was on day 6.⁸ In the present experiments we found that IL-13 could be detected in the lungs of *STAT1* KO-RSV mice as early as day 4 and as late as day 10 after infection, with the peak at day 8 (Fig 2, A), a time point at which cytokines were not measured in the earlier report. IL-13 could not be detected in the *STAT1* KO-MOCK, WT-MOCK, and WT-RSV groups at any time. Similarly, we previously found that lung levels of IFN- γ were greater in *STAT1* KO-RSV mice than in WT-RSV mice. In the present experiment lung levels of IFN- γ were greater on day 6 in the *STAT1* KO-RSV mice than in the WT-RSV mice and were still increased in the *STAT1* KO-RSV mice on day 8, a day when this cytokine could not be detected in the WT-RSV mice. IFN- γ was undetectable in the lungs of the *STAT1* KO-MOCK and WT-MOCK groups at any time. IL-17 has recently been reported to induce the expression of Muc5ac.²⁴ Because we saw an induction of Muc5ac in the *STAT1* KO-RSV group, we hypothesized that IL-17 would be increased in this group of mice when compared with the WT-RSV, WT-MOCK, and *STAT1* KO-MOCK groups. Fig 2, A, confirms a significant induction of IL-17 in the lung supernatants of the *STAT1* KO-RSV group, whereas levels of IL-17 in the WT-RSV, WT-MOCK, and *STAT1* KO-MOCK groups were undetectable. IL-17 was first detectable at day 6 in the lungs of the *STAT1* KO-RSV mice and was present at the same level on day 8 but was not detectable at earlier or later times. Therefore RSV infection in the setting of deficient *STAT1* signaling induces

IL-17 expression. Because IL-17 production has been recently reported to be increased in response to IL-23, we measured the expression of the IL-23-specific p19 subunit in the lungs of *STAT1* KO-RSV and WT-RSV mice, as well as in uninfected *STAT1* KO and WT animals by means of quantitative real-time PCR (Fig 2, B). We found that there was increased expression of the IL-23-specific p19 subunit in the lungs of noninfected WT mice compared with that in noninfected *STAT1* KO mice ($P < .01$); however, the IL-23-specific p19 subunit expression did not increase as a result of RSV infection. However, there was a significant increase in the IL-23-specific p19 subunit expression in the *STAT1* KO-RSV mice on days 4, 6, and 8 after infection compared with that in the noninfected *STAT1* KO mice ($P < .005$). Thus RSV infection induces the expression of IL-23 p 19 in *STAT1* KO mice but not in WT mice.

STAT1 KO mice have higher RSV titers in the lung both early and later in the course of infection

To determine whether the differences in physiologic measurements, airway mucus expression, and cytokine production might have been related to differences in viral loads between the WT and *STAT1* KO mice, we performed a time course experiment in which we measured viral titers daily between days 3 and 7 after infection (Fig E5 in the Online Repository in the online version of this article at www.mosby.com/jaci). We found that *STAT1* KO mice had significantly higher viral titers than similarly infected WT mice on day 3 (4.20 ± 0.21 vs 3.47 ± 0.11 log₁₀ PFU/g, $P < .05$), day 4 (4.84 ± 0.21 vs 3.36 ± 0.02 log₁₀ PFU/g, $P < .05$), and day 7 (4.89 ± 0.11 vs 4.24 ± 0.09 log₁₀ PFU/g, $P < .05$). There was no difference in viral titers between the RSV-infected *STAT1* KO and WT mice on days 5 and 6, with day 6 being the day of peak viral titers for both groups.

DISCUSSION

STAT1 KO mice have defective signal transduction in response to both type I (α/β) and type II IFNs.²⁵ In our

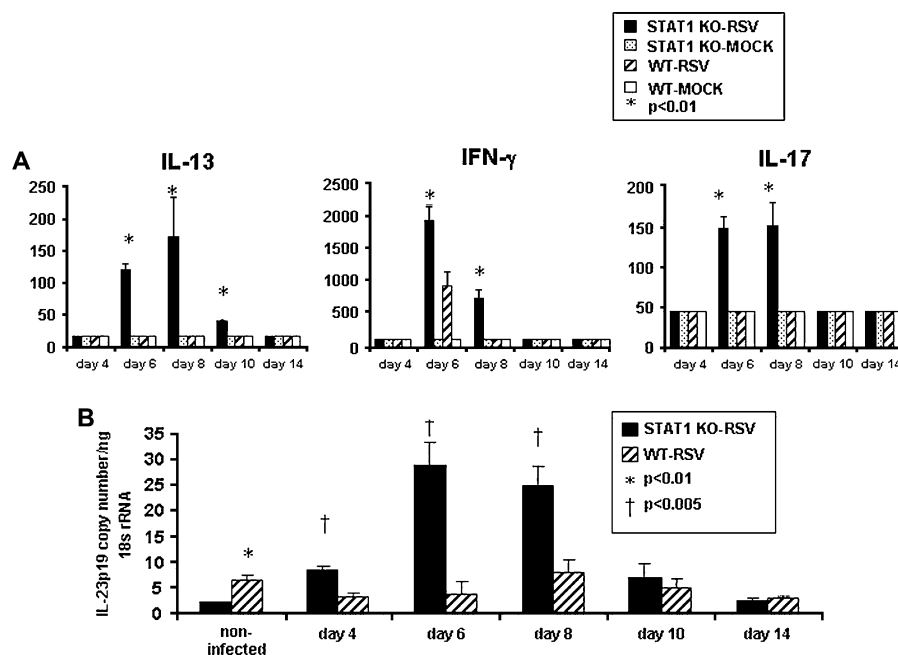


FIG 2. A, Measurements of cytokines levels in ground lung supernatants obtained at days 4, 6, 8, 10, and 14. * $P < .01$. B, Lung expression of the IL-23-specific p19 subunit in *STAT1* KO and WT mice. * $P < .01$ versus noninfected *STAT1* KO mice; † $P < .005$ versus noninfected *STAT1* KO mice.

experiments we found that 14 days after RSV infection, *STAT1* KO mice had significantly greater baseline lung resistance and AHR than WT mice, whereas RSV-infected WT mice had neither increased baseline lung resistance nor AHR compared with that seen in noninfected WT or *STAT1* KO mice. This increase in baseline lung resistance and AHR in the RSV-infected *STAT1* KO mice was associated with a significant T_H2 immune response characterized by pulmonary eosinophilia. In addition, RSV infection in *STAT1* KO mice induced mucus production by the airway epithelium that was not present in the WT-RSV mice. This production of mucus was associated with the induction of both *Muc5ac* and *gob-5*. IL-17, a cytokine recently shown to induce *Muc5ac* expression,²⁴ was increased as a result of RSV infection in the lungs of *STAT1* KO mice but not in the WT mice. It is not clear whether the observed pathology and AHR in the *STAT1* KO-RSV mice is a result of a lack of normal IFN function or, alternatively, is caused by abnormal cytokine signaling that occurs in the absence of *STAT1*.

Airway obstruction is a key component of severe RSV-induced bronchiolitis in infants and young children.⁴ The turbulent airflow that results from airway obstruction creates wheezing with respiratory effort, a common finding in these children with RSV-induced respiratory compromise.²⁶ Pathologic changes that occur with severe RSV-induced bronchiolitis include submucosal inflammation and airway obstruction by mucus and necrotic epithelial cells.⁴ Although epithelial necrosis is not seen in murine RSV infection,⁹ in our studies *STAT1* KO-RSV, but not WT-RSV, mice had the other pathophysiologic hallmarks associated with severe RSV-induced bronchi-

olitis in children. AHR was increased in the *STAT1* KO-RSV mice but not in WT-RSV mice. AHR is defined as abnormally large airflow limitation in response to a nonspecific agonist, such as methacholine, and is a hallmark of asthma. The differences in the AHR between the *STAT1* KO-RSV and WT-RSV groups could possibly be a result of more RSV in the lung in the *STAT1* KO mice compared with the WT mice because we found that peak titers in the *STAT1* KO mice were higher than in WT mice on days 3 and 4 after infection when both groups are challenged with the same inoculum (10^5 PFU). Even though the viral titers are higher in the *STAT1* KO mice, these mice have a fundamentally different inflammatory response to RSV infection compared with that seen in the WT mice. For instance, we found that a 2-log higher inoculum (10^7 PFU) did not lead to T_H2 cytokine production, mucus expression, or AHR in WT mice at days 7, 14, 21, or 28 after infection.^{13,14,27,28}

Most reports detailing that RSV causes AHR in BALB/c mice have measured AHR by using a system that measures airway resistance through the entire respiratory tract,²⁹⁻³³ which might include a nasal contribution to obstruction as a result of virally induced rhinitis. Two other groups have found that RSV results in AHR by using invasive systems,^{34,35} and we are currently working with the investigators from one of these groups to understand the reason for the difference in our results. However, other investigators have found results similar to ours. Shardonofsky and Colasurdo (personal communication, March 2005) found that methacholine challenge performed in BALB/c mice in a mechanically ventilated system did not cause AHR when RSV strain A2 was

administered at either 10^5 , 10^6 , or 10^7 PFU. Reasons for these differences might include the dose of viral inoculum administered, the source of the virus, and the supplier of the BALB/c mice used in the experiments.³⁶ We found that AHR in the *STAT1* KO-RSV mice was associated with increased IL-5 and IL-13 levels, cytokines not found in WT-RSV mice. IL-5 is a critical eosinophil growth, differentiation, and survival factor that blocks the inhibitory muscarinic M2 receptor on postganglionic parasympathetic nerves and leads to smooth muscle constriction.^{37,38} IL-13 is a critical mediator of AHR, modulating airway epithelial mucus production, influencing eosinophil migration into the lung, and perhaps acting as a direct smooth muscle spasmogen.^{39,40}

IFN- γ has been proposed as a potential mediator of RSV-induced airway dysfunction.³¹ Another group found that RSV-infected WT mice had a small but significant increase in AHR compared with either IFN- γ KO mice or mice treated with anti-IFN- γ antibodies, suggesting that IFN- γ might induce AHR in that system.³¹ Our results imply that IFN- γ might not be the sole determining factor in RSV-induced airway dysfunction because IFN- γ signaling is disrupted in *STAT1* KO mice, whereas AHR is exaggerated. Instead, our data suggest a role for the type I IFNs in inhibiting airway dysfunction. However, experiments would need to be performed in mice that specifically lack the ability to signal through IFN- α/β to definitively prove this point.

We also found that RSV infection in *STAT1* KO mice induced the production of IL-17 in the lungs, whereas IL-17 was not detectable in WT-RSV mice. IL-17 regulates MUC5AC expression *ex vivo*, whereas the T_H2 cytokines IL-4, IL-9, and IL-13 do not.²⁴ In human, monkey, and mouse tracheobronchial epithelial cells, IL-17 stimulated MUC5AC expression after 16 hours of culture.²⁴ IL-17 is produced solely by activated T lymphocytes.⁴¹ Binding of IL-17 to its receptor activates extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase pathways, resulting in upregulation of IL-6, IL-1, and nuclear factor κ B.⁴¹ The correlation of increased mucus production with augmented IL-17 levels in *STAT1* KO-RSV mice suggests a pathogenic role for this cytokine in the observed heightened mucus production. However, the possibility that an IL-17-independent pathway increases mucus production in the *STAT1* KO-RSV group has not been tested because of our inability to effectively block IL-17 activity *in vivo*. It is important to note that IL-17 levels were undetectable in the WT-RSV mice, a finding that correlated with the lack of mucus expression in these mice. In exploring the mechanism by which RSV infection increases IL-17 in *STAT1* KO mice, we found that RSV induced the expression of IL-23 in the *STAT1* KO mice but not in the WT mice. IL-23 is a novel member of the IL-12 cytokine family and is a heterodimer comprised of IL-12p40 and the IL-23-specific p19 subunit.⁴² IL-23 is produced by activated dendritic cells and promotes IL-17 secretion by T lymphocytes.^{42,43} It has similar, but not identical, activities as IL-12 in that it induces IFN- γ

production and T_H1 differentiation. At this point, we do not know the mechanism by which the inability to signal through *STAT1* induces IL-23 production. Other factors that might regulate mucus production in the *STAT1* KO-RSV mice include IL-4 and IL-13, which have also been shown to induce mucus production.^{39,44} Although we did not find that RSV infection induced airway mucus expression in WT BALB/c mice, others have reported that it does.³²⁻³⁵ The reasons are probably similar to the difference in AHR results found by our group and others.

An important question is whether the results of these experiments are relevant to human disease. Their relevance is supported by the fact that infants hospitalized with RSV-induced bronchiolitis have an immune response similar to that in RSV-infected *STAT1* KO mice.⁴⁵⁻⁴⁸ Increased eosinophils and CD4⁺ lymphocytes in the blood of infants with RSV-induced bronchiolitis correlates with the number of days of wheezing.⁴⁷ Additionally, concentrations of eosinophil cationic protein (ECP) are greater in nasopharyngeal secretions from children with RSV-induced respiratory tract infection than in children with non-RSV-induced upper respiratory tract illness.⁴⁵ High levels of ECP in nasopharyngeal secretions predict the development of bronchiolitis at the time of RSV infection, and ECP concentrations are inversely correlated with initial oxygen saturation.⁴⁶ In children hospitalized for severe bronchiolitis, increased concentrations of ECP in nasopharyngeal secretions were predictive of bronchiolitis during wheezing.⁴⁸ However, the finding of an association of a T_H2 immune response with RSV-induced bronchiolitis similar to that that we found with RSV infection in *STAT1* KO mice has not been universal.⁴⁹

Recently, 2 unrelated infants were described in whom *STAT1* alleles were mutated and in whom neither IFN- α/β nor IFN- γ activated *STAT1* containing transcription factors.⁵⁰ Recombinant IFN- α/β did not inhibit viral replication in cell lines created from both individuals. Both children died of viral infection. Thus in human subjects, as in mice, inherited impairment of the *STAT1*-dependent response to IFN- α/β has been shown to increase susceptibility to severe viral infection.

In conclusion, we have found that RSV infection in *STAT1* KO mice leads to airway resistance, AHR, mucus production (which is regulated at least in part by Muc5ac and gob-5), and induction of IL-17 protein expression. These results suggest that defects in *STAT1* signaling might explain the immune-mediated airway dysfunction in a subset of infants with severe RSV-induced bronchiolitis.

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