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# Ribavirin and cysteinyl leukotriene-1 receptor blockade as treatment for severe bronchiolitis

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**Abstract**

In this work we have evaluated the clinical responses of pneumovirus-infected mice to combination therapy with the antiviral agent, ribavirin, and the CysLT1 cysteinyl leukotriene receptor antagonist, montelukast. We observed substantial virus replication in our mouse model of pneumovirus infection and significant accumulation of cysteinyl leukotrienes in lung tissue, the latter detected at levels that correlate directly with granulocyte recruitment to the airways. While administration of the nucleoside analog, ribavirin, reduced virus replication ~2000-fold, the clinical outcomes as measured by morbidity and mortality, in response to ribavirin monotherapy were indistinguishable from those of the no-treatment controls. Similarly, montelukast therapy alone did not reduce granulocyte recruitment nor did it improve the clinical outcome. However, combined therapy with ribavirin and montelukast resulted in a significant reduction in morbidity and a substantial reduction in mortality (50% survival at $t = 14$ days and onward, compared to 10–20% survival in response to montelukast alone or to ribavirin alone, respectively, $p < 0.01$). These findings define further the independent contributions made by virus replication and by the ensuing inflammatory response to the detrimental sequelae of pneumovirus infection in vivo.


*Keywords:* Pneumovirus; Chemokine; Montelukast; Ribavirin; Mice

## 1. Introduction

Cysteinyl-leukotrienes (leukotrienes $C_4$, $D_4$ and $E_4$) are leukocyte-derived lipid proinflammatory mediators that make prominent contributions to the pathophysiology of bronchial asthma (Bigby, 2000; McMillan, 2001). Among these contributions, cysteinyl leukotrienes promote bronchospasm, wheezing and enhanced eosinophil recruitment in response to allergen provocation. Leukotriene antagonists, which function as selective, competitive inhibitors of these leukotrienes at serpentine G protein coupled CysLT1 receptors found in bronchial smooth muscle, lung macrophages, and peripheral blood cells, provide significant clinical benefit (James and Sampson, 2001) and are already approved for clinical use in the management of this disease.

The clinical similarities between acute episodes of bronchial asthma and symptoms associated with primary respiratory syncytial virus infection (RSV; family *Paramyxoviridae*, subfamily pneumovirus) suggested the possibility of pathophysiologic and biochemical similarities. Several groups have reported on cysteinyl-leukotriene synthesis and release and its association with RSV infection and its related symptomatology (Volovitz et al., 1988; VanSchaik et al., 1999; Behera et al., 1998), and a recent randomized clinical trial suggested that leukotriene antagonist therapy provided after emergence of primary RSV symptoms could result in significant reduction in reactive airways disease developing secondary to severe infection in infants (Bisgaard, 2003).

As part of an overall study of the pathogenesis of pneumovirus infection in vivo, we have developed a model of respiratory virus infection in mice that replicates the signs and symptoms of the most severe forms of RSV infection in human infants (Domachowske et al., 2000a, 2000b; Bonville et al., 2003). The natural rodent pathogen, pneumonia virus of mice (PVM; also family *Paramyxoviridae*, subfamily pneumovirus) is among the closest known phylogenetic relatives of RSV, but, unlike RSV when utilized in mouse models, PVM can replicate effectively in mouse lung tissue and infection results in significant morbid-

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ity and mortality. Similar to severe RSV infection in humans, PVM infection in mice is accompanied by a profound, acute inflammatory response, which includes a prominent pulmonary eosinophilia. We have identified the CC-chemokine MIP-1α (CCL3) as the crucial mediator of the antiviral inflammatory response to PVM (Domachowske et al., 2000b), analogous to what has been inferred for RSV infection in human infants (Harrison et al., 1999; Garofalo et al., 2001).

Using the PVM-infection model, we have recently reported on the clinical utility of combined antiviral and specific anti-MIP-1α immunomodulatory therapy, findings that underscore the independent contributions of both virus replication and the inflammatory response to the pathogenesis of this respiratory virus infection in vivo (Bonville et al., 2003, 2004). Here we present our findings on the responses of PVM-infected mice to antiviral therapy when it is administered in conjunction with cysteinyl-leukotriene receptor blockade. Our results suggest a role for cysteinyl-leukotrienes in promoting the detrimental sequelae of this infection, and as a target for development of novel rational therapies.

## 2. Materials and methods

### 2.1. Mouse and virus stocks

C57Black/6 mice were obtained from Taconic Laboratories, Germantown, NY. Mouse-passaged stocks of PVM (strain J3666, $\sim 10^6$ pfu/ml, original stock virus obtained from Dr. A.J. Easton, University of Warwick, Coventry, U.K.) were obtained from clarified mouse lung homogenates as described previously (Domachowske et al., 2000a, 2000b) and stored in liquid nitrogen. Virus stocks were defrosted and diluted in phosphate buffered saline (PBS) immediately prior to intranasal inoculation.

### 2.2. Establishing PVM infections in mice and treatment interventions

Six- to 8-week-old mice were used in all experiments. Mice subjected to brief isoflurane anesthesia were inoculated intranasally with 60 plaque-forming units (pfu) of mouse-passaged PVM strain J3666 in a 50 ul volume of PBS at day 0. This viral inoculum was chosen because it produces moderate to severe clinically symptoms in 100% of infected mice, and at least 80% mortality if therapeutic intervention is not initiated. Animals were weighed and observed daily. Clinical scoring of infected mice was as initially devised by Cook et al. (1998) with modifications as previously described (Bonville et al., 2003). This clinical scoring system is based on observed symptoms using a scale from 1 to 6: 1, healthy; 2, ruffled fur at neck; 3, piloerection and difficulty breathing, less alert; 4, lethargic with labored breathing; 5, pre-morbid, with emaciation and cyanosis; 6, death. Mice were sacrificed on days 0 through 7 post-inoculation for bronchoalveolar lavage fluid analysis and total lung chemokine and cysteinyl leukotriene concentrations. Lung PVM titers were determined from total lung homogenates obtained on days 0, 3, 5, and 7. Experiments used to collect clinical scores and mortality included four groups of 10 mice each. To collect virologic, biochemical and histologic data, identical experiments were performed using 60 additional mice. Six mice were sacrificed on day 0, then six mice in each treatment arm were sacrificed on days 3, 5 and 7 for viral lung titers, chemokine (MIP-1α and MCP-1) concentrations, and cysteinyl leukotriene concentrations. Using appropriate diluent controls, Group 1 received daily intraperitoneal montelukast (10 mg/kg; generously provided by Merck & Co., Inc.) and twice daily doses of intraperitoneal ribavirin (37.5 mg/kg/dose; ICN Pharmaceuticals), group 2 received montelukast, group 3 received twice daily ribavirin and group 4 received diluent injections only. For all experiments, treatments were initiated on day 3 post-infection and continued until day 14. All procedures were reviewed and approved by the Committee on the Humane Use of Animals, SUNY Upstate Medical University.

### 2.3. Bronchoalveolar lavage, differential cell counts and pulmonary histology

At time points indicated, bronchoalveolar lavage (BAL) fluid was harvested from six mice by trans-tracheal instillation and removal of pre-chilled phosphate-buffered saline with 0.25% bovine serum albumin (BSA; 2 × 0.80 ml instillation with recovery of 1.2–1.5 ml per mouse). Total and differential leukocyte counts were obtained by light microscopic quantitative analysis of methanol-fixed cytospin preparations stained with Diff Quik (Fisher Scientific, Pittsburgh, PA). For histologic evaluation, lungs were inflated with 10% formalin, dissected en bloc, set in paraffin, and sectioned onto glass slides.

### 2.4. Lung homogenates, chemokine, cysteinyl leukotriene and plaque assays

Mice were sacrificed as described above (six mice per condition per time point) and lungs were removed and transferred into 1 ml pre-chilled Iscove's Modified Dulbecco's Medium. Lung tissue suspensions were subjected to blade homogenization (Tissumizer, Tekmar, Cincinnati OH) and cellular debris was removed by low speed centrifugation (500 × *g* at 4 °C). Clarified supernatants were flash frozen in a dry ice and ethanol slurry and stored at −80 °C or liquid nitrogen prior to analysis. Assays for mouse MIP-1α, mouse MCP-1, and cysteinyl leukotrienes were performed as per the manufacturer's instructions (R&D Systems) and results were corrected for total protein as determined by the Bradford colorimetric assay using bovine serum albumin standards. Viral recovery was determined by standard plaque assay on the BS-C-1 epithelial cell line (American Type Culture Collection, Manassas VA).

### 2.5. Statistical analysis

Data points represent the average ± S.E.M. of samples from six mice in two or more separate trials. Fisher's exact test was employed for categorical (clinical) data. Pearson's correlations were performed for paired sets of continuous data. One-tailed *t*-tests were used to compare continuous data. Kaplan Meier

analyses were performed using Statistica Software (StatSoft, Tulsa, OK), all other statistics were per the algorithms of the Microsoft Excel data analysis program.

## 3. Results

### 3.1. PVM induced cellular, chemokine and leukotriene responses

Table 1 documents the cellular inflammatory responses seen during acute, severe PVM infection for each of the four treatment groups. The results shown here are consistent with those reported previously (Domachowske et al., 2000a, 2000b). The inflammatory response to acute infection with PVM is virtually 100% granulocytic, with eosinophils representing 10–15% of the total leukocyte count at the earliest time points. No differences were noted among the treatment groups in the magnitude or composition of cellular influx, or in the patterns of increased leukotriene concentrations. A correlation between cysteinyl leukotriene concentration (pg/ml) and total granulocyte count (cells/ml) was observed ($r^2 = 0.70$; Fig. 1A) together with a similar correlation between cysteinyl leukotriene concentration and BAL eosinophils ($r^2 = 0.74$; Fig. 1B).

### 3.2. Ribavirin treatment inhibits PVM replication in mouse lung

Table 2 documents virus titers on days 0–7 determined from total lung homogenates obtained from mice inoculated on day 0 with 60 pfu PVM. In the absence of ribavirin, pulmonary virus titers increased to $1.3 \pm 0.7 \times 10^8$ pfu/g lung tissue by day 7. Twice daily ribavirin administration decreased lung virus titers nearly 2000-fold, to $6.8 \pm 1.8 \times 10^4$ pfu/g lung tissue when used alone ($^*p < 0.01$ compared to PBS or montelukast only con-

Table 1
Total and differential leukocyte counts determined in bronchoalveolar lavage fluid, and leukotriene concentrations in lung homogenates from mice inoculated with 60 pfu of PVM on day 0

| Day | Total cells ($\times 10^3$/ml) | Neutrophils ($\times 10^3$/ml) | Eosinophils ($\times 10^3$/ml) | Lymphocytes ($\times 10^3$/ml) | Leukotriene (pg/ml) |
|---|---|---|---|---|---|
| PBS | | | | | |
| 0 | <1 | <1 | <1 | <1 | 94 ± 4 |
| 1 | <1 | <1 | <1 | <1 | 90 ± 6 |
| 2 | 58 ± 6 | 46 ± 8 | 12 ± 4 | <1 | 117 ± 6[a] |
| 3 | 660 ± 190 | 560 ± 230 | 97 ± 32 | <1 | 200 ± 19[a] |
| 4 | 1580 ± 290 | 1440 ± 340 | 143 ± 40 | <1 | 243 ± 18[a] |
| 5 | 1390 ± 390 | 1280 ± 420 | 111 ± 28 | <1 | 218 ± 21[a] |
| 6 | 1350 ± 310 | 950 ± 380 | 56 ± 24 | 340 ± 130 | 186 ± 9[a,b] |
| 7 | 1190 ± 270 | 850 ± 300 | 7 ± 5 | 327 ± 103 | 173 ± 17[a,b] |
| Montelukast only | | | | | |
| 0 | <1 | <1 | <1 | <1 | 65 ± 29 |
| 1 | <1 | <1 | <1 | <1 | 82 ± 14 |
| 2 | 36 ± 9 | 26 ± 18 | 10 ± 5 | <1 | 98 ± 12 |
| 3 | 714 ± 140 | 630 ± 230 | 84 ± 28 | <1 | 242 ± 36[a] |
| 4 | 1648 ± 330 | 1510 ± 290 | 138 ± 38 | <1 | 245 ± 27[a] |
| 5 | 1652 ± 400 | 1540 ± 510 | 112 ± 23 | <1 | 184 ± 30[a] |
| 6 | 1325 ± 270 | 880 ± 250 | 45 ± 18 | 400 ± 140 | 207 ± 18[a] |
| 7 | 1054 ± 360 | 690 ± 270 | 4 ± 2 | 360 ± 90 | 211 ± 36[a,b] |
| Ribavirin only | | | | | |
| 0 | <1 | <1 | <1 | <1 | 88 ± 9 |
| 1 | <1 | <1 | <1 | <1 | 93 ± 7 |
| 2 | 22 ± 3 | 16 ± 9 | 6 ± 2 | <1 | 124 ± 14 |
| 3 | 540 ± 220 | 490 ± 230 | 47 ± 10 | <1 | 188 ± 16[a] |
| 4 | 1530 ± 270 | 1400 ± 310 | 130 ± 30 | <1 | 200 ± 41[a] |
| 5 | 1280 ± 420 | 1170 ± 510 | 111 ± 28 | <1 | 238 ± 33[a] |
| 6 | 850 ± 510 | 500 ± 280 | 42 ± 18 | 310 ± 100 | 172 ± 35[a] |
| 7 | 910 ± 470 | 620 ± 280 | 3 | 290 ± 88 | 189 ± 48[a,b] |
| Ribavirin and montelukast | | | | | |
| 0 | <1 | <1 | <1 | <1 | 90 ± 8 |
| 1 | <1 | <1 | <1 | <1 | 92 ± 4 |
| 2 | 12 ± 2 | 10 ± 2 | 2 ± 2 | <1 | 111 ± 12 |
| 3 | 430 ± 150 | 380 ± 140 | 54 ± 14 | <1 | 208 ± 14[a] |
| 4 | 1370 ± 340 | 1230 ± 320 | 142 ± 20 | <1 | 252 ± 40[a] |
| 5 | 1250 ± 480 | 1120 ± 500 | 130 ± 40 | <1 | 230 ± 22[a] |
| 6 | 900 ± 88 | 560 ± 120 | 62 ± 38 | 275 ± 68 | 179 ± 16[a] |
| 7 | 750 ± 310 | 430 ± 350 | <1 | 315 ± 90 | 185 ± 9[a] |

Data are expressed as the mean ± S.E. from $n = 6$ mice.
[a] $p < 0.01$ compared to day 0.
[b] Concentrations from $n = 2$ mice only.

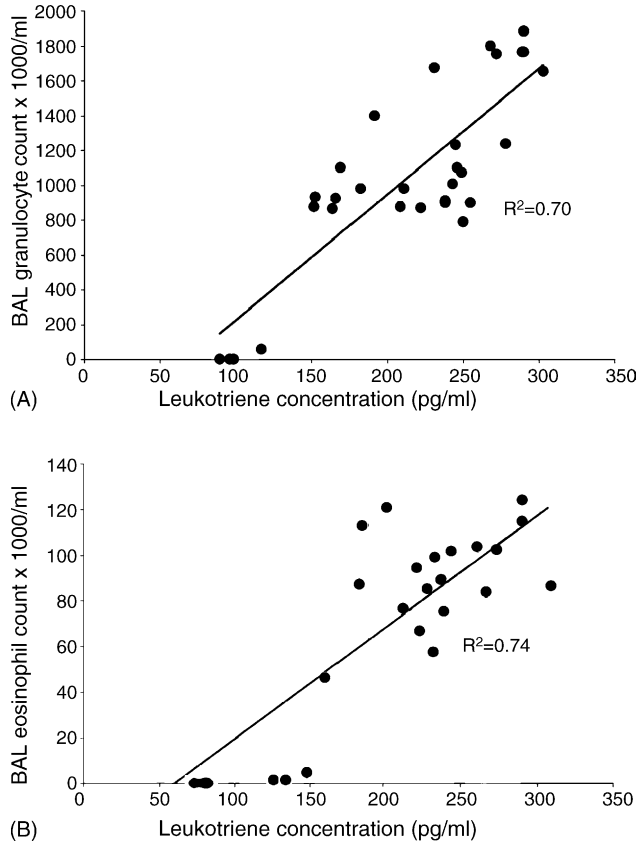


Fig. 1. Bivariate scattergram of cysteinyl leukotriene concentrations (pg/ml) vs. bronchoalveolar lavage (BAL) fluid (A) granulocyte counts (cells/ml) and (B) eosinophil counts. Regression lines with Pearson coefficients are indicated.

trols) and to $8.2 \pm 2.4 \times 10^4$ ($^*p < 0.01$ compared to PBS or montelukast only controls) when used in combination with montelukast. We conclude that montelukast alone has no effect on virus replication.

### 3.3. Ribavirin treatment reduces virus-induced pulmonary chemokine release

Concentrations of the proinflammatory chemokines MIP-1α and MCP-1 detected in PVM-infected mouse lungs over time are documented in Table 3. When compared to PBS-treated mice, mice treated with ribavirin had lower mean pulmonary MIP-1α and MCP-1 concentrations on day 7 post-inoculation (both two-fold reductions, $^*p < 0.01$). This is also consistent with previous findings (Bonville et al., 2003), and can be contrasted to the ~2000-fold drop in lung virus titer. Mice that received both ribavirin and montelukast had the lowest overall mean pulmonary MIP-1α and MCP-1 concentrations ($24 \pm 4.5$ pg/ml mg and $160 \pm 70$ pg/ml mg lung protein on day 7 post-infection, respectively) despite the observation that montelukast alone had no inhibitory effect on the production of either chemokine. None of the intervention combinations tested had a measurable effect on total lung cysteinyl leukotriene concentrations at the time points tested (Table 1).

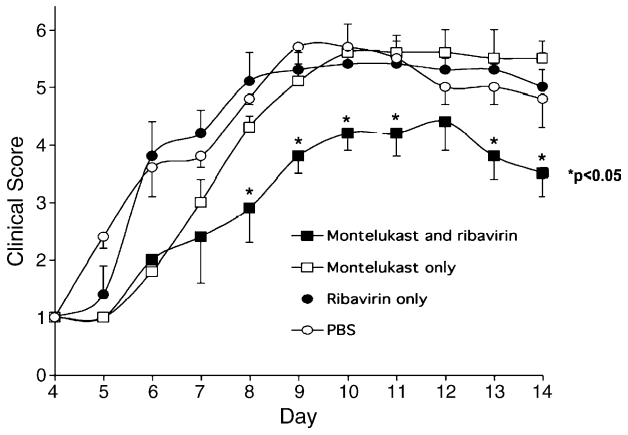


Fig. 2. Mean clinical scores of mice ($n = 6$ per point) infected with pneumonia virus of mice (PVM, strain J3666) on day 0 and treated with twice-daily intraperitoneal ribavirin (37.5 mg/kg/dose × 2 doses/day, filled circles), once daily montelukast (10 mg/kg, open squares), both ribavirin and montelukast (filled squares) or diluent control (PBS, open circles) beginning on day 3. Error bars indicate ± standard error of the mean (S.E.). Clinical scores in the ribavirin plus montelukast treatment arm were lower compared to each of the other three treatment arms on day 8 and thereafter ($p < 0.05$), except for the PBS arm on day 12 ($p = 0.13$ compared to montelukast plus ribavirin).

### 3.4. Co-administration of montelukast and ribavirin results in reduced severity of clinical symptoms and improved long term survival

Shown in Fig. 2 are the mean clinical scores from two separate experiments that included 10 mice in each treatment arm (drugs and doses as per Table 3). While clinical scores were similar in mice receiving no therapy, montelukast alone, or ribavirin alone, mice treated with both ribavirin and montelukast had overall a much less severe clinical course, statistically significant by day 8 and thereafter ($^*p < 0.05$ as shown). Percent

Table 2
Virus titers (pfu/g lung tissue) from mice ($n = 6$ per point) inoculated on day 0 with 60 pfu of PVM strain J3666

| Day | PBS | Montelukast only | Ribavirin only | Fold reduction | Montelukast and ribavirin | Fold reduction |
|---|---|---|---|---|---|---|
| Virus titer ($\times 10^4$ pfu/g lung tissue) | | | | | | |
| 0 | ND | ND | ND | – | ND | – |
| 3 | ND | ND | ND | – | ND | – |
| 5 | $900 \pm 200$ | $1000 \pm 70$ | $1.2 \pm 0.3$[a] | 750 | $2.1 \pm 0.3$[a] | 430 |
| 7 | $13000 \pm 700$ | $16000 \pm 1200$ | $6.8 \pm 1.8$[a] | 1900 | $8.2 \pm 2.4$[a] | 1600 |

Montelukast (10 mg/kg) was administered once daily; ribavirin (37.5 mg/kg/dose), twice daily. All treatments were initiated on day 3 post-inoculation. Data are expressed as the mean ± S.E.

[a] $p < 0.01$ compared to PBS-treated control, fold reduction calculated vs. PBS-treated control. ND is not detected.

Table 3
Detection of proinflammatory chemokines MIP-1α and MCP-1 in lung tissue homogenates from mice ($n = 6$ per point) inoculated on day 0 with 60 pfu of PVM strain J3666

| Day | PBS | Montelukast only | Ribavirin only | Fold reduction | Montelukast and ribavirin | Fold reduction |
|---|---|---|---|---|---|---|
| MIP-1α (pg/ml mg lung protein) | | | | | | |
| 0 | 0 | 0 | 0 | – | 0 | – |
| 3 | 14 ± 3 | 10 ± 0.4 | 8 ± 0.8 | – | 12 ± 8 | – |
| 5 | 79 ± 28 | 87 ± 36 | 73 ± 11 | – | 26 ± 7[a,b] | 3 |
| 7 | 191 ± 16 | 239 ± 21 | 98 ± 21[a] | 2 | 24 ± 4[a,b] | 8 |
| MCP-1 (pg/ml mg lung protein) | | | | | | |
| 0 | 47 ± 9 | 54 ± 6 | 39 ± 11 | – | 37 ± 5 | – |
| 3 | 108 ± 19 | 97 ± 6 | 92 ± 13 | – | 54 ± 6[a] | 2 |
| 5 | 1250 ± 200 | 1610 ± 320 | 255 ± 31[a] | 5 | 89 ± 18[a,b] | 14 |
| 7 | 1750 ± 45 | 2080 ± 300 | 458 ± 27[a] | 2 | 160 ± 70[a,b] | 11 |

Montelukast (10 mg/kg) was administered once daily; ribavirin (37.5 mg/kg/dose), twice daily. All treatments were initiated on day 3, and continued until day 14 post-inoculation. NA, is not assayed. Data expressed ± S.E.M. from six mice at each time point from two separate experiments.
[a] $p < 0.01$ compared to PBS-treated controls.
[b] $p < 0.01$ compared to the ribavirin only group.

mean weight loss in the control arm was 11 ± 3% original body weight, 9 ± 4% in the ribavirin alone group, 12 ± 5% in the montelukast alone group, while only 2 ± 1% in the ribavirin plus montelukast group ($^{*}p < 0.01$). Results from a separate survival study ($n = 10$ per treatment arm) shown in Fig. 3 demonstrated 10% survival in the montelukast alone group, 20% survival in the control and ribavirin only groups, with 50% long-term survival in the montelukast and ribavirin group, statistically significant at $^{*}p < 0.01$ when compared to each of the other groups. Observed pulmonary histology on day 5 is shown in Fig. 4. Compared to uninfected lungs (Fig. 4A), untreated, PVM-infected lungs (Fig. 4B) show intense granulocytic inflammation. The granulocytic response characteristic of PVM infection continues to be observed when both ribavirin and montelukast are administered as therapeutics (Fig. 4C).

## 4. Discussion

We have shown that during acute, severe PVM infection, pulmonary cysteinyl leukotriene concentrations increased two-fold in direct correlation with absolute bronchoalveolar lavage fluid granulocyte counts. These results are analogous to those obtained by VanSchaik et al. (1999), who reported a substantial increase in cysteinyl leukotriene concentrations in respiratory secretions of infants and children infected with RSV. As cysteinyl-leukotrienes are potent pro-inflammatory medi-

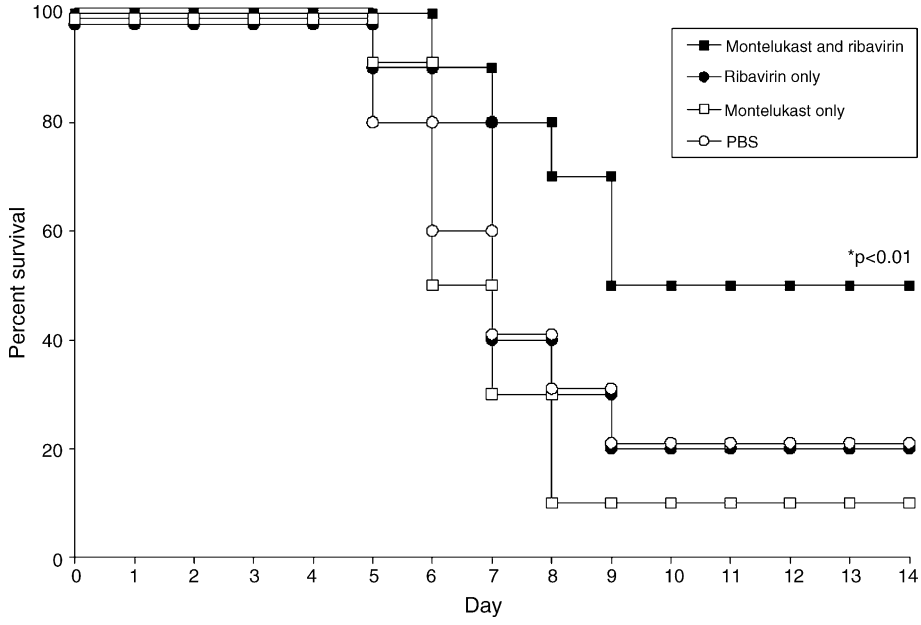


Fig. 3. Survival analysis of mice inoculated with 60 pfu PVM on day 0, and treated with ribavirin (37.5 mg/kg/dose × 2 doses/day, filled circles), montelukast (10 mg/kg, open squares), both ribavirin and montelukast (filled squares) or diluent control alone (same volumes, open circles) beginning on day 3; $n = 10$ mice per group. Significantly improved survival of combined ribavirin and montelukast-treated mice ($^{*}p < 0.01$) was observed when compared independently to each of the other three groups.

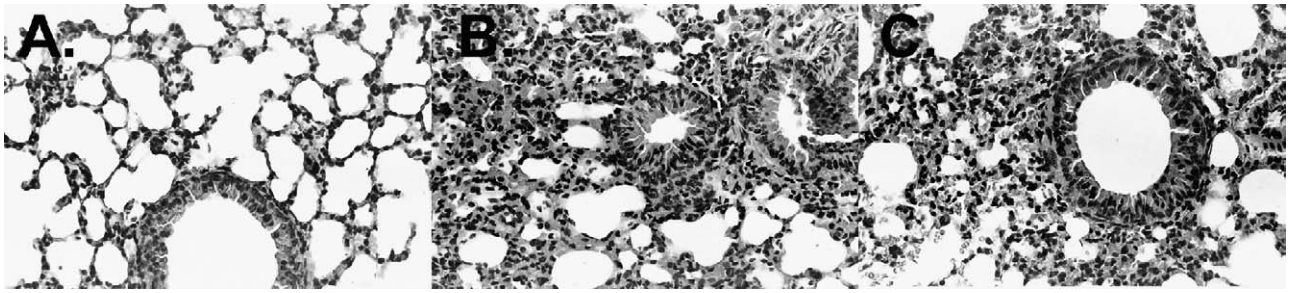


Fig. 4. Microscopic pulmonary anatomy in (A) uninfected lung, (B) PVM-infected lung (untreated), and (C) PVM-infected lung (treated with both montelukast and ribavirin). Original magnification ×400, stained with hematoxalin and eosin.

ators known to cause bronchial obstruction, mucosal edema, eosinophil recruitment, and increased bronchial hyperresponsiveness, they represent a rational target for the treatment of acute viral bronchiolitis.

While administration of montelukast or ribavirin alone resulted in no clinical benefit in our mouse model of acute, severe viral bronchiolitis, the co-administration of ribavirin and montelukast resulted in an overall decrease in symptom severity and a reduction in mortality. These results are consistent with our earlier studies (Bonville et al., 2003; Bonville et al., 2004) in which we documented the reduction in morbidity and mortality in response to co-administration of ribavirin and MIP-1α signaling blockade with one major difference. MIP-1α blockade consistently and reliably blocks the cellular inflammation seen during the acute phase of PVM infection, while administration of montelukast (with or without ribavirin) does not have any measurable effect on granulocyte recruitment to the infected lung. In contrast, when MIP-1α gene-deleted mice are challenged with PVM, they do not develop a granulocytic response (Domachowske et al., 2000). Similarly, we demonstrated that granulocytic influx is blocked when pharmacologic (Bonville et al., 2003) or immunologic (Bonville et al., 2004) blockade of MIP-1α is utilized during PVM infection. Despite the lack of granulocytic inflammation in these contexts, the PVM-infected mice develop the same degree of morbidity and mortality as the untreated controls unless ribavirin is co-administered. In our current study, we showed that combination therapy with montelukast and ribavirin affords similar benefits in terms of morbidity and mortality despite the observation that montelukast does not interfere with PVM-associated recruitment of granulocytes. As such, we were not surprised to find that when we treated MIP-1α gene-deleted mice with both montelukast and ribavirin, we did not observe any improvement in clinical outcome measures that were superior to those determined from administration of only ribavirin to MIP-1α deficient mice (Domachowske et al., unpublished data). Since MIP-1α deficient mice lack a cellular inflammatory response to PVM, eosinophils, the major source of the cysteinyl leukotrienes, are not recruited to the lung during infection. In the absence of eosinophil recruitment, montelukast treatment becomes unnecessary. These results define the independent contributions of virus replication and the ensuing inflammatory response to the pathogenesis of respiratory virus infection in vivo, and similarly, they explain why ribavirin therapy alone, although clearly effective at reducing virus replication, is of limited clinical benefit overall in the pathogenesis of RSV infection in infants.

The hypothesis that pneumovirus pathogenesis requires both active replication of virus, and virus-induced inflammatory responses, follows from the results presented. Effective therapeutics for severe forms of infection will logically require blocking virus replication and interfering with virus-induced inflammatory responses. Combination therapy with ribavirin and monoclonal anti-RSV F antibody in the cotton rat model of human RSV infection resulted in clearance of virus within 24 h, but had no effect on early pulmonary pathology. Treatment with the glucocorticoid, triamcinalone, reduced virus-associated inflammation, but delayed virus clearance. When the two interventions were combined, an antiviral and anti-inflammatory effect was appreciated (Prince et al., 2002).

When we tested the potential anti-inflammatory properties of glucocorticoids in the PVM model, we noted that the hydrocortisone-treated group exhibited elevated rates of viral replication in the lung tissue and accelerated mortality, suggesting that hydrocortisone suppresses elements crucial to the host defense against the viral infection (Domachowske et al., 2001). Patients that develop moderate to severe RSV bronchiolitis in infancy are prone to the development of recurrent wheezing and asthma in childhood (Pullan and Hey, 1982; Carlsen et al., 1987; Long et al., 1995; Folkerts et al., 1998). The mechanism(s) responsible for this phenomenon are unknown, and many have speculated as to whether RSV bronchiolitis actually induces reactive airways disease or whether underlying and as-yet-undefined host factors predispose some infants to the development of both conditions. In an earlier publication we presented the possibility that asthma and reactive airways disease represent dysregulation of what were designed as essential, beneficial host responses to respiratory virus replication in lung tissue. Whatever causes this dysregulation to be established—whether it is infection with a specific respiratory virus at a specific developmental stage in a specifically genetically and/or environmentally susceptible individual or all or none of the above—it is intriguing to consider the possibility that the inflammatory responses characteristic of the asthmatic state represent the dysregulation of the responses designed to promote innate antiviral host defense.

In addition, we note that the levels of pro-inflammatory chemokine concentrations (MIP-1α and MCP-1) present in PVM-infected mice were reduced substantially by co-

administration of montelukast and ribavirin. Although there is no immediate explanation for this observation, we suggest that this relates in some way to the as yet incompletely characterized immunomodulatory properties of ribavirin.

As alluded to earlier, results from the first published randomized trial of montelukast treatment in the setting of post RSV bronchiolitis wheezing support a role for leukotriene receptor modifiers during the subacute and convalescent phases of RSV disease (Bisgaard, 2003). The infants that received montelukast were free of any respiratory symptoms 22% of the days and nights during the follow-up period, while the infants that received placebo were symptom free only 4% of the time. The study design aimed to evaluate the potential benefit of montelukast following RSV bronchiolitis rather than on the acute infection itself. Treatment was delayed by a median of 3 days from hospitalization and up to 7 days from the onset of symptoms, but it is important to note that all of the patients continued to have acute symptoms until day 13. The authors suggest that montelukast treatment may have offered additional benefit if started earlier in the course of inflammation. While this study in infants supports a role for montelukast therapy following RSV bronchiolitis, the present results from our mouse model of severe bronchiolitis support a dual role for montelukast and ribavirin during the acute phase of severe infection. Our current PVM model does not allow study of the effects of montelukast during convalescence because without dual therapy with montelukast and ribavirin, the infection is nearly always fatal. Our recent efforts to develop a non-lethal PVM challenge model, and to incorporate whole body plethysmography into outcome measures appear promising (Bonville et al., 2005, unpublished observation).

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