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Persistent Airway Obstruction After Virus Infection Is Not Associated With Airway Inflammation*

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Background: This study examined the contribution of airway inflammation to the delayed lung function recovery that occurs in some people following virus-induced asthma exacerbations.

Methods: Subjects (n = 40) were recruited at hospital admission for acute asthma exacerbation. Respiratory virus infection was diagnosed by viral nucleic acid detection and/or cell culture, using induced sputum, nasal, or throat swabs. Data collected included lung function, answers to common cold and asthma control questionnaires, and induced sputum cellular profiles. Subjects were reexamined 4 to 6 weeks postexacerbation and were compared with stable asthmatic subjects (n = 26) who had been recruited from ambulatory care clinics.

Results: Persistent airway obstruction, defined as lung function improvement at follow-up (ie, change in FEV₁ percent predicted [Å%FEV₁]) of <15%, was observed in 10 subjects (25%). Airway recovery (Å%FEV₁, ≥ 15%) was observed in the remaining subjects (30 subjects; 75%). During the acute episode, the airway-recovery group had increased total cell count (p = 0.019), increased number of neutrophils (p = 0.005), and increased percentage of neutrophils (p = 0.0043) compared to the group of stable subjects with asthma. Postexacerbation, the airway-recovery group had reduced numbers of neutrophils and an increased percentage of eosinophils. In contrast, during exacerbation, subjects with persistent airway obstruction showed no differences in inflammatory cell counts compared to stable subjects with asthma, nor did cell counts change postexacerbation. Symptoms improved in both groups postexacerbation. However, in the persistent-airway-obstruction group, asthma remained uncontrolled.

Conclusion: Persistent airway obstruction and uncontrolled asthma are observed in some people after viral asthma exacerbations. These abnormalities are not associated with inflammatory cell influx into the airway lining fluid during the exacerbation and may reflect the involvement of noncellular elements. Further work should explore other mechanisms leading to incomplete airway recovery.

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Key words: airway inflammation; airway obstruction; asthma exacerbation; virus

Severe exacerbations of asthma are responsible for a large burden of illness in Australia and throughout the world. Viral infection is the main cause of asthma exacerbation requiring hospitalization, with respiratory viruses being isolated from 81% of patients with asthma exacerbations requiring hospitalization in adults and children. The mechanisms driving viral asthma exacerbations are clearly different from the well-characterized pathogenesis of allergen-induced asthma, which is driven by an interleukin (IL)-5-mediated eosinophil infiltrate. Viral asthma exacerbations involve a marked neutrophil infiltration together with eosinophil degranulation. The chemokine RANTES (or regulated on activation, normal T cell expressed and secreted), which promotes eosinophil degranulation, and the
cytokine IL-10, which suppresses eosinophil cellular infiltration, may be important in this inflammatory response, as the gene expression of these mediators is up-regulated in patients with virus-induced asthma.11

Despite optimal medical therapy, incomplete recovery of airway function occurs in a relatively high proportion of people after an acute asthma exacerbation. Corne et al12 observed that in people with asthma who were infected with human rhinovirus (RV), lower respiratory tract symptoms were experienced more often, were more severe, and were of longer duration than in nonasthmatic people. Persistent lower respiratory symptoms in people with asthma extended up to 35 days, which is well beyond the 7-day expected duration of common cold symptoms. Chang et al13 described a group with prolonged episodes of persistent asthma that lasted months to years and was triggered by symptoms of viral infection in 89% of cases. Incomplete recovery of peak expiratory flow (PEF) has also been reported after acute episodes of asthma.14 In another study,15 there was a trend for asthmatic subjects with human RV infection to have lower PEF measurements during convalescence. Delayed recovery of lung function has also been reported following COPD exacerbations, with 25% of subjects not recovering to baseline PEF levels after 5 weeks.16,17 Thus, persistent airway abnormality after respiratory exacerbation is a well-documented clinical problem, occurring both in patients with asthma and with COPD.

In addition to the delayed recovery of lung function after acute asthma episodes, the inflammatory response has been shown to persist. Pizzichini et al10 studied adults who had experienced asthma exacerbations triggered by viral infection (n = 6) vs those triggered by nonviral causes (n = 2). They observed a persistent reduction in FEV1 (73% vs 93% predicted, respectively) and persistent sputum neutrophilia (53% vs 20% of cells, respectively) at follow-up in the virus-infected group. El-Radhi et al18 reported the persistent elevation of serum IL-5, soluble CD25, and eosinophilic cationic protein (ECP) after oral corticosteroid treatment for acute asthma in children.

We hypothesize that the delayed recovery of lung function after acute asthma episodes may be due to an altered inflammatory response. The aim of this study was to elucidate the role of airway inflammation in the persistence of airway obstruction that occurs in some people following virus-induced asthma exacerbations. We have carefully characterized subjects whose condition does not improve following a virus-induced asthma exacerbation, comparing their airway inflammatory profile to that of subjects who do improve postexacerbation.

Materials and Methods

Subjects

Patients admitted to John Hunter Hospital (Newcastle, NSW, Australia) who were experiencing an acute exacerbation of asthma were recruited into the study between February 2001 and May 2005; a subset of these patients has previously been described.13 Induced sputum samples were collected after ultrasonic nebulization of isotonic saline solution, as previously described.9,10,20 The selection criteria were the presence of acute asthma with positive viral infection, age > 7 years, FEV1 > 40% predicted, and completion of a follow-up visit. The subjects were studied again 4 to 6 weeks postexacerbation. Participants provided nasal/throat swabs, and underwent skin allergen testing and spirometry. Participants also completed the common cold questionnaire (CCQ)21 and the asthma control questionnaire (ACQ).22 The ACQ is a seven-item questionnaire that has been validated to measure the goals of asthma management (ie, minimization of daytime and nighttime symptoms, activity limitation, β2-agonist use, and bronchoconstriction). Analysis of asthma status using this scoring system has determined that well-controlled asthma has an optimal cut point of < 0.75, while a score of > 1.50 indicates inadequately controlled asthma.22

At follow-up, subjects were categorized into one of the following two groups: the persistent-airway-obstruction group, defined as subjects with an improvement in lung function (ie, change in FEV1 percent predicted [Δ%FEV1]) <15%; and the airway-recovery group, defined as a Δ%FEV1 of ≥15%. Stable subjects with asthma who were recruited within the same study period were included as a comparison group. They met American Thoracic Society criteria23 for asthma diagnosis, had experienced no change in asthma activity or respiratory infections in the last 4 weeks, and were recruited from John Hunter Hospital ambulatory care clinics. Written informed consent was obtained from all participants for this study, which was approved by the Hunter Area Health Service and University of Newcastle Human Research Ethics Committees.
Specimen Processing

Selected portions of induced sputum samples were allocated to (1) a lytic solution (Buffer RLT; Qiagen; Hilden, Germany) for RNA extraction and subsequent messenger RNA expression analysis and virus polymerase chain reaction (PCRs), (2) in vitro culture in virus-permissive human epithelial cell lines (HEI and HEp-2) for outgrowth of respiratory viruses, and (3) sequential dithiothreitol and phosphate-buffered saline solutions for cellular dispersion and profiling. This involved selecting sputum samples from saliva, processing it in a dithiothreitol solution, then filtering the dispersed suspension and performing a total cell count of leukocytes. Cytospins were also prepared and stained (May-Grünwald Giemsa stain), and a differential cell count was obtained from 400 nonsquamous cells. Nasal swabs and throat swabs were also immersed in the lytic solution (Buffer RLT; Qiagen), and extraction and purification of the sputum sample, clarification of the in vitro culture supernatant, and swab RNA were performed using a standard commercially available kit (RNeasy kit; Qiagen) per the instructions of the manufacturer. RNA was then reverse-transcribed to total complementary DNA using random primers and a standard commercially available kit (Superscript II RT kit; Invitrogen; Carlsbad, CA).

Virus Identification

Patient samples were assayed for the presence of RV, enterovirus (EV), influenza virus types A and B, respiratory syncytial virus (RSV) types A and B, non-severe acute respiratory syndrome coronavirus, and metapneumovirus (MPV) virus RNA transcripts. Due to the application of advances in real-time PCR technology, the initial gel-based PCR assays for RV, EV, RSV, and MPV were replaced with real-time PCR assays (TaqMan; Applied Biosystems) proceeded using 12.5% of the complementary DNA product and a commercially available kit (HotMaster Taq DNA PCR; Eppendorf AG) and 40 cycles of 95°C for 15 s followed by 1 min at 60°C (ABI 7500 cycler; Applied Biosystems; Foster City, CA). RNA was then reverse-transcribed to total complementary DNA using random primers and a standard commercially available kit (Superscript II RT kit; Invitrogen; Carlsbad, CA).

Table 1—Clinical Description of Background Asthma in Subjects With Airway Recovery vs Persistent Airway Obstruction Following Viral Asthma Exacerbation

| Variables                        | Stable Asthma Subjects (n = 26) | Airway-Recovery Group (n = 30) | Persistent–Airway-Obstruction Group (n = 10) | p Value |
|----------------------------------|---------------------------------|-------------------------------|-----------------------------------------------|---------|
| Age, yr                          | 13.0 (9.4–43.0)                 | 10.9 (9.3–16.8)               | 15.6 (11.4–50.5)                              | 0.137   |
| Sex                              | 0.103†                          |                               |                                               |         |
| Male                             | 12                              | 14                            | 1                                             |         |
| Female                           | 14                              | 16                            | 9                                             |         |
| Atopy                            | 0.521†                          |                               |                                               |         |
| Duration of asthma, yr since diagnosis | 7.2 (5.5–10.2)                  | 7.06 (3.6–12.1)               | 14.1 (8.0–33.5)                               | 0.054†  |
| Maintenance dose of ICSs, µg/d✿ | 650 (400–1,000)                 | 650 (400–1,000)               | 2,000 (2,000–2,000)§                         | 0.042†  |

*Values are given as the median (IQR) or No. (%), unless otherwise indicated.
†Fisher exact test.
‡Kruskal-Wallis test.
§p < 0.008 compared to airway-recovery group.
ment, there was no clinical difference between the groups, suggesting that the severity of the exacerbations was similar. However, the change in clinical parameters postexacerbation was markedly different (Table 3, Fig 1). In the airway-recovery group, there was a highly significant $\Delta \% \text{FEV}_1$, resulting in values at recovery that were similar to those for stable subjects with asthma. In the persistent–airway-obstruction group, however, FEV$_1$ percent predicted did not improve. In the airway-recovery group, both the CCQ and the ACQ scores showed highly significant improvements, with visit 2 values similar to those for stable subjects with asthma. For the persistent–airway-obstruction group, there was improvement in the CCQ and the ACQ scores; however, asthma continued to be uncontrolled postexacerbation.

At visit 1, subjects in the persistent–airway-obstruction group had a similar inflammatory cell profile to that in stable subjects with asthma. In contrast, the airway-recovery group had an increase in total cell count (Fig 2, top, A) and neutrophil count (Fig 2, bottom, B) compared to those in stable subjects with asthma. On recovery, neutrophil counts in the airway-recovery group decreased significantly (Table 4). In contrast, subjects in the persistent-airway-obstruction group showed no significant decreases in inflammatory cell counts postexacerbation.

**Discussion**

Persistent airway obstruction following acute asthma exacerbation is a significant clinical problem. Approximately one in four people recovering from a severe asthma exacerbation with virus infection experience minimal improvement in lung function. They continue to experience persistent airway obstruction and poor asthma control. This study is the first to examine the role of lower airway inflammation in this phenomenon. We have determined that subjects with persistent airway obstruction following virus exacerbation had, at the time of the exacerbation, levels of total inflammatory cells, eosinophils, macrophages, and lymphocytes in the airway that were similar to those in stable subjects with asthma. Furthermore, the inflammatory cell profile in the lower airways of these individuals did not change postexacerbation, despite some improvement in symptoms. This is a clinically important group, as, despite the absence of inflammatory cell influx into the airways during exacerbation, their asthma symptoms were uncontrolled during the exacerbation, and, although symptoms improved 4 to 6 weeks postexacerbation, they continued to be uncontrolled.

While both the airway-recovery and persistent-airway-obstruction groups experienced acute asthma exacerbations of apparently similar severity (Table 2), the two groups were clinically very different at follow-up. Table 3 indicates that during the exacerbation the CCQ was elevated in both the airway-recovery and the persistent–airway-obstruction groups; then, at 4 to 6 weeks postexacerbation, a similar improvement in virus symptoms was seen in both groups (percentage change in CCQ) [Fig 1]. Table 3 also describes inadequate asthma control during exacerbations in both the airway-recovery and persistent–airway-obstruction groups, as indicated by an asthma control score of $> 1.5$. However, while there was a highly significant improvement in ACQ score in the airway-recovery group, who demonstrated well-controlled asthma (ACQ score, < 0.75) at visit 2, a more modest improvement was seen in persons in the persistent–airway-obstruction group, who continued to have inadequately controlled asthma (ACQ score, $> 1.5$) postexacerbation. The poor lung function (percent predicted FEV$_1$) observed during the acute episode significantly improved in the airway-recovery group, with values returning to levels of stable asthma patients postexacerbation. However, there was no significant
## Table 3—Clinical Outcomes in Airway-Recovery and Persistent-Airway-Obstruction Groups at Visit 1 and Visit 2 vs Stable Subjects With Asthma

| Variables       | Airway-Recovery Group          | Persistent-Airway-Obstruction Group   | Stable Asthma Subjects |
|-----------------|--------------------------------|--------------------------------------|------------------------|
|                 | Visit 1                        | Visit 2                              | p Value                |                        |
| FEV<sub>1</sub> L | 1.34† (1.06–1.56)              | 2.03† (1.52–2.49)                    | < 0.001                | 1.72 (1.22–1.95)       | 1.75† (1.40–1.88)       | 0.919                  | 2.15 (1.70–2.94)       |
| % predicted     | 61.50† (43.00–66.40)           | 89.24 (81.70–92.86)                  | < 0.001                | 72.50† (64.00–84.00)   | 69.43† (68.18–78.33)   | 0.799                  | 90.00 (81.00–103.00)   |
| CCQ Total score | 7.50 (4.50–12.50)              | 3.00 (1.00–6.00)                     | 0.002                  | 14.50† (3.0–19.0)      | 3.00 (0.00–11.00)      | 0.075                  | 2.00 (0.00–9.00)       |
| General symptoms| 0.5 (0–2.5)                    | 0 (0–1)                              | 0.061                  | 2.5 (0–6)             | 0 (0–0)                | 0.041                  | 0 (0–0)                |
| Nasal symptoms  | 3 (1.5–4)                      | 2 (0–3)                              | 0.039                  | 4.5 (0–7)             | 1.5 (0–4.5)            | 0.399                  | 0 (0–2)                |
| Throat symptoms | 1 (0–2)                        | 0 (0–1)                              | 0.002                  | 2 (0–3)               | 0 (0–0)                | 0.028                  | 0 (0–1)                |
| Chest symptoms  | 2 (1–4)                        | 0 (0–1)                              | < 0.001                | 4 (2–6)               | 2 (1–2)                | 0.024                  | 1 (0–2)                |
| ACQ Average score| 2.29† (1.43–3.43)              | 0.57 (0.29–1.71)                     | < 0.001                | 2.79† (1.43–3.86)     | 1.57 (0.86–2.43)       | 0.045                  | 0.57 (0.14–1.0)        |
| Nocturnal asthma| 2 (0.5–4)                      | 0 (0–1)                              | < 0.001                | 2.5 (2–5)             | 0.5 (0–2)              | 0.018                  | 0 (0–0)                |
| Morning symptoms | 2 (0–3)                       | 0 (0–1)                              | 0.001                  | 1.5 (0–3)             | 0.5 (0–2)              | 0.123                  | 0 (0–2)                |
| Activity limitation | 2 (0–3)                    | 0 (0–2)                              | 0.001                  | 2.5 (1–5)             | 2 (0–3)                | 0.122                  | 0 (0–0)                |
| Shortness of breath | 2 (1–4)                   | 0 (0–1)                              | < 0.001                | 3 (0–4)               | 2 (0–3)                | 0.489                  | 1 (0–1)                |
| Wheeze in past week | 2 (1–3.5)                 | 0 (0–2)                              | < 0.001                | 2.5 (1–4)             | 2 (0–4)                | 0.502                  | 0 (0–1)                |
| Rescue β<sub>2</sub>-agonist use | 3 (1–4)         | 1 (0–2)                              | < 0.001                | 3.5 (3–5)             | 2 (0–3)                | 0.058                  | 1 (0–2)                |
| FEV<sub>1</sub>, % predicted | 4 (4–6)               | 2 (1–2)                              | < 0.001                | 3 (2–4)               | 3 (2–4)                | 0.752                  | 1 (0–2)                |

*Values are given as the median (IQR), unless otherwise indicated.
†p < 0.05 compared to stable subjects with asthma.
‡Stable subjects with asthma, n = 5.
improvement in %FEV\textsubscript{1} in the persistent–airway-obstruction group (Fig 1, Table 3). Thus, while the two groups were clinically similar during exacerbations, they were distinctly different at recovery. This may reflect differences in the groups before or as a result of viral infection. Due to the design of this study, preexacerbation data are not available. However, the higher dose of maintenance ICSs used in the persistent–airway-obstruction group suggests that this group had more severe illness before the exacerbation, and it is possible that they may have had preexisting airflow obstruction.

Incomplete clinical recovery from exacerbations has been reported\textsuperscript{16} previously in COPD patients, with 25% of patients experiencing exacerbations not recovering to baseline lung function at 5 weeks. Seemungal et al\textsuperscript{16} related exacerbation length to the magnitude of acute deterioration. While the extent of acute deterioration cannot be assessed by our study design, the data in Table 2 suggest that acute episodes were of similar severity in the two groups. Another longitudinal study\textsuperscript{17} in COPD patients found that, over time, clinical recovery to baseline levels (FEV\textsubscript{1} and symptoms) after an exacerbation took longer. The mechanisms by which recovery rate slows over time are unclear, but it has been suggested that this may be associated with postviral increases in airway and systemic inflammation.\textsuperscript{17} Our data indicated that subjects in the persistent-airway-obstruction group had a longer duration of asthma. If this group had been followed up for a longer time, a return to baseline FEV\textsubscript{1} may eventually have been observed. An alternate study design, which allowed the assessment of airflow obstruction preexacerbation, would be required to test this hypothesis.

During asthma exacerbation, the airway-recovery group showed elevated total inflammatory cell counts, which were driven by increased numbers of neutrophils (Fig 2) that decreased postexacerbation (Table 4). This is consistent with the known effects of viral infection in the airway.\textsuperscript{11} Conversely, patients in the persistent–airway-obstruction group showed a lower airway inflammatory profile during exacerbations similar to those with stable asthma (Fig 2), and this did not change significantly postexacerbation (Table 4). This is surprising, considering the well-established role of the neutrophil in driving an innate immune response to viral infection. However, the apparent insensitivity of lower airway inflammation to the presence of virus suggests that there is a suppression of the innate immune response in these subjects. The persistent–airway-obstruction group had been using a higher maintenance dose of ICSs than the airway-recovery group (Table 1). It is possible that high doses of ICSs may be inhibiting the generation of inflammatory mediators\textsuperscript{32} such as...
tumor necrosis factor-α, IL-1, and IL-6, thereby preventing the infiltration of inflammatory cells into the airways.33 There are a number of mechanisms associated with viral infection that may lead to the persistence of abnormal lung function and poor asthma control postexacerbation that are not dependent on inflammatory cell influx. Virus-induced eosinophil activation may increase the release of mediators such as ECP34 and leukotriene C4,35,36 which may worsen asthma symptoms. Activated eosinophils may also drive neurogenic inflammation by releasing eosinophil major basic protein, which is an endogenous antagonist for M2 muscarinic receptors. These receptors normally inhibit the release of acetylcholine, but when blocked by major basic protein, acetylcholine is released, resulting in airway muscle hyperresponsiveness.37 Viral induction of vascular leakage has also been reported, with mediators such as IL-8 and ECP38 being exuded into the airway lumen, which may sustain and perpetuate the inflammatory process.39 Viral activation of mast cells, leading to increases in histamine release, may also contribute to worsened asthma.40 Mucus hypersecretion has also been linked to viral infection.41,42 Thus, there are a range of different virus-induced mechanisms, which may exacerbate a variety of the physiologic features that characterize asthma but do not involve infiltration of the lower airways with inflammatory cells. Finally, it is possible that the exacerbations in the persistent–airway-obstruction group are driven by upper rather than lower airway inflammation. This is supported by the data in Table 3, which indicate that airway inflammation is more pronounced in the upper airways than in the lower airways.

In conclusion, we have observed that approximately one in four people with asthma continue to have persistent airway obstruction and uncontrolled asthma symptoms at 4 to 6 weeks post-viral asthma exacerbation. We have thoroughly described the phenomenon and have determined that it does not involve the influx of inflammatory cells into the lower airways. Other mechanisms, such as the activation of resident airway cells and enhanced upper airway inflammation, should be further explored. The number of people who experience persistent airway obstruction postexacerbation represent a significant proportion of asthmatic patients (25%), and these people should be followed up postexacerbation as the continuation of uncontrolled asthma will lead to a significant loss of quality of life.

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**Table 4—Airway Inflammation in Airway-Recovery and Persistent–Airway-Obstruction Groups at Visit 1 and Visit 2 vs Stable Subjects With Asthma**

| Variables                              | Airway-Recovery Group Visit 1 | Visit 2 | p Value | Persistent–Airway-Obstruction Group Visit 1 | Visit 2 | p Value | Stable Asthma Subjects |
|----------------------------------------|--------------------------------|---------|---------|---------------------------------------------|---------|---------|------------------------|
| Total cell count, × 10⁶ cells/mL        | 5.36† (2.70–15.17)             | 4.37 (1.89–4.95) | 0.600   | 2.16 (0.54–8.64)                            | 2.03 (0.72–4.41) | 0.273   | 1.94 (1.71–3.24)       |
| Neutrophils, × 10⁴ cells/mL             | 265.32 (107.01–752.73)         | 127.80 (66.69–182.22) | 0.023   | 85.66 (5.47–181.94)                         | 85.66 (5.47–181.94) | 0.655   | 16.22 (6.17–70.3)      |
| Eosinophils, × 10⁴ cells/mL             | 1.74 (0.00–16.45)              | 6.12 (1.04–46.36)  | 0.182   | 0.00 (0.00–1.94)                            | 3.44 (0.00–7.04)  | 0.180   | 2.67 (0.31–4.35)       |
| Macrophages, × 10⁴ cells/mL             | 162.23 (114.75–306.00)         | 140.13 (79.22–398.78) | 0.938   | 44.50 (4.14–303.06)                         | 105.40 (62.10–243.90) | 0.655   | 166.83 (106.35–251.42) |
| Lymphocytes, × 10⁴ cells/mL             | 3.71 (0.88–6.75)               | 1.47 (0.00–11.17)  | 0.906   | 0.00 (0.00–8.10)                            | 2.88 (1.15–5.33)  | 0.180   | 2.54 (1.46–4.94)       |
| Columnar epithelial cells, × 10⁴ cells/mL | 5.24 (0.00–12.05)             | 3.08 (0.00–3.43)  | 0.224   | 4.42 (0.29–9.97)                            | 3.40 (1.00–5.44)  | 0.180   | 5.03 (2.39–6.71)       |

*Values are given as the median (IQR), unless otherwise indicated.

†p < 0.05 vs stable subjects with asthma.
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