Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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

Lisa G. Wood, PhD; Heather Powell, MMedSci; Terry Grissell, BSc; Thuy T.D. Nguyen, MD; Darren Shafren, PhD; Michael Hensley, MBBS; and Peter G. Gibson, MBBS

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 FEV_1 percent predicted [$\Delta\%FEV_1$]) of <15%, was observed in 10 subjects (25%). Airway recovery ($\Delta\%FEV_1$, ≥ 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.** ***(CHEST 2007; 131:415–423)***

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

Abbreviations: ACQ = asthma control questionnaire; CCQ = common cold questionnaire; ECP = eosinophilic cationic protein; EV = enterovirus; $\Delta\%FEV_1$ = change in FEV_1 percent predicted; ICS = inhaled corticosteroid; IL = interleukin; IQR = interquartile range; MPV = metapneumovirus; PCR = polymerase chain reaction; PEF = peak expiratory flow; RSV = respiratory syncytial virus; RV = rhinovirus

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,[1] with respiratory viruses being isolated from 81% of patients with asthma exacerbations requiring hospitalization in adults and children.[2] 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.[3–5] Viral asthma exacerbations involve a marked neutrophil infiltration together with eosinophil degranulation.[2,6–10] 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 al[12] 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 al[13] 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 al[10] 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 FEV_1 (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 al[18] 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.[11] Induced sputum samples were collected after ultrasonic nebulization of isotonic saline solution, as previously described.[9,19,20] The selection criteria were the presence of acute asthma with positive viral infection, age > 7 years, $FEV_1 > 40\%$ 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, β_2-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 FEV_1 percent predicted [$\Delta\%FEV_1$]) $<15\%$; and the airway-recovery group, defined as a $\Delta\%FEV_1$ of $\geq 15\%$. Stable subjects with asthma who were recruited within the same study period were included as a comparison group. They met American Thoracic Society criteria[23] 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.

*From the School of Medicine and Public Health (Drs. Wood, Nguyen, Hensley, and Gibson, Ms. Powell, and Mr. Grissell), University of Newcastle, Newcastle, NSW, Australia; and Picornaviral Research Unit (Dr. Shafren), Royal Newcastle Hospital, Newcastle, NSW, Australia.
Dr. Wood was the recipient of a Postdoctoral Fellowship from the National Health and Medical Research Council, Australia. Dr. Gibson was the recipient of a Practitioner Fellowship from the National Health and Medical Research Council, Australia. This study was funded by a National Health and Medical Research Council of Australia Project Grant.
The authors have reported to the ACCP that no significant conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.
Manuscript received April 20, 2006; revision accepted August 11, 2006.

Correspondence to: Lisa Wood, PhD, Hunter Medical Research Institute, Respiratory and Sleep Medicine, Level 3, HMRI, John Hunter Hospital, Newcastle, NSW 2305, Australia; e-mail: lisa.wood@newcastle.edu.au
DOI: 10.1378/chest.06-1062

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 (PCR), (2) *in vitro* culture in virus-permissive human epithelial cell lines (HEL 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.[24,25] 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.[11] 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 probe; Applied Biosystems; Foster City, CA) [RV,[26] EV,[27] RSV types A and B,[28] human MPV,[29] and coronavirus[30]] during the later stages of this study, and real-time PCR also was used to confirm prior gel-based viral detection results. All PCR assays (TaqMan; Applied Biosystems) proceeded using 12.5% of the complementary DNA product and a commercially available kit (RealMasterMix Probe ROX kit; Eppendorf AG; Hamburg, Germany), all with the same cycling parameters, namely, 2 min at 95°C to activate the PCR assay (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). Subjects were considered to be positive for virus if the presence of a virus was determined by either direct molecular detection (*ie*, gel PCR or real-time PCR) in sputum, swab (nasal or throat), or saliva specimens or by post-cell culture molecular detection (gel PCR or real-time PCR) in sputum or saliva specimens.

Statistical Analysis

Subjects with virus-positive acute asthma at visit 1 were classified at visit 2 according to their improvement in FEV_1 (in liters) [$< 15\%$ or $\geq 15\%$] as having persistent airway obstruction or airway recovery, respectively. Analysis was performed using a statistical software package (Stata, version 7; Stata Corporation; College Station, TX), with results presented as the median (interquartile range [IQR]) or No. (%). Significance ($p < 0.05$) was determined from a nonparametric Kruskal-Wallis test or Fisher exact test and χ^2 test. *Post hoc* analysis was applied to all significant variables using the Kruskal-Wallis test (Kruskal-Wallis2 test; Stata Corporation). Paired analyses for the change from visit 1 to visit 2 were conducted using the Wilcoxon signed rank test.

Results

Viruses were detected in 40 subjects during acute exacerbations. These viruses included the following: RV, 30 subjects (75%); EV, 17 subjects (42.5%); influenza virus type A, 4 subjects (10%); influenza virus type B, 1 subject (2.5%); and RSV, 1 subject (2.5%). Eleven subjects had dual viruses, and 1 subject tested positive for three viruses. Following the acute exacerbation, persistent–airway-obstruction was observed in 10 subjects (25%) and airway recovery was observed in 30 subjects (75%). Characteristics of these groups are described in Table 1. There was no significant difference in atopy between groups. However, the persistent–airway-obstruction group used a significantly higher maintenance dose of inhaled corticosteroid (ICS) preexacerbation and had a longer duration of asthma.

Details of the acute asthma episode for the persistent–airway-obstruction group compared with those for the airway-recovery group are described in Table 2. On examination in the emergency depart-

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	20 (76.9)	24 (88.9)	9 (90)	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.

Table 2—*Details of the Acute Asthma Episode

Variables	Airway-Recovery Group	No.	Persistent–Airway-Obstruction Group	No.	p Value
Physical exhaustion	10 (33.3)	30	2 (20.0)	10	0.693†
Altered consciousness	0	30	0	10	
Talks in		20		7	0.333†
Words	4 (20.0)		0		
Phrases	4 (20.0)		3 (42.9)		
Sentences	12 (60.0)		4 (57.1)		
Length of stay, d	2 (1–3)	30	2 (1–4)	10	0.962‡

*Values are given as No. (%) or median (IQR), unless otherwise indicated.
†Fisher exact test.
‡Wilcoxon rank sum test.

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 $\Delta\%FEV_1$, 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.[31] 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)[31] 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	Visit 1	Visit 2	p Value	
FEV_1							
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_2-agonist use	3 (1–4)	1 (0–2)	< 0.001	3.5 (3–5)	2 (0–3)	0.058	1 (0–2)
FEV_1, % 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.

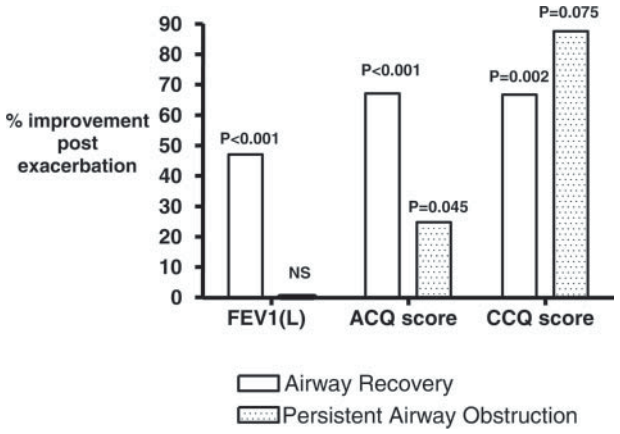

FIGURE 1. Improvement in clinical markers from visit 1, in the airway-recovery and persistent–airway-obstruction groups. The p values refer to the change between visit 1 and visit 2.

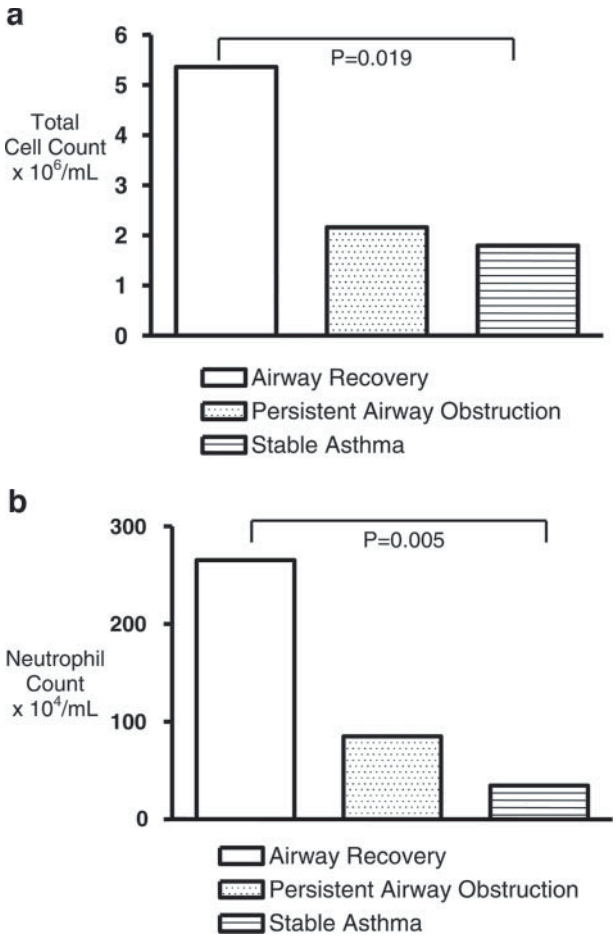

FIGURE 2. Total cell count (*top*, *a*) and neutrophil count (*bottom*, *b*) in induced sputum samples obtained during an acute exacerbation (visit 1) from persons in the airway-recovery group and the persistent–airway-obstruction group, and from stable patients with asthma. *Post hoc* testing was performed using the Kruskal-Wallis 2 test.

improvement in $\%FEV_1$ 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[16] previously in COPD patients, with 25% of patients experiencing exacerbations not recovering to baseline lung function at 5 weeks. Seemungal et al[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[17] in COPD patients found that, over time, clinical recovery to baseline levels (FEV_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.[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_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.[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[32] such as

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			Persistent-Airway-Obstruction Group			Stable Asthma Subjects
	Visit 1	Visit 2	p Value	Visit 1	Visit 2	p Value	
Total cell count, $\times 10^6$ 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, $\times 10^4$ cells/mL	265.32† (107.07–872.73)	127.80 (66.69–182.22)	0.023	85.06 (27.72–220.81)	85.66 (5.47–181.94)	0.655	16.22 (6.17–70.3)
Eosinophils, $\times 10^4$ cells/mL	1.74 (0.00–16.48)	6.12 (1.04–46.58)	0.182	0.00 (0.00–1.94)	3.44 (0.00–7.04)	0.180	2.67 (0–31.43)
Macrophages, $\times 10^4$ cells/mL	162.23 (114.75–306.00)	140.13 (78.22–389.78)	0.938	44.50 (4.14–303.06)	105.40 (62.10–243.90)	0.655	166.85 (106.88–251.42)
Lymphocytes, $\times 10^4$ cells/mL	3.01 (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, $\times 10^4$ cells/mL	5.24 (0.00–12.05)	3.08 (0.00–3.43)	0.224	4.42 (0.28–9.97)	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.

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 ECP[34] 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 ECP[38] 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 minimal changes in asthma symptoms at visit 2. This is further supported by the observation that throat symptoms were one of the domains that showed the greatest improvement postexacerbation.

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.

ACKNOWLEDGMENT: Assistance with the collection of samples and clinical data were received from Naomi Timmins and

Joanne Smart from Department of Respiratory and Sleep Medicine, Hunter Medical Research Institute, John Hunter Hospital, Newcastle, NSW, Australia.

References

1 Wark PA, Gibson PG. Asthma exacerbations: 3. Pathogenesis. Thorax 2006; 61:909–915
2 Wark PA, Johnston SL, Moric I, et al. Neutrophil degranulation and cell lysis is associated with clinical severity in virus-induced asthma. Eur Respir J 2002; 19:68–75
3 De Monchy JG, Kauffman HF, Venge P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985; 131:373–376
4 Wark PAB, Simpson J, Hensley MJ, et al. Airway inflammation in thunderstorm asthma. Clin Exp Allergy 2002; 32: 1750–1756
5 Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. Am J Respir Crit Care Med 1999; 160:640–647
6 Busse WW, Vrtis RF, Dick EC. The role of viral infections in intrinsic asthma: activation of neutrophil inflammation. Agents Actions Suppl 1989; 28:41–56
7 Teran LM, Johnston SL, Schroder JM, et al. Role of nasal interleukin-8 in neutrophil recruitment and activation in children with virus-induced asthma. Am J Respir Crit Care Med 1997; 155:1362–1366
8 Norzila MZ, Fakes K, Henry RL, et al. Interleukin-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma. Am J Respir Crit Care Med 2000; 161:769–774
9 Twaddell SH, Gibson PG, Carty K, et al. Assessment of airway inflammation in children with acute asthma using induced sputum. Eur Respir J 1996; 9:2104–2108
10 Pizzichini MM, Pizzichini E, Efthimiadis A, et al. Asthma and natural colds: inflammatory indices in induced sputum: a feasibility study. Am J Respir Crit Care Med 1998; 158:1178–1184
11 Grissell TV, Powell H, Shafren DR, et al. Interleukin-10 gene expression in acute virus-induced asthma. Am J Respir Crit Care Med 2005; 172:433–439
12 Corne JM, Marshall C, Smith S, et al. Frequency, severity and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. Lancet 2002; 359:831–834
13 Chang KC, Vollmer WM, Marrett MJ, et al. Prolonged episodes of persistent asthma: a distinct clinical pattern with characteristic clinical features. Chest 2000; 117:944–949
14 Coyle YM, Hynan LS, Gruchalla RS, et al. Predictors of short-term clinical response to acute asthma care in adults. Int J Qual Health Care 2002; 14:69–75
15 Ferreira A, Williams Z, Donninger H, et al. Rhinovirus is associated with severe asthma exacerbations and raised nasal interleukin-12. Respiration 2002; 69:136–142
16 Seemungal TAR, Donaldson GC, Bhhowmik A, et al. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000; 161:1608–1613
17 Donaldson GC, Seemungal TAR, Patel IS, et al. Longitudinal changes in the nature, severity and frequency of COPD exacerbations. Eur Respir J 2003; 22:931–936
18 El-Radhi AS, Hogg CL, Bungre JK, et al. Effect of oral glucocorticoid treatment on serum inflammatory markers in acute asthma. Arch Dis Child 2000; 83:158–162
19 Gibson PG, Wlodarczyk J, Hensley M, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. Am J Respir Crit Care Med 1998; 158:36–41
20 Wark PAB, Simpson JL, Hensley MJ, et al. Safety of sputum induction with isotonic saline in adults with acute severe asthma. Clin Exp Allergy 2001; 31:1745–1753
21 Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. BMJ 1993; 307:982–986
22 Juniper EF, O'Byrne PM, Guyatt GH, et al. Development and validation of a questionnaire to measure asthma control. Eur Respir J 1999; 14:902–907
23 American Thoracic Society. Standardization of spirometry: 1987 update; statement of the American Thoracic Society. Am Rev Respir Dis 1987; 136:1285–1298
24 Wark PA, Simpson JL, Hensley MJ, et al. Safety of sputum induction with isotonic saline in adults with acute severe asthma. Clin Exp Allergy 2001; 31:1745–1753
25 Cai Y, Carty K, Henry RL, et al. Persistence of sputum eosinophilia in children with controlled asthma when compared with healthy children. Eur Respir J 1998; 11:848–853
26 Deffernez C, Wunderli W, Thomas Y, et al. Amplicon sequencing and improved detection of human rhinovirus in respiratory samples. J Clin Microbiol 2004; 42:3212–3218
27 Nijhuis M, van Maarseveen N, Schuurman R, et al. Rapid and sensitive routine detection of all members of the genus enterovirus in different clinical specimens by real-time PCR. J Clin Microbiol 2002; 40:3666–3670
28 van Elden LJ, van Loon AM, van der Beek A, et al. Applicability of a real-time quantitative PCR assay for diagnosis of respiratory syncytial virus infection in immunocompromised adults. J Clin Microbiol 2003; 41:4378–4381
29 Maertzdorf J, Wang CK, Brown JB, et al. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. J Clin Microbiol 2004; 42:981–986
30 van Elden LJ, van Loon AM, van Alphen F, et al. Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. J Infect Dis 2004; 189:652–657
31 Juniper EF, Bousquet J, Abetz L, et al. Identifying "well-controlled" and "not well-controlled" asthma using the Asthma Control Questionnaire. Respir Med 2006; 100:616–621
32 Noah TL, Wortman IA, Becker S. The effect of fluticasone propionate on respiratory syncytial virus-induced chemokine release by a human bronchial epithelial cell line. Immunopharmacology 1998; 39:193–199
33 Schleimer RP. Innate immune responses and chronic obstructuve pulmonary disease: "Terminator" or "Terminator 2." Proc Am Thorac Soc 2005; 2:342–346
34 Dimova-Yaneva D, Russell D, Main M, et al. Eosinophil activation and cysteinyl leukotriene production in infants with respiratory syncytial virus bronchiolitis. Clin Exp Allergy 2004; 34:555–558
35 Kimpen JLL, Garofalo R, Welliver RC, et al. Activation of human eosinophils *in vitro* by respiratory syncytial virus. Pediatr Res 1992; 32:160–164
36 Gentile DA, Fireman P, Skoner DP. Elevations of local leukotriene C4 levels during viral upper respiratory tract infections. Ann Allergy Asthma Immunol 2003; 91:270–274
37 Jacoby DB, Costello RM, Fryer AD. Eosinophil recruitment to the airway nerves. J Allergy Clin Immunol 2001; 107:211–218
38 Greiff L, Andersson M, Svensson C, et al. Allergen challange-induced acute exudation of IL-8, ECP and α_2-macroglobulin in human rhinovirus-induced common colds. Eur Respir J 1999; 13:41–47

39 Greiff L, Andersson M, Erjefalt JS, et al. Airway microvascular extravasation and luminol entry of plasma. Clin Physiol Funct Imaging 2003; 23
40 Skoner DP, Gentile DA, Fireman P, et al. Urinary histamine metabolite elevations during experimental influenza infection. Ann Allergy Asthma Immunol 2001; 87:303–306
41 Londhe V, McNamara N, Lemjabbar H, et al. Viral dsRNA activates mucin transcription in airway epithelial cells. FEBS Lett 2003; 553:33–38
42 Hashimoto K, Graham BS, Ho SB, et al. Respiratory syncytial virus in allergic lung inflammation increases Muc5ac and gob-5. Am J Respir Crit Care Med 2004; 170:306–312