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CHAPTER 24

The Prevalence of Common Respiratory Viruses in Human Lungs

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INTRODUCTION

Viral infections probably initiate a large percentage of childhood and adult asthmatic attacks based on a history of preceding ‘cold’, high rates of viral isolation during attacks with lower rates of isolation during symptom-free intervals, and peaks of hospital admissions for asthma coinciding with viral epidemics (1–3). Although PCR technology provides a much more sensitive and specific method for detecting viral nucleic acids, its role in diagnosing active viral infection has been only partially explored (4–8). The purpose of this chapter is to present our preliminary findings using a PCR screening procedure for common respiratory viruses and review our data concerning the possible role of latent infection with the adenovirus and persistent viral infection with respiratory syncytial virus (RSV) in the pathogenesis of chronic lung inflammation.

The PCR screening procedure for nine common respiratory viruses was performed on lung tissue obtained from both asthmatic and non-asthmatic patients. The lung histology and immunohistochemistry of the inflammatory reaction in these cases was the subject of a recent report (9). Six additional cases (four lifetime non-smokers and two with remote minimal smoking histories) were added to control for the fact that both the asthmatics and non-asthmatics smoked heavily. We then used data from two viruses (the adenovirus and RSV) to discuss the possible role of latent and persistent viral infection in the pathogenesis of chronic lung inflammation.

EXPERIMENTAL APPROACH

Patients

The age, sex, smoking history, evidence for pre-operative infection and available lung function of the patients from whom the lung tissue was obtained are summarized in Table I. Cases 1–3 are autopsy cases where sudden death unrelated to the lung resulted
| Case | Age | Sex | Cig. years | Pre-op inf (wks) | TLC | FRC | RV | DLCO | FEV₁ | FVC | FEV₁/FVC |
|------|-----|-----|------------|-----------------|-----|-----|----|------|------|-----|----------|
| Minimal smoker |     |     |            |                 |     |     |    |      |      |     |          |
| 1    | 42  | M   | 20         | ND              |     |     |    |      |      |     |          |
| 2    | 17  | M   | 0          | ND              |     |     |    |      |      |     |          |
| 3    | 24  | M   | 0          | ND              |     |     |    |      |      |     |          |
| 4    | 46  | M   | 0          | 4               | 105 | 115 | 114| 111  | 104  | 97  | 84       |
| 5    | 50  | F   | 0          | 8               | 89  | 84  | 97 | 75   | 85   | 86  | 82       |
| 6    | 80  | M   | 15         | ND              |     |     |    |      |      |     |          |
| Average | 43  | 5/1 | 6          |                 | 97  | 100 | 106| 93   | 91   | 88  | 82       |
| SE   | 20  |     | 20         |                 | 8   | 16  | 8  | 18   | 9    | 7   | 2        |
| Asthma |     |     |            |                 |     |     |    |      |      |     |          |
| 7    | 48  | F   | 540        | 4               | 119 | 112 | 140| 91   | 112  | 101 | 73       |
| 8    | 33  | M   | 340        | 8               | 29  | 30  | 29 | 79   | 114  | 113 | 82       |
| 9    | 63  | F   | 650        | ND              |     |     |    |      |      |     |          |
| 10   | 47  | M   | 780        | ND              | 113 | 84  | 113| 84   |      |     |          |
| 11   | 82  | M   | 500        | 3               | 126 | 116 | 126| 38   | 52   | 71  | 56       |
| Average | 55  | 3/2 | 562        |                 | 97  | 91  | 102| 73   | 87   | 98  | 70       |
| SE   | 17  |     | 148        |                 | 39  | 26  | 43 | 21   | 23   | 17  | 9        |
| Non-asthma |     |     |            |                 |     |     |    |      |      |     |          |
| 12   | 62  | F   | 420        | ND              | 129 | 148 | 149| 82   | 91   | 120 | 61       |
| 13   | 62  | M   | 2080       | ND              | 109 | 121 | 144| 72   | 67   | 90  | 59       |
| 14   | 47  | F   | 660        | ND              | 105 | 88  | 71 | 89   | 108  | 122 | 75       |
| 15   | 50  | M   | 600        | ND              | 97  | 98  | 98 | 85   | 98   | 98  | 81       |
| 16   | 61  | M   | 280        | ND              | 102 | 126 | 131| 78   | 57   | 80  | 56       |
| 17   | 61  | M   | 920        | ND              | 100 | 112 | 112| 93   |      |     |          |
| 18   | 72  | F   | 740        | 4               | 120 | 124 | 144| 76   | 105  | 106 | 75       |
| Average | 59  | 3/4 | 1089       |                 | 109 | 117 | 121| 80   | 88   | 101 | 69       |
| SE   | 8   |     | 680        |                 | 11  | 18  | 27 | 6    | 18   | 14  | 9        |

TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; DLCO, diffusion capacity for carbon monoxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ND = not determined.

*Values shown are percent predicted.
in the subject being selected as a lung transplant donor. The remaining cases (4–18) are surgical, where the patients underwent lung resection for tumour. These are part of a long-term study of lung structure and function based at St Paul’s Hospital in Vancouver which has entered more than 400 patients since 1979 (10). A review of hospital records identified that six of the 15 surgical cases (4, 5, 7, 8, 11 and 18) had a flu-like illness or severe cold in the 3–8 weeks prior to surgery.

Tissue Preparation
The lung tissue was processed using a method previously described in detail (9, 11). Briefly, the resected specimens were allowed to collapse and the tumour was palpated through the pleural surface, bisected and sampled for histology. The regional lymph nodes were then dissected and the bronchial resection margins sampled to obtain the material needed to complete a standard surgical pathology report. The incision made in the pleural surface was then sutured closed, and the lung filled with a cryoprotective agent and suspended over liquid nitrogen on aluminium foil in a closed styrofoam container until it was frozen solid. The frozen lung was then cut into 1.5 cm thick slices on a band saw in the transverse plane and these slices were kept frozen on dry ice while they were sampled with a power-driven hole saw. The cores of tissue obtained by this procedure were stored at −70°C until used. The lung specimens obtained at autopsy were processed in a similar manner without the necessity of cutting the pleural surface.

Nucleic Acid Extraction
Fragments obtained from the cores of frozen tissue were homogenized using a Polytron probe. RNA was extracted from this homogenate using the RNeasy Kit (Qiagen GMBH, Germany) with overnight tissue digestion in digestion buffer containing 200 μg/ml of proteinase K (samples 1–7) or by Trizol (Gibco BRL, Burlington, Ontario, Canada) (samples 8–18). DNA was extracted using the QIA amp Tissue Kit (Qiagen GMBH, Germany) after overnight digestion with a buffer containing proteinase K.

Reverse Transcription
The RNA extracted from the tissue homogenate was reverse transcribed at 37°C for 1 hour in a 50 μl reaction consisting of 10 mM Tris HCl pH 8.3, 50 mM KCl, 5 mM MgCl₂, 50 μM each of dNTPs, 50 units of RNAase inhibitor (RNAguard, Pharmacia), 5 μg of random hexamers (Pharmacia) and 250 units of cloned Moloney murine leukaemia virus reverse transcriptase (Gibco BRL).

PCR Amplification
cDNA product in 5 μl for the reverse transcription reaction, or a fraction of the extracted DNA in the case of adenovirus, was subsequently used for amplification in 50 μl PCR containing 10 mM Tris HCl pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 50 μM each of dNTPs, 1.5 units of Taq DNA polymerase (Gibco BRL) and 50 pmol each of upstream- and downstream-specific primers (Table II). Samples were subjected to denaturation at 94°C for 4 minutes, followed by 35 cycles of amplification in a thermal Robocycler-40 (Stratagene, La Jolla, CA, USA), each cycle consisting of denaturation at 94°C for
TABLE II
Control PCR Primers and Product Size

DNA: α1-antitrypsin
AATP1: 5'-CTACCAGGAATGGCcTTGTCC-3'
AATP3: 5'-GTGTGGGAACAGTCGcGTATCC-3'
Product size: 205 bp

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AATP3: 5'-GTGTGGGAACAGTCGcGTATCC-3'
Product size: 205 bp

RNA
Human β-actin
S primer: 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' (1038–1067)
AS primer: 5'-CTAGAAGCATTTGCGGTGGACGATGcG-3' (1876–1905)
Product size: 661 bp

RNA
Human β-actin
A primer: 5'-TCATCACCATTGGCAATGAG-3'
B primer: 5'-CACTGTGTTGGCGTACAGGT-3'
Product size: 154 bp

RNA
Human glyceraldehyde 3-phosphate dehydrogenase (G3PDH)
5 primer: 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3'
3 primer: 5'-CATGTGGGCCATGAGGTcACCAC-3'
Product size: 983 bp

S, sense; AS, antisense.

1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1.5 minutes. For the last cycle, the extension was for 8 minutes. After amplification, the PCR products were resolved by electrophoresis on ethidium bromide-stained 1.5% agarose gels. For adenovirus, 2 μl of amplified products were reamplified with nested primers in 50 μl of the same reaction mixture, and the resulting products underwent electrophoresis on 2% agarose gels; the ethidium bromide-stained DNA was visualized under ultraviolet light.

Positive Controls

The controls for the PCR reaction consisted of nucleic acids extracted from virus-infected cell cultures. The following viruses were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA): RSV Long strain type A, human rhinovirus (HRV) type 1B, coronavirus subtypes 229E and OC43, influenza A/Weiss/43, influenza B/Lee/40, influenza C/Taylor 1233/47 and adenovirus serotype 2.

RSV was grown on HEP-2 (ATCC) cell monolayers in minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). HRV was grown on MRC-5 (ATCC) cell monolayers in Eagle’s basal medium containing 5% FBS. All the influenza and coronaviruses were grown on MK-2 monkey kidney cell monolayers in medium 199 supplemented with 1% horse serum (HyClone, Logan, UT, USA). For influenza viruses, 0.01% trypsin was added to the culture. Adenovirus serotype 2 was grown on monolayers of A549 cells in MEM with 5% FBS. All culture media contained 50 μg/ml of gentamicin.

For the assessment of successful DNA extraction, we examined the α1-antitrypsin gene. For RNA extraction, both the β-actin and G3PDH RNA were examined (Table
### TABLE III
Viral PCR Primers, Product Size and Probes

| Virus            | Specific primer/probe | Target gene | Product size (bp) |
|------------------|-----------------------|-------------|-------------------|
| RSV              | AS: 5'-GCGATGTCTAGGTTAGGAAGAA-3' S: 5'-GCTTCCCTGGTAGTAAGCCT-3' Probe: 5'-TAGCTCAGAATAGTAAGCCT-3' | Nucleocapsid | 410              |
| HRV              | AS: 5'-CGGACACCCAAAGTAG-3' S: 5'-GCACCTTGTTCCTCCC-3' Probe: 5'-GCATGCGGGGCGGAG-3' | 5' non-coding region of HRV type 1B | 380              |
| Influenza A      | AS: 5'-GCTCTGTCATGTTATTTGGATC-3' S: 5'-CAGAGACTGAGAGATGTCTTTGC-3' Probe: 5'-TCCTGTCACTCTGACTAAGGGGATTTTG-3' | Matrix protein | 212              |
| Influenza B      | AS: 5'-AGCGTTCTCATTTATTTCTGC-3' S: 5'-GAAAATTAACCTGTGGTGGTGCGG-3' Probe: 5'-TTCTAGCTGAGAGAAAAATGAGAATG-3' | Matrix protein | 365              |
| Influenza C      | AS: 5'-GCCAAGTAAATCCACGCAATCTC-3' S: 5'-CCCTAATGTCTTGGAGAAGCCAC-3' Probe: 5'-TCGAAATAGGAACACACAACCTCAGTTGTT-3' | Matrix protein | 425              |
| Coronavirus 229E | AS: 5'-TGCACTAGGGTTAATGGAAGAG-3' S: 5'-GGTACTCCTAAGCCTTTCTCG-3' Probe: 5'-GACTATCAAGACAGCATGACG-3' | Nucleocapsid | 370              |
| Coronavirus OC43 | AS: 5'-TGCAAAAGATGGAAGACTGTGGG-3' S: 5'-AGGAAGGTCTGTCTCTAATTTCC-3' Probe: 5'-GTCTGAGCAAATCTGCAAG-3' | Nucleocapsid | 450              |
| PIV              | AS: 5'-TGCACTAGGGTTAATGGAAGAG-3' S: 5'-GGTACTCCTAAGCCTTTCTCG-3' Probe: 5'-GACTATCAAGACAGCATGACG-3' | F gene 5' non-translated region | 205              |
| Adenovirus       | Outer primers AS: 5'-CAGACACGGCGGAGATCTCAAGATG-3' S: 5'-GGCAGCTGTTGCTCCTGAACTACAT-3' Nested primers AS: 5'-TTGTAGCAGATAGCAGGTATCCTGCGGT-3' S: 5'-GCCACCGAGACGTTACCTAGCCTG-3' | Hexon | 300              |

S, sense; AS, antisense.

The integrity of the extracted RNA was difficult to assess because amplification of the cDNA from cytoplasmic mRNA transcripts of housekeeping genes (β‐actin 1 and 2 and G3PDH) was confused by amplification of a corresponding pseudogene in DNA contaminating these samples (12). As an alternative measure, we based the quality of our RNA samples on the integrity of the 18 and 28S of ribosomal RNA in these samples as determined by their appearance on an agarose gel.

Table III summarizes the features of the specific primers and probes used in these studies. All except the primer and probe for influenza C which was designed from Genbank® sequences, have been previously published (4–8). All of these reagents were prepared using an automated DNA synthesizer at a commercial source (University of Calgary, Calgary, Alberta, Canada). The primers selected for HRV also detect other picornaviruses (3) and those used for the adenovirus detect 47 serotypes of this virus (8).
Viral contamination during nucleic acid extraction was controlled for by simultaneous extraction of porcine lung tissue known to be free of these human viral agents. Tubes containing diethylpyrocarbonate (DEPC) water instead of nucleic acid were added every 8–10 samples in the PCR runs to ensure that no contamination occurred during this step.

Southern Blot Analysis

After Southern transfer on to Hybond-N nylon membranes (Amersham, Arlington Heights, IL, USA) and cross-linking with ultraviolet light, PCR products were hybridized with the oligonucleotide probe that was end-labelled with \((\gamma^{32}P)\) ATP using T4 polynucleotide kinase (Gibco BRL) following the manufacturer's instructions. Hybridization was carried out in a Hybaid hybridization oven (Stratagene) at 65°C for 18 hours in a solution consisting of 6 × sodium chloride/sodium citrate (SSC) (0.9 M NaCl, 0.09 M sodium citrate, pH 7.0), 5 × Dernhardt's solution [0.1% Ficoll 400, 0.1% bovine serum albumin (fraction V), 0.1% polyvinylpyrrolidone], 0.5% sodium dodecyl sulfate and 100 µg/ml fragmented salmon sperm DNA. After hybridization, membranes were washed three times for 5 minutes each in 6 × SSC at room temperature and for 30 minutes at 65°C before exposure to Kodak X-ray film.

Statistics

The proportion of positive to total cases was tabulated for each virus and differences relating to asthma, smoking and infection status were examined using the Fisher exact test, with corrections for multiple comparisons.

RESULTS

Table I shows the age, sex distribution, smoking history and history of recent infection for the minimal smokers (MS) \((n = 6)\), asthmatic \((n = 5)\) and non-asthmatic \((n = 7)\) groups. The asthmatics were all moderate smokers with an average history of 562 ± 148 cigarette years (approximately 28 pack years) of consumption. The smoking group without asthma tended to have a higher exposure, 1089 ± 680 cigarette years (approximately 54 pack years) of exposure, but this did not reach statistical significance because of the wide variance between subjects. The group with the lowest cigarette exposure contained four lifetime non-smokers and two cases (1, 16) with minimal remote smoking histories. The available lung function in the group with little smoking history was within normal limits and there was no statistically significant difference in lung function between the patients who were smokers and either did or did not have asthma.

Figure 1 shows representative autoradiographs of an agarose gel containing the results of the PCR analysis from 10 samples, plus the positive and negative control for each virus tested. These data show that RSV, influenza A, influenza C and adenovirus were the most prevalent viruses in these 10 cases. All of the cases except number 10 contained at least one virus; several (4, 5, 6, 8, 9) contained more than one and case 5 contained nucleic acid from four viruses.
At least one virus was isolated in 17 of 18 cases examined. The most common viruses found were adenovirus (11/18), influenza A (9/18), influenza C (6/18), RSV (3/18) and human rhinovirus (3/18) (Table IV). More than one virus was detected in 13/18 cases, with two found in six cases, three in four cases and four in three cases. There was no statistically significant difference in the proportion of positive tests for any virus between asthmatics and the smoking and MS control groups ($p > 0.05$). There was also no difference in either the number or type of virus detected between the cases that had a clinical episode suggesting a recent infection (cases 4, 5, 7, 8, 11 and 18, Table I) and the remainder of the cases in the study ($p > 0.05$).

**DISCUSSION**

The infection of tissue by a virus involves adherence of a virus to the host cell, penetration of the cell, replication of viral nucleic acid, production of structural proteins, assembly of a complete virus and release of the assembled virus from the host cell by
### TABLE IV

| Virus no. | RSV | HRV | Infl A | Infl B | Infl C | Cor 229E | Cor 043 | ADV | Total |
|-----------|-----|-----|--------|--------|--------|----------|---------|-----|-------|
| **Minimal smokers without asthma**<br>\(n = 6\) | 2/6 | 1/6 | 5/6 | 0/6 | 3/6 | 0/6 | 0/6 | 2/6 | 13/48 |
| **Smokers with asthma**<br>\(n = 5\) | 1/5 | 0/5 | 2/5 | 0/5 | 3/5 | 0/5 | 0/5 | 3/5 | 9/40 |
| **Smokers without asthma**<br>\(n = 8\) | 0/7 | 2/7 | 2/7 | 2/7 | 0/7 | 1/7 | 0/7 | 6/7 | 13/56 |
| \(p^*\) | NS | NS | NS | NS | NS | NS | NS | NS | NS |

* Fisher exact test corrected for multiple comparisons; NS, not significant.

either shedding from the cell surface or host cell lysis. The tests required to establish that each of these steps has occurred include culture to document viral replication, *in situ* hybridization or PCR to detect viral nucleic acids, immunocytochemistry or immunohistochemistry to demonstrate viral proteins, and electron microscopy to establish that a complete virus has been assembled. These changes are usually associated with seroconversion in the host indicating that there has been an immune response to the virus. Data based solely on the PCR of viral nucleic acid must be interpreted cautiously because demonstration of viral nucleic acid does not necessarily mean that the results of culture, *in situ* hybridization, immunohistochemistry or electron microscopy will also be positive. A previous PCR-based study from this laboratory showed excellent concordance between culture and PCR in samples where culture was positive (5) but in these cases PCR results frequently showed the presence of more than one type of viral nucleic acid whereas the culture rarely showed the presence of more than one replicating virus.

The results reported in Fig. 1 and Table IV establish that the nucleic acid from several respiratory viruses was present in the lower respiratory tract of these subjects. Six of the 15 patients undergoing lung resection gave a history of a flu-like illness or upper respiratory infection in the 3–8 weeks prior to surgery. Others may have had asymptomatic infections but there was no difference in either the number or type of virus detected between the groups with and without symptomatic illness. Replicating virus is shed from the upper airways for only 5–7 days following acute infections (13) but viral nucleic acid may remain long after the virus ceases to replicate. Recent animal studies reported from our laboratory have shown that RSV can be demonstrated by culture two months after infection and by RT–PCR four months after an acute infection (14), and PCR has shown similar results in guinea pig adenoviral bronchiolitis (15). It is much more difficult to establish viral persistence in humans because many respiratory viruses have several serotypes and infection by a different serotype could easily be mistaken for persistence. For example, the screen used for adenovirus in the study is based on a sequence for a hexon protein that is common to all adenoviral serotypes. This makes it a sensitive test for the presence of adenovirus but it is not a useful test for persistent adenoviral infection because it cannot differentiate between serotypes.

Acute viral infections of the respiratory tract cause prolonged increases in bronchial reactivity in asthmatic patients (16). This might result from previous sensitization of the
host to viral proteins during a lytic infection, with reinforcement of the sensitization by protein produced either by persistent low level replication of the virus or by the expression of latent viral genes remaining after replication has ceased. The importance of the host response was illustrated by the early attempts to develop RSV vaccines where fatal bronchiolitis sometimes occurred when the vaccinated children were next exposed to a wild type virus (17). More recent studies of healthy individuals infected with rhinovirus showed that the severity of the upper respiratory infection could be diminished by administration of neutralizing antibodies to non-atopic subjects but was without effect in atopic subjects. A report of suppression of virally induced asthmatic attacks with prolongation of viral shedding in animals with parainfluenza virus infection receiving corticosteroid therapy (19) is consistent with symptoms being caused by the response to the virus rather than to direct tissue damage caused by the virus. This concept was further supported by a recent report showing that the airways of patients who die from asthma are infiltrated with CD8+ T lymphocytes, suggesting that the cellular immune response of the host contributes to fatal asthma attacks (20). All of these studies suggest that the host immune response to the virus is important in inducing and/or amplifying the airway inflammatory process responsible for asthma. Therefore, although the relevance of the viral nucleic acid demonstrated in the present study is unclear, we believe that the mechanisms whereby persistent or latent viral infection might contribute to chronic airway inflammation deserves to be investigated.

Recent studies of adenoviral infection indicate that latent adenoviral DNA expresses a protein capable of amplifying an asymptomatic inflammatory response into one capable of causing chronic airways obstruction (21). Several laboratories have shown that the protein coded by an early adenoviral gene (E1A) dysregulates gene expression by interacting with the DNA-binding sites of transcription factors (22). This same protein can also interfere with the function of suppressor genes such as the retinoblastoma gene product (23) and interfere with gene expression by binding to the CREB-binding protein (CBP) (24). Our studies have established that excessive amounts of adenovirus E1A DNA are present in lungs from patients with obstructive lung disease (25) and that E1A protein can be demonstrated in human lungs (26). Very recent studies of transfected A549 cells have shown that E1A is capable of upregulating IL-8 and ICAM-1 when these cells are challenged (27). Our working hypothesis is that latent adenoviral infection is capable of amplifying the cigarette smoke-induced airway inflammation present in all smokers, to produce airway obstruction in the minority of patients who develop this complication. The relevance of this hypothesis to asthma remains to be determined but Macek et al. (28) have provided an interesting lead by showing that children with bronchiolitis who subsequently develop steroid-resistant asthma frequently show evidence of the adenoviral hexon protein in their BAL fluid.

RSV, parainfluenza virus (PIV) and mumps virus are paramyxoviruses that are characterized by a single-stranded, negative polarity RNA genome with a heavily glycosylated envelope. In children, RSV and PIV are, respectively, the most common causes of acute bronchiolitis and croup. In addition to these serious acute lower respiratory tract infections, epidemiological studies have clearly established that many children develop sequelae of recurrent wheezing and asthma, typically in the context of allergy (29–31). Animal studies have reported that experimental RSV and PIV lower respiratory tract infections can produce increases in airway responsiveness that persist for weeks to months after acute infection (32, 33). Histological examination of the lungs of RSV-infected guinea pigs after resolution of acute bronchiolitis reveals evidence of
chronic inflammation and the presence of viral proteins in the airways (12, 33). Chronic airway inflammation, mast cell hyperplasia of the airway wall and increased expression of cytokines such as TGF-β have also been reported as sequelae of PIV infection in rats that have persistent airway hyper-responsiveness (34). These observations suggest that persistent viral lung infections may cause structural changes to the airways, which could result in airway hyper-responsiveness, a hallmark of asthma.

Viral respiratory tract infections may also play an indirect role in the pathogenesis of asthma. Several lines of evidence have implicated viral infections in triggering initial sensitization to environmental allergens in susceptible children (31) and experimental animals (35). The development of RSV-specific IgE antibodies in children with post-bronchiolitis wheezing is consistent with RSV being a ‘marker’ of an allergic diathesis. Our laboratoruty has recently reported that airway hyper-responsiveness produced in ovalbumin-sensitized guinea pigs is exaggerated by acute RSV infection, in part mediated by virus-induced increases in airway epithelial necrosis (36). These observations are consistent with the paradigm that allergic individuals are not more susceptible to viral respiratory tract infections but do develop more severe symptoms of infection than non-allergic subjects (1). A better understanding of the relative importance of direct viral damage to the airways and indirect viral damage via allergic mechanisms could lead to valuable new strategies for treatment and prevention of viral respiratory infections and their sequelae.

In summary, our results show a widespread prevalence of common respiratory viral nucleic acid in human lung tissue obtained from patients undergoing lung resection. This suggests that the lung may serve as a reservoir of viral nucleic acids that enter the host via the respiratory tract. We postulate that some of these viral residua may amplify the airway inflammatory process responsible for asthma and chronic obstructive pulmonary disease.

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**DISCUSSION**

Schleimer: It looked like NF-κB was increased in the E1A-positive cells, but then you also suggested that those genes that were induced in the E1A-positive cells were ICAM-1 and IL-8 which had a unique NF-κB-binding site. What is the mechanism of the increase in NF-κB? Does the NF-κB promoter have that unique sequence or is there another mechanism?

Hogg: I don’t know. The pathway seems to be involved.

Raz: I think a paper published in *Nature* two or three years ago claims that the adenovirus utilizes another early gene that includes protosome processing and one of them is I-kB. So you get translocation of NF-κB and it has activating potential.

Gelfand: The E1A will activate the Ras-dependent pathway and it will also activate P53 or RB, the stress-activated kinases, so you upregulate a number of the transcription factors including NF-κB and AP-1.

Hogg: In these particular cells we didn’t get any evidence that the AP-1 was upregulated.

Gelfand: As you mentioned, a lot of this affects cell proliferation as well as cell survival. Do you know whether the changes in culture were associated with significant changes in either apoptotic death or survival?

Hogg: We don’t know that. That’s an obviously important thing to look at.

Bienenstock: How long after the first infection can the E1A still be found in tissue?

Hogg: That’s controversial. Most people believe that it’s episomal so it should disappear as cells divide. Dr. Hayashi in our group is trying to test the hypothesis that it may be integrated into host DNA. She has begun with the Graham 293 cell because these cells are permanently transfected with adenoviral E1A. By choosing primers in E1A DNA that allow the inverse PCR technique to be applied, she has amplified the unknown DNA adjacent to the E1A. She has preliminary results including sequence data extending across the junction of adenoviral and human DNA. The next step is to attempt to determine whether there is integration in the human lung, but this will be a greater challenge because the E1A is much more widely dispersed.

Busse: You did all your stimuli with LPS. You also mentioned TNF that will activate the A549 cells. Did you see the same pattern and the same degree of cytokine production and did you see any other cytokines other than IL-8?

Hogg: The answer to the first question is no. Others have used TNF, we haven’t. The list that I showed gives the ones that we have looked at; a search in the literature showed that they were produced by the A549 cells. Of those, an effect was seen only with ICAM and the IL.

Platts-Mills: We interpret positive PCR for rhinovirus in acute asthmatics as meaning the patient has recently been infected, but you find this RNA all over the place in patients. Could we be fooled, and that actually there is upregulation of rhinovirus by some other event?

Hogg: We too were surprised. My colleague, Dr. Hegele, has shown that in guinea pigs RSV can persist for a very long time after the infection by low level replication in alveolar macrophages. Perhaps these infections persist for much longer than we think with very low level replication that doesn’t have any effect. The other possibility is that the viral RNAs are resistant to the RNAases that are present in humans and that viral nucleic acid just hangs around for longer periods. We have done our best to make sure that the finding isn’t an artefact. We believe the viral nucleic acids are there. How they get there, is another question. Although birds provide the reservoir for the influenza virus, the reservoir for RSV between outbreaks is not known. The human lung could provide such a reservoir, possibly with persistence by low level replication of the virus.

Raz: There is a long debate in immunology: what is the mechanism of immunological memory? I think you have shown that constant viral presence probably is responsible for this memory, which is very beneficial. It keeps the lung system in shape.
Hogg: I would like to stress that we looked at the nucleic acid. The only protein we've looked at so far is the E1A protein; you can't assume just because the nucleic acid is there that anything else happens. I think you have to show that it really happens.

Schwartz: The airway epithelium is the primary target in the initial infection and then you just get non-replicative viral nucleic acid retained. Then you'd expect that the asthmatic airway with its increased turnover of epithelium discards the infected epithelial cells more rapidly over time and shows less. Is this correct?

Hogg: That's correct, unless the viral DNA gets integrated into the host cell DNA. At present, we don't know if the virus first entered the lungs in the remote past or in the week before the sample was obtained. When we compared cases that gave us a history of recent infection to cases that did not have this history, there was no difference in the number of viruses found.

Grant: There is a group of patients that I see every winter that I've always been very curious about. These are people that seem to develop some respiratory virus infection and then cough for about six months. We've always assumed it would be impossible for a viral infection to continue. I don't believe there has ever been an adequate explanation for this problem. Some are smokers, some are not, some have asthma, some don't, some have rhinitis and some don't. So, I think in the clinic there is some evidence of some persistence of a phenomenon, and perhaps you have some explanation for it.

Hogg: The NIH Lung Health Study has shown that many of the people who develop chronic obstructive lung disease do so without any cough, sputum production or any other symptoms. If the adenovirus were to produce an infection and remain in the lung for a long period of time, it is reasonable to think that the residual adenoviral E1A protein expressed in the latent period when there is no viral replication might amplify the initial inflammatory response to the infection. But that's very speculative. In our model of latent adenoviral infection in the guinea pig, the animals with latent infection show persistent airways inflammation and an enhanced inflammatory response to a single dose of cigarette smoke in the latent period.

Gleich: You showed an epithelial cell and the presence of adenovirus. Do you have any sense as to the differential localization of adenovirus amongst cells in the lung?

Hogg: I contacted colleagues from around the world who'd autopsied cases of children who died from adenovirus infection. These cases were all proven to be due to adenoviral infections using viral culture to prove replication and electron microscopy to show complete viral assembly. We studied those with in situ hybridization and the cells with high copy numbers of the virus were mostly airway epithelial cells. Because the majority of these cells were epithelial type 2 cells, which serve as the repair cell for the alveolar surface, we believe the virus may readily infect cells that are dividing. This strongly suggests that the epithelial cells are the primary target but other cells such as peripheral blood lymphocytes and tonsils have been shown to carry latent infection by other investigators.

Gleich: Have you tried to do these studies on induced sputum?

Hogg: No, we have not.