Is chronic rhinosinusitis caused by persistent respiratory virus infection?

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Background: Many chronic rhinosinusitis (CRS) patients recall an upper respiratory tract infection as the inciting event of their chronic illness. Viral infections have been shown to cause obstruction of the osteomeatal complex, which is likely to be a critical step in the development of CRS. There is clear overlap between the pathogenesis of CRS and asthma. Infections with respiratory viruses in childhood increase the risk of subsequently developing asthma. Viral infections in established asthmatics are associated with acute exacerbations. We sought to determine whether respiratory viruses could be detected within the sinonasal mucosa of CRS patients using polymerase chain reaction (PCR) techniques.

Methods: Sinus mucosa was sampled from 13 patients with CRS and 2 patients with normal sinuses. PCR was used to look for common respiratory viruses (parainfluenza 1, 2, and 3; respiratory syncytial virus [RSV]; human metapneumovirus [hMPV]; adenovirus [ADV]; rhinovirus; coronavirus; bocavirus [BoV]; cytomegalovirus [CMV]; and influenza A and B).

Results: No respiratory viruses were detected in any of the samples.

Conclusion: Persistence of respiratory viruses within the sinonasal mucosa is unlikely to be a cause of ongoing inflammation in CRS. The possibility remains that a transient viral infection provides the initial inflammatory stimulus.

Key Words: adult; chronic disease; humans; infection; nasal mucosa; nasal polyps; polymerase chain reaction; rhinitis; sinusitis; virology

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Despite being 1 of the most common chronic diseases in the Western world the pathogenesis of chronic rhinosinusitis (CRS) remains poorly understood. As a consequence of this, the significant morbidity and financial burden associated with this disease persist.1 A wide variety of pathogenic mechanisms, mostly related to microorganisms, have been investigated extensively, including bacterial and fungal biofilms,2,3 intracellular bacteria,4 and aberrant immune responses to both fungal allergens5 and staphylococcal superantigens.6

Two of the key events in the development of CRS are thought to be obstruction of the osteomeatal complex7 and the development of epithelial cell dysfunction.8 It is common for CRS patients to report that their symptoms initially developed after a viral infection and it is noteworthy that viral infections have been shown to cause obstruction of sinus ostia,9 production of inflammatory mediators by nasal epithelial cells,10 and damage to epithelial cells and cilia.11 Rhinovirus has also been shown to induce persistent changes in the local cytokine milieu12 and to increase bacterial adherence to nasal epithelial cells,13 with both mechanisms potentially providing a lasting effect following transient infection.

The link between asthma and CRS, particularly in those with nasal polyps, is well-established14 and potential parallels in pathogenesis of these conditions exist. Childhood infection with respiratory syncytial virus (RSV) increases
the probability of an affected child developing asthma for at least a decade afterward.15 It may be that the virus induces a persisting change in the mucosa before it is cleared or that viral particles persist within the mucosa, there being clear evidence that respiratory viruses are capable of establishing latent infections in human tissue.16 It has also been demonstrated that many acute exacerbations of asthma relate to infection with respiratory viruses.17

Several studies have used polymerase chain reaction (PCR) techniques to look for the presence of respiratory viruses in samples from CRS patients. Ramadan et al.18 reported that 20% of patients had evidence of RSV in their mucosa but no patients had evidence of adenovirus (ADV). They did not, however, have a control group nor report whether samples were collected from patients during the winter months when a significant proportion of the general population are affected by respiratory viruses.19 Jang et al.20 published a similar study that avoided these problems, finding rhinovirus in 21% of epithelial cell samples from CRS patients and none in controls during the summer months. However, the number of virus species sought was limited and samples were collected from the inferior turbinates rather than sinus mucosa.

Hypotheses concerning a role for viruses in the pathogenesis of CRS appear to fall into 3 groups. Viruses have been considered as potentially causative in the initial development of inflammation, the ongoing stimulus of inflammation, or the cause of acute exacerbations in symptoms.

This study was designed to evaluate whether evidence could be found to implicate respiratory virus persistence in the ongoing inflammation seen in CRS.

**Patients and methods**

**Patients**

A total of 15 adult patients who were undergoing endoscopic sinus surgery in the tertiary practice of the senior author (R.G.D.) either for CRS or for access to skull-base lesions were prospectively recruited. CRS patients fulfilled agreed diagnostic criteria for CRS21 and had failed a prolonged trial of medical therapy.1 All had sufficiently extensive disease to merit dissection of all their paranasal sinuses and there were no exclusion criteria. Normal sinonasal mucosa was sampled from 1 patient with a nonfunctioning pituitary adenoma and 1 patient with a malignant lesion of the pterygopalatine fossa. Neither of these patients had symptoms of CRS or evidence of CRS on endoscopy or imaging. Patients were recruited during the southern hemisphere summer and early autumn months (February-April, 2010). Recruitment was ceased at an agreed time when in previous years the rate of detection of respiratory viruses in the general population had been seen to rise. The regional ethics committee (Northern Regional Ethics Committee Ref: NTX/08/12/126) and the hospitals involved gave prior approval of the study and informed written consent was given by all patients.

**Clinical data**

Patient demographics as well as relevant past medical and surgical history were recorded. Patients were also asked to quantify how long they had had symptoms from their nose and sinuses (Table 1). Patients were classified on the basis of the presence (CRSwNP) or absence (CRSsNP) of nasal polyps as per published guidelines.21 No patients had aspirin exacerbated respiratory disease. Subjective and

**TABLE 1. Patient details**

| No. | Diagnosis | Age (years) | Sex | Ethnicity | Comorbidities | Duration of symptoms (years) | Revision surgery |
|-----|-----------|-------------|-----|-----------|---------------|----------------------------|-----------------|
| 1   | CRSwNP    | 46          | F   | European  |               | 35                         | Yes             |
| 2   | CRSwNP    | 41          | M   | Tongan    |               | 15                         | No              |
| 3   | CRSwNP    | 54          | M   | European  |               | 15                         | No              |
| 4   | CRSwNP    | 69          | F   | Maori     |               | 5                          | No              |
| 5   | CRSwNP    | 38          | M   | Algerian  |               | 4                          | No              |
| 6   | CRSsNP    | 18          | F   | Maori     | Bronchiectasis | 10                         | No              |
| 7   | CRSsNP    | 53          | F   | European  | Asthma        | 2                          | No              |
| 8   | CRSsNP    | 39          | M   | European  |               | 4                          | No              |
| 9   | CRSsNP    | 55          | F   | European  | Asthma        | 2.5                        | No              |
| 10  | CRSsNP    | 18          | M   | European  | Cystic fibrosis | 2                          | Yes             |
| 11  | CRSsNP    | 44          | F   | European  | Asthma        | 4                          | No              |
| 12  | CRSsNP    | 19          | F   | European  |               | 2                          | No              |
| 13  | CRSsNP    | 52          | F   | European  |               | 5                          | Yes             |
| 14  | Normal    | 33          | F   | European  |               | N/A                        | N/A             |
| 15  | Normal    | 65          | M   | European  |               | N/A                        | N/A             |
**TABLE 2. Primers and probes**

| Primers and probes | Oligonucleotide sequence (5′-3′) | Target gene |
|--------------------|----------------------------------|-------------|
| Inf A F            | GAC CRA TCC TGT CAC CTC TGA C    | M           |
| Inf A R            | AGG GCA TTY TGG ACA AAK CGT CTA  |             |
| Inf A P            | FAM-TGC AGT CCG TCA CTG GGC ACG-BHQ1 |             |
| Inf B F            | TCC TCA AYT CAC TCT TCG AGC G    | NC          |
| Inf B R            | CCG TGC TCT TGA CCA AAT TGG      |             |
| Inf B P            | 6FAM-CCA ATT CGA GCA GCT GAA ACT GGC GTG-BHQ1 |             |
| PIV1 F             | GTT GTC AAT GTC TTA ATT CGT ATC AAT T | HN         |
| PIV1 R             | GTC GCC TMC CTG CGG CAC CTA A     |             |
| PIV1 P             | FAM-TAG GCC AAA GAT TGT TGG CGA GAC TAT TCC AA-BHQ1 |             |
| PIV2 F             | GCA TTT CCA ATC TTC AGG ACT ATG A | HN          |
| PIV2 R             | ACC TCC TGG TAT AGC GAT GAC TGA AC |             |
| PIV2 P             | CAL FOS60-CCA TTT ACC TAA GTG ATG GAA TCA ATC GCA AA-BH |             |
| PIV 3 F            | CCA GGG ATA TAY TAY AAA GGC AAA A | HN          |
| PIV 3 R            | CGG GGR CAC CCA GTT GTG           |             |
| PIV 3 P            | FAM-TGG RTG TTC AAG ACC GCC ATA YCC GAG AAA-BHQ1 |             |
| ADV F              | GCC CCA GTG GTC TTA CAT GCA CAT C | Hexon       |
| ADV R              | GCC AGG GTG GGG TTT CTA AAG TT    |             |
| ADV P              | FAM-TGC ACC AGA CCC GGG GTC AGG TAC TCC GA-BHQ1 |             |
| RSV F              | AAT ACA GCM AAA TCT AAC CAA CTT TAC A | L          |
| RSV R              | GCC AAG GAA GCA TGC AAT AAA      |             |
| RSV P1             | FAM-TGC TAT TGT GCA CTA AAG-BHQ1 |             |
| RSV P2             | CAL FOS60-CAC TAT TCC TTA CTA AAG ATG TC-BHQ1 |             |
| hMPV F             | CATATAAGCATGCTATATTTAAAGAGTCTTC | NS          |
| hMPV R             | CCTTCTCTGACGATATTTGTGAACTCAG    |             |
| hMPV P             | FAM-TGY AAT GAT GAG GGT GTC ACT GGC TGG G-BHQ1 |             |
| CMV F              | CCG GCA AGC TCT TTA TGC A        | Phosphoprotein 65 |
| CMV R              | TGG GAC ACA ACA CCG TAA AGC      |             |
| CMV P              | FAM-CCG CAAC CCC TCC AT-BHQ1     |             |
| RV F               | GCA CTT CTG TTT CCC C            | 5′ noncoding region |
| RV R               | GGC AGC CAC GCA GGC T            |             |
| RV P1              | FAM-AGC CTC ATG TGC CAG GTC TA-BHQ1 |             |
| RV P2              | CAL FOS60-AGC CTC ATC CAC CAA ACT A-BHQ1 |             |
| hBoV F             | TGC AGA CAA CGC YTA GT TGT TT    | NS1         |
| hBoV R             | CTG TCC CGC CCA AGA TAC A        |             |
| hBoV P             | 6FAM-CCA GGA TTG GGT GGA ACC TGC AAA-BHQ1 |             |
| OC43 + HKU1        |                                  | Polymerase 1b |
| CoV F1             | TGG TGG CTG GGA CGA TAT GT       |             |
| CoV R1             | GGC ATA GCA CGA TCA CAC TTA GG  |             |
| CoV P1             | 6-FAM-ATA ATC CCA ACC CAT RAG-BHQ1 |             |
| NL63               |                                  | Polymerase 1b |
| CoV F2             | TTT ATG GTG CTT GGA ATA ATA TGT TG |             |
| CoV R2             | GGC AAA GCT CTA TCA CAT TTT G    |             |
TABLE 2. Continued

| Primers and probes | Oligonucleotide sequence (5′-3′) | Target gene |
|--------------------|---------------------------------|-------------|
| CoV P1             | FAM-ATA ATC CCA ACC CAT RAG-BHQ1 |             |
| 229E               |                                 | Polymerase 1b |
| CoV F3             | TGG CGG GTG GGA TAA TAT GT       |             |
| CoV R3             | GAG GGC ATA GCT CTA CAC TTA GG   |             |
| CoV P2             | CAL FO560-ATA GTC CCA TCC CAT CAA-BHQ1 |             |
| RnaseP F           | AGA TTT GGA CCT GCG AGC G       | Human ribonuclease P |
| RnaseP R           | GAG CGG CTG TCT CCA CAA GT       |             |
| RnaseP P           | FAM-GAG CGG CTG TCT CCA CAA GT-BHQ1 |             |

F = forward; HN = hemagglutinin-neuramidase; L = RNA polymerase large subunit; M = matrix; NC = nucleocapsid; NS = nonstructural gene; P = probe; R = reverse.

objective measures of disease severity in the form of the Lund-MacKay score22 and preoperative symptom scores23 were recorded. Our practice is to ask patients to give the 5 main symptoms of CRS (obstruction, anterior rhinorrhea, posterior rhinorrhea, hyposmia, and midface congestion) a score from 0 to 5 based on their severity in the preceding 2 weeks.

Sample collection
Representative mucosal samples were collected from the ethmoid or sphenoid sinuses and immediately placed into sterile normal saline. They were then transferred to the laboratory where analysis was undertaken by a technician blinded to clinical details.

Nucleic acid extraction
Tissue pieces approximately 5 mm in diameter were predigested in 50 μL proteinase K 20 mg/μL and 150 μL of tissue lysis buffer at 55°C until completely dissolved (Roche High Pure PCR Template Preparation kit; Roche, Mannheim, Germany), then extracted using MagNA Pure LC automatic extractor and Total Nucleic Acid High Performance kit according to the manufacturer’s recommendations. Total nucleic acid was eluted with 100 μL elution buffer. Extracted samples yielded on average 50–200 ng/μL nucleic acids.

For each patient, between 2 and 6 tissue pieces were processed.

Respiratory panel
A total of 15 independent PCR assays were designed with identical assay protocols and PCR platforms. These included influenza A, influenza B, parainfluenza 1, multiplex parainfluenza 2 and 3, multiplex RSV A and B, human metapneumovirus (hMPV), ADV, cytomegalovirus (CMV), bocavirus (BoV), multiplex rhinovirus (RV) 1 and 2, coronavirus OC43 and HKU1, coronavirus NL63, coronavirus 229E, and human RnaseP (which acted as the extraction control).

Sequences were adapted from the Centers for Disease Control and Prevention Protocol for Detection and Characterization of Influenza (www.cdc.gov) and from published reports.24–31 The CMV assay was designed in-house. All assays used TaqMan hydrolysis probes labeled with fluorophore FAM or CAL Fluor Orange 560 at the 5′ end and no fluorescent Black Hole Quencher 1 (BHQ1) at the 3′ end. All primers and probes were synthesized by Biosearch Technologies (Novato, CA); sequences are listed in Table2.

Reagents
Reactions were carried out in 25-μL reaction mixtures containing 1 × reaction mix (Invitrogen SS III Platinum One-Step Quantitative RT-PCR System; Invitrogen, Carlsbad, CA) and 0.5 μM enzyme mix Superscript III/Platinum Taq Polymerase; in case of ADV, CMV, and BoV, Platinum Taq Polymerase was used, 0.8–0.9 μM forward and reverse primers, 0.2 μM fluorescent probe, and 5 μL extracted ribonucleic acid (RNA)/DNA.

PCR mixes without enzyme were prepared in large volumes and stored in single use aliquots at −20°C. Before use, aliquots for each PCR were thawed, mixed with enzyme, and aliquotted onto a reaction plate. RNA/DNA was then added. Positive controls were aliquotted last to minimize possible contamination.

Controls
Each run contained a no template control (NTC) water, extraction blank controls, and positive controls.

Positive controls consisted of RNA extracted from confirmed tissue culture isolates. ADV type 5 (Ad-5) strain and CMV AD169 were obtained commercially from Advanced Biotechnologies (Columbia, MD). BoV control consisted of a cloned amplified isolate, confirmed by sequencing.

All these assays (except BoV) are subjected to annual quality control programs as distributed by Quality Control for Molecular Diagnostics (Glasgow, Scotland) and RCPA Australia.

Amplification
Assays were carried out on the Roche Light Cycler 480 using a 96-well microplate format. Amplification conditions were 50°C for 20 minutes (reverse transcription), 95°C for
2 minutes (initial DNA polymerase activation), then 45 cycles of denaturation at 95°C for 15 seconds, followed by 45 seconds at 55°C (annealing/extension). Results were analyzed in the FAM channel and Cal Orange 560 separately. Assays were considered valid if RNaseP amplification was positive.

Results
In the CRS patients, the median duration of symptoms reported was 4 years (range, 2–35 years). The median preoperative symptom score was 17 out of 25 (range, 10–21). The median Lund-Mackay score was 16 out of 24 (range, 10–22).

No respiratory viruses were detected in any of the samples.

Since conventional respiratory viruses were not found, further assays were performed looking for viruses known to be capable of establishing persistent infection, namely human herpes-6 (HHV-6) and Epstein-Barr virus (EBV) using PCR techniques with published primers. Low-titer HHV-6 was found in samples from 3 of 8 CRSsNP patients, 4 of 5 CRSwNP patients, and 1 of 2 normal subjects. Low-titer EBV was found in 1 of 8 CRSsNP patients, 4 of 5 CRSwNP patients, and 0 of 2 normal subjects. The low titers of virus present suggest latent rather than active infection.

Conclusion
It seems likely that CRS has a multifactorial pathogenesis, and that the inflammatory stimuli differ at different stages of the disease and in different subgroups. Respiratory viruses can however cause florid sinonasal symptoms and may generate a long-lasting effect on respiratory mucosa. Respiratory viruses have also been shown to be capable of establishing latency in human tissue. The role of respiratory viruses in the ongoing inflammation seen in CRS therefore warrants further investigation.

In this series of patients all CRS patients had active inflammation and despite using sensitive tests for an extensive panel of respiratory viruses we did not identify any evidence of respiratory viral presence in our patients. It may be that CRS is a spectrum of disease and our small sample size may not therefore include all variants but our clearly negative results do suggest that persistent respiratory virus infection is not responsible for the chronic inflammation seen in the major variants of this disorder. Evidence of latent EBV infection was seen in CRS mucosa in 42% of cases but in the small number of patients examined, EBV was not found in the controls. The significance of this is unclear.

Despite not finding respiratory viruses in our series it is noted that animal and in vitro models have shown that the deleterious effect of respiratory virus infection may persist well beyond the time when the virus particles have been cleared.

Our rate of detection of viruses in CRS is lower than in other published series. This may in part be due to methodological differences such as the collection of sinus mucosa rather than inferior turbinate samples and collection of specimens during the summer months when respiratory viruses are far less prevalent.

A study of whether viral infections are responsible for acute exacerbations in CRS symptoms could be designed in a similar fashion but undertaken during the winter months. A large group of negative control patients would be required to establish what the background rate of viral infection is at that time of year.

The chronic nature of CRS implies that presentation to specialist care is remote from the development of the condition, which makes study of the initiating factors more difficult. Transient respiratory virus infection causing damage to the epithelial surface could be the process that allows bacterial biofilms to form and adhere to the mucosal surface or could cause long-lasting changes in the inflammatory milieu to occur that are critical to the subsequent development of CRS. We found, however, no evidence of persisting respiratory virus infection in diseased sinus mucosa.

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