Nasal IgA response in wheezy infants

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Abstract
It is unknown why some infants wheeze during upper respiratory tract infections. One possibility is that secretory IgA, which has a major role in mucosal defence against viral infection, might be deficient in wheezy infants. The nasal IgA response to upper respiratory tract infection in 32 wheezy infants (median age 5·8 months) was compared with nine siblings (median age 2·6 years) who had nasal symptoms only. Nasal lavage was performed during infections and on follow up when free from symptoms, using inulin as a marker of dilution to determine absolute concentrations of IgA in the nasal secretions.

The two groups showed a similar increase in total IgA and total protein levels during infection, but secretory IgA concentrations were unchanged. This study shows that wheezy infants have a normal nasal IgA response to infection and that the increase in total IgA during early infection is due to plasma exudation rather than increased production of secretory IgA.

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Most infant wheezing is associated with acute viral infection of the respiratory tract.[1-4] It is unknown, however, why many infants wheeze during their upper respiratory tract illness whereas others simply have coryzal symptoms. The exact mechanism whereby viruses induce airway narrowing is not clear, but it must be dependent on the interaction between host defence and viral pathogenicity.[5 6]

Immunoglobulin A (IgA) is the predominant immunoglobulin in respiratory secretions and has an important role in protecting the mucosa from infection. IgA directly neutralises free virus, prevents adherence to the mucosal surface, and blocks entry of the virus into epithelial cells. Within 24 hours of infection IgA concentrations in nasal secretions start to increase. Levels increase for two to three weeks and may exceed twice the baseline levels.[7] Specific IgA is not produced for the first two weeks in most viral infections, however,[8-10] though it appears earlier with respiratory syncytial virus.[11] Secretory IgA is produced locally in the submucosal tissues and does not result from plasma exudation.[12]

It has been shown that infants who mount a greater non-specific nasal IgA response have fewer respiratory infections[13] and children prone to wheezing have an increased incidence of viral infection.[14] A deficient IgA response might allow viruses to breach the mucosal defence and cause inflammation and airway narrowing. Alternatively, wheezy infants might show an exaggerated IgA response similar to the hyperactive, virus specific IgE response that many of these infants exhibit.[15 16]

The nasal IgA response of wheezy infants has not been studied, though it is known that older children with asthma have normal baseline concentrations of IgA in their nasal secretions.[17] We hypothesised that wheezy infants may have an abnormal nasal IgA response to infection. In this study we compared the nasal IgA response of infants who wheezed with infection, with children who simply had rhinorrhoea and local symptoms.

Subjects and methods

SUBJECTS
Patients were recruited from the paediatric ward at Northwick Park Hospital. They had all been admitted to hospital with an acute episode of wheezing and clinical evidence of upper respiratory tract infection (URTI). Children were excluded if they had other chronic respiratory disorders or had received corticosteroids. Thirty seven children were enrolled but five did not return for follow up. There were 23 male and nine female wheezy infants whose ages ranged from 4 weeks to 2·6 years (median 5·8 months). Seven children were studied over more than one episode of wheezing.

The comparison group were patients' siblings who had clinical signs of upper respiratory tract infection but had never wheezed. If they had an URTI at the same time as the wheezing sibling they were studied while the wheezy infant was still an inpatient, otherwise the parents contacted us by telephone when the child developed a cold and they were seen the same day. There were six boys and three girls, whose ages ranged from 4 weeks to 4·2 years (median 2·6 years).

STUDY DESIGN
Nasal lavage was performed on the subjects during the acute illness and then repeated when the children had recovered, having been free of symptoms for at least one week. Timing of symptoms relative to sampling was recorded in detail. Lavage was carried out between 10 am and 4 pm, avoiding the effects of diurnal variation on nasal secretion.[18 19] Second wheezing episodes were studied if the parents contacted the research team. A single sample of blood was taken from the patients during the acute illness.

The study was approved by the hospital ethics committee. Parents were given written information and gave verbal consent.

NASAL LAVAGE TECHNIQUE
The nasal wash solution consisted of phosphate buffered saline with added inulin (0·45 mg/ml) as a marker of dilution. The wash was warmed to room temperature before use. By measuring the inulin concentration in the wash solution and in

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the lavage fluid obtained, the proportion of nasal lavage fluid consisting of nasal secretions can be calculated. This allows the IgA and protein recovered in the lavage fluid to be expressed for each unit volume of nasal lining fluid.

The sampling apparatus was a standard mucus extractor connected to wall suction. A soft rubber Jaques catheter was used, size 8 FG for infants and size 10 FG for older children.

The subject was held in a supine position. A 2 ml volume of wash was instilled gently into each nostril while simultaneously the fluid mixed with secretions was aspirated from the anterior nares. The whole procedure took only a few minutes and no adverse effects were suffered by any children.

Lavage fluid was centrifuged within 10 minutes at 3000×g for 20 minutes. The mucus pellet was removed and the supernatant was divided into aliquots and stored at −70°C. Samples were also tested for the presence of blood with Labstix (Ames) and discarded if positive.

Nasopharyngeal aspirate was collected by standard methods for detection of respiratory syncytial virus.

LABORATORY ASSAYS
Total IgA and secretory IgA were measured by enzyme linked immunosorbent assays (ELISAs) adapted from previously described methods.[20 21] In the former assay, plates were coated with antihuman IgA α chain whereas in the latter an antihuman IgA secretory piece was used to capture the antibody. The coefficient of variation of replicate control readings for the total IgA assay was 8%, and for secretory IgA 20%.

Total protein was measured by an adaptation of the standard Lowry method[22] using 10% human albumin as standard. Inulin was measured by an adaptation of the method described by Heyrovsky.[23]

Serum IgA was assayed by laser nephelometry and serum IgE by standard ELISA.

Identification of respiratory syncytial virus was determined by standard immunofluorescence. The polymerase chain reaction was used to amplify and detect RNA from rhinovirus in the nasal wash, as previously described.[24] Attempts were not made to identify other viruses.

STATISTICAL METHODS
Statistical analysis was performed using Minitab statistical software (Minitab Inc). Response to infection within a group was analysed using a one sample Wilcoxon signed rank test. Absolute concentrations and responses to infection in patients and siblings were compared using the Mann-Whitney U test. Values of $p<0.05$ were considered significant. Correlation analysis was performed on the Fig P (Biosoft) computer program. The coefficient of repeatability for non-infected samples measured on two occasions was calculated and related to the mean value for these samples.[25]

Results
Nasal lavage fluid contained a mean (SD) proportion of 25·7 (8·8)% of nasal secretions. Amounts varied little between the healthy and infected states, or wheezy infants and siblings.

There were increased levels of nasal total IgA during respiratory infections in the two groups (fig 1A). In a third of all subjects, however, the IgA concentration was higher in the follow up samples than during infection. There was no difference between wheezy infants and siblings in terms of absolute values during infection or recovery, nor was there any difference in response to infection between the two groups. Response to infection was taken as the difference in IgA concentrations between infection and recovery. Seven children were studied over two episodes of wheezing. There was no significant difference in the nasal IgA response to infection between the two episodes, though the response tended to be greater during the first episode. When baseline (recovery) IgA levels were measured on two occasions, great individual variability was shown by a high coefficient of repeatability (132%).

Secretory IgA concentrations were not significantly increased during infection in either wheezy infants or siblings (fig 1B). The ratio of secretory to total IgA was significantly lower
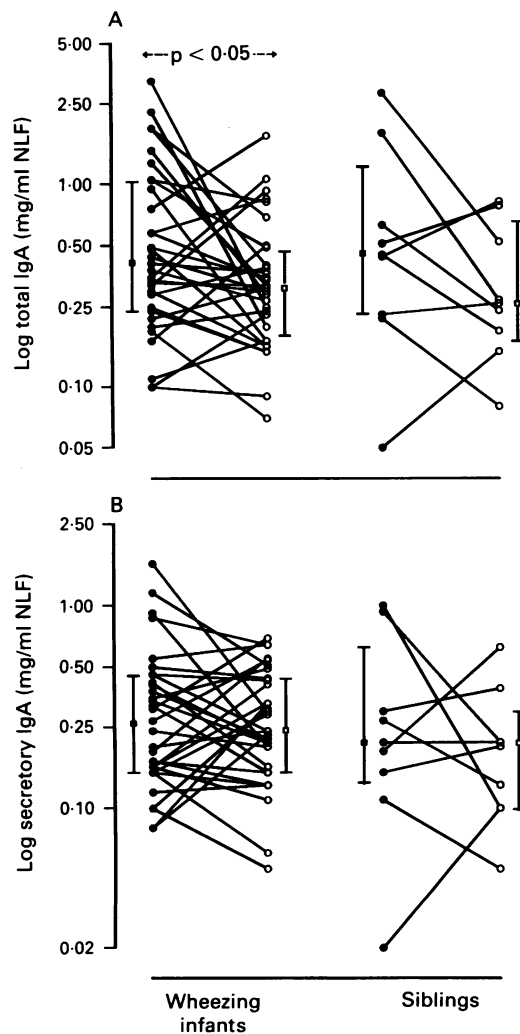

Figure 1 (A) Total IgA and (B) secretory IgA in nasal lining fluid (NLF) of wheezing infants and siblings during respiratory infection (closed circles) and after recovery (open circles). Median values with interquartile ranges are also shown (squares and bars).

during infection than after recovery in the wheezy infants, with a mean (SEM) of 62(5)% *v* 81(6)% (p<0·005). The ratio was also lower in the siblings (52(4)% *v* 59(7)%), but this did not reach statistical significance.

Total protein concentrations in the nasal lining fluid increased during infection (fig 2A), and protein levels tended to be higher in the wheezy infants. The ratio of total IgA to total protein in the nasal lining fluid was unchanged during infection but there was a significant difference (p<0·005) between the wheezy infants and siblings during infection, with those who wheezed having relatively less IgA (fig 2B). The good correlation of total IgA with total protein concentrations was similar during infection ($r=0·87$) and health ($r=0·83$) and was better than the correlation of secretory IgA with total protein ($r=0·75$ during infection and $r=0·51$ during health).

Serum IgA concentrations were normal in all wheezy children in relation to published reference ranges[26] except for one child of 11 months who had an increased serum IgA (1·6 g/l). There was no correlation between serum and nasal IgA concentrations and in over half the subjects the nasal IgA concentration exceeded that in the serum sample.

Serum IgE was high (>500 kU/l) in three children, all of whom had eczema. All other children had concentrations less than 100 kU/l (in 22 children it was <10 kU/l). Four other children also had eczema but had IgE <10 kU/l. The children with eczema tended to have higher baseline nasal IgA concentrations than the other wheezing infants (p=0·06), but their response to infection was significantly less marked (p<0·05).

There was no difference in the IgA response to infection of those who were positive for respiratory syncytial virus (18 subjects), those positive for rhinovirus (three subjects), or those negative for the two viruses (11 subjects). Respiratory syncytial virus was detected from the recovery samples of three infants who had been completely free of symptoms for 15–21 days. Rhinovirus was not detected in any of the recovery samples.
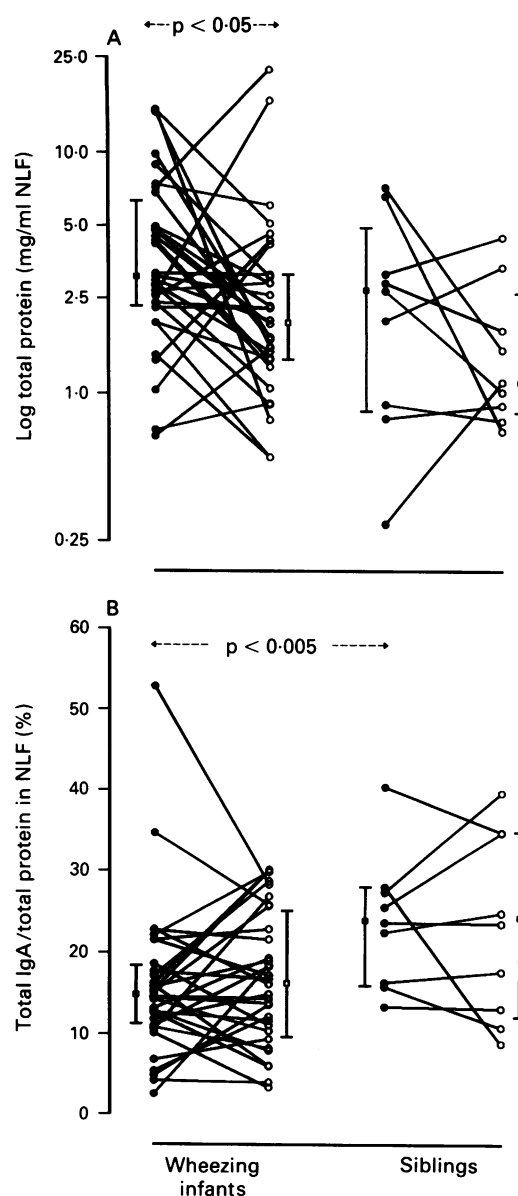

Figure 2   (A) Total protein concentrations and (B) total IgA to total protein percentages in nasal lining fluid (NLF) of wheezing infants and siblings during respiratory infection (closed circles) and after recovery (open circles). Median values with interquartile ranges are also shown (squares and bars).

Discussion

We have shown that infants who wheeze with viral upper respiratory tract infections have a similar nasal IgA response to children who only have nasal symptoms. IgA concentrations obtained during periods of respiratory infection and after recovery were similar to previously published results for non-wheezing infants.[13 27]

Total IgA concentrations were higher during infection but secretory IgA remained unchanged, which implies that the increased total IgA in nasal secretions was due to plasma exudation. This is confirmed by the increased total protein levels during infection and the fact that the total IgA to total protein ratio was unaltered between infection and recovery. During acute infection the protein composition of the nasal secretions bears a closer resemblance to an inflammatory exudate,[28] as respiratory viral infections selectively increase the vascular permeability of mucosal blood vessels in the

nose.[29] IgA concentrations in the nasal secretions continue to increase after infection after all evidence of exudation has disappeared.[30] This may reflect the production and active secretion of virus specific IgA, which is known to appear after a delay of at least 10 days. The delayed appearance of specific antiviral and non-specific IgA may be due to virus induced downregulation of IgA production by mucosal plasma cells. Hence the early IgA response is confined to IgA originating from plasma exudation.

Total protein was more closely correlated with total IgA than with secretory IgA. This reflects the fact that total IgA concentrations depend on plasma exudation, as does the total protein, whereas secretory IgA is independent of the exudate as it is produced locally and actively secreted.

The lack of a secretory IgA response to infection may imply that nasal secretory IgA has a less important role in initial host defence

against an acute viral infection than do immunoglobulins that reach nasal secretions via the plasma exudate. This probably only applies to primary viral infection of the respiratory mucosa as secondary subclinical infection may be associated with increased local mucosal production of specific and total IgA. It has been proposed that plasma exudation should be considered a normal and important part of specific first line defence in the airways, rather than a secondary effect of vascular mucosal leakage due to damage to the epithelial lining.[31] The importance of serum immunoglobulins to defence against viral respiratory infections is confirmed by the reduction in respiratory symptoms in patients with primary agammaglobulinaemia after regular infusions of IgG,[32] and the reduction in viral shedding in infants with respiratory syncytial virus infection given intravenous immunoglobulin.[33]

Another less likely but possible explanation for our failure to detect an increase in secretory IgA concentrations lies in the differences in the antisera used in the two ELISA assays. In the secretory IgA assay the antiserum captures only IgA that is bound to the secretory component (in addition to any free secretory component present), whereas the antiserum used in the total IgA assay will detect all forms of IgA as it binds to the α chain. Before secretion from the mucosal plasma cells, monomeric IgA complexes with a glycoprotein J chain to become dimeric. Secretory component is synthesised within the mucosal epithelial cells where it binds to dimeric IgA and facilitates active transport of the functional secretory IgA onto the mucosal surface. During the inflammatory state it is possible that the airway epithelium no longer forms a tight barrier.[31] This would allow exudation of dimeric IgA that is not bound to secretory component into the mucosal secretions. This locally produced dimeric IgA would be measured in our total IgA assay, but would not be detected as secretory IgA due to the lack of secretory component.

In all instances the episodes of wheezing were associated with clinical features suggesting an infection of the upper respiratory tract, and in 66% a virus was isolated at the time, even though we did not make extensive attempts at viral isolation. Virus positive and virus negative children were clinically indistinguishable and there was no difference in their IgA responses, so we have assumed viral aetiology even when a virus was not isolated. This assumption is supported by previous work on the aetiology of respiratory infections accompanying acute wheezing in infants.[1 2]

We used patient's siblings as the normal comparison group as it was likely that they would be infected simultaneously with the same virus as their wheezy sibling. Unfortunately, not as many siblings were enrolled as we had hoped for, as there was reluctance by some parents to let their non-wheezy children undergo nasal lavage. There was also a difference in age between the two groups. This should not influence our interpretation of the results as nasal IgA response has been shown to be independent of age in children under 4 years.[13]

Atopy is difficult to determine in infancy so conclusions could not be made about differences in nasal IgA response between atopic and non-atopic children. There were three children with high serum IgE but this is too few to make statistical comparisons. We did find that the seven children with eczema had a less marked IgA response than the others, but eczema alone is not an accurate marker of atopy as it is easily confused with other common infant rashes. Skin testing was not performed as in this age group results are often inconclusive unless a strong reaction is elicited. Long term follow up will determine which of our patients can be defined as atopic.

The true nasal IgA response was determined by using inulin in the nasal wash solution as an exogenous marker of dilution. Previous studies that have not used markers could only express the IgA as a ratio to total protein.[7 17] This may be misleading because when the ratio of two substances changes it is impossible to distinguish which has increased or decreased. In our own study, we might have concluded that wheezy infants had a relative deficiency of nasal IgA when looking at the IgA to total protein ratios, yet the absolute IgA concentrations were normal when expressed per volume of nasal secretion.

We chose inulin because it is inert, safe, and easy to measure. A potential problem, however, is that it is a small molecule which may be absorbed by the nasal mucosa, giving an overestimate of the proportion of secretions in the lavage fluid and hence an underestimate of IgA concentrations. When comparing IgA concentrations during infection and health it is possible that the amount of inulin absorbed varied depending on the inflammatory state and permeability of the nasal mucosa. Radiolabelled albumin is said to be a more reliable marker[34] but is unacceptable to most parents despite the low radiation levels, and using urea as an endogenous marker is unreliable in the nose.[35]

A problem with measuring IgA in nasal secretions is that the timing of sampling is critical. IgA concentrations depend on the length of time from the onset of infection, increasing over two to three weeks. Although the history of symptoms is a guide, it remains uncertain when the child acquired the infection. From the detailed history, however, there were minimal differences in mean times from onset of symptoms to initial sampling between the wheezing infants and siblings. The children who had lavage performed over two episodes of wheezing had an apparently lower IgA response with the second episode. This may be because they were sampled sooner after the onset of the second infection. Marked day to day variability of nasal IgA has been shown in children who had repeated sampling over one year.[13] This would introduce an element of chance in terms of measuring the baseline IgA concentration in any study and this was confirmed by the high coefficient of repeatability when we made repeat measurements of non-infected IgA levels in seven children.

We studied IgA responses in the nose as nasal lavage is less traumatic than bronchoalveolar lavage and it is assumed that the study of secretions in the nose serves as a model for lower

airway secretions.[29][36] Caution is warranted, however, as although this may be true, evidence of correlation is lacking and although IgA is predominant in the upper respiratory secretions, it is IgG that is found in greatest concentration further down the tracheobronchial tree.[37][38] This shows differences in the relative importance of IgG and IgA to host defence at different sites along the airways and may reflect variability in IgA responses throughout the respiratory system.

In conclusion, we have found that infants who wheeze with viral respiratory infections do not have a major deficit of nasal IgA production, nor do they show a hyperactive mucosal humoral immune response. We have found that the increased nasal total IgA concentrations during early infection were not due to increased production of secretory IgA but were due to increased plasma exudation.

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