PostScript

LETTERS

Response to: Bacterial co-infection and the interpretation of immunological data from BAL fluid specimens in severe RSV bronchiolitis (*Thorax* 2006;61:1098)

Eisenhut expresses a valid consideration that pertains to many of the studies investigating inflammatory mediators in respiratory syncytial virus (RSV) bronchiolitis. Many RSV immunopathogenesis studies presume a pure RSV aetiology. Co-infection (viral and/or bacterial) in severe bronchiolitis is not uncommon.¹⁻³ A phagocytic (neutrophils and macrophages) and natural kill cell response is common to both viral and bacterial lower respiratory infections.^{4,5}

Analysis of the blood differential leucocyte counts in our study group failed to discriminate between the RSV-only and bacterial co-infection groups (Mann-Whitney-Wilcoxon test: neutrophils $p = 0.2$; lymphocytes $p = 0.2$; monocytes $p = 0.9$), as did the C-reactive protein ($p = 0.9$).³ However, we only evaluated total number of leucocytes/white cells, bacterial growth density and microorganism species in the broncho-alveolar lavages, and not white cell type. Therefore we are unable to comment on potential differences in concentrations of lower respiratory tract neutrophils and alveolar macrophages between the groups.

The interaction between the phagocytic effector cells, the pathogen and endogenous immune molecules orchestrate the inflammatory response.³ Cytokine and chemokine reactions can therefore be customised by the triggering pathogen(s).⁶ Consequently it is vital to identify the correct perpetrator when assigning responsibility for these inflammatory effects.

K Thorburn

Paediatric Intensive Care, Royal Liverpool Children's Hospital, Eaton Road, Liverpool L12 2AP, UK; kent.thorburn@rlc.nhs.uk

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Assessing fitness to fly in young infants and children

Commercial aircrafts cruise between 9150 and 13000 m above sea level, with a cabin pressure equivalent to 1530-2440 m at which passengers breathe the equivalent of 15-17% of fractional inspired oxygen (FiO_2) at sea level.

British Thoracic Society recommendations for passengers with chronic respiratory disorders planning air travel¹ suggest that infants and young children unable to perform spirometry, with a history of neonatal respiratory disease consult a paediatrician and a hypoxia test be considered. The recommended hypoxia test method in young children is to place the child in a body plethysmograph while seated on the parent's lap and introduce nitrogen until FiO_2 equals 15%. This method relies on equipment not readily available outside the tertiary hospital setting. We have reported a review of hypoxia testing in infants and young children with a history of neonatal lung disease.²

In our study, the hypoxia test was performed by exposing the children to high flow (15 l/min) 14% oxygen in nitrogen (Air Liquide, Perth, Western Australia, Australia), via a mask, for 20 min. Here we report the validation of this hypoxia test method in a group of infants and young children.

Seven children aged 2-51 months (3 boys) underwent a hypoxia test as described earlier. In addition, a gas sampling line was fixed under the nares of the child, permitting real-time recording of respired gas concentrations (Sensormedics Spectra V.12.3; Viasys, Yorba Linda, California, USA). A two-point calibration procedure encompassing the respirable range was performed before testing and accepted at an accuracy of 0.05%. Once the hypoxia test commenced, two separate periods of 30 s of quiet breathing were recorded and results saved for retrospective analysis. The median FiO_2 for each inspiration was obtained and median FiO_2 for all breaths calculated for each child. Table 1 shows the anthropometric and FiO_2 data. The study was approved by the

Princess Margaret Hospital ethics committee and parental written consent obtained.

Group median FiO_2 is comparable to the 15-17% encountered at cruising altitudes. Several factors may have influenced our median FiO_2 values. The mask applied to the infant does not provide a tight-fitting seal, and room air entrainment, proportional to inspiratory flow, may result in increased FiO_2 . We attempted to account for this influence by using a challenge gas of 14% O_2 , a gas concentration lower than recommended, and a high delivery flow of 15 l/min. Two infants had low median FiO_2 , with values of about 13%. After the investigation of these children, it was noted the median FiO_2 was 13.5% (standard deviation 0.2%), giving a potential lower limit of 13.3%. Follow-up with the gas supplier showed that the normal titration procedure used during cylinder filling has an error of 0.5%, with the final concentration confirmed with an error of 0.2%. Despite these variations in FiO_2 , the median FiO_2 of 15.1% equates well to the upper limit of cruising altitude. Although the confirmation that hypoxia challenge tests mimic the hypobaric conditions experienced during flight has been reported in adults,³ a study on adolescent children with cystic fibrosis found that the hypoxia test only poorly predicted subsequent in-flight oxygen requirements.⁴ Hypoxia tests on infants and young children have yet to be validated as reliably reproducing the hypobaric conditions experienced during air travel, and further work in this discipline is urgently required.

In summary, we present a simple, easily-applied hypoxia test method for use in infants and young children, which produces a median FiO_2 similar to that encountered during flight.

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G L Hall

School of Paediatrics and Child Health, University of Western Australia, Perth, Western Australia, Australia

M Verheggen, S M Stick

Department of Respiratory Medicine, Princess Margaret Hospital for Children, Perth, Western Australia, Australia

S M Stick

School of Paediatrics and Child Health, University of Western Australia, Perth, Western Australia, Australia

Correspondence to: Dr G Hall, Department of Respiratory Medicine, Princess Margaret Hospital, GPO Box D184, Perth, WA 6840, Australia; graham.hall@health.wa.gov.au

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Competing interests: None.

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Table 1 Anthropometric and fractional inspired oxygen concentrations

Participant	Age (months)	Sex	History	FiO_2 (%)*
1	51	F	ILD	15.7 (12.87-16.58)
2	41	M	H	15.04 (13.19-17.08)
3	4	F	H	13.42 (12.98-13.71)
4	10	F	H	13.14 (11.96-14.55)
5	2	M	H	15.66 (12.79-16.59)
6	11	M	BPD	16.05 (14.44-16.58)
7	3	F	H	15.08 (12.73-16.59)
Group	10 (2.2-49.0)			15.08 (13.19-15.98)

BPD, bronchopulmonary dysplasia; F, female; FiO_2 , fractional inspired oxygen concentration; H, healthy; ILD, interstitial lung disease; M, male.

*Continuous data are expressed as median (10th-90th centiles).

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Toll-like receptors 2 and 4 and innate immunity in neutrophilic asthma and idiopathic bronchiectasis

We read with interest the article by Simpson *et al*¹ relating parameters of innate immunity, particularly expression of toll-like receptors (TLR)2, TLR4, CD14, SP-A and cytokines interleukin (IL)8 and IL1 β , to disease in neutrophilic asthma and bronchiectasis. We agree that there is much to be gained from further analysis of innate mechanisms in both diseases. This is especially so in light of our findings implicating a role for natural killer (NK) cells in idiopathic bronchiectasis and the work from Umetsu's laboratory (Harvard Medical School, Boston, USA) on NKT cells in asthma.^{2,3}

It may be useful to add one caveat and additional data to the case for innate mechanisms in neutrophilic asthma and idiopathic bronchiectasis.

Although it is self-evident that TLR2, TLR4 and CD14 activation may well indicate differences in endotoxin stimulation, considerable attention has recently focused on expression of TLR2 and TLR4 by T lymphocytes. This has implications both for the interface between innate and adaptive immunity, and for the regulation of the T cell response itself: a body of evidence now suggests that TLR2 is expressed by regulatory T cells (Treg), that TLR2 activation has a role in driving Treg expansion and that, in some cases, the ligands driving this may be endogenous rather than microbial.^{4,5} In the light of such findings, it is important not to interpret TLR activation solely in terms of innate, microbial activation, particularly if it has not been possible to define the cell type responsible for the increase in mRNA. Put simply, changes in expression of TLR2 and TLR4 can, in some cases, bear on alterations in populations of regulatory and effector T cells, rather than differences in microbial exposure. In the sputum samples analysed by Simpson *et al* from patients with asthma, the relatively low lymphocyte counts might make this unlikely; in their bronchiectasis samples, where the lymphocyte counts are higher, it is impossible to clarify the situation without further analysis—for example, by multiparameter flow cytometry.

Simpson *et al* ask whether polymorphisms in TLRs may account for different phenotypes observed in disease studies. Single nucleotide polymorphisms of TLRs have been linked to susceptibility to infectious diseases.⁶ With such a hypothesis in mind, we recently compared the frequency of the TLR2 Arg753Gln and the TLR4 Asp299Gly and Thr399Ile polymorphisms in patients with idiopathic bronchiectasis and controls. The Asp299Gly TLR4 polymorphism (and the co-segregating Thr399Ile polymorphism) is, as indicated by Simpson *et al*, proven to be bona fide, with respect to both effect on endotoxin binding and disease associations. Similarly, the TLR2 Arg753Gln polymorphism is functional and has been associated with altered susceptibility to several infectious diseases including herpes simplex virus, cytomegalovirus and rheumatic

Table 1 Toll-like receptor (TLR)2 and TLR4 restriction fragment length polymorphism analysis*

Gene polymorphism	Restriction enzyme	Restriction fragment length (bp)	Primer sequence
TLR2 Arg753Gln	Msp 1	Wild type (G allele): 104 + 25 Arg753Gln (A allele): 129	F: cattccccagcgcttctgcaagctcc R: ggaacataggaactatcagcagctc
TLR4 Asp299Gly	Nco 1	Wild type (A allele): 188 Asp299Gly (G allele): 168 + 20	F: agcatactagactactactccatg R: gagagatttgagttcaatgtggg
TLR4 Thr399Ile	Hinf 1	Wild type (C allele): 124 Thr399Ile (T allele): 98 + 26	F: gggtgtctgttcaaaagtgtttgggagaa R: ggaaatccagatgttctagtgttctaagcc

Gene polymorphism	Allele	Controls n = 86 (TLR2) n = 85 (TLR4)	Idiopathic bronchiectasis n = 94	Odds ratio (95% CI)	p Value
TLR2 Arg753Gln	G allele	169 (98.2)	180 (95.7)	—	—
	A allele	3 (1.8)	8 (4.3)	2.50 (0.65 to 9.59)	NS
TLR4 Asp299Gly	A allele	162 (95.3)	177 (94.1)	—	—
	G allele	8 (4.7)	11 (5.9)	1.26 (0.49 to 3.21)	NS
TLR4 Thr399Ile	C allele	163 (95.9)	177 (94.1)	—	—
	T allele	7 (4.1)	11 (5.9)	1.45 (0.56 to 3.82)	NS

F, forward; R, reverse; TLR, toll-like receptor.

The table shows the frequencies of TLR2 and TLR4 polymorphisms in patients with idiopathic bronchiectasis and in controls.

*As described previously by Folwaczny *et al*.⁷

fever. The polymorphism was initially described in the context of a possible enhanced risk of staphylococcal sepsis.⁶

A total of 94 unrelated individuals with a diagnosis of idiopathic bronchiectasis recruited at the Royal Brompton Hospital, London, UK, and 86 heart/lung transplant donor controls from the Harefield Hospital, London, UK, were studied. The ethics committee of the Royal Brompton & Harefield & NHLI approved the study and all patients gave written informed consent for participating in the study. A diagnosis of idiopathic bronchiectasis was made where there was bilateral, predominately lower lobe bronchiectasis on CT, chronic rhinosinusitis and all known underlying causes had been excluded.² Genomic DNA was extracted from peripheral blood using a high-salt technique. Typing of the TLR2 Arg753Gln, TLR4 Asp299Gly and TLR4 Thr399Ile polymorphisms was carried out using polymerase chain reaction restriction fragment length polymorphism analysis as described previously. Allele frequency was determined and statistical analysis carried out using the Simple Interactive Statistical Analysis. Allele frequency comparisons were made using the Fisher's exact test. Only minor differences in gene frequency were observed, none of which were statistically significant (table 1).

In summary, we make the following points, building on the case made by Simpson *et al*. First, although it seems likely that the pathogenesis in both asthma and idiopathic bronchiectasis has a strong innate immunity component, it is important to note that the components contributing to TLR changes can be complex, including events at the innate-adaptive immune response interface, as well as interactions between effector T cells and Tregs. Specifically, it can be hard to interpret increases in the expression of TLR in the absence of data on the particular cell type(s) accounting for the change. Second, faced with clinical phenotypes of this level of complexity, it is perhaps not surprising that, as has been the case in analysis of sepsis and other complex disease end points, the effects of TLR polymorphisms are not striking.

C Reynolds

Lung Immunology Group, National Heart & Lung Institute, Imperial College, London, UK

L Ozerovitch, R Wilson

Department of Respiratory Medicine, Royal Brompton and Harefield NHS Trust, London, UK

D Altmann

Human Disease Immunogenetics Group, Department of Infectious Diseases and Immunity, Imperial College, London, UK

R Boyton

Lung Immunology Group, National Heart & Lung Institute, Imperial College, London, UK

Correspondence to: Dr R Boyton, Lung Immunology Group, National Heart & Lung Institute, Sir Alexander Fleming Building, South Kensington Campus, Faculty of Medicine, Imperial College, London SW7 2AZ, UK; r.boyton@imperial.ac.uk

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