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Enhanced IL-4 responses in children with a history of respiratory syncytial virus bronchiolitis in infancy

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ABSTRACT: Infants who recover from respiratory syncytial virus (RSV)-induced bronchiolitis are at high risk of developing asthma and recurrent wheezing. It is not known whether severe RSV infection itself causes persistent effects or is a marker of a "wheezy" predisposition. To determine the long-term immunological correlates of infantile bronchiolitis, interleukin (IL)-4 and interferon (IFN)-γ responses to a panel of antigens were studied in a well-characterised cohort of 7–8-yr-old children with a history of severe RSV bronchiolitis in infancy.

Peripheral blood lymphocytes from 37 children who were hospitalised with RSV bronchiolitis in infancy and from 69 age-, sex- and location-matched controls were stimulated *in vitro* with RSV, house-dust mite, birch and cat antigens. Cellular proliferation, and enzyme-linked immunoSPOT IFN-γ and IL-4 production were measured.

IL-4 producing T-cells responding to RSV and cat antigens were significantly more frequent in exbronchiolitics. Other responses (including the IFN-γ response to RSV) were equally strong in exbronchiolitics and controls.

Respiratory syncytial virus infection primes memory T-cells that make interferon-γ, but virus and aeroallergen-specific and interleukin-4 producing T-cells are also frequently primed in bronchiolitics. Respiratory syncytial virus bronchiolitis in infancy may increase the risk of allergic sensitisation by providing a local interleukin-4-rich environment, in which airborne allergens are first encountered.

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Most children are infected with respiratory syncytial virus (RSV) in the first year of life, but only 0.5–2% suffer severe respiratory tract disease. RSV causes ~70% of cases of viral bronchiolitis [1], and recurrent wheeze is frequent in children after recovery [2]. In some studies, the risk of asthma, atopy and allergy *i.e.* the propensity to allergic sensitisation, also appears to be increased in exbronchiolitics [3, 4].

Several mechanisms could explain the association between RSV bronchiolitis, wheezing and atopy. There is convincing evidence from animal studies linking interleukin (IL)-4 production with enhanced lung disease during RSV infection [5]. Such type 2 cytokine responses are also a feature of asthma and atopy [6]. Serum eosinophil cationic protein is raised in children with asthma and in children with acute bronchiolitis, suggesting degranulation of activated eosinophils in both conditions [7].

Mice exposed to airborne antigen during acute viral lung infections develop enhanced sensitisation to the aeroantigen, and show anaphylactoid responses to antigen challenge. Mice exposed to antigen in the absence of viral infection show no such sensitisation [8, 9].

In the present study, the hypothesis that severe RSV bronchiolitis in infancy is associated with enhanced IL-4 responses to RSV in childhood was tested. Single cell production of interferon (IFN)-γ, IL-4 and proliferative responses to RSV antigens and nonviral allergens were measured *in vitro* in peripheral blood cells from 7–8-yr-old children with and without a history of hospitalisation due to bronchiolitis. The results show antigen-specific enhancement of IL-4 production in children with a history of bronchiolitis which may explain the association between bronchiolitis, sensitisation to aeroallergens and recurrent wheezing in later childhood.

Methods

Study participants

Recruitment has been described previously [3]. Briefly, 47 hospitalised children with immunoassay verified RSV bronchiolitis were recruited at a mean age of 116 days (range 30–307 days). These index cases and controls, matched for age, sex and location were followed prospectively and restudied at age 1, 3 and 7.5 yrs. The ethics committee of the medical faculty of Gothenburg University approved the study. Informed consent in writing was obtained from all parents.

All 140 children reported for the 7.5 yr follow-up; 46 children in the RSV bronchiolitis group and 89 controls also attended for clinical examination.

Clinical definitions

These have been described previously [3, 4]. Briefly, asthma was defined as at least three episodes of episodic bronchial obstruction verified by a physician. Wheeze was defined as a history of any kind of bronchial obstructive symptoms with or without verification by a physician, asthma included [4].

Peripheral blood mononuclear cells

Blood was obtained from 41 cases and 79 controls who consented to the extended venous blood sampling required for this study. Peripheral blood mononuclear cells (PBMC) were separated from 5–10 mL heparinised venous blood by sedimentation on a Ficoll-Hypaque gradient. PBMC were resuspended in 90% human serum and 10% dimethyl sulfoxide (DMSO) and cryopreserved in liquid nitrogen. All samples were coded so that the investigator performing the proliferation and enzyme-linked immunoSPOT (ELISPOT) assays could not know their clinical status. After thawing, 14 samples were rejected before unblinding and statistical treatment because of poor recovery (<50% viable cells upon thawing), lack of response to phytohaemagglutinin-L (PHA) or nonspecific responses. Of 106 samples with analysable responses, 37 were index cases and 69 were controls. The mean age of the index group was 7.5 yrs (90% range 7.2–7.8 yrs), of which 21 were female. The mean age of the control group was 7.5 yrs (90% range 7.1–7.8 yrs), of which 39 were female. The mean age at which index cases included in the analysis developed RSV infection was 112.8 days (90% range 4–256 days). The age at which RSV infection first occurred in controls was not ascertained. Nine of the 37 exbronchiolitics and 19 of the 69 controls had indoor cats in the family during the first year of life.

Antigens

FG recombinant fusion protein comprising amino acids 1–526 of F (RSV SS2) and amino acids 69–298 of G (RSV A2) (patent WO 98/18819) was kindly provided by SmithKline Beecham Biologicals (Rixensart, Belgium). Tetanus toxoid was purchased from Calbiochem (San Diego, CA, USA). PHA was purchased from Sigma (Sigma-Aldrich, Gillingham, Dorset, UK). Der p (mite), Bet v (birch) and Fel d (cat) antigens were kindly donated by ALK Abelló (Hørsholm, Denmark). RSV A2 virus was grown in HEp-2 cells. RSV was inactivated by exposure to 1.2 joules of ultraviolet (UV) radiation in a Stratalinker 2400 (Stratagene, Europe, Amsterdam, the Netherlands). Both virus and cells were mycoplasma free as assayed by GEN-PROBE test kits (GEN-PROBE Incorporated, San Diego, CA, USA).

Proliferation assay

Thawed PBMC were incubated in round bottom 96-well plates in triplicate cultures at $5\times10^5\cdot mL^{-1}$ in 200 μL Roswell Park Memorial Institute (RPMI) 1640 medium with 5% human serum AB, L-glutamine, penicillin and streptomycin supplements and optimal concentrations of antigens (determined in pilot experiments). These were: recombinant, chimeric F and G glycoprotein of RSV (FG): 1 $\mu g\cdot mL^{-1}$, tetanus toxoid: 1 $\mu g\cdot mL^{-1}$, PHA: 10 $\mu g\cdot mL^{-1}$, Der p: 50 $\mu g\cdot mL^{-1}$, Bet v: 50 $\mu g\cdot mL^{-1}$, Fel d: 50 $\mu g\cdot mL^{-1}$. RSV stock grown in HEp-2 cells and equivalent amounts of mock-infected HEp-2 cells used as controls were UV irradiated and included at a nominal multiplicity of infection of five. After 7 days ^{3}H-thymidine was included for the last 18 h at 1 microcurie ($\mu Ci)\cdot well^{-1}$. Proliferation was expressed as a stimulation index.

Enzyme-linked immunoSPOT assays for interferon-γ and interleukin-4

Thawed PBMC were stimulated as described for proliferation assays, at densities of 2.5×10^5 and 1.5×10^6 cells$\cdot mL^{-1}$ for IFN-γ and IL-4 ELISPOTs, respectively, in Millipore MAIP S45 10 Immobilon plates precoated with antihuman IFN-γ antibody 1-D1K or antihuman IL-4 clone 82.4 (Mabtech AB, Nacka, Sweden). After 48 h at 37°C, the wells were washed extensively with phosphate-buffered saline (PBS) and distilled water to remove all cells, then incubated at room temperature for 4 h with 1 $\mu g\cdot mL^{-1}$ of biotinylated antibodies clone 7-B6-1 and clone 12.1 (antiIFN-γ and IL-4, respectively; Mabtech). After further washes, 100 μL streptavidin-alkaline phosphatase conjugate (Sigma E-2636) diluted 1:1000 in PBS was added for 2 h; after further washes, 100 $\mu L\cdot well^{-1}$ of bromochloroindoil phosphate/nitro blue tetrazolium (BCIP/NBT) substrate (Sigma B-5655) was added for 15 min and the reaction stopped by washing in tap water. Spots were counted manually with a dissection microscope at a magnification of 25x. Proliferation assays and ELISPOT assays were run in parallel. All observations were performed in a blinded fashion on coded samples.

Statistical analysis

The proportions of individuals with asthma or wheeze were compared between exbronchiolitics and control groups using Fisher's Exact Test. Median cytokine ELISPOT responses in exbronchiolitics and controls were compared using the nonparametric Mann-Whitney U-test adjusted for ties. Relationships between frequencies of cells producing different cytokines were evaluated using Spearman's correlation coefficient. Logistic regression analysis was employed to evaluate the associations between each atopic disease and each cytokine response. Nested logistic regression models were compared to investigate the involvement of IL-4 and IFN-γ responses in the association between severe RSV bronchiolitis in infancy and development of asthma and wheeze.

Results

Within the subset of 106 children evaluated in this study, current prevalence of asthma (1997) was 27% in index children (*i.e.* exbronchiolitics) and 1% in controls ($p<0.0005$), while current prevalence of wheeze was 46% in index cases and 1% in controls ($p<0.0005$). Current allergic sensitisation, as measured by skin-prick test and serum IgE antibodies to common inhaled allergens, was seen in 43% in the RSV group and 25% of the controls (p=0.08).

Interleukin-4 responses

Frequencies of IL-4 secreting cells to antigens ranged between 0 and 340·million^{-1}·PBMC^{-1} (excluding responses to PHA, which were often too high to quantify). The highest mean frequencies were 36.4 and 17.6·million^{-1}·PBMC^{-1} for tetanus toxoid and RSV antigens, respectively.

Comparison of cytokine responses in index cases and controls showed significantly higher frequencies of RSV-specific cells producing IL-4 in index cases (Mann-Whitney p=0.04) (fig. 1). Stratification of the two groups by level of response (defined arbitrarily after examination of the distribution of the data as: negative; low: ⩽10 IL-4 producing cells·million^{-1}·PBMC^{-1}; high: >10 IL-4 producing cells·million^{-1}·PBMC^{-1}) showed that only 28.6% of controls were high responders, compared to 43.2% of the index cases (fig. 2).

IL-4 responses to Fel d were also significantly lower in controls than in bronchiolitics (Mann-Whitney p=0.04), while differences in the response to FG were not significant (Mann-Whitney p=0.5). There was no relationship between the presence of a cat at home during the first year of life and IL-4 responses to Fel d in the whole group of 106 children although there was a nonsignificant trend towards higher levels of IL-4 responses to Fel d in the exbronchiolitic children with a cat at home (data not shown).

Index cases and controls did not differ significantly for IL-4 responses to other unrelated antigens, such as tetanus toxoid, Der p and Bet v (data not shown).

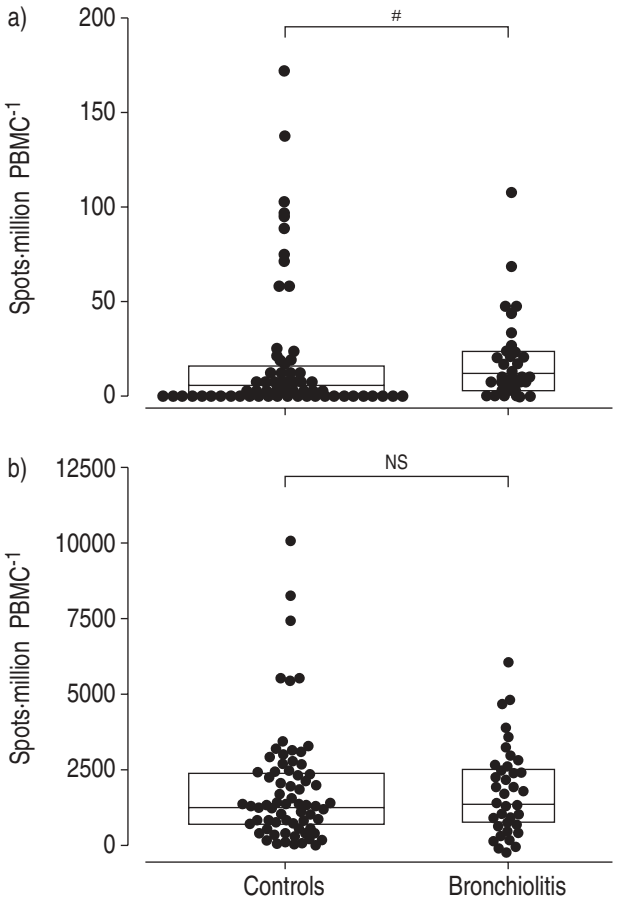

Fig. 1. – Frequencies of respiratory syncytial virus (RSV)-specific T-cells producing a) interleukin-4 or b) interferon-γ. Peripheral blood mononuclear cells (PBMC) were stimulated with ultraviolet irradiated RSV and incubated in enzyme-linked immunoSPOT assays. The median frequencies of cytokine producing cells are indicated by horizontal lines, while the boxes indicate the Q1–Q3 interquartile range. Index cases (n=37) and controls (n=69) were compared using the Mann-Whitney U-test adjusted for ties. #: p=0.04. NS: nonsignificant.

More than 60% of individuals had high frequencies of tetanus toxoid-specific IL-4-secreting T-cells, with only ~15% of nonresponders, but these were similar in index cases and controls (fig. 2).

Interferon-γ responses

The frequency of IFN-γ secreting cells was about 100-fold higher than that of IL-4 secreting cells (maximal responses were seen with RSV stimulation) but did not correlate with the case/control status or asthma (fig. 1). Recombinant chimeric FG (which contains only a subset of RSV epitopes) stimulated a higher percentage of T-cells producing IFN-γ than IL-4, but this was not significantly different between exbronchiolitics and controls (data not shown). Frequencies of RSV specific cells producing IL-4 or IFN-γ were not significantly correlated over the total study population (Spearman's correlation coefficient= 0.17, p=0.08, data not shown). As expected, there was a good correlation between IFN-γ responses to RSV

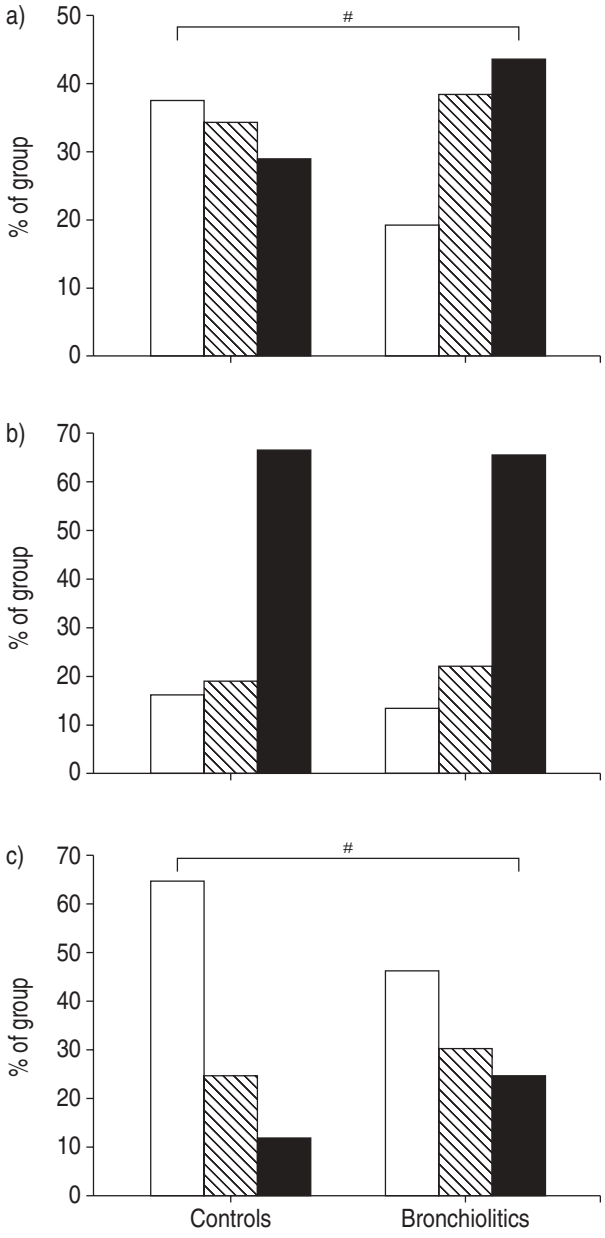

Fig. 2. – Distribution of interleukin (IL)-4 responders in bronchiolitics (n=37) and controls (n=69). Peripheral blood mononuclear cells (PBMC) were stimulated with a) respiratory syncytial virus (RSV), b) tetanus toxoid and c) Fel d, and the frequency of IL-4-secreting cells was measured in enzyme-linked immunoSPOT assays. Individuals were categorised as negative (no IL-4 spots, □), low responders (1–10 IL-4 spots·million·PBMC^{-1}, ▨) or high responders (>10 IL-4 spots·million·PBMC^{-1}, ■). $^{\#}$: p=0.04.

and to FG (Spearman′s correlation coefficient=0.72, p<0.00005).

Involvement of interleukin-4 and interferon-γ responses in the enhanced risk of asthma and wheeze in exbronchiolitics

Logistic regression analysis was used to investigate the involvement of IL-4 and IFN-γ responses to viral antigens and allergens in the correlation between previous RSV bronchiolitis and asthma and wheeze. Two nested models were compared. In model one ("unadjusted" analysis) the odds ratio (OR) for asthma was separately correlated with previous bronchiolitis or an increase in the frequency of IL-4 and IFN-γ producing cells specific for RSV, FG, Der p, Bet v and Fel d. In model two ("adjusted" analysis), the OR for asthma is related to the combined effects of previous bronchiolitis and cytokine responses. A reduction in the effect of bronchiolitis in model 2 compared to its effect in model 1 would imply that the cytokine included in model 2 can account for a portion of the unadjusted association between bronchiolitis and asthma. Just how much of the association that cytokine is able to account for is indicated by the size of the reduction.

In agreement with previous studies [3, 4], the unadjusted analysis showed an OR of 25.19 (95% confidence interval (CI) 3.07–206.73) for asthma in exbronchiolitics (table 1). In addition, 10 unit increases in the frequency of IL-4 producing T-cells specific for FG were associated with a small increase in the ORs for asthma (OR 1.05, 95% CI 0.71–1.56), suggesting that these responses have a small, not significant, effect on the risk of asthma. IL-4 responses to whole RSV were also not associated with an increased OR for asthma or wheeze. Interestingly, there was an effect of IL-4 responses to Fel d on the OR for asthma of 1.73 (95% CI 1.06–2.84) and wheeze (OR 1.70, 95% CI 1.14–2.55) (table 1). Thus higher frequencies of Fel d-specific IL-4-producing cells increase the risk of asthma or wheeze. IFN-γ responses to the same antigens did not show an effect on the OR for asthma or wheeze (table 1). Adjustment for IL-4 responses to Fel d (in model 2) reduced the bronchiolitis OR for asthma from 25.19 to 21.36. This implies that the IL-4 response to this antigen accounts for ~15% of the association between RSV bronchiolitis in infancy and asthma. The OR for wheeze was similarly decreased from 57.80 to 51.86, a reduction of ~10%. Adjustments for other IL-4 responses, and for the IFN-γ responses, provided no evidence that these responses were responsible for the bronchiolitis/asthma association.

Proliferative responses

Proliferative responses to individual antigens correlated with frequencies of cytokine-producing cells, although not for all possible comparisons (table 2). Thus IL-4 responses to RSV showed a small but significant correlation with proliferation to RSV (Spearman′s rho=0.23, p=0.02), but IFN-γ responses to RSV did not (Spearman′s rho=0.04, p=0.69). Conversely, responses to FG showed correlation between IFN-γ responses and proliferation (Spearman′s rho=0.26, p=0.007) but not between IL-4 responses and proliferation (Spearman′s rho=0.15, p=0.13). By contrast, tetanus toxoid induced very strong proliferative responses, which correlated with high frequencies of IL-4-secreting cells (rho=0.65, p<0.00005) and with the frequency of IFN-γ producing cells (rho=0.30,

Table 1. – Unadjusted effects of cytokine responses and bronchiolitis on asthma and wheeze

	Asthma			Wheeze		
	OR	95% CI	p-value	OR	95% CI	p-value
IL-4 FG	1.05	(0.71–1.56)	0.81	1.08	(0.78–1.49)	0.64
IL-4 RSV	1.16	(0.77–1.77)	0.48	1.11	(0.79–1.56)	0.54
IL-4 Fel d[#]	1.73	(1.06–2.84)	0.03	1.70	(1.14–2.55)	0.01
IL-4 Bet v	1.53	(0.97–2.41)	0.07	1.35	(0.92–1.99)	0.12
IL-4 Der p	1.04	(0.63–1.73)	0.87	0.95	(0.61–1.48)	0.83
IFN-γ FG	1.00	(0.70–1.43)	0.99	1.06	(0.78–1.44)	0.70
IFN-γ RSV	1.25	(0.65–2.40)	0.51	1.17	(0.71–1.93)	0.55
IFN-γ Fel d	1.09	(0.79–1.50)	0.61	0.91	(0.72–1.16)	0.45
IFN-γ Bet v	0.87	(0.67–1.13)	0.30	0.82	(0.66–1.02)	0.08
IFN-γ Der p	1.13	(0.85–1.52)	0.40	1.03	(0.83–1.29)	0.78
Bronchiolitis[#]	25.19	(3.07–206.37)	0.003	57.80	(7.24–461.44)	0.0005

Logistic regression analysis was used to identify the odds ratios (OR) associated with a history of bronchiolitis, or current interleukin (IL)-4 or interferon (IFN)-γ responses of peripheral blood mononuclear cells stimulated with the viral antigens FG, respiratory syncytial virus (RSV), and airborne allergens Fel d, Bet v and Der p. The ORs shown represent the increase in odds for a one log unit increase of antigen specific IL-4 secreting cells or IFN-γ secreting cells. CI: confidence interval. [#]: significant risk factors ($p<0.05$).

Table 2. – Correlations between proliferative and interleukin (IL)-4 or interferon (IFN)-γ responses

Antigen	IL-4		IFN-γ	
	rho	p-value	rho	p-value
FG	0.15	0.13	0.26[#]	0.007[#]
RSV	0.23[#]	0.019[#]	0.04	0.69
Fel d	0.07	0.47	0.26[#]	0.008[#]
Bet v	0.09	0.35	0.15	0.13
Der p	0.20[#]	0.040[#]	0.07	0.47
TT	0.65[#]	0.00005[#]	0.30[#]	0.002[#]

Correlations between proliferative responses stimulation index (SI) and IL-4 or IFN-γ responses (frequencies of cytokine secreting cells) were evaluated using Spearman's rho correlation coefficient. RSV: respiratory syncytial virus; TT: tetanus toxoid. [#]: significant correlations ($p<0.05$).

p=0.002). Proliferative responses did not show significant differences between exbronchiolitics and controls, with the only exception of responses to Bet v, which were significantly stronger in controls (exbronchiolitics: median=2.80, interquartile range (IQR) (1.02–5.92) 90% range (0.56–12.33); controls: median=4.80, IQR (1.86–7.81) 90% range (0.42–22.24); Mann-Whitney U-Test, cases *versus* controls: p=0.025).

Discussion

The studies reported here show that 7–8-yr-old children with a history of RSV bronchiolitis in infancy, severe enough to cause hospitalisation, have enhanced frequencies of IL-4 secreting T-cells, not only to whole RSV, but also to the purified cat antigen Fel d. Using logistic regression analysis, enhanced frequencies of IL-4-secreting cells specific for Fel d were associated with small but significant increases in the asthma risk. The OR for wheeze was also significantly increased by higher IL-4 responses to Fel d. By contrast, IL-4 responses to whole RSV or FG did not correlate significantly with an increased OR for asthma or wheeze.

Previous studies of the T-cell responses to viral antigens in children with RSV bronchiolitis [10, 11] were performed in the acute phase of viral infection and are difficult to interpret because of possible concentration of relevant antiviral cells at sites of disease and depletion from the peripheral blood. This is the first study to use the ELISPOT method to count individual cytokine producing cells in exbronchiolitic children, and to demonstrate immunological differences between index cases and controls during later childhood. Not only are these studies unique in the extended follow-up period, but also in the detail in which the index cases and controls were studied [3, 4]. It is the only study so far in which both children with RSV bronchiolitis and controls were followed prospectively and in the same way from infancy.

The current observations may be explained as follows. During normal, mild RSV infection children (like mice [12]) develop a predominant T-helper (Th) 1 antiviral response that clears the virus. In this case, T-cell memory responses are dominated by IFN-γ production, rather than IL-4. However, children with severe lung disease (RSV bronchiolitis) not only develop strong IFN-γ responses, but also IL-4 responses accompanied by excessive pulmonary accumulation of antiviral T-cells with peribronchial infiltration and respiratory impairment. After resolution, both IL-4 and IFN-γ producing T-cells are maintained in the peripheral T-cell pool, recirculating in the blood and forming part of the memory T-cell response boosted by reinfections with RSV. It is these T-cells that were detected using ELISPOT assays on 7–8-yr-old children.

The enhanced IL-4 responses to cat allergen in the exbronchiolitics are harder to explain. Only a non-significant trend towards higher levels of IL-4 responses in the exbronchiolitics with a cat at home during the first year of life was found, compared to exbronchiolitics without a cat at that time. It could be that in humans (as in mice [8, 9]) the inflammatory

environment in which inhaled antigen is first encountered influences the immunological memory response to that antigen. Since RSV infection appears to induce unusually prolonged pulmonary inflammation [13], it is possible that postbronchiolitic children are sensitised to inhaled antigens more efficiently than those who do not suffer from viral bronchiolitis. The implication from logistic regression analysis is that currently enhanced IL-4 responses to cat allergen and FG are contributing a significant share of the association between previous RSV bronchiolitis and asthma or wheeze. It is possible that IL-4 responses to other environmental antigens, which were not tested, might account for more or all of the association. In this scenario, early RSV bronchiolitis would be the crucial factor biasing the cytokine response to antigens encountered subsequently. Given the wide CI (due to low numbers of children developing asthma and wheeze), these findings should be confirmed in a larger study.

Proliferative responses appeared to correlate with either IFN-γ or IL-4 responses for RSV, FG, Der p and Fel d, both IFN-γ and IL-4 for tetanus toxoid and neither cytokine for Bet v. This might simply reflect the intensity of the responses elicited by the antigens. Proliferative responses were not significantly different between exbronchiolitics and controls, with the exception of those induced by Bet v, which were stronger in controls than in bronchiolitics. Given the weak responses obtained with this antigen, this finding should be investigated further.

There are two major hypotheses concerning RSV bronchiolitis and subsequent asthma and allergy [14, 15]. It is debated whether RSV bronchiolitis by itself predisposes infants to the development of asthma and allergy or if an inherent predisposition is required which gives rise to both the bronchiolitis and postbronchiolitic symptoms. Accordingly, an alternative interpretation to the current findings is that children who suffered RSV bronchiolitis have an inherent predisposition to enhanced IL-4 responses.

The authors think this unlikely for two reasons: first, family history of asthma or atopic disease did not differ between the children with RSV bronchiolitis and the controls in this study population [4]; second, equally high frequencies of IL-4-producing cells recognising tetanus toxoid were seen in index cases and controls, reflecting vaccination of Swedish children with tetanus toxoid in *alum adjuvant* at 3, 5 and 12 months of age. A general trend towards enhanced IL-4 production to all antigens in the exbronchiolitic children was not found, suggesting that the propensity to enhanced IL-4 production is specific and acquired.

Ideally, an interventional study using specific monoclonal antibody to delay RSV infection in a cohort of children should be undertaken to resolve the longstanding difficulty in demonstrating a causal role for RSV infection in initiating immunological abnormalities and in the pathogenesis of later asthma and other wheezing disorders [14, 15]. Intriguingly, there is some preliminary evidence that children in whom polyclonal antiviral antibody therapy was used to delay RSV infection have improved lung function, are less likely to take time off school because of chest problems and are less likely to become asthmatic or atopic than control children [16].

The concept that some infections in early childhood prevent asthma and atopy (the "hygiene hypothesis" [17]) is hotly debated [18, 19]. Recently, it has been shown that respiratory allergy is infrequent in military recruits who had been previously heavily exposed to orofaecal and foodborne infections [20]. By contrast, lower respiratory tract infections appear to have no protective effect [21].

In man, neonatal immune responses are skewed towards Th2 cytokine production, a pattern that matures towards predominant Th1 responses in early childhood. In children predisposed to atopic disease, this maturation is impaired or defective [22]. It may therefore be that the age at which RSV infection occurs in part determines the type of immune response that develops. Therefore, the route of infection, the site at which initial priming occurs, antigen load and the age of the host are likely to be among the factors that determine the pattern of cytokines produced by antigen specific memory T-cells.

In conclusion, the results here are compatible with the hypothesis that viral bronchiolitis in infancy enhances the risk of asthma/recurrent wheezing in later childhood by increasing the likelihood of T-helper 2 sensitisation to subsequent respiratory infections and to inhaled allergens.

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