

Peripheral blood T and B lymphocyte subpopulations in infants with acute respiratory syncytial virus bronchiolitis

Raes M, Peeters V, Alliet Ph, Gillis Ph, Kortleven J, Magerman K, Rummens JL. Peripheral blood T and B lymphocyte subpopulations in infants with acute respiratory syncytial virus bronchiolitis. *Pediatr Allergy Immunol* 1997; 8: 97–102. © Munksgaard 1997.

Most data concerning immunopathogenetic mechanisms involved in respiratory syncytial virus (RSV) infection are derived from animal studies. In infants with RSV bronchiolitis the target organ i.e. the airway is hard to explore. We looked for specific alterations in peripheral blood lymphocyte subpopulations in infants hospitalized for RSV bronchiolitis. Flow cytometric analysis with a large panel of monoclonals was performed on peripheral blood lymphocytes in thirty-two infants (mean age: 4.9 months) admitted for RSV bronchiolitis. Data collected on admission were compared with age-matched control values and also with results obtained at the end of the first week of hospitalization. Differences between age-groups (older or younger than 4 months) and between clinical subgroups (clinical severity score more or less than 6) were looked for. In the group of infants as a whole, regardless of age and clinical score the number of CD4⁺ cells on admission was significantly elevated compared to normal values for age ($p < .0001$) including a high fraction of the naive suppressor-inducer subpopulation (CD4⁺/CD45RA⁺) and a low fraction of the reciprocal memory helper-inducer subpopulation (CD4⁺/CD29⁺). Within the CD8⁺ cell population the number of T cells with cytotoxic activity (CD8⁺/S6F1⁺) was significantly elevated ($p < .0001$) as were other types of cytotoxic cells. A significant decrease ($p < .0001$) in the proportion of the precursor/suppressor-effector subpopulation (CD8⁺/S6F1[–]) was seen. Absolute numbers and percentages of CD19⁺ B cells were significantly elevated ($p < .0001$) with a significant increase in the CD5⁺ subfraction ($p < .0001$) as well as in the CD10⁺ subfraction ($p < .0001$). In the older age group immunophenotypic cytotoxicity was more pronounced with increased clinical score. During recovery the CD45RA⁺:CD29⁺ ratio tended to normalize within the CD4⁺ T cells. Within the B lymphocyte subsets significant increase in the CD19⁺/CD5⁺ fraction ($p < .05$) was seen. We conclude that there are significant changes in the number of peripheral blood lymphocyte subsets in infants with RSV bronchiolitis as compared to age-related controls. We hope that present data could be useful in further exploration of RSV immunology in humans. A possible link between RSV bronchiolitis and the subsequent development of atopy is mentioned.

**Marc Raes¹, Veerle Peeters²,
Philippe Alliet¹, Philippe Gillis¹,
Jan Kortleven², Koen Magerman²
and Jean Luc Rummens²**

¹Department of Pediatrics and the ²Clinical Laboratory of Hematology and Immunology, Virga Jesse Ziekenhuis, Hasselt, Belgium

Dr. M. Raes, Department of Pediatrics, Virga Jesse Ziekenhuis, Stadsomvaart 11, B-3500 Hasselt, Belgium

Key words: atopy; infants; peripheral T and B lymphocytes; RSV bronchiolitis

Received November 15, 1996
Accepted May 26, 1997

The immune response to respiratory syncytial virus infections has been studied for many years. Cell-mediated immunity to RSV has recently been evaluated in animal models and *in vitro* cell culture systems. However, evidence that T cells play an important role in the pathogenesis of RSV-induced disease is based almost exclusively on data from experimentally infected animals (1). Only scant data exists about the role of different inflammatory cell types in the immune process of acute bronchiolitis in hu-

mans. Recently, Everard et al. (2) challenged the animal concept and the major role of lymphocyte mediated cytotoxicity in the pathology of RSV bronchiolitis in infants. Their results suggest that neutrophils rather than lymphocytes predominantly contribute to the cellular response in the airway of these RSV-infected infants.

The need for invasive techniques to obtain information on the mucosal inflammatory process in the airway following RSV infection prohibits large

scale studies in infants. However, in asthma, a disease believed to be the result of a specialised form of cell-mediated immunity mainly restricted to the airway, specific patterns of T lymphocyte activation in peripheral blood are described both in adults (3) and in infants (4). We therefore decided to study the cellular immune response in the peripheral blood of infants with RSV infection in order to provide additional data on the RSV immunological response in humans.

Lymphocyte cell populations in peripheral blood samples of infants hospitalised with acute RSV bronchiolitis were analysed.

Materials and methods

Study population

We studied 32 infants with the classical clinical features of acute bronchiolitis (5) admitted to our hospital during one winter epidemic (1994–1995). Mean age was 4.9 months (range 1–12 months). Nineteen infants were younger than four months of age. Family history revealed at least one atopic parent in 12 cases.

Symptoms at the time of admission and daily during the hospital stay were assessed by a clinical scoring system using a scale from 0 to 12 (6).

Treatment varied according to disease severity. Adequate fluid intake was assured. In 26 patients, oxygen was administered via a head box. No infant needed mechanical ventilation. All infants received nebulised salbutamol and ipratropium bromide. In the case of a clinical score of 6 or more, intravenous hydrocortisone was given. Ribavirin was not administered.

Methods

On admission nasopharyngeal secretions, for RSV antigen detection by direct immunofluorescence (7) were obtained in all 32 patients. In 19 patients RSV antigen detection was repeated one week after admission.

Peripheral blood samples were taken on admission and at the end of the first week of hospitalisation for leucocyte count, differential analysis, CRP, total IgE and electrolytes. Parental consent was obtained.

For lymphocyte phenotyping all samples were prepared immediately on a Q-prep workstation (Coulter Corp., Hialeah, Fla.) according to the manufacturer's recommendations. Flow cytometric analysis was done within 24 hours on an Epics Profile II flow cytometer (Coulter), using a two-colour immunofluorescent method with fluorescein isothiocyanate (FITC) and phycoerythrin (PE) fluorescent staining on gated lymphocytes (10,000 total events).

Mouse IgG-isotype controls (FITC/PE) were used to exclude non-specific binding and autofluorescence. Purity of the gated lymphocytes was evaluated using a dual-colour combination of CD45/CD14.

Monoclonal antibodies (Table 1) were purchased from Coulter, except for CD57 and CD16 (Becton Dickinson Immunocytometry Systems Cockeysville, Md.). Normal values for lymphocyte subpopulations were those routinely used in our laboratory. These data were derived from age-matched controls from our own study (8), verified and completed with data from literature (9, 10).

Statistical analysis

Because distributions were asymmetric for most of the lymphocyte subsets, data were analysed by non-parametric statistical methods. In order to test the difference between the median of the normal population and the RSV population the one sample sign test was used.

Comparison of the clinical subgroups and of the different age groups was by the Mann–Whitney two sample (non-matched) test.

Comparison of the data obtained at admission and at the end of the first week after admission was by the Wilcoxon test for matched pairs.

Results

On admission the clinical score ranged from 0 to 5 in eighteen (subgroup I, mean age: 3.4 months) and from 6 to 12 in fourteen infants (subgroup II, mean

Table 1. Panel of monoclonal antibodies for defining lymphocyte subpopulations

	CD*	Positivity on peripheral blood lymphocytes
T cells	CD2	all T cells
	CD3	mature T cells
	CD4	helper T cells
	CD4/CD45RA+	naive, suppressor-inducer T cells
	CD4/CD29+	memory, helper-inducer T cells
	CD8	cytotoxic & suppressor T cells
	CD8/S6F1+	cytotoxic-effector T cells
	CD8/S6F1–	precursor/suppressor-effector T cells
	CD3/CD16+ CD56+	T cells with natural killer (NK) activity
	CD2/CD57+	cytotoxic T cells with low NK cell activity
B cells	CD19	all B cells
	CD19/CD5+	mature B cells
	CD19/CD10+	subpopulation of precursor B cells
Others	CD2–/CD57+	non-T cytotoxic cells
	CD3–/CD16+ CD56+	non-T natural killer cells
	CD57	subpopulation of NK cells, subpopulation of T cells, some B cells
	CD25	activated T and B cells, interleukin-2 receptor
	HLA DR	activated T and B cells, class II histocompatibility antigen

*: Cluster of differentiation.

age: 5.8 months). No difference in the family history of atopy was seen between subgroup I and II. At the end of the first week of hospitalisation all infants had a score below 7.

All 32 infants had positive RSV antigen nasopharyngeal secretions on admission. Only one out of 19 infants, in whom a control test for RSV antigen detection was performed after one week, was still positive.

Results of blood analyses on admission are shown in Table 2 and compared with the normal values. The median white blood cell count was 11.800/mm³ (range, 8.3–14.4), not significantly different from controls. Total lymphocyte counts were significantly elevated ($p < .0001$) with an increase of total numbers of CD3+ cells ($p < .0001$) due to a significantly higher number of CD4+ cells ($p < .0001$) and an elevated number of CD8+ cells ($p < .005$). The CD4:CD8 ratios were also significantly elevated ($p < .0001$). In comparison to literature data in both adults (11) and children (10), the suppressor-inducer subpopulation (CD4+/CD45RA+) within the CD4+ cell population was significantly high ($p < .0001$) and the helper-inducer subpopulation (CD4+/CD29+) was significantly low with a median CD45RA+:CD29+ ratio of 4.99 (range, 3.38–9.02). The percentages of CD4+ T lymphocytes expressing the activation markers CD25 and HLA-DR were 4.6% and 2.3% respectively. Peripheral cytotoxicity, expressed by CD2-/CD57+, CD3-/CD16+CD56+ and CD57+ cells was elevated in all the infants regardless of age and clinical score. In comparison with our own data on day 5 after birth (8), determination of CD8+ subsets revealed a significantly higher fraction of CD8+/S6F1+ cells (cytotoxic T lymphocytes) ($p < .0001$) and a significantly lower fraction of CD8+/S6F1- cells (precursor/suppressor-effector T cells) ($p < .0001$). Absolute numbers and percentages of B cells (CD19+) were significantly elevated ($p < .0001$). In the CD19+ cell population the CD5+ and CD10+ subsets were increased ($p < .0001$). In healthy adults CD19+/CD5+ cells account for < 2% of the total number of lymphocytes (11). When both clinical subgroups were compared, cytotoxicity was highest in subgroup II. Percentages of CD57+ (median: 1.1% v 0.3%, $p < .05$), CD2+/CD57+ (median: 0.3% v 0.1%, $p < .05$) CD3-/CD16+CD56+ (median: 8.3% v 4.7% $p < .05$) and of CD8+/S6F1+ cells (median: 14.5% v 11.9%, $p < .05$) were significantly higher in subgroup II than in subgroup I. In the older age group (> 4 months of age) percentages of CD2+/CD57+ (median: 1.5% v 0.1%, $p < .05$) and of CD8+/S6F1+ cells (median: 16.6% v 11%, $p < .05$) were significantly higher in subgroup II. In the younger age group (< 4 months of age) percentages of CD3-/CD16+CD56+ cells (median: 10.9% v 4.6%, $p < .005$) were also significantly higher in subgroup

II. No other significant differences could be seen between the clinical subgroups. In infants under 4 months of age the total number of CD3+ cells was significantly higher in than in infants above 4 months of age (median: 4.85 v 3.85, $p < .01$). The differences were mainly due to a greater elevation in CD4+ cells (median: 3.98 v 2.76, $p < .025$). The percentage of CD2+/CD57+ cells was significantly lower in the younger age-group (median: 0.1% v 0.6%, $p < .025$).

Results of blood analysis performed at the end of the first week are compared to those on admission in Table 3. A significant decrease ($p < .025$) in total leucocyte count was seen. Percentages of CD3+ cells were decreased ($p < .05$) with a decrease in both the percentage ($p < .01$) and the total number of CD4+ cells ($p < .05$). The CD4:CD8 ratio was only slightly decreased. Within the CD4+ cell population a significant decrease ($p < .001$) of the suppressor-inducer

Table 2. Absolute counts and percentages of lymphocytes and lymphocyte subsets in infants with RSV bronchiolitis on the day of admission. Comparison with control data

	Controls	RSV bronchiolitis on admission	p value
Lymphocytes (%)	47 (35–59)	65.5 (52–75.5)	< .005
Absolute count	4.1 (2.7–5.4)	6.29 (5.34–7.68)	< .0001
CD3+ (%)	64 (58–67)	69.6 (58.1–77.2)	< NS
Absolute count	2.2 (1.7–3.6)	4.43 (3.44–5.55)	< .0001
CD3+/CD16+CD56+ (%)	0.1 (0.1–0.2)	0.5 (0.2–0.8)	< .0001
CD4+ (%)	41 (38–50)	49.5 (41–46)	< .005
Absolute count	2.2 (1.7–2.8)	3.53 (2.53–4.22)	< .0001
CD4+/CD45RA+			
Absolute count	0.87 (0.26–3.38)	3.08 (0.98–6.78)	< .0001
(%CD4+)	66.6 (33.9–82.8)	88.7 (70.3–96.3)	< .0001
CD4+/CD29+			
Absolute count	0.39 (0.22–0.90)	0.58 (0.08–1.46)	NS
(%CD4+)	29.6 (12.1–51.8)	17.5 (2.7–42)	< .001
CD45RA+:CD29+ ratio	2.25	4.99 (3.38–9.02)	< .0001
CD4+/HLA DR+ (%)		2.3 (0.2–4.6)	
CD4+/CD25+ (%)		4.6 (3.9–5.2)	
CD8+ (%)	21 (18–25)	17.4 (13.8–19.4)	< .0001
Absolute count	0.9 (0.8–1.2)	1.06 (0.78–1.42)	< .005
CD8+/S6F1+ (%)	5.3 (3.8–8.7)	14.4 (12–16.2)	< .0001
CD8+/S6F1- (%)	14.9 (11.9–18.7)	2.1 (1.6–3.1)	< .0001
CD8+/HLA DR+ (%)		1 (0.1–5.1)	
CD8/CD25+ (%)		0.4 (0.2–0.6)	
CD4+:CD8+ ratio	1.9 (1.5–2.9)	3.17 (2.13–3.97)	< .0001
CD19+ (%)	6 (1.4–10.4)	21.7 (13.4–27.6)	< .0001
Absolute count	0.21 (0.06–0.43)	1.21 (0.8–1.85)	< .0001
CD19+/CD5+ (%)	0.6 (0.3–1.1)	5.9 (3.3–10.8)	< .0001
CD10+ (%)	0.3 (0.2–0.5)	4.5 (1.5–6.9)	< .0001
CD2+/CD57+ (%)	0.1 (0.0–0.1)	0.2 (0.1–0.6)	< NS
CD2-/CD57+ (%)	0.1 (0.0–0.1)	0.3 (0.1–0.4)	< .0001
CD3-/CD16+CD56+ (%)	4.8 (2.3–7.0)	6.9 (4.4–9.4)	< .05
CD57+ (%)	0.1 (0.1–0.3)	0.6 (0.3–1.5)	< .0001

Values are median, with ranges from 25th to 75th percentiles in parentheses. Values for absolute counts are expressed in the following unit of measure: ($\times 10^9/L$). Percentages are % of total lymphocytes, unless otherwise specified.

subpopulation (CD4+/CD45RA+) was seen with an increase in the number of CD4+/CD29+ cells. The CD45RA+:CD29+ ratio was significantly decreased ($p < .05$). A significant decrease ($p < .025$) in the number of CD4+/CD25+ cells was seen, without a significant change in the CD4+/HLADr+ fraction. Within the CD8+ cell population the only significant change was the decrease in the CD8+/S6F1-subfraction ($p < .05$). Percentages of CD19+ cells increased significantly ($p < .005$), without a significant change in the absolute count. A significant increase in the CD19+/CD5+ subfraction was seen ($p < .05$). No changes were seen in the other cytotoxic lymphocyte populations. When clinical subgroups were compared one week after admission, no significant difference was seen in any lymphocyte subpopulation except for the CD8+/S6F1+ cells. The percentage of CD8+/S6F1+ cells was significantly higher in subgroup II (median: 16% v 11.8%, $p < .02$).

On clinical improvement, CRP and total IgE concentration decreased significantly ($p < .005$) and ($p < .05$) respectively, whereas total eosinophylic counts increased significantly ($p < .005$).

Discussion

In this study peripheral blood lymphocyte flow cytometry was performed on infants hospitalised with acute RSV bronchiolitis.

On admission, in the acute phase, absolute leucocyte counts were within the normal range for age. However, there was a significantly elevated lymphocytosis. The high peripheral lymphocytosis was largely attributable to T helper (CD3+/CD4+) cells. T suppressor cells (CD3+/CD8+) increased only slightly in absolute numbers producing with an increase in the CD4+:CD8+ ratio. Similar data were reported by Welliver et al. (12) although they report a slightly higher CD8+ fraction. This finding is in contrast to the picture of CD8+ predominance in peripheral blood in the acute stage of other viral infections in humans (13, 14, 15). In experimentally infected mice (16) an increase in CD8+ cell was seen in the peripheral blood during the first week after inoculation. CD4+ cells increased slightly with a decrease in the CD4+:CD8+ ratio following an initial increase on day 1.

We found a manifest rearrangement within the peripheral blood CD4+ and CD8+ cell populations. In comparison with control data (10), there is a striking imbalance between the CD4+/CD45RA+ and the CD4+/CD29+ subpopulations. The predominance of CD4+/CD45RA+ cells during the acute phase of RSV infection could be due to a defect of this subpopulation to differentiate into CD4+/CD29+ cells, a phenomenon previously believed to be unique to atopic diseases (10). Whether this

CD45RA+ predominance, seen in the acute phase of RSV bronchiolitis, plays a role in the introduction of atopic diathesis in some children can be questioned. In newborns the degree of immaturity of the immune system, reflected by a high number of CD45RA+ (naive) and a low number of CD45RO+ (memory) T cells, is linked to an increased allergic potential in the infant (17). In a recent prospective study, RSV bronchiolitis during infancy was postulated to be an important trigger for allergic sensitisation and subsequent atopic diseases in childhood (18).

As suggested by de Jong et al. (19) CD45RA+ T cells could further proliferate and differentiate into cytotoxic T lymphocytes, provided that a high density of antigen-presenting cells are present. The significant increase of the cytotoxic T lymphocytes within the CD8+ T cells (CD8+/S6F1+), seen in our study, together with the increase in other T cell populations and NK cells (CD57+, CD3+NK+, NK+, CD2-/CD57+) known to represent peripheral cyto-

Table 3. Absolute counts and percentages of leucocytes, lymphocytes and lymphocyte subsets in infants with RSV bronchiolitis on admission and after one week

	On admission	After one week	p value
Leucocytes			
Absolute count	11.8 (8.3–14.4)	9.5 (7.2–13)	< .025
Lymphocytes (%)	65.5 (52–75.5)	65 (51–72)	NS
Absolute count	6.29 (5.34–7.68)	6.4 (4.02–7.27)	NS
CD3+ (%)	69.6 (58.1–77.2)	62.4 (58.5–68.9)	< .05
Absolute count	4.43 (3.44–5.55)	3.81 (2.58–4.74)	NS
CD3+/CD16+CD56+ (%)	0.5 (0.2–0.8)	0.3 (0.2–0.5)	NS
CD4+ (%)	49.5 (41–64)	46.1 (39.9–54.5)	< .01
Absolute count	3.53 (2.53–4.22)	2.69 (1.74–3.52)	< .05
CD4+/CD45RA+ (%)	44.7 (33.3–57.7)	38.1 (31.1–46.2)	< .001
CD4+/CD29+ (%)	9 (5.5–12.6)	12 (9.7–16.4)	NS
CD45RA+:CD29+ ratio	4.99 (3.38–9.02)	3.14 (2.51–4.31)	< .05
CD4+/CD25+ (%)	4.6 (3.9–5.2)	3.6 (3.5–4.0)	< .025
CD4+/HLA DR+ (%)	2.3 (0.2–4.6)	2.4 (0.6–3.7)	NS
CD8+ (%)	17.4 (13.8–19.4)	15.4 (13.4–17.9)	NS
Absolute count	1.06 (0.78–1.42)	0.96 (0.69–1.18)	NS
CD8+/S6F1+ (%)	14.4 (12–16.2)	13 (11.4–16.1)	NS
CD8+/S6F1- (%)	2.1 (1.6–3.1)	1.7 (1.3–2.3)	< .05
CD8+/CD25+ (%)	0.4 (0.2–0.6)	0.5 (0.1–0.7)	NS
CD8+/HLA DR+ (%)	1.0 (0.1–5.1)	0.7 (0.2–1.8)	NS
CD4+:CD8+ ratio	3.17 (2.13–3.97)	2.83 (2.37–3.87)	NS
CD19+ (%)	21.7 (13.4–27.6)	23.1 (21.1–33.5)	< .005
Absolute count	1.21 (0.80–1.85)	1.67 (0.99–2.08)	NS
CD19+/CD5+ (%)	5.9 (3.3–10.8)	13.4 (5.7–21.5)	< .05
CD10+ (%)	4.5 (1.5–6.9)	6 (0.8–10.7)	NS
CD2-/CD57+ (%)	0.2 (0.1–0.6)	0.2 (0.1–0.7)	NS
CD2-/CD57+ (%)	0.3 (0.1–0.4)	0.3 (0.2–0.5)	NS
CD3-/CD16+CD56+ (%)	6.9 (4.4–9.4)	5.5 (4.2–7.4)	NS
CD57+ (%)	0.6 (0.3–1.5)	0.6 (0.6–1.6)	NS

Values are median, with ranges from 25th to 75th percentiles in parentheses. Values for absolute cell counts are expressed in the following unit of measure ($\times 10^9/L$). Percentages are % of total lymphocytes. NS: $p > .05$.

toxicity, support this hypothesis. The role that cytotoxic T cells play in the pathogenesis of RSV infection has been investigated in animals (20). Human data remains scarce. In a small number of children, Isaacs et al. (21) found a cell-mediated cytotoxic response to RSV only in the peripheral blood of infants under the age of 4 months with mild RSV infection. Chiba et al. (22) found that peripheral blood lymphocytes exhibited cellular cytotoxicity to RSV in over 65% of infants 6–24 months of age and in about 35% of infants under 5 months of age.

The significant decrease of the CD8+ precursor/suppressor-effector subpopulation (CD8+/S6F1–) may be the phenotypic equivalent of the defect in histamine-induced suppressor-cell function demonstrated by Welliver et al. (12) in infants with RSV infection. In view of the growing interest in the possible role of the CD8+ T cell in the regulation of the allergic immune response (23), our findings add to the accumulating evidence showing a possible link between RSV bronchiolitis and subsequent atopy (18). Certainly, more prospective evaluation of the different clinical subgroups of RSV infection and the determination of different T cell subpopulations and cytokine production is needed.

Although we did not measure antibody responses, the significant increase of CD19+ B cells with a significant expansion of CD5+ and CD10+ subsets may imply that there is also a humoral immune mechanism involved.

During recovery, one week after admission, CD4+ subsets tended to normalise, reflected in a decline of the CD4+/CD45RA+ fraction and a trend to normalisation of the CD45RA+:CD29+ ratio. The increase in CD4+/CD29+ cells, representing primed cells, suggests memory induction after primary RSV infection (24), although significant differences in activation markers was limited to CD25 expression on CD4+ cells.

The further significant increase in the CD19+/CD5+ subfraction is compatible with an ongoing humoral immune response, during clinical recovery.

Steroid therapy given to all patients with more severe illness might have influenced these results, complicating the comparisons between admission and subsequent measurements. Although the study design did not allow us to exclude this possibility it seems unlikely that steroids would have influenced all the lymphocyte subpopulations, resulting in the absence of any significant difference between clinical subgroups, except for the CD8+/S6F 1 + fraction.

In conclusion, flow cytometric analysis on peripheral blood lymphocytes in infants hospitalised with acute RSV bronchiolitis shows a significant increase in CD4+ T helper cells in contrast to the CD8+ predominance seen in other viral infections in

humans. One could argue that this is only a reflection of the bronchotropic nature of the RS virus and that peripheral blood findings are not representative of the immune process at the site of infection within the respiratory tract. Indeed simultaneous determination of changes in peripheral blood and respiratory tract samples would be needed to solve this issue. Nevertheless the rearrangements within the CD4+ and CD8+ cell populations not only suggest an increase in cytotoxicity but also imply a relationship between bronchiolitis in infancy and the development of allergic sensitisation and subsequent asthmatic disease.

Although it seems rational to look for changes within the target organ itself, i.e. the airway, looking for changes at distance, i.e. peripheral blood, is more practical in these very sick infants. More data on immune parameters in infants with acute RSV bronchiolitis are needed to further elucidate the immunopathogenic mechanisms involved and confirm the usefulness of animal data (25). Data on peripheral blood cells might help in assessing the effect of different treatment regimens and aid vaccine research. The comparison of data in different disease states might help to explain the prevalence of subsequent recurrent respiratory and allergic symptoms in some infants following RSV infection.

Acknowledgements

The authors are grateful to Prof. P. Brock (University of Leuven, Belgium) for linguistic revision of the manuscript. They also wish to acknowledge the financial support received from Milupa, Belgium.

References

1. HUSSELL T, SPENDER LC, GEORGIU A, O'GARRA A, OPENSHAW PJM. Th1 and Th2 cytokine induction in pulmonary T cells during infection with respiratory syncytial virus. *J Gen Virol* 1996; 77: 2447–55.
2. EVERARD ML, SWARBRICK A, WRIGHTHAM M, et al. Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection. *Arch Dis Child* 1994; 71: 428–32.
3. VIRCHOW JC, OEHLING A, BOER L, et al. Pulmonary function activated T cells, peripheral blood eosinophilia, and serum activity for eosinophil survival *in vitro*: A longitudinal study in bronchial asthma. *J Allergy Clin Immunol* 1994; 94: 240–9.
4. GEMOU-ENGESAETH V, KAY AB, BUSH A, CORRIGAN CJ. Activated peripheral blood CD4 and CD8 T-lymphocytes in child asthma: correlation with eosinophilia and disease severity. *Pediatr Allergy Immunol* 1994; 5: 170–7.
5. RUUSKANEN O, OGRA PL. Respiratory syncytial virus. *Curr Probl Pediatr* 1993; 23: 50–79.
6. TAL A, BAVILSKI C, YOHAI D, BEARMAN JE, GORODISCHER R, MOSES SW. Dexamethasone and salbutamol in the treatment of acute wheezing in infants. *Pediatrics* 1983; 71: 13–18.
7. MLINAVIC-GALINOVIC G, UGRIC I, CVETKOVIC M, PENDE B, IVANKOVIC D. Rapid detection of respiratory syncytial

- virus in clinical specimen. *Acta Virol* 1987; 31: 410–16.
8. RAES M, ALLIET Ph, GILLIS Ph, et al. Lymphocyte subpopulations in healthy newborn infants: Comparison of cord blood values with values five days after birth. *J Pediatr* 1993; 123: 465–7.
9. ERKELLER-YUKSEL FM, DENEYS V, YUKSEL B, et al. Age-related changes in human blood lymphocyte subpopulations. *J Pediatr* 1992; 120: 216–22.
10. SCHAUER U, JUNG T, HEYMANN J, RIEGER CHL. Imbalance of CD4+CD45R+ and CD4+ CD29+ T helper cell subsets in patients with atopic diseases. *Clin Exp Immunol* 1991; 83: 25–9.
11. VUILLIER F, SCOTT-ALGARA D, DIGHERO G. Extensive analysis of lymphocyte subsets in normal subjects by three-color immunofluorescence. *Nouv Rev Fr Hematol* 1991; 33: 31–8.
12. WELLIVER RC, KAUL TN, SUN M, OGRA PL. Defective regulation of immune responses in respiratory syncytial virus infection. *J Immunol* 1984; 133: 1925–30.
13. DE WAELE M, THIELEMANS C, VAN CAMP BKG. Characterization of immunoregulatory T cells in EBV-induced infectious mononucleosis by monoclonal antibodies. *N Engl J Med* 1981; 304: 460–2.
14. YONEYAMA A, NAKAHARA K, HIGASHIHARA M, KUROKAWA K. Increased levels of soluble CD8 and CD4 in patients with infectious mononucleosis. *Br J Haematol* 1995; 89: 47–54.
15. CARNEY WP, RUBIN RH, HOFFMAN RA, HANSEN WP, HEALY K, HIRSCH MS. Analysis of T lymphocyte subsets in cytomegalovirus mononucleosis. *J Immunol* 1981; 126: 2114–16.
16. KIMPEN JLL, RICH GA, MOHAR CK, OGRA PL. Mucosal T cell distribution during infection with respiratory syncytial virus. *J Med Virol* 1992; 36: 172–9.
17. MILES EA, WARNER JA, LANE AC, JONES AC, COLWELL BC, WARNER JO. Altered T lymphocyte phenotype at birth in babies born to atopic parents. *Pediatr Allergy Immunol* 1994; 5: 202–8.
18. SIGURS N, BJARNASON R, SIGURBERGSSON F, KJELLMAN B, BJÖRKSTÉN B. Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: a prospective cohort study with matched controls. *Pediatrics* 1995; 95: 500–5.
19. DE JONG R, BROUWER M, MIEDEMA F, VAN LIER RAW. Human CD8+ T lymphocytes can be divided into CD45RA+ and CD45RO+ cells with different requirements for activation and differentiation. *J Immunol* 1991; 146: 2088–94.
20. GRAHAM BS, BUNTON LA, WRIGHT PF, KARZON DT. Role of T lymphocyte subsets in the pathogenesis of primary infection and rechallenge with respiratory syncytial virus in mice. *J Clin Invest* 1991; 88: 1026–33.
21. ISAACS D, BANGHAM CRM, MCMICHAEL AJ. Cell-mediated cytotoxic response to respiratory syncytial virus in infants with bronchiolitis. *Lancet* 1987; ii:769–71.
22. CHIBA Y, HIGASHIDATE Y, SUGA K, HONJO K, TSUTSUMI H, OGRA PL. Development of cell-mediated cytotoxic immunity to respiratory syncytial virus in human infants following naturally acquired infection. *J Med Virol* 1989; 28: 133–9.
23. KEMENY DM, NOBLE A, HOLMES BJ, DIAZ-SANCHEZ D. Immune regulation: a new role for the CD8+ T cell. *Immunol Today* 1994; 15: 107–10.
24. KIMPEN JLL, OGRA PL. Mucosal T cells recovered from mice after infection with respiratory syncytial virus display a memory/activation phenotype. *Pediatr Allergy Immunol* 1995; 6: 119–23.
25. OPENSHAW PJM. Immunopathological mechanisms in respiratory syncytial virus disease. *Springer Semin Immunopathol* 1995; 17: 187–201.