Infection with respiratory syncytial virus (RSV) may induce asthma-like symptoms and RSV-specific IgE in infected infants as a result of Th2-like response to RSV. The effect of RSV infection on the expression of B cell antigens CD21 and CD23, putative participants in Th2 responses, was investigated. Samples from bronchiolitic infants (n = 19) were tested by three-color immunofluorescence flow cytometry during the acute phase of infection and 4–6 weeks later. In 6 of 10 RSV-positive infants, the percentage of CD23+ B cells was higher than in 9 RSV-negative children and in controls. Both CD21+ and CD21− B cells exhibited a higher percentage of CD23. The group with increased expression of CD23 antigen had RSV-specific IgE and IgG4 antibodies. These findings corroborate the hypothesis that RSV could provoke a Th2-type response, but the relationship between CD23 antigen and RSV infection must be determined.

Subjects and Methods

Subjects. We studied 19 hospitalized infants with bronchiolitis (16 boys, 3 girls) ages 2–11 months (mean, 4). All were examined by a study team member. Bronchiolitis was defined as wheezing and a prolonged expiratory phase. Pneumonia, if suspected, was excluded by radiographic examination. Children with suspected anatomic cardiopulmonary abnormalities were excluded from the study. Clinical studies, such as auscultatory examination of the lung, frequency of respiration, and 24-h monitoring of oxygen saturation, were done daily and recorded with details of each child’s illness. At the onset of acute illness and within 6 days thereafter, samples of nasopharyngeal secretions, sera, and heparinized blood were obtained simultaneously. With the cause of
disease still unknown, peripheral blood cell surface markers were examined by flow cytometry.

We obtained blood specimens from 17 children during the convalescent stage 3–5 weeks after the onset of illness, as described above.

The control group of 9 children (8 boys, 1 girl) ages 1.5–10 months (mean, 6) provided blood and sera. The controls were hospitalized for minor surgery and were free of manifest allergic, immunologic, and hematologic disorders. Their total IgE levels were within the range of healthy controls.

Virus identification. The presence of RSV infection was documented by detection of the virus in nasopharyngeal secretion using monoclonal antibodies (MAbs) in the immunofluorescence test. RSV was confirmed by isolation in cell cultures by standard techniques [21, 22].

Analysis of surface markers by flow cytometry. We used the following murine antibodies to human lymphocyte cell-surface antigens: anti-CD20 (PerCP [peridinin chlorophyll protein]-conjugated) and phycoerythrin (PE)-conjugated anti-CD23 (both from Becton Dickinson, Heidelberg, Germany) and fluorescein isothiocyanate (FITC)–conjugated anti-CD21 (Immunotech, Marseille, France). Each experiment involved FITC-conjugated IgG1, PE-conjugated IgG2a, or PerCP-conjugated IgG1 controls for the determination of nonspecific bindings. To minimize the volume of peripheral blood drawn from children, we followed the standard procedure of triple direct immunofluorescence whole blood staining. Briefly, 50 µL of heparinized blood was incubated for 30 min in the dark at 4°C with 10 µL of FITC-, PE- and PerCP-conjugated antibody. The lysis of erythrocytes was done by adding 2 mL of a 10% fluorescence-activated cell sorter lysing solution (Becton Dickinson, San Jose, CA) for 10 min at room temperature in the dark. After a wash step, the cells were resuspended in 0.5 mL of the staining solution. Control suspensions were prepared by the same procedure, with the fluorochrome-conjugated normal mouse immunoglobulins of the same isotype as the corresponding MAbs.

Cell fluorescence was analyzed by flow cytometer (FACScan; Becton Dickinson). Correlated analysis of forward and right angle scatter was used to establish a lymphocyte gate with a minimum of 20,000 cells/sample counted for the three-color immunofluorescence. Data were analyzed by FACScan research and lysis software.

Determination of total serum IgE levels. Total serum IgE levels were determined by commercial ELISA kit (Abbott Laboratories, Abbott Park, IL).

Determination of RSV-specific IgG4 and IgE. RSV-specific IgG4 and IgE antibodies were determined by immunoblot as described previously [23]. A semipurified RSV (strain Long) grown in HEp-2 cells was used as antigen. Sera diluted 1:10 were tested simultaneously on strips with virus-specific proteins and on those prepared from mock-infected HEp-2 cells. All incubation steps were at room temperature. Biotin-labeled mouse MAbs against human IgG4 and IgE (γ- and ε-specific; Southern Biotechnology Associates, Birmingham, AL) were used as conjugates. Membrane-bound biotinylated antibodies were visualized using a blotting detection kit for human antibodies (Amersham International, Amersham, UK) containing streptavidin–alkaline phosphatase, nitroblue tetrazolium, and 5-bromo-4-chloro-3-indolyolphosphate in dimethyl formamid.

Molecular weights of the proteins recognized by antibodies were determined by densitometric comparison with co-electrophoresed biotinylated standard proteins of known molecular weights. As controls, a rabbit pre- and RSV-immune serum, a human convalescent serum (RSV complement-fixing titer, 1:128) and a mouse MAb directed against the 35-kDa protein (provided by C. Orvell, Dept. of Virology, Karolinska Institute, Stockholm) were included in the assays.

Statistical analysis. Intergroup comparison was made by using the Mann-Whitney nonparametric U test. With Wilcoxon’s parametric test, values for the same group of children tested during the acute phase of infection were compared with results at convalescence.

Results

Children with bronchiolitis were divided into 2 groups according to their virologic test results (table I). The first group comprised the 10 RSV-positive infants. The 9 RSV-negative infants, whose bronchiolitis, as we presumed, was caused by other viruses, were in group 2. We compared the CD23 antigen expression on B lymphocytes of the 2 groups and of the control group. In contrast with the RSV-negative children with bronchiolitis and with the control group, all RSV-infected children had significantly higher percentages of CD23+ B lymphocytes in the total B lymphocyte population (figure 1), while the percentage of CD21+ B lymphocytes remained unchanged (data not shown). This higher percentage was due to the higher number of CD23+ B lymphocytes observed in 6 of 10 RSV-positive children. This increase in CD23 antigen expression was observed in the subset of CD21+ and CD21− B cells (figure 2), but it was not accompanied by elevated total serum IgE. In 6 of 8 infants in the RSV-negative group, the percentage of CD23 on CD20+ CD21+ B cells was increased when tested 3–5 weeks after the onset of illness. Virus-specific IgE and IgG4 antibodies were detectable by immunoblot in sera of the 6 RSV-positive children with increased percentages of CD23+ B cells collected during the acute phase of their illness and were present in convalescent sera of 3 children (figure 3). IgE and IgG4 antibodies were predominantly directed against the RSV 35-kDa phosphoprotein, the 42-kDa nucleoprotein, and the 48-kDa protein.

No correlation was found between the percentage of CD23+ B lymphocytes and the clinical course of the disease. Thus, the clinical status of RSV-positive patients who did not manifest increased CD23 expression was not different from the 6 infants with increased CD23 expression (table 1). Only 1 RSV-infected infant had an atopic predisposition or a prior allergic disease, but he had expression of CD23 antigen comparable to that in the control group. This suggests, in agreement with other reports, that atopy might not be an important predisposing factor for a severe course of RSV infection.

In blood samples from 9 of 10 RSV-positive infants 3–6 weeks after onset of disease, the percentage of CD23 antigen on both the CD21+ and CD21− B cell subpopulation was com-
Table 1. Comparison of clinical score, degree of increase in CD23 antigen, and appearance of RSV IgE and RSV IgG4 antibodies in infants during acute phase of bronchiolitis caused by RSV and other respiratory viruses.

<table>
<thead>
<tr>
<th>Virus, infant, age (months)</th>
<th>No. days of wheezing</th>
<th>Duration of desaturation/O₂ supplementation (days)</th>
<th>% MOS*</th>
<th>MRR/min</th>
<th>DFA</th>
<th>Culture</th>
<th>% increase in RSV CD23</th>
<th>IgE</th>
<th>IgG4</th>
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<td>85.4</td>
<td>76</td>
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<td>+</td>
<td>40</td>
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<td>+</td>
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<td>4</td>
<td>77</td>
<td>74</td>
<td>+</td>
<td>−</td>
<td>50</td>
<td>+</td>
<td>+</td>
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<td>22</td>
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<td>+</td>
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<td>2</td>
<td>90.2</td>
<td>68</td>
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<td>+</td>
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<td>71</td>
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<td>87.7</td>
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NOTE: DFA, direct fluorescence assay; ND, not done.
* MOS = minimal oxygen saturation measured by percutan oxymetry.
† MRR, maximal respiratory rate/min (normal, <50/min for 2–12 months).

parable to those in the RSV-negative and control groups (figure 2), indicating that the change observed in the B lymphocytes was transient.

Discussion

To our knowledge, this is the first study to clearly demonstrate that RSV infection is capable both of increasing CD23 antigen expression and inducing the production of RSV-specific IgE and IgG4 antibodies in children. This CD23 antigen increase in B lymphocytes was transient: Convalescence values were within normal limits. So far, the rise in CD23 antigen in B cells was reported in several pathologic conditions, such as allergic disease, autoimmune disorders, Kawasaki disease, and Epstein-Barr virus (EBV) infections [14, 15, 24–26]. Factors in these disorders that might contribute to an increased expression of CD23 antigen in B lymphocytes have been extensively explored. In patients with allergic asthma, increased CD23 antigen in B lymphocytes is mainly regulated by soluble factors (IL-4 and IL-13) or by binding of the CD40 molecule [13, 27]. However, the effect of EBV on the expression of the CD23 molecule is mediated by an expression of the two latent EBV proteins (EBNA-2 and LMP-1) [17, 28].

Of interest, in addition to binding antigen CD21, antigen CD23 binds EBV, C3dg, and IFN-α. Bonnefoy et al. [17] recently reported that binding the peptide from EBV gp350/220 inhibits CD23 binding to CD21 and IL-4–induced IgE and IgG4 production. The transformation of B lymphocytes with EBV in conjunction with IL-4 has been considered a very potent system for the induction of IgE synthesis [29]. Unlike EBV, during natural infections, RSV attacks not only lymphocytes but monocytes/macrophages as well [28]. While the receptor for RSV is unknown, some still undefined intrinsic factors seem to govern the infection of mononuclear cells. Domurat et al. [30] found that the youngest infants with bronchiolitis [30] had the most commonly identified RSV antigen in circulating mononuclear cells. In our study, the CD23 molecule induced by RSV could not be observed in every RSV-positive infant, regardless of age. We therefore presume that the induction of CD23 antigen in B cells in some RSV-positive infants could be a consequence of a direct effect of RSV on B cells as seen in EBV infection.

Because CD4+ T lymphocytes specific for RSV attachment glycoprotein G were recently reported to be of the Th2 type (predominantly producing IL-4) [8], we hypothesized that the occurrence of this T cell subpopulation in the course of natural infection could be responsible for the greater expression of CD23 antigen in B lymphocytes. Undoubtedly, this Th2 T cell subpopulation plays an essential role in the induction of IgE synthesis [10]. By providing both physical contact (via CD40/
CD40L interaction) and cytokine production (IL-4 or IL-13), these cells drive B cells to produce IgE. An interaction between other ligand pairs, such as CD23/CD21, enhances T to B cell interaction [17, 19]. On the other hand, cytokines produced by Th1 cells (IFN-γ, IL-12, and IL-2) are preferentially important for the T cell cytotoxic antiviral response [10]. IFN-γ downregulates the expression of CD23 antigen [13]. In 6 of 8 infants in the RSV-negative group, the percentage of CD23 on CD20+CD21+ B cells was lower in the acute phase of the disease than in healthy controls and RSV-positive infants. This could result from IFN-γ produced by T cells during an antiviral response to non-RSV.

CD23+ B cells in RSV Infection

We previously showed that the expression of CD23 antigen was significantly stronger in a subpopulation of CD20+CD21+ B cells in children with allergic asthma than in controls [15]. In vitro experiments have shown that the co-occurrence of stimulatory anti-CD40 MAbs and recombinant (r) IL-4/rIL-13 in highly purified CD19+ B cell cultures leads to the generation of CD21+CD23+ B lymphocytes [27] (unpublished data). Therefore, the higher percentage of CD23 antigen in CD21+ B lymphocytes during the acute stage of an RSV infection indirectly illustrates that RSV in those children stimulates the immune system in a Th2-type fashion, which is advantageous for viral replication. Isaacs et al. [31] found a cytotoxic cellular immune response in the peripheral blood of infants with a mild RSV infection but not in those with the most severe infection. We are currently exploring the possibility of an aberrant response to RSV, possibly resulting from the immaturity of an infected infant’s immune system. The generation and regulation of IgG4-blocking antibodies are controlled in a manner similar to IgE production [11]. In our study, we observed the simultaneous induction of RSV-specific IgE and IgG4 antibodies, which suggests that the production of blocking IgG4 antibodies controls unwanted IgE allergic symptoms (probably by a mechanism of competitive inhibition of binding to viral peptides).

Although RSV induces RSV-specific IgE response along with the induction of CD23 antigen in B cells, this effect is transient, as is the period of bronchial obstruction (i.e., wheezing). Although in very young infants, other viruses could also induce wheezing [32], we did not notice any difference in the expression of CD23 antigen among RSV-negative infants.

Welliver et al. [33] hypothesized that RSV infection in infancy and possibly other viral infections could induce a state of bronchial hyperreactivity through immunologic mechanisms resembling those found in asthma. Although wheezing was

Figure 1. Increased % of CD23+ B lymphocytes in infants during acute phase of RSV low respiratory tract infection (RSV+) compared with healthy controls and non-RSV infections (RSV-). P calculated by Mann-Whitney U test.

Figure 2. Expression of CD23 antigen on CD20+CD21+ (A) and CD20+CD21- B cell (B) subpopulation in acute (A) and convalescent (R) phases of infection in RSV-infected (RSV+) and noninfected (RSV-) infants. Each □ represents 1 patient; hatched areas are mean ± SD of CD23+ B lymphocytes in controls.
present in all sick infants in our study, it was not mediated by IgE antibodies in each infant, since we found no difference between the 2 groups of RSV-infected infants. Therefore, neither the ability of RSV to induce an RSV-specific IgE and IgG4 response nor the induction of CD23 antigen in 6 of 10 RSV-positive infants could be ascribed to a history of atopy. It is not clear from our results whether the higher expression of CD23+ B cells within the RSV-positive group had a role in the immunopathology of RSV infection. Furthermore, our data did not show a relationship between changes in CD23+ B cell distribution to the outcome of disease during RSV infection. We are currently testing our hypothesis that the determination of CD23, in addition to measuring the magnitude of an RSV-specific immune response during RSV-caused bronchiolitis [33], might be a useful prognostic indicator for recurrent wheezing later in life.

In conclusion, the present study indirectly shows that RSV infection in infants induces a Th2-type immune response. More studies are underway to establish the predominant cytokine profile in CD4+ T lymphocytes in RSV-positive infants.

References


