Frequency of lower respiratory tract infections in relation to adaptive immunity in children with Down syndrome compared to their healthy siblings

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ABSTRACT
Aim: Children with Down syndrome (DS) experience respiratory tract infections (RTIs) more frequently than healthy children. We investigated whether this is related to different immunological characteristics associated with DS.

Methods: The study group consisted of 22 children with DS and 22 of their healthy, age-range matched siblings. Data were collected on infections and hospitalizations because of lower RTIs. Immunoglobulin and IgG subclass levels in blood, as well as lymphocyte and T cell (subset) counts, were determined.

Results: The children with DS had a significantly higher frequency of lower RTIs and related hospitalization than their siblings. We also found significantly reduced IgG2 levels as well as significantly lower counts of total lymphocytes, CD4+ T lymphocytes, CD4+ invariant natural killer (iNKT) cells and regulatory T cells in the DS group.

Conclusion: In children with DS, reduced levels of IgG2, total lymphocytes, T lymphocytes, iNKT cells and regulatory T cells might contribute to their higher susceptibility to lower RTIs.

INTRODUCTION
The prevalence of Down syndrome (DS), or trisomy 21, in the Netherlands is 16 per 10 000 live births (1). In addition to mental retardation, children with DS have a higher incidence of physical disorders, such as congenital heart disease (CHD), gastrointestinal malformations, hypothyroidism and leukaemia, and are also more likely to develop autoimmune disease than children without this condition. Children with DS are also prone to infections, especially respiratory tract infections (RTIs) (2,3). This higher susceptibility can be due to anatomical (4,5), neurological (6) or immunological abnormalities as concluded in many reviews (7,8).

To date, most immunological studies of children with DS have reported defects in both the innate and adaptive immune systems, such as a deficiency of mannann-binding lectin (9), a high number of proinflammatory CD14dim CD16+ monocytes (10), changes in T and B lymphocyte counts (8,11,12), an intrinsic defect of T and B lymphocytes (8,13,14), and deficiencies in the IgG2 and IgG4 subclasses (8,14,15). However, in one study, DS children were found to have normal immunoglobulin levels and normal lymphocyte counts (total lymphocytes, CD4+ T cells, CD8+ T cells, and natural killer cells) (16). A possible reason for these conflicting results might be the fact that different types of control group were used in the studies. Different studies have used historical controls, healthy controls, children with mental retardation who do not have DS or no controls.

Key notes
- Children with Down syndrome experience more lower respiratory tract infections and related hospitalization than their healthy siblings.
- Children with Down syndrome show significantly diminished invariant natural killer T cells and regulatory T cells.
- The diminished subpopulations of T cells might contribute to their higher susceptibility to lower RTIs.

Abbreviations
CHD, Congenital heart disease; DS, Down syndrome; iNKT, Invariant natural killer T; mAb, monoclonal antibody; RTI, Respiratory tract infection; Th, T helper; Treg, Regulatory T cell.
at all; thus, the control groups had different environmental and genetic backgrounds (11,15,16).

The aim of the present study was to investigate the role of adaptive immunity in relation to lower RTIs in children with DS. To minimize genetic, environmental and age-related differences, we used their healthy, age-ranged matched siblings as a control group. Given that 50% of children with DS have CHD (17) and CHD may also lead to an increase in RTIs (18), we also analysed the occurrence of RTIs in relation to the presence of CHD in these children.

PATIENTS AND METHODS

Patient evaluation

Twenty-two children with DS and 22 of their healthy siblings were recruited from the DS outpatient clinic at the VU University Medical Center in Amsterdam, the Netherlands. Children with DS who met the following criteria were included: diagnosis of DS proven by chromosome analysis, age >3 months, and no infection present on the day the blood sample was taken. Siblings of the children in the DS group were included if they met the following criteria: age >3 months and no infection present on the day the blood sample was taken. Immunoglobulin levels and lymphocyte counts vary with age; thus, we chose age categories on the basis of the literature (19). A DS-sibling pair was included in the study only when both the child with DS and the matched sibling were in the same age category.

To rule out the presence of an infection on the day of venipuncture, parents were interviewed about their child’s recent health and a physical examination of the child was performed. The appointment was postponed if the interview, physical examination or laboratory results (including leucocyte count, leucocyte differentiation, and C-reactive protein) revealed an infection.

One of the investigators (C.J.M. Broers, paediatrician) conducted a structured interview with the parents about the total number of infections that their children had had since birth and any related hospitalization. To confirm these data, we asked the parents for written consent to obtain a copy of their children’s medical data. From the medical records, the frequency and type of infection were retrieved. Lower RTIs were defined as: subglottic laryngitis, pneumonia, bronchitis and bronchiolitis. The medical records of all the participants were checked for the frequency of infections, presence of CHD, any history of heart surgery and remaining heart defects, and in children with DS, the results of chromosome analysis.

Immunological evaluation

Immunoglobulin and IgG subclass analysis was performed using immunonephelometry (IMMAGE 800 Immunochemistry System; Beckman Coulter, Fullerton, CA, USA) in a routine diagnostic clinical chemistry laboratory.

Peripheral blood lymphocytes were phenotyped by monoclonal antibody (mAb) staining of whole blood and flow cytometric analysis after lysing red cells with Lysing Solution (BD Biosciences, San Jose, CA, USA) and fixing with 1% paraformaldehyde. Flow cytometric analysis was performed using a four-colour FACS Calibur flow cytometer (BD Biosciences).

Absolute numbers of T lymphocytes were determined by adding fixed volumes of FlowCount fluorospheres (Beckman Coulter) to the pretreated cell sample, just before flow cytometric evaluation. Lymphocyte gates in the scatter diagram were determined in each blood sample by CD4/CD45 mAb staining (BD Biosciences). T helper (Th) cells and cytotoxic T cells were characterized using the markers CD3, CD4 and CD8. CD25 on T cells was also evaluated (all mAb of BD Biosciences).

T regulatory cells (Tregs) were identified on the basis of coexpression of CD3 (fluorescein isothiocyanate (FITC)-labelled mAb), CD4 [phycoerythrin (PE)-labelled mAb], CD45 [peridinin chlorophyll protein complex (PerCP)-labelled mAb] and high expression of CD25 [allophycocyanin (APC)-labelled mAb] (all mAb of BD Biosciences).

V$^\text{24+}$V$^\beta_{11}^+$ invariant natural killer T (iNKT) cells were determined by flow cytometry using a FITC-labelled monoclonal antibody (mAb) against human V$^\text{24}$, a PE-labelled mAb against human V$^\beta_{11}$ (Immunotech, Marseille, France), a PerCP-Cy5.5-labelled mAb against CD5 and an APC-labelled mAb against CD4 (BD Biosciences). V$^\text{24+}$V$^\beta_{11}^+$ iNKT cells were evaluated as a fraction of CD3$^+$ lymphocytes in the blood. A minimum of 100 000 viable lymphocytes were acquired from each patient for the determination of iNKT cells.

Data analysis

Fisher’s exact test (SPSS version 15.0, IBM, Armonk, NY, USA) was used to analyse the response categories in the questionnaires. The paired t test (SPSS version 15.0) was used to analyse the data for lymphocytes and immunoglobulins. A p-value <0.05 was considered significant.

Table 1  The number of children with at least one infection and the number of children with hospitalization because of infection [Down syndrome (DS) vs. sibling group]

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Down syndrome (n = 22)</th>
<th>Sibling (n = 22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% male)</td>
<td>15/22 (68%)*</td>
<td>8/22 (36%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>10.0 ± 1.3†</td>
<td>10.4 ± 1.2</td>
<td>0.82</td>
</tr>
<tr>
<td>History of infections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower RTI</td>
<td>13/22 (59%)*</td>
<td>2/22 (9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>2/22 (9%)</td>
<td>2/22 (9%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>9/22 (41%)</td>
<td>10/22 (45%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hospitalization†</td>
<td>11/22 (50%)</td>
<td>2/22 (9%)</td>
<td>0.007</td>
</tr>
<tr>
<td>Hospitalization‡</td>
<td>7/22 (32%)</td>
<td>0/22 (0%)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*Number (%).
†Mean (SEM).
‡Because of infection.
§Because of lower respiratory tract infection (RTI).
**Ethical approval**
The study was approved by the Ethics Committee of the VU University Medical Center in Amsterdam.

**RESULTS**
All 22 children with DS had trisomy 21 (no translocation or mosaic trisomy 21). The number of DS–sibling pairs in each age category was as follows: 5 DS-5 siblings in age category 2–5 years; 7 DS-7 siblings in age category 5–10 years; 6 DS-6 siblings in age category 10–16 years; and 4 DS-4 siblings in age category >16 years.

Children with DS had significantly more lower RTIs in comparison with their siblings, and they also were significantly more hospitalized because of infection (Table 1). Concerning RTIs, the level of agreement between the data obtained from the parental interview and from the medical records was 95%.

As seen in Figure 1, levels of IgM, IgG2 and IgG4 were significantly lower in the DS group than in the sibling group,
whereas levels of IgG1 and IgG3 were significantly higher in the DS group. There was no significant difference in total IgG and IgA levels between the two groups. In the sibling group, all immunoglobulin levels were within the normal range.

As seen in Figure 2, the numbers of total lymphocytes, CD4⁺ T lymphocytes, CD4⁺ iNKT cells and CD4⁺ CD25^{high} T cells (Tregs) were all significantly lower in the DS group than in the sibling group. In the sibling group, all counts for total lymphocytes, T lymphocytes, CD4⁺ T lymphocytes and CD8⁺ T lymphocytes were within the normal range. No normal values were available for CD4⁺ iNKT cells or Tregs. The CD4/CD8 T cell ratio was significantly lower in the DS group than in the sibling group (data not shown).

In the DS group, 10 of 22 children had CHD and cardiac surgery for CHD was performed in five patients. At the time of our study, four of the 10 patients with CHD had a remaining minor heart defect, either post-surgery or because of an incomplete spontaneous closure. No significant difference was found between the number of hospitalizations because of lower RTIs in the 10 DS children with CHD as compared with the 12 DS children without CHD.

In addition, no significant difference was found between the four patients with a remaining heart defect and six patients without a remaining heart defect in terms of the number of hospitalizations related to lower RTIs (data not shown). None of the 22 siblings had CHD.

DISCUSSION
The results of the study demonstrated that the children with DS had a significantly higher frequency of lower RTIs and related hospitalization than their siblings. Immunological defects associated with DS are reported frequently as a cause of RTI. To date, the interpretation of immunological studies in children with DS has been hampered by the fact that control groups have been lacking or have consisted of historical controls (11,16). One strength of the present study is that we included healthy, age-range matched siblings as controls, thereby circumventing environmental, genetic and age-related differences between the patient and control groups as much as possible.

For the most part, the immunoglobulin levels that were found in the children with DS in the present study...
confirmed previously reported results (15), namely increased levels of IgG1 and IgG3 and reduced levels of IgM, IgG2 and IgG4. In general, reduced IgG2 levels can cause an increase in RTIs (20).

The results of the study clearly demonstrate that reduced numbers of lymphocytes and T cells are associated with DS, especially CD4<sup>+</sup> T cells, CD4<sup>+</sup>iNKT cells and Tregs. iNKT cells constitute a subset of T lymphocytes that have a hybrid phenotype of NK cells and conventional T cells and can be both of thymic and extrathymic (e.g. bone marrow or blood) origin (21). The majority of iNKT cells express CD4, but most of the remaining cells express neither CD4 nor CD8, although humans contain a small subset of CD8<sup>+</sup>iNKT cells (22,23). The T cell receptors of iNKT cells recognize glycolipid antigens that are presented through CD1d molecules on antigen-presenting cells (21). iNKT cells play an important role in the defence to bacteria and viruses, and after activation CD4<sup>+</sup>iNKT cells can produce both Th1-type and Th-2 type cytokines (22,23).

In the present study, we found that children with DS had more lower RTIs than their healthy siblings. It is well known that respiratory syncytial and influenza A viruses are important causes of severe viral disease in DS (2,24). Enhanced production of pro-inflammatory cytokines, which leads to excessive inflammation, and a more severe clinical course may be an explanation for the increased severity of influenza A infection in DS children (25,26). Also iNKT cells play an important role in the defence against influenza A virus by enhancing the maturation of dendritic cells, and the antigen-specific antibody response by B cells, and regulating CD8<sup>+</sup> T cell functions through the production of interferon-γ (21). Lower counts of iNKT cells in children with DS, as we and others found (10) might also contribute to the severity of influenza A infection.

Tregs are a naturally occurring subpopulation of CD4<sup>+</sup>CD25<sup>high</sup> T cells, which are produced by the thymus. Forkhead box protein 3 is a transcription factor that is expressed in CD4<sup>+</sup>Tregs and is required for Treg development and function. Tregs suppress host immune responses against self or nonself antigens and thus play a crucial role in the prevention of autoimmune disease and in the modulation of immune responses to bacterial and viral pathogens. During infection, Tregs suppress the inflammatory response via the production of anti-inflammatory cytokines, such as transforming growth factor β and interleukin-10 (27). During infection with swine-origin H1N1 influenza virus in children, low percentages of Tregs are present in patients with complications, which suggests dysregulation of the host immune response (28). The low number of Tregs that we found in children with DS could also lead to a hyperinflammatory response and therefore increased severity of influenza A infection.

Another important cause of RTI in children with DS is the presence of CHD in up to 33% (3,29). Children with DS and CHD were more often hospitalized for RTI and needed more often ventilatory support than children with DS but no CHD (3,30). In our study, the presence of CHD in children with DS did not enhance the frequency of lower RTI, but we have to take into account that the subgroups (CHD vs. non-CHD) are small and thus the subgroup analysis might be underpowered.

The limitations of our study are that we only had access to a small, however, useful group of children with DS and their siblings and that our study design was retrospective as far as the history of infections was concerned.

In conclusion, children with DS have reduced levels of IgG2 and reduced numbers of lymphocytes, CD4<sup>+</sup> T cells, CD4<sup>+</sup>iNKT cells and Tregs, as compared with their healthy siblings. This could contribute to an increased frequency of lower RTI and related hospitalization.

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CONFLICT OF INTEREST

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