

# Down Syndrome B-Lymphocyte Subpopulations, Intrinsic Defect or Decreased T-Lymphocyte Help

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**ABSTRACT:** Down syndrome (DS) is known for increased incidence of respiratory infections and autoimmune diseases, indicating impaired immunity. Until now, attention has been mainly focused on T lymphocytes. Therefore, we determined B-lymphocyte subpopulations in 95 children with DS compared with 33 age-matched control (AMC) children. DS serum immunoglobulin levels were compared with 962 non-DS children with recurrent infections. The results were combined with clinical data. Transitional and naive B lymphocytes are profoundly decreased in the children with DS. This could be caused by an intrinsic B-lymphocyte defect resulting in (partial) failure of B-lymphocyte generation, decreased antigen-induced proliferation and/or increased apoptosis, or by decreased proliferation due to deficient T-lymphocyte help, or a combination of these. The decreased CD27<sup>+</sup>, CD21<sup>high</sup>, and CD23<sup>+</sup> cells are reminiscent of common variable immunodeficiency and suggestive of disturbed peripheral B-lymphocyte maturation. Immunoglobulin levels in DS are abnormal—as has been described before—and different from non-DS children with recurrent infections. We conclude that the humoral immune system is abnormal in DS, but could not find a relation between B-lymphocyte subsets, immunoglobulins and clinical features of the children with DS in our cohort, nor could we answer the question whether DS lymphocytes are truly intrinsically deficient, or could all findings be explained by deficient T-lymphocyte help. (*Pediatr Res* 67: 563–569, 2010)

Down syndrome (DS) is associated with recurrent—mainly respiratory—infections (1,2), decreased responses to vaccination (3–8), a higher frequency of hepatitis B surface antigen carriers (3), and autoimmune diseases such as celiac disease and hypothyroidism (9–11). These features are suggestive of immunodeficiency. Until now, medical attention has been mainly focused on the thymic alterations and decreased absolute numbers of T lymphocytes in peripheral blood (12,13). We recently showed that a striking B lymphocytopenia is present from the very beginning in patients with DS (14). This B lymphocytopenia could be due to an intrinsic B-lymphocyte defect, a deficient T-lymphocyte help, or a combination of these. An intrinsic B-lymphocyte defect could be due to (partial) failure of B-lymphocyte generation, decreased antigen-induced proliferation, and/or increased apoptosis. Deficient T-lymphocyte help could lead to disturbed B-lymphocyte activation and proliferation. Despite the B lymphocytopenia, a considerable hypergammaglobulinemia of IgA and IgG after the age of 5 y, with high levels of IgG<sub>1</sub> and IgG<sub>3</sub> and low levels of IgG<sub>2</sub> and IgG<sub>4</sub>, is described (3,15,16).

This combination of profound B lymphocytopenia and hypergammaglobulinemia favors a disturbance in T-lymphocyte help, with the possibility that immunoglobulins are oligoclonal in DS, and specific T-cell-dependent antibody responses inadequate. The latter has indeed been described (3,15). However, the T-cell-independent antibody response to pneumococcal polysaccharide antigen is also decreased in DS (4), suggesting an intrinsic B-lymphocyte defect is also present. We studied B-lymphocyte subpopulations in relation to relevant clinical features in 95 children with DS, to further unravel this question.

**METHODS**

**Study population.** From 95 noninstitutionalized children with DS (49 boys; Fig. 1), either visiting the Jeroen Bosch Hospital, 's-Hertogenbosch or the Rijnstate Hospital, Arnhem, the Netherlands, an extra 3 mL of EDTA and 7 mL of blood without additive was drawn during routine follow-up of thyroid function after parental informed consent. All children were otherwise healthy at the time of sampling. Leftover EDTA blood from 33 healthy age-matched control (AMC) children who underwent venipuncture, e.g. preoperative screening for minor surgery, was used as control.

## METHODS

We retrospectively collected the titers of serum IgG, IgA, and IgM that were determined for diagnostic purposes in 962 non-DS patients suffering from recurrent infections (younger than 21 y) between January 2006 and July 2008 in the Jeroen Bosch Hospital, 's-Hertogenbosch, and the Bernhoven Hospital, Oss/Veghel, The Netherlands. In 285 of the 962 patients, IgG-subclasses were also determined. The study was approved by the local Medical Ethics Committees of both hospitals.

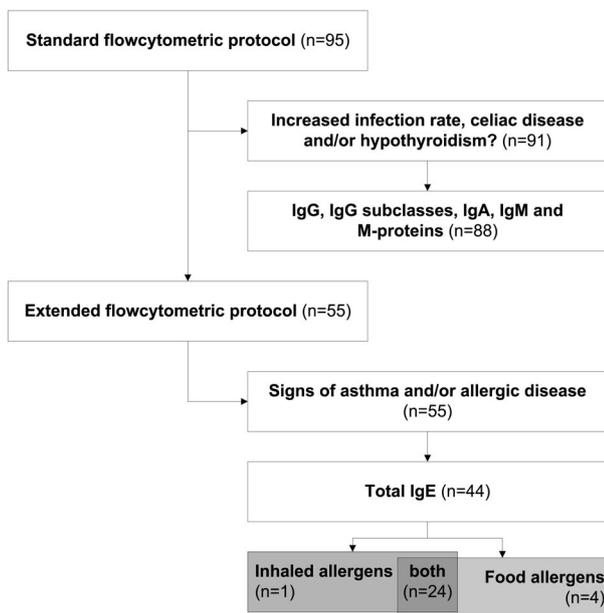
**Immunophenotyping.** Three-color flow cytometric immunophenotyping was performed to determine B-lymphocyte subpopulations in children with both DS and AMC using the lysed whole-blood method. FITC, phycoerythrin (PE), and PE-cyanine 5 (PE-Cy5)-conjugated antibodies were used with the following antigen specificity: CD3 (PE-Cy5; Immunotech, Marseille, France), CD5 [FITC; Becton Dickinson (BD), San Jose, CA], CD10 (FITC; BD), CD16/CD56 (FITC; BD), CD14 (PE; BD), CD15 (FITC; IQProducts, Groningen, The Netherlands), CD19 (PE-Cy5; Immunotech), CD20 (PE; BD), CD21 (PE; BD), CD23 (PE; BD), CD27 (FITC; BD), CD38 (PE; BD), CD45 (PE-Cy5; Immunotech), κ (PE; Dako, Carpinteria, CA), and λ (PE; Dako). In all children, T lymphocytes (CD3<sup>+</sup>), B lymphocytes (CD19<sup>+</sup>), natural killer (NK) cells (CD16<sup>+</sup> and/or CD56<sup>+</sup>CD3<sup>-</sup>), and CD21 and CD5 expression on CD19<sup>+</sup> B lymphocytes were determined. An extended protocol was used in the last 55 included children. In this group, CD10, κ and λ expression, CD27 and CD20 expression, CD27 and CD38 expression, and CD27 and CD23 expression on CD19<sup>+</sup> B lymphocytes were also analyzed.

**Abbreviations:** AMC, age-matched control; BD, Becton Dickinson; DS, Down syndrome; NK, natural killer; PE, phycoerythrin; PE-Cy5, PE-cyanine 5

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**Figure 1.** Patient flow diagram. Flow diagram showing group size of patients with Down syndrome in relation to assessed variables.

Aliquots were incubated for 15 min at room temperature in the dark with different combinations of optimally titrated antibodies. Only for the samples that were incubated with anti- $\kappa$  or anti- $\lambda$  antibodies, the aliquots were washed three times with 0.5% BSA/PBS before incubation. Erythrocytes were lysed using FACSlysing solution (BD) according to the manufacturer's protocol. The remaining cells were washed twice with BSA/PBS and analyzed by flow cytometry after calibration with the SPHERO CaliFlow kit (Spherotech, Libertyville, IL) as recommended by the European Society for Clinical Cell Analysis (17). A FACScan or FACSCalibur flow cytometer (BD) was used in combination with CellQuest or CellQuest Pro software (BD). The lymphocyte gate was checked with a CD15/CD14/CD45 triple labeling and considered correct if  $<5\%$  contamination was present. T lymphocytes and NK cells were used to check whether the "lymphosum" (B + T + NK) equaled  $100 \pm 5\%$ . Absolute leukocyte counts were determined with a Sysmex SE-9500 hematology analyzer (Sysmex, Kobe, Japan). Absolute numbers of B-lymphocyte subpopulations were calculated by multiplying the absolute leukocyte count ( $\times 10^9/L$ ) by the relative total lymphocyte size (%) and relative size of the lymphocyte subpopulation (%).

**Immunoglobulins.** For 88 of the 95 children with DS, serum IgG, IgG1, IgG2, IgG3, IgG4, IgA, and IgM were studied; in seven children, serum was not available. IgG, IgA, and IgM were determined by kinetic nephelometry (Beckman Coulter Array 360, Beckman Coulter, Fullerton, CA); IgG-subclasses were assessed by kinetic nephelometry using a human IgG subclass nephelometry kit (Sanquin Reagents, Amsterdam, The Netherlands).

Qualitative M-proteins were assessed by serum electrophoresis on alkaline-buffered (pH 9.2) agarose gels by a Hydrasys system (Sebia, Norcross, GA). In cases of uncertainty, additional serum electrophoresis using immunofixation with monovalent antiserum was performed.

IgE was measured in 44 of the 55 children included in the extended protocol using a sandwich chemiluminescent immunoassay (Immulite 2500; DPC/Siemens, Deerfield, IL); the volume of serum available was insufficient in 11 children. Specific IgE testing (Immulite 2500) of food and inhaled allergens was performed in 28 and 25 children, respectively. When insufficient serum was available, we tested for food allergens only for children aged younger than 2 y and inhaled allergens only for children aged older than 2 y. The Fp5 food allergen panel (DPC/Siemens) contained egg white, cow's milk, codfish, soya, peanut, and wheat allergen. The AlaTOP inhaled allergen panel (DPC/Siemens) contained house mite (*Dermatophagoides pteronyssinus*), cat dander epithelium, dog dander, Bermuda grass, timothy grass, *Penicillium notatum*, *Alternaria tenuis*, birch, Japanese cedar, common ragweed (*Ambrosia artemisiifolia*), and English plantain and *Parietaria officinalis* allergen. To interpret the IgE results, we used our laboratory cutoff values of  $<50$  U/mL for children aged younger than 10 y and  $<90$  U/mL for children aged older than 10 y and adults.

**Review of medical files.** The medical files of 91 of the 95 children with DS were reviewed retrospectively; four files were unavailable. The 91 children

were divided into four groups: 1) no increased infection rate, 2) increased infection rate (age at inclusion younger than 8 y), 3) increased infection rate (age at inclusion older than 8 y), and 4) increased infection rate until, but not after, the age of 8 y. The presence of celiac disease or autoimmune hypothyroidism was noted. In addition, the 55 patients of the extended protocol were also divided into positive or negative for symptoms of asthma and/or allergic disease (recurrent cough, persistent wheeze, admission on a pediatric ward for asthma exacerbation, clinical response to bronchodilators and/or inhalation corticosteroids, and clinical signs of allergic disease).

**Statistical analysis.** An analysis of variance (completely randomized two-factorial design;  $p < 0.05$ ) was applied to the data. For this analysis, we excluded those age groups for which the number of AMC data was too low. The two fixed factors in the analysis of variance were age (three age groups: 2–5, 5–10, and 10–16 y) and the difference between DS and AMC children. Levene's test for equality of error variances was used, and the results are mentioned in the text only when  $p < 0.05$ . The one sample *t* test ( $p < 0.05$ ) was used to compare the Ig values of children with DS and non-DS children suffering from recurrent infections with the mean of age-matched reference values and each other (18,19). All analyses were performed with SPSS 16.0 for Windows.

## RESULTS

**B-lymphocyte subpopulations.** The absolute and relative numbers of CD19<sup>+</sup> B-lymphocyte subpopulations with results of statistical analyses can be found in Table 1, clinically relevant data are presented in Table 2. We did not find a relation between any of the determined B-lymphocyte subpopulations and the incidence of infections or of allergic complaints and/or asthma in these children with DS. The values for CD19<sup>+</sup> B lymphocytes were reported before (14): the CD19<sup>+</sup> B-lymphocyte count is decreased significantly in all age groups in DS compared with AMC, and the enormous expansion, which is found in healthy children in the first years of life, is lacking (20). The effect of age on the CD19<sup>+</sup> B-lymphocyte count is significantly different between DS and AMC children (interaction for absolute values; Table 1), so this finding is highly significant. The  $\kappa/\lambda$  ratio is slightly increased in older children with DS. CD5<sup>+</sup> and CD5<sup>-</sup> B lymphocytes follow the pattern of total CD19<sup>+</sup> B lymphocytes in children with DS. There is no evident increase of "immature" B lymphocytes in DS: CD10<sup>+</sup> and CD20<sup>-</sup> B lymphocytes do not clearly differ between DS and AMC children.

CD27<sup>-</sup>CD38<sup>dim</sup> naive B lymphocytes and CD27<sup>-</sup>CD38<sup>+</sup> transitional B lymphocytes follow the pattern of total CD19<sup>+</sup> B lymphocytes in DS as well. Unfortunately, we cannot differentiate between CD27<sup>+</sup>CD38<sup>dim</sup>IgD<sup>+</sup> marginal zone and CD27<sup>+</sup>CD38<sup>dim</sup>IgD<sup>-</sup> memory B lymphocytes because the expression of IgD was not determined. Absolute and relative numbers of total CD27<sup>+</sup> B lymphocytes are decreased in DS compared with AMC children; the absolute numbers show a slight increase during the CD19<sup>+</sup> B-lymphocyte expansion in the first years of life, which is less prominent in DS than in AMC children. The CD27<sup>+</sup>CD38<sup>++</sup> plasma cell population is small in peripheral blood, but—unexpectedly—not different between DS and AMC children. The relative and absolute numbers of B lymphocytes with high expression of CD21 (CD21<sup>high</sup>) are significantly decreased in children with DS; the absolute numbers decline with age, but more so in AMC than in children with DS due to a higher initial peak in the former. The same holds true for CD23. The relative expression of CD23 within the CD19<sup>+</sup> B-lymphocyte population shows a far wider range in DS than in AMC children.

**Table 1.** Absolute and relative numbers of B-lymphocyte subpopulations in Down syndrome children compared with age-matched control children

Total CD19 <sup>+</sup> B lymphocytes	9-15 mo		15-24 mo		2-5 y		5-10 y		10-16 y		Older than 16 y		DS vs AMC, p*	Age effect for DS + AMC, p†	Interaction DS and AMC, p‡
	n	AMC	n	AMC	n	AMC	n	AMC	n	AMC	n	AMC			
DS	18 (9-26)	0.46 (0.19-1.14)	14 (8-24)	0.33 (0.16-0.76)	13 (6-24)	0.28 (0.13-0.55)	16 (9-22)	0.19 (0.06-0.45)	38 (19-58)	0.11 (0.05-0.58)	19 (6-37)	0.11 (0.04-0.12)	<0.001	<0.001	0.001
AMC	20	1.47	33	0.91	23 (18-34)	0.75 (0.33-0.96)	10 (12-21)	0.45 (0.32-0.66)	8	0.32 (0.19-0.60)	11 (10-17)	0.26 (0.23-0.29)	<0.001	<0.001	NS
CD19 <sup>+</sup> CD5 <sup>+</sup>															
DS	49 (31-74)	0.22 (0.06-0.88)	37 (23-55)	0.14 (0.04-0.30)	30 (20-76)	0.08 (0.04-0.29)	16 (9-22)	0.06 (0.01-0.15)	38 (16-47)	0.03 (0.02-0.22)	19 (19-24)	0.02 (0.01-0.02)	<0.001	<0.001	0.003
AMC	43	0.64	43	0.40	36 (15-59)	0.26 (0.08-0.55)	9 (21-38)	0.13 (0.1-0.22)	8	0.08 (0.02-0.28)	11 (10-17)	0.05 (0.04-0.06)	NS	0.007	NS
CD19 <sup>+</sup> CD10 <sup>+</sup>															
DS	0.02 (0.01-0.07)	0.01 (0.01-0.06)	0.01 (0.01-0.06)	0.01 (0.00-0.01)	0.02 (0.00-0.07)	0.01 (0.00-0.02)	7 (4-20)	0.01 (0.00-0.02)	19 (0.00-0.02)	0.00 (0.00-0.00)	13 (0.00-0.01)	0.01 (0.00-0.01)	NS	0.003	0.031
AMC	0.5 (0.2-2.3)	0.4 (0.2-3.4)	0.3	0.4 (0.2-3.4)	0.5 (0.1-3.5)	0.2 (0.04-1.4)	7 (2-39)	0.2 (0.04-1.4)	19 (0.06-1.1)	0.1 (0.06-1.1)	2 (2-2)	0.3 (0.04-0.5)	NS	NS	0.048
CD19 <sup>+</sup> CD20 <sup>-</sup>															
DS	5 (1-8)	0.00 (0.00-0.00)	6 (3-18)	0.00 (0.00-0.01)	5 (1-12)	0.00 (0.00-0.01)	7 (4-20)	0.00 (0.00-0.01)	19 (0.00-0.01)	0.00 (0.00-0.00)	13 (0.00-0.00)	0.00 (0.00-0.00)	NS	NS	NS
AMC	2	0.00	2	0.00	2 (1-4)	0.00 (0.00-0.01)	10 (2-33)	0.00 (0.00-0.00)	7 (1-8)	1 (0-8)	4 (3-5)	2 (2-2)	<0.001	NS	NS
CD19 <sup>+</sup> CD21 <sup>-</sup>															
DS	7 (1-16)	0.04 (0.01-0.08)	5 (3-26)	0.03 (0.01-0.06)	9 (4-22)	0.03 (0.01-0.10)	16 (11-23)	0.02 (0.00-0.17)	38 (9-42)	0.01 (0.00-0.05)	19 (6-34)	0.00 (0.00-0.02)	NS	<0.001	NS
AMC	3	0.04	0	0.00	6 (2-10)	0.05 (0.01-0.08)	9 (3-18)	0.02 (0.00-0.04)	8 (1-8)	2 (1-4)	5 (5-5)	0.02 (0.01-0.02)	<0.001	NS	NS
CD19 <sup>+</sup> CD21 <sup>low</sup>															
DS	22 (6-41)	0.09 (0.03-0.19)	20 (10-29)	0.07 (0.04-0.17)	14 (6-24)	0.03 (0.02-0.09)	16 (11-23)	0.03 (0.01-0.09)	38 (14-42)	0.01 (0.00-0.07)	19 (13-24)	0.01 (0.01-0.02)	0.05	<0.001	NS
AMC	15	0.22	6	0.06	8 (3-18)	0.06 (0.01-0.08)	9 (6-21)	0.04 (0.02-0.06)	8 (6-10)	6 (5-10)	9 (7-10)	0.03 (0.02-0.03)	<0.001	NS	NS
CD19 <sup>+</sup> CD21 <sup>high</sup>															
DS	69 (48-93)	0.25 (0.15-0.98)	70 (52-86)	0.25 (0.09-0.65)	77 (62-84)	0.22 (0.11-0.43)	16 (11-23)	0.14 (0.04-0.33)	38 (77-92)	0.09 (0.03-0.48)	19 (81-88)	0.08 (0.03-0.10)	<0.001	<0.001	0.006
AMC	82	1.22	87	0.87	86 (72-95)	0.68 (0.25-0.76)	9 (82-93)	0.42 (0.34-0.62)	8 (88-95)	30 (0.15-0.56)	11 (86-85-88)	0.23 (0.21-0.25)	<0.001	NS	NS
CD19 <sup>+</sup> CD23 <sup>+</sup>															
DS	66 (28-80)	0.36 (0.08-0.56)	73 (26-88)	0.23 (0.07-0.53)	61 (47-98)	0.24 (0.08-0.37)	7 (53-57)	0.10 (0.04-0.26)	19 (75-94)	0.08 (0.03-0.32)	13 (73-83)	0.06 (0.02-0.09)	<0.001	<0.001	0.017
AMC	92	1.38	64	0.60	82 (66-98)	0.62 (0.29-0.93)	10 (82-94)	0.38 (0.27-0.60)	8 (27-32)	0.27 (0.15-0.52)	11 (20-24)	0.20 (0.16-0.24)	<0.001	NS	NS
CD19 <sup>+</sup> CD27 <sup>+</sup>															
DS	1.2 (0.9-2.3)	0.04 (0.03-0.06)	1.5 (1.0-2.2)	0.04 (0.03-0.06)	1.9 (1.0-2.6)	0.05 (0.02-0.08)	7 (6.6-3.4)	0.03 (0.01-0.06)	19 (1.0-2.6)	0.01 (0.00-0.07)	13 (0.6-0.8)	0.01 (0.01-0.01)	<0.001	<0.001	0.041
AMC	0.06	0.08	0.08	0.08	0.12 (0.05-0.18)	0.12 (0.05-0.18)	10 (0.8-5.0)	0.08 (0.05-0.11)	8 (0.4-0.15)	0.04 (0.01-0.15)	0.05 (0.04-0.06)	0.05 (0.04-0.06)	<0.001	<0.001	NS
CD19 <sup>+</sup> CD27 <sup>dim</sup>															
DS	64 (41-85)	0.35 (0.15-0.79)	63 (48-82)	0.17 (0.08-0.56)	66 (60-71)	0.25 (0.08-0.37)	7 (58-49-80)	0.10 (0.04-0.25)	19 (67-55-82)	0.07 (0.03-0.44)	13 (70-57-82)	0.06 (0.02-0.09)	<0.001	<0.001	NS
AMC	73	1.07	63	0.58	57 (47-67)	0.50 (0.18-0.56)	10 (31-24-0.42)	0.31 (0.24-0.42)	8 (71-59-77)	0.22 (0.14-0.43)	11 (72-68-77)	0.20 (0.18-0.22)	NS	0.001	0.014

(Continued)

Table 1. Continued

	9–15 mo	15–24 mo	2–5 y	5–10 y	10–16 y	Older than 16 y	DS vs AMC, <i>p</i> *	Age effect for DS + AMC, <i>p</i> †	Interaction DS and AMC, <i>p</i> ‡
CD19 <sup>+</sup> CD27 <sup>-</sup> CD38 <sup>+</sup>									
DS	0.13 (0.04–0.24) 27 (7–49)	0.06 (0.03–0.09) 17 (8–36)	0.06 (0.02–0.08) 15 (7–21)	0.02 (0.01–0.10) 13 (5–30)	0.01 (0.00–0.05) 8 (5–25)	0.01 (0.00–0.01) 7 (5–9)	<0.001 NS	<0.001 0.031	NS NS
AMC	0.33 22	0.23 25	0.10 (0.05–0.31) 18 (7–36)	0.05 (0.02–0.15) 12 (5–22)	0.04 (0.01–0.09) 12 (3–21)	0.04 (0.04–0.04) 14 (12–16)			
CD19 <sup>+</sup> CD27 <sup>+</sup> CD38 <sup>++</sup>									
DS	0.02 (0.00–0.03) 3 (0–7)	0.02 (0.00–0.03) 5 (2–15)	0.01 (0.00–0.03) 3 (1–8)	0.01 (0.00–0.03) 3 (1–17)	0.00 (0.00–0.01) 2 (0–8)	0.00 (0.00–0.00) 0 (0–1)	NS	<0.001 NS	NS NS
AMC	0.01 0.4	0.01 1	0.01 (0.01–0.02) 1 (1–4)	0.01 (0.00–0.01) 1 (0–3)	0.00 (0.00–0.01) 1 (0–1)	0.01 (0.00–0.01) 2 (1–4)	0.005	NS	NS
CD19 <sup>+</sup> CD27 <sup>+</sup> CD38 <sup>dim</sup>									
DS	0.02 (0.01–0.03) 3 (2–7)	0.01 (0.01–0.02) 5 (2–7)	0.03 (0.01–0.04) 6 (4–10)	0.01 (0.00–0.03) 7 (2–11)	0.01 (0.00–0.03) 6 (1–17)	0.01 (0.00–0.01) 9 (5–12)	<0.001 <0.001	<0.001 NS	0.018 NS
AMC	0.07 5	0.06 7	0.13 (0.01–0.20) 17 (1–22)	0.07 (0.04–0.10) 14 (11–20)	0.03 (0.01–0.16) 10 (5–28)	0.02 (0.01–0.03) 8 (5–11)			
CD19 <sup>+</sup> κ <sup>+</sup>									
DS	0.32 (0.16–0.38) 59 (57–64)	0.18 (0.15–0.37) 57 (54–60)	0.23 (0.06–0.31) 60 (50–66)	0.10 (0.03–0.21) 62 (51–68)	0.07 (0.04–0.41) 63 (58–72)	0.05 (0.02–0.07) 62 (58–67)	<0.001 0.001	<0.001 NS	NS NS
AMC	0.85 58	0.49 56	0.44 (0.19–0.55) 57 (51–62)	0.23 (0.18–0.39) 56 (50–64)	0.18 (0.10–0.35) 58 (54–65)	0.15 (0.13–0.16) 55 (55–55)			
CD19 <sup>+</sup> λ <sup>+</sup>									
DS	0.19 (0.11–0.25) 39 (33–42)	0.13 (0.09–0.25) 40 (37–41)	0.12 (0.06–0.24) 39 (36–45)	0.06 (0.02–0.14) 36 (29–48)	0.04 (0.02–0.17) 36 (31–42)	0.03 (0.01–0.05) 38 (35–42)	<0.001 <0.001	<0.001 NS	0.04 NS
AMC	0.55 39	0.41 46	0.37 (0.12–0.43) 45 (33–52)	0.20 (0.16–0.24) 44 (38–50)	0.13 (0.08–0.25) 42 (31–45)	0.11 (0.09–0.12) 44 (42–46)			
κ <sup>+</sup> /λ <sup>+</sup> ratio									
DS	1.5 (1.4–2.0)	1.4 (1.4–1.6)	1.5 (1.1–1.8)	1.7 (1.2–2.1)	1.7 (1.4–2.3)	1.6 (1.6–1.7)	<0.001	NS	NS
AMC	1.5	1.2	1.2 (1.0–1.9)	1.3 (1.1–1.6)	1.4 (1.2–2.1)	1.3 (1.2–1.3)	<0.001	NS	NS

Upper line: absolute numbers of B-lymphocyte subpopulations (10<sup>9</sup> cells/L), lower line: relative numbers (%). Range is mentioned between brackets.

\* Analysis of variance, 2–16 y, DS vs AMC children.

† Analysis of variance, DS and AMC children together (2–16 y), effect of age.

‡ Analysis of variance, interaction effect between DS and AMC children aged 2–16 y.

**Table 2. Clinical features**

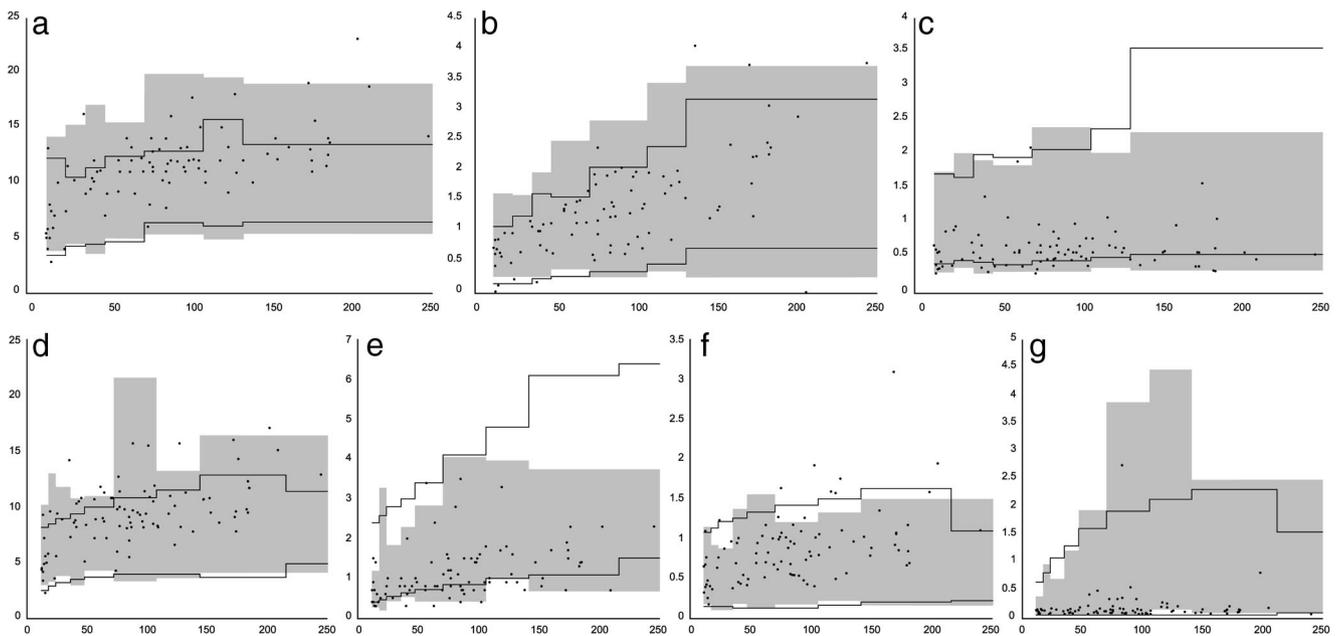
	9–15 mo (n = 11)	15–24 mo (n = 4)	2–5 y (n = 16)	5–10 y (n = 38)	10–16 y (n = 19)	Older than 16 y (n = 3)	Total (n = 91)
No increased infection rate (all ages)	n = 5		n = 6	n = 5	n = 1	n = 2	n = 19
Increased—mainly respiratory—infection rate (age at inclusion younger than 8 y)	n = 6	n = 4	n = 10	n = 20			n = 40
Increased—mainly respiratory—infection rate until, but not after, the age of 8 years				n = 6	n = 12	n = 0	n = 18
Increased—mainly respiratory—infection rate (age at inclusion older than 8 y)				n = 7	n = 6	n = 1	n = 14
Celiac disease	0/11*	1/4	1/16	2/38	1/19	0/3	5/91
Autoimmune hypothyroidism	0/11	1/4	0/16	1/38	1/19	0/3	3/91

	9–15 mo (n = 8)	15–24 mo (n = 6)	2–5 y (n = 7)	5–10 y (n = 19)	10–16 y (n = 13)	Older than 16 y (n = 2)	Total (n = 55)
Asthma	4/8†	3/6†	0/7	3/19	0/13	0/2	10/55
Allergy	1/8	1/6	0/7	0/19	0/13	0/2	2/55
Total IgE increased	0/6	1/3	1/5	2/17	2/12	0/1	6/44
Specific IgE inhaled allergens present	0/2	0/0	0/3	0/12	0/7	0/1	0/25
Specific IgE food allergens present	0/3	0/2	0/4	0/12	0/6	0/1	0/28

\* 0/11 means 0 of 11 patients tested, etc.

† It is doubtful whether these children will continue to have the diagnosis of asthma in later years (21).



**Figure 2.** Immunoglobulin values in Down syndrome compared with reference values and children suffering from recurrent infections. Values of a) IgG, b) IgA, c) IgM, d) IgG<sub>1</sub>, e) IgG<sub>2</sub>, f) IgG<sub>3</sub>, and g) IgG<sub>4</sub> obtained in 88 children with DS are shown as *black dots*. *x* axis age in mo; *y* axis immunoglobulin levels (g/L). The gray areas represent the values between the p2.5 and p97.5 of the determined immunoglobulin levels per age group in patients suffering from recurrent infections. Age-matched reference values (p2.5 and p97.5) are shown as a *black line*.

**Immunoglobulins.** The serum levels of IgG, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA, and IgM found in the children with DS and in non-DS children with increased infection rates (see Methods section) in comparison with age-matched reference values are shown in Figure 2 (18,19). In the DS group, mean IgG and IgG<sub>1</sub> are already higher than the age-matched reference values from the ages of 2 and 3 y onwards, respectively (one-sample *t* test; *p* < 0,05). Mean IgA and IgG<sub>3</sub> are normally distributed,

but mean IgM and IgG<sub>2</sub> are lower in children with DS in all age groups. IgG<sub>4</sub> values are consistently very low in children with DS. Mean Ig serum levels in the non-DS children with increased infection rates are similar to the children with DS for IgA and IgG<sub>2</sub>, but mean IgG is higher in DS children in some of the older age groups, and mean IgG<sub>1</sub> and IgG<sub>3</sub> are higher in DS children from the ages of 3 and 2 y onwards, respectively. Mean IgM and IgG<sub>4</sub> are lower in children with

DS than in the non-DS children with increased infection rates in the older age groups. We did not find any mono- or oligoclonal M-proteins in the 88 children with DS tested. IgE is increased in six of the 44 DS children tested; five showed high relative percentages of CD23<sup>+</sup> B lymphocytes, which are within the range of the AMC group (one with asthma; data not shown). Specific IgE testing of food and inhaled allergens is negative in all children tested.

## DISCUSSION

The profound B-lymphocytopenia in DS children, with decreased transitional and naive B lymphocytes compared with AMC children, is the most striking result of our study. There are no indications for release of unusual numbers of “immature” B lymphocytes from the bone marrow (CD10<sup>+</sup>, CD20<sup>-</sup>), the cells show the normal phenotype of the transitional and naive stages of peripheral B-lymphocyte development (22). As stated before, this could be caused by decreased B-lymphocyte proliferation due to a disturbance in T-lymphocyte help, an intrinsic B-lymphocyte defect, or a combination of these.

Interestingly, the distribution of B-lymphocyte subpopulations is reminiscent of the situation found in patients with common variable immunodeficiency (CVID)(23–25): CD27<sup>+</sup> cells, CD21<sup>high</sup> cells, and CD23<sup>+</sup> cells are decreased in absolute and relative numbers in the children with DS. These findings are suggestive of an intrinsic defect in B-lymphocyte maturation in the periphery.

CD21 is the complement type 2 receptor; it has a role in the response to polysaccharide antigens such as pneumococcal capsular elements. These antigens form a complex with CD21 on B lymphocytes causing a T-cell-independent response. The lower response to unconjugated pneumococcal vaccination and the increased rate of respiratory infections in DS could be related to this decreased expression of CD21. Interestingly, a subgroup of patients with CVID with relatively increased CD21<sup>low</sup> B lymphocytes is more likely to develop splenomegaly, autoimmune diseases, and lower respiratory tract infections (26); the latter two are frequently found in DS as well.

CD23 is the low-affinity IgE-receptor (FcεRII), it is a ligand of CD21. Together, they stimulate B-lymphocyte proliferation and differentiation (27). CD23 expression is increased just before the class switch from IgM to IgG, IgA, or IgE (27). Besides, CD23 is involved in both positive and negative feedback loops for IgE-homeostasis (27). Interestingly, both asthma incidence (RR: 0.4; 95% CI: 0.2–0.6) and IgE-levels are decreased in DS (28,29), which is consistent with our findings. Our results suggest that increased IgE production is associated with a higher level of CD23 expression in children with DS.

The serum Ig values in children with DS—with or without recurrent infections—and non-DS children with recurrent infections are both abnormal but differ from each other. Decreased IgG2 is a well-known abnormality in children with recurrent infections; this coincides with our findings in children with DS. However, the increased IgG, IgG1, and IgG3

and decreased IgM and IgG<sub>4</sub> levels are found in the children with DS only.

In conclusion, we found that the humoral immune system is disturbed in children with DS. We could not differentiate between an intrinsic B-lymphocyte defect and disturbed T-lymphocyte help as the most important cause based on our present data. This question remains unanswered, and further studies are needed to solve it.

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