Chapter 2

Increased risk of respiratory tract infections in children with Down syndrome: the consequence of an altered immune system

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Abstract

Down syndrome is the most common chromosomal abnormality among live-born infants. Respiratory tract infections are the most important cause of mortality in individuals with Down syndrome at all ages. In recent decades several studies have been performed to elucidate abnormalities of the immune system in Down syndrome. However, the influence of the immune system on the occurrence of respiratory tract infections in these children has never been reviewed.

1. Introduction

Down syndrome (DS) is the most common chromosomal abnormality among live-born infants. In Europe DS accounts for 8% of all registered cases of congenital anomalies. In a recent study conducted by our Down Syndrome Study Group the prevalence of DS in the Netherlands was determined to be 16 per 10,000 live births and thus is much higher than suggested in previous literature.[1] DS is characterized by a variety of dysmorphic features and congenital malformations, including congenital heart disease (CHD) and gastrointestinal disease. In addition, DS is associated with various immunological impairments. Leukemia and auto-immune diseases like hypothyroidism, celiac disease and diabetes mellitus are more prevalent in these children.[2] Respiratory tract infections (RTIs) in children with DS are a common problem encountered in daily clinical practice but evidence in literature is sparse.[3-6]

Life expectancy in children with DS has increased significantly in the last decade. However, neonatal and infant mortality in DS in the Netherlands is still 5 and 8 times higher than in children without DS, respectively (1.65% vs. 0.36% and 4% vs. 0.48%).[1] Important causes for increased mortality in DS are congenital heart disease, other congenital anomalies (e.g. of nervous system, respiratory system, gastrointestinal tract, genito-urinary system and musculoskeletal system), leukemia, testicular cancer and sepsis. In addition RTIs are still the most important cause of mortality in DS at all ages.[7-11]

Over the years, it has been suggested that the increased incidence of leukemia, celiac disease, hypothyroidism, and diabetes mellitus might be explained by an impaired immune system in patients with DS. A high incidence of respiratory morbidity in children with DS might be (partially) explained by an aberrant immune system as well. We have reviewed the literature on RTIs to support the clinical finding of a high incidence of respiratory morbidity in children with DS. Next, we have reviewed the immunologic literature in children with DS to clarify the role of the immune system in the respiratory morbidity in this specific population.

2. Respiratory tract infections

Children with DS have an increased risk of RTIs. RTIs can be divided in infections of the upper respiratory tract (URTI) (e.g. sinusitis, middle ear infections, rhinitis, tonsillitis, pharyngitis, laryngitis subglottica) and lower respiratory tract (LRTI) (e.g. pneumonia, bronchiolitis), which can be of diverse pathogenic origin (e.g. viral, bacterial, fungal or a combination of these).

Although data on the frequency of URTI in children are not exactly known and may vary in different studies because of different definitions and criteria by which they are assessed, the frequency of URTIs in children with DS seems increased compared to healthy controls: 12% have more than 3 URTIs in 12 months.[5] The most frequently described infections include pharyngitis in 27% and otitis media with effusion in 55%.[5,6] Abnormal anatomy of the upper respiratory tract may predispose children with DS to (chronic) URTIs. Stenotic ear canals, present in 40–50% of the newborns with DS, result in cerumen impaction.[12] Midface hypoplasia is common in these children as well, with smaller and abnormally inserted Eustachian tubes, and smaller nasal area as well as nasal sinuses. This, in combination with dysfunction of Eustachian tubes, may lead to accumulation of middle ear fluid and obstruction of airflow, making these children prone to otitis media. Hypoplasia of the nose and sinuses contributes to nasal obstruction, rhinorrhea and sinusitis. Hyperproduction of mucus was shown for most children with DS in a study performed by Piatti et al., but the ultrastructure and functions of the nasal cilia were normal.[13]

Lower respiratory tract pathology is the main cause of hospitalization and the most frequent cause for admission to the pediatric intensive care unit in children with DS.[4] Some children with DS and LRTI require intubation and mechanical ventilation. Children with DS were reported to have a higher incidence of acute lung injury and acute respiratory distress syndrome when they are mechanically ventilated in acute LRTI.[14] Acute lung injury is known to be associated with elevated rates of apoptosis of leukocytes and epithelial cells.[15] In DS an increase in the apoptosis of granulocytes has been observed[16] which might be a factor in DS contributing to a higher rate of acute lung injury.

A few case reports have been published on LRTIs in children with DS that were caused by uncommon microorganisms or that showed an uncommon course of disease: Cant et al. described four cases of bacterial tracheitis in children with DS, of which three were caused by *Haemophilus influenzae*.[17] These children were severely ill and had to be intubated and mechanically ventilated. One report described a child with DS who died because of pneumonia caused by *Bordetella bronchiseptica*, which normally causes RTIs only in animals.[18] Finally, Orlicek has reported on three children with DS under the age of 5 with a severe course of pneumonia caused by *Mycoplasma pneumoniae*, a microorganism that in the general population uncommonly produces such a serious infection.[19]

Besides these few case reports on rare pathogens or uncommon course of disease, there have been hardly any studies on the association of more common respiratory pathogens and

severe LRTI in children with DS. Respiratory syncytial virus (RSV) is the most important cause of severe LRTI in infants and young children worldwide, leading to hospitalization in many cases. DS is an independent risk factor for severe RSV-LRTI, resulting in a 10-fold increase in the risk of hospitalization for RSV-LRTI.[3] CHD, present in 40–60% of children with DS, is associated with an increased risk of hospitalization for RTIs, of which RSV is the most common pathogenic cause.[20] Children with DS with hemodynamically significant CHD have a more than twofold higher risk of hospital admission because of RTIs compared to controls with hemodynamically significant CHD without DS.[21]

In addition to the upper respiratory tract, anatomical abnormalities of the lower respiratory tract, such as laryngo- and tracheomalacia have been shown as well in children with DS.[22] Two groups have reported disturbed lung growth in children with DS that results in alveolar and pulmonary hypoplasia.[23-25] These abnormalities might lead to a different airway physiology with increased susceptibility to RTIs in children with DS compared to controls.

Children with DS are known to suffer from generalized hypotonia that may result in swallowing dysfunction and subsequently silent (micro) aspiration.[26] Recurrent aspiration of thin fluids is associated with an increased incidence of LRTIs. However, a study in children with neurologic impairment performed by Weir et al. showed that the diagnosis of DS was significantly associated with pneumonia, but swallowing dysfunction in these children did not have an additive effect on the risk of pneumonia.[27]

In conclusion, children with DS have an increased incidence of RTIs which might be associated with congenital heart disease, abnormal airway anatomy and physiology, hypotonia, and aspiration.

3. Immunology

In the last thirty to forty years several studies have focused on the immune system of patients with DS in an attempt to clarify the clinical problems frequently seen in this specific population. Although multiple congenital abnormalities are associated with DS, RTIs are an important cause of morbidity frequently seen in these children in daily clinical practice. In the following paragraphs, we have attempted to increase our understanding of the functions of the immune defense systems in children with DS and to translate it to their role in respiratory morbidity.

4. Innate immunity

4.1. Cell numbers

The innate immunity is very important in the first-line defense against microorganisms. Although children with DS have a high incidence of RTIs, innate immune responses have only been partially studied. Over the years different cell surface molecules have been used to describe different innate immune cells, which make it difficult to compare more recent with previous studies. The number of $CD16^+CD56^+$ natural killer (NK) cells is decreased in children with DS.[28,29] By contrast, in adults with DS this subset was shown to be significantly increased.[28] The exact function of CD57 on NK cells is not fully clear, but $CD16^-CD57^+$ cells were suggested to have low NK activity compared to $CD16^+CD56^+$ cells. In both children and adults with DS $CD16^-CD57^+$ cells are significantly increased.[28] Although functional studies have been performed with other innate cells such as polymorphonuclear granulocytes and monocytes, none of them have focused on absolute numbers. Neutrophils are reported to normally express surface markers (e.g. CD11a, CD11b, CD16 and CD18) in DS.[30] Invariant natural killer T-cells (iNKT-cells) have never been studied in DS up to date.

In conclusion, accurate information on numbers of most innate immune cells is not available, except for $CD16^+CD56^+$ NK cells which are decreased in individuals with DS compared to controls.

4.2. Cell function

Chemotactic migration of polymorphonuclear leukocytes (PMN) and mononuclear phagocytes is found reduced in DS.[16,30-32] This finding was suggested to be secondary to either an intrinsic defect of the leukocytes of DS (due to a shorter half-life), or enzymatic defects, or shifts in the migrating subpopulations of leukocytes. In contrast, random mobility, without chemotactic gradient, is normal in DS for leukocytes and mononuclear phagocytes.[30-32]

Some authors describe that PMN phagocytosis in children with DS is comparable to controls.[30,33] Others, like Rosner and Kozinn have shown decreased in vitro phagocytic ability of peripheral blood neutrophils to ingest live Candida albicans and decreased neutrophil adhesiveness in DS compared to controls.[34] No differences in the oxidative burst of PMN leukocytes have been established in children with DS compared to controls.[30] Peroxidase and periodic-acid/Schiff activity in leukocytes is normal as well.[34] Although a small decre-

ase in superoxide production by isolated neutrophils has been described.[16] Adults with DS have increased percentages of apoptotic neutrophils and eosinophils, both spontaneously and anti-Fas antibody induced.[16] GM-CSF and IL-5, cytokines that are reported to support the survival and activation of granulocytes, have less protective effect on apoptosis in DS than in controls. Fas and bcl-2 expression did not show any differences between DS and controls.

Studies on NK activity in DS have also shown contrasting results. Nurmi et al. showed slightly higher NK activity in adult patients with DS, both in peripheral blood mononuclear cells (PBMCs) and monocyte depleted PBMCs.[35] Lower NK cytotoxic activity compared to controls has been shown in children and adults with DS by others.[28,36,37] Nair and Schwartz have shown that during NK-cytotoxicity assays in children with DS lower levels of IFN are produced by lymphocytes against target cells in vitro.[37] This NK activity could be up regulated in DS by adding IL-2 or PHA to the culture, but not up to levels of healthy controls. However, in adults with DS NK activity could reach similar levels compared to controls by using peripheral blood lymphocytes (PBLs) preincubated with IL-2, IFNβ or IFNγ.[28] In DS subjects, no correlation between numbers of NK cells and NK activity has been observed.

IFNAR1 and 2, the genes encoding for the interferon α/β receptor, which binds type I interferons, are located on chromosome 21.[38,39] Because of an increased expression of the IFN receptor, trisomy 21 patients may have enhanced sensitivity to the antiviral effects of interferon. Trisomic fibroblasts were shown to have a three times higher response to both virus-induced and PHA-induced human interferon.[38] Together with an antiviral effect, interferon has several quite diverse effects as well. Epstein et al. have shown an increased sensitivity of DS monocytes to the inhibiting action of interferon on lysosomal enzyme activity, a measurement of monocyte maturation.[40] Although the number of patients in the study was small, this effect was hypothesized to override the antiviral effect and therefore to result in a reduced rather than an increased ability to respond to infectious agents in DS.

In conclusion these studies show conflicting results on a more or less disturbed PMN function resulting in decreased chemotaxis, normal or decreased phagocytosis and increased apoptosis. Overall most studies in DS suggest a decreased NK-cell activity in vitro, although this depends on the presence of cytokines and seems to improve with age. The results might be conflicting due to the age of patients studied, techniques used to separate cell populations and variations in DS individuals as well.

5. Adaptive immunity

5.1. T-cells

5.1.1. Thymus

Several groups have proposed that T-cell abnormalities found in children with DS might be explained by an abnormal thymic function and suggested that this dysfunction was the consequence of early senescence of the immune system. Over the years, several studies have been performed focusing on thymic deficiency in DS. Morphological and immunohistochemical studies of DS thymus have shown comparable histologic alterations.[41-45] Children with DS, differing in age from 1 day old up to 4 years of age, showed moderate to severe cortical thymocyte depletion with decreased thickness of the cortex compared to children without DS and from a poor demarcation to a complete disappearance of the corticomedullary junction. In addition, all studies showed enlarged Hassal's corpuscles in DS with cystic changes in the majority of cases and fibrosis in 46–77%. Levin et al. concluded that age was not a determining factor, since newborns already showed marked alterations.[41] In contrast, Larocca et al. defined three different groups according to severity of the thymic lesions and found that this was roughly related to age.[45] No definite conclusions can be drawn since groups were small and age distribution was not equal. No extrinsic factors (e.g. stress) have been found to explain differences between children with and without DS when thymus pathology was combined with clinical data.[41,42] In conclusion, these histopathologic results are compatible with accelerated involution and atrophy of the thymus as seen in elderly.

Thymic hormones have been studied to provide additional evidence of thymic impairment in DS. Activity of serum thymic factor (FTS), which is suggested to be essential for further differentiation into fully immunocompetent T lymphocytes, has been found to be lower in DS.[46,47] Lack of this factor was proposed as the primary cellular immune defect in DS. Fabris et al. showed lower FTS activity as well, with additional higher FTS inhibitory activity in DS compared to controls.[48] However, they also showed that this loss of activity could be restored by zinc-suppletion, and therefore do not support the hypothesis of primary thymic dysfunction.

Studies of thymocytes and subpopulations also have shown marked alterations in children with DS. Proportions of $CD1^+$, $CD3^+$, $CD4^+$ and $CD8^+$ thymocytes are decreased in children with DS.[43,44] Double positive $CD1^+CD3^+$ thymocytes are significantly lower compared to controls. In children with DS, a different distribution of cells with high expression of CD3

(CD3 bright) and low expression (CD3 dim) has been found. Compared to controls, these children have lower percentages of CD3 bright cells (18% versus 43%) and higher percentages of CD3 dim cells (58% versus 36%).[49] In addition, normal proportions of total CD3 expressing cells are found. Further maturation of thymocytes yields surprising differences.[50] Children with DS have higher percentages of CD8 bright thymocytes with lower percentages of CD8 dim cells, compared to controls. However, DS CD4 bright and dim cells are equal to controls. Total percentages of double negative and single positive CD4 and CD8 thymocytes are slightly decreased while the double positive thymocytes are slightly increased. However, differences have not been shown to be significant, probably due to the size of the groups (n=8). These studies suggest that in DS the process of T-cell commitment to either CD4 or CD8 single positive T-cells is present, although perhaps somewhat incomplete. Whether these different levels of expression will result in functional changes in DS has not been studied.

DS thymocytes are able to express TCRαβ. However, a significantly lower proportion of TCRαβ bright cells and a higher proportion of TCRαβ dim cells have been described in these children. The total percentage of thymocytes expressing TCRαβ is slightly lower in children with DS as well.[43,44,49,50] In contrast, normal proportions of TCRγδ expressing thymocytes in DS are described.[44] The findings of a higher percentage of mature single positive thymocytes expressing lower levels of TCRαβ and CD3 suggest a dysfunctional maturation in DS. These studies are partially indicative of a delayed maturation of T-cells within the thymus of DS.

Children with DS show slightly lower proliferative responses of thymocytes to IL-4 in vitro.[51] However, TCRγδ thymocytes, the thymocytes most responsive to IL-4, are normal in DS.

IFNγ and TNF have important inhibitory effects of IL-4 induced thymocyte proliferation.[52] In children with DS both IFNγ and TNFα expression are increased, with higher sensitivity to inhibition of the IL4-induced response in vitro. Consequently, T-cell differentiation and maturation might be impaired in DS.

Although the previous studies suggest dysfunctional maturation of thymocytes, this is not reflected directly in PBLs. The expression of both CD3 and TCRαβ on PBL in DS is comparable to children without DS. However, both the percentage and absolute numbers of TCRαβ expressing cells is decreased in DS. In contrast, the proportion and absolute numbers of TCRγδ is increased.[53]

In recent years attempts have been made to quantify thymic output by using T-cell receptor excision circle (TREC) content of cells. Lower percentages of TREC positive lymphocytes

have been shown in children with DS, which showed an age-related decrease in contrast to healthy controls.[54,55] Low TREC content, however, might rather be explained by lower percentage of naïve T-cells in DS, the main cell type containing TRECs. Higher plasma levels of IL-7, a primary cytokine in T-cell survival and maturation, and IL-15, a primary cytokine in the regulation of CD8 T-cells, are found in children with DS, with normal expression of IL-7Rα. Although DS T-cells show normal responses upon IL-7 stimulation, the increased IL-7 plasma levels do not result in higher proliferation rates of DS T-cells.

In conclusion, studies on DS thymus have provided evidence of accelerated involution of the thymus, an altered pattern of maturation of thymocytes and indications of inefficient thymic output.

5.1.2. T-cell numbers

Absolute total leukocytes and lymphocytes are significantly lower in children with DS at all ages.[29,56-58] As a consequence absolute numbers of T-cells are lower as well, especially in the first two years of life. With increasing age differences become smaller. As expected, absolute counts of $CD4^+$ and $CD8^+$ T-cell subsets are lower as well. Looking at distribution of lymphocytes, normal percentages of $CD3^+$ T-cells are reported.[56-58] In contrast, increased percentages of $CD8^+$ and decreased $CD4^+$ subsets are described,[57] although, according to Cocchi et al. only beyond the age of 3 years.[56] The $CD4^+/CD8^+$ ratio is stable with age, but lower in DS than in healthy controls. Further differentiation of $CD4^+$ and $CD8^+$ subsets reveals lower percentages and absolute counts of naïve T-cells in both subsets and increased percentages of memory subsets.[53,55] One study has reported increased percentages of activated (Ia^+) T-cells in DS.[59] This finding might be explained by the fact that all these patients had known auto-immune diseases such as hypothyroidism and diabetes mellitus.

It can be concluded that children with DS have decreased absolute numbers of actually all $CD4^+$ and $CD8^+$ T-cell subsets, especially in the first two years of life, with a relative increase of $CD8^+$ T-cells and memory subsets.

5.1.3. T-cell function

As described above, most of the $CD3^+$ T-cells in the peripheral blood of DS have a mature phenotype. These cells would be expected to proliferate normally after anti-CD3 stimulation. In contrast, proliferation upon stimulation with anti-CD3 is depressed in children with DS.[60,61] Scotese et al. have shown that children with DS have an aberrant pattern of the signaling pathway after CD3 cross-linking, characterized by the absence of tyrosine phosphorylation

of part of the proteins involved in the cascade. In contrast, the gamma chain of the IL-2-receptor is normally expressed and properly phosphorylated during cell activation in DS.

In addition to anti-CD3, several studies on proliferation of T-cells upon mitogenic stimulation and stimulation with recall antigens in DS have been performed. Use of patients of different ages combined with different mitogenic stimuli, such as phytohaemagglutinin (PHA), concanavalin (ConA), and pokeweed (PWM) make it difficult to compare most studies. Contrasting results are reported for responses upon stimulation with PHA in DS, being either normal[57,62-64] or significantly decreased.[47,58,65-68] Burgio has shown a clear effect of age, with normal response in children up to ten years of age and afterwards a significant decrease with increasing age. This is in contrast with Lockitch who has shown a small, not significant, increase of the response with age. Lower mitogenic responses are found in DS upon stimulation with PWM and ConA.[58,67,68] However, Lockitch et al. have shown a reverse age effect with higher proliferation in DS than healthy controls upon PWM stimulation below six years of age but lower proliferation beyond six years. Stimulation with a recall antigen (Purified Protein Derivative, PPD) is followed by normal proliferation in DS.[68] A decreased proliferation in DS in response to the specific viral antigens of influenza A and B, and also to tetanus toxoid has been described.[69,70] The decreased proliferation in response to influenza B in DS is mainly due to a decreased response of CD4 T-cells. This effect is partially overcome in the presence of monocytes and B-cells.

PHA stimulation induces normal IL-2 production in DS.[65] Expression of IFNγ, encoded by chromosome 21, is found to be normal upon stimulation, but with a higher basal level.[71] No correlation with age is described. One study of serum levels of IFNγ in otherwise healthy patients with DS, aged 22–58 years, showed marked and significantly increased levels.[72] Epstein and Epstein have shown that stimulation of T-cells with PHA results in production of normal amounts of interferon in DS and normal proliferation.[64] The addition of exogenous interferon to stimulation with PHA inhibits proliferation equally in both DS and controls. However, when ConA was used as stimulus, DS appeared to be significantly more sensitive to inhibition of proliferation by interferon. A reciprocal relationship was shown between stimulation with tetanus toxoid and the effect of interferon on this stimulation. Low levels of toxoid-stimulated proliferation results in increased stimulation by interferon in DS, while in controls at high levels, interferon has a more inhibitory effect on proliferation. It was suggested by Epstein et al. that this might be more the result of an effect of the toxoid than of the trisomic state. In addition, responses of different T-cell subsets may be involved in the differences in effect of the two mitogens used.

Antibody-dependent cell-mediated cytotoxicity (ADCC) is lower in children with DS compared to healthy controls.[37,47,67,73] Autologous mixed lymphocyte reaction (MLR) with T-cells and irradiated non-T-cells has been shown to be much lower in children with DS compared to healthy controls.[47] This result could not be confirmed by Gupta et al., who reported a normal response on autologous MLR in DS.[74] Age could be a possible explanation for this discrepancy in results, since the group studied by Franceschi et al. was older. Allogeneic MLR resulted in either equal[47,74] or lower[36] activity in DS compared to controls. Dissociation between autologous and allogeneic MLR has been observed in auto-immune diseases, such as SLE, and normal aged individuals. $CD4^+$ T-cells are described to be the cells proliferating during MLR. The lower autologous MLR might therefore be explained by lower $CD4^+$ T-cell counts in DS.

Lymphocyte functional antigen-1 (LFA-1) is expressed on the cell surface of lymphocytes. LFA-1 is a heterodimeric molecule consisting of α (CD11a) and β chains (CD18), which is encoded on chromosome 21. Since LFA-1 has a role in intercellular adhesion, it has been suggested that overexpression of this antigen (due to a gene dosage effect in DS), would lead to increased aggregation of cells and subsequently causes cellular immune dysfunction. It has indeed been shown that LFA-1 is overexpressed on lymphoid cells of DS.[75,76] However, the overexpression could be explained by an abnormal distribution of lymphocyte subsets in DS that express different levels of LFA-1.[75,77] In addition, it was shown that DS children under 2 years of age have increased levels of LFA-1, but consecutively lack an age-associated increase beyond the age of 2 in $CD4^+$, $CD8^+$, $CD45RO^-$ and $CD45RO^+$ subsets compared to controls resulting in comparable expression at older age.[77] Although LFA-1 levels were comparable at older age, significantly lower binding of T-cells to intercellular adhesion molecule 1 (ICAM-1) was shown in vitro in children with DS. This was also in apparent contrast to a previous study showing general increased adhesion of lymphocytes in DS.[77,78] A possible explanation for this defective binding by LFA-1 could be either abnormal T-cell activation or defective intracellular signal transduction in DS. These findings therefore do not support the hypothesis of lymphocyte hyperadhesiveness as the cause of immunologic problems seen in DS.

An important function of T-cells is to regulate the immune response. Differentiation into $CD4^+$ T-helper (Th) subsets is an important step in selecting effectors functions. Cytokines are major contributors to a Th1 or Th2 type of response. While Th1 cytokines promote a cellular immune reaction, Th2 cytokines drive humoral immune responses. In children with DS no studies have been performed on this specific subject. However, a second type

of T-cells with a regulatory function (Tregs, $CD25^+$ $FoxP3^+$ $CD4^+$ T-cells) has been reported in DS. One study described a relative increase of Tregs in DS, but no functional tests have been performed.[55]

In conclusion, different functional assays have been performed in DS, revealing decreased proliferation and cytotoxicity of T-cells in most of them. These combined studies lead to the hypothesis that accelerated thymic involution in children with DS results in both decreased numbers and dysfunction of T-cells.

5.2. B-cells

5.2.1. B-cell numbers

Children with DS were found to have decreased counts of B-lymphocytes compared to healthy children.[57,58] De Hingh et al. confirmed this and reported that the primary expansion of B- lymphocytes as seen in healthy children in the first years of life does not occur in children with DS.[29] Throughout childhood, the B lymphocyte population remains severely decreased. It was suggested that this is caused by an intrinsic abnormality of the adaptive immune system. One of the possible causes is a decreased maturation of B-lymphocytes in DS. There is one early report however in 1975 by Burgio et al. that in 83 DS children the B-cell counts were normal, compared to 76 controls.[63]

Overall, the number of B-cells is reported to be decreased.

5.2.2. B-cell function

Several studies have been done in children with DS in which serum levels of immunoglobulin A, M and G and/or IgG subclasses were measured.[56,58,63,79-82] Cocchi et al. showed normal IgA and IgG levels in children with DS,[56] while in other studies elevated serum levels of IgA and IgG compared to controls were found.[58,63,80,82] This hyperglobulinemia might be explained by a slower elimination of infectious agents in DS, which may cause overstimulation of the immune system and overproduction of antibodies. IgM levels in DS are reported to be diminished[56,58,63] or normal.[80-82] In DS the IgG1 and IgG3 levels are often elevated, whereas the IgG2 and IgG4 levels are diminished.[79,81] Lower IgG2 and IgG4 levels may partially explain the increased susceptibility of children with DS to infections with encapsulated bacteria. In 1990 Anneren et al. reported an increase in serum concentrations of IgG2 and IgG4 in DS after a selenium supplement of 10 microgram/kg/day during 6 months.[83] The parents reported spontaneously a reduced infection rate during this treatment. This study

suggests that selenium might have an immunoregulatory effect. Costa-Carvalho et al. in 2006 evaluated the production of antibodies to a 23-valent pneumococcal vaccine in 17 children with DS (age 6–13 years).[80] Before the vaccination, these children had normal IgA and IgM levels, but they had elevated levels of total IgG, and the IgG subclasses of IgG1 and IgG3 and lower levels of IgG subclasses IgG2 and IgG4 than the controls. All DS children had a significant increase in the levels of antibodies (IgM and IgG2) to all serotypes of the vaccine, although these levels were lower than in the controls. Their advice is that this 23-valent pneumococcal vaccine could be of benefit in children with DS.

In conclusion, B-cell production of IgM antibodies is normal or decreased in children with DS, while IgG and IgA are normal or even increased.

6. Early senescence of the immune system

The abnormal findings of thymic immunohistology and function in DS have lead to the concept of early senescence of the immune system in this specific population. Ageing not only results in thymic involution, but also in changes of telomere length and apoptotic rate of immune cells. Telomere shortening has a causal role in cellular ageing. With ageing shortening of telomere length is seen in lymphocytes.[84] In individuals with DS the rate of loss of telomeres of peripheral blood leukocytes is significantly increased compared to healthy controls. This loss of telomeric length is comparable in subpopulations of T-cells, B-cells and neutrophils of DS. Several explanations for the increased loss of telomeres in DS have been suggested; individuals with DS might have an increased cell division because of immunologic abnormalities. The rate of telomere loss could also be increased if the expression of genes involved in telomere length regulation is altered due to trisomy 21. Holmes et al. studied fetuses with DS to determine if accelerated telomere loss is associated with a stem cell deficiency, predisposing children with DS to clonal changes.[85] They found that leukocytes of fetuses with DS already showed significantly decreased telomere length. In addition, they showed that both fetuses and children with DS had significantly reduced numbers of hematopoietic stem cells, mostly from the myeloid lineage. In contrast, the erythroid progenitors were not affected in fetuses with DS.

The decreased numbers of immune cells seen in children with DS might be the result of increased loss due to apoptosis. In addition to increased apoptosis of granulocytes, Corsi et al. describe a higher proportion of $CD3^+$ T-cells in DS that express CD95, also known

as the Fas-receptor that induces apoptosis upon Fas-ligand binding.[86] Purified T-cells of children with DS showed a higher percentage of cells positive for Annexin-V compared to controls (33.2% vs. 29.8%). However, cells positive for both propidium iodide and Annexin-V, indicative of necrotic cells, were decreased in DS (1.8% vs. 3.7%). In addition, Roat et al. studied apoptosis after in vitro treatment of PBMC with apoptogenic drugs.[87] Children with DS showed similar tendency to undergo apoptosis compared with controls, both unstimulated and after stimulation with apoptogenic drugs.

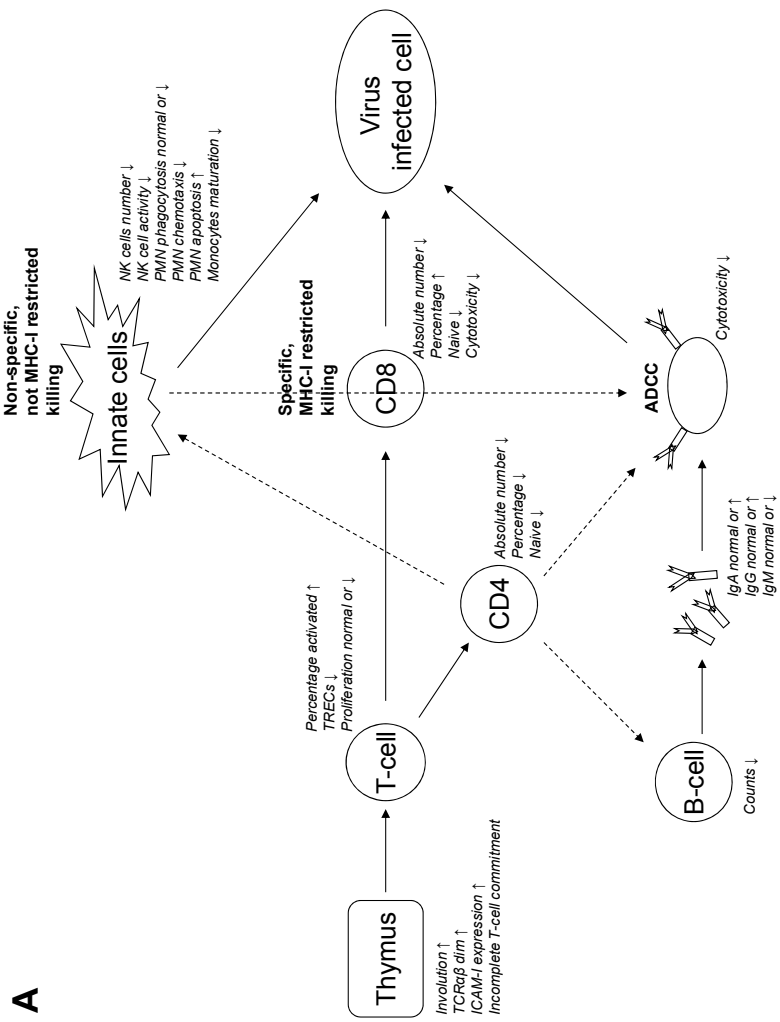
From these studies we conclude that in individuals with DS telomere shortening occurs already in fetal life, it continues after birth and in the myeloid lineage might be influenced by progenitor cell deficiency. With respect to apoptosis, an increased apoptotic rate probably does not play a role in the decreased numbers of immune cells in children with DS.

7. Immunologic mechanisms underlying increased incidence of respiratory tract infections in DS

The previously described immunologic abnormalities in DS have been presented in diagrammatic form in Figure 2.1 in an attempt to clarify the role of the immune system in the respiratory morbidity in children with DS. The first-line defense against bacteria and viruses, the innate immunity, seems clearly disturbed in children with DS. Both quantitative and qualitative abnormalities have been shown for children with DS. Decreased numbers of NK cells, decreased NK-function, phagocytosis and chemotaxis of PMNs and monocytes might result in decreased direct killing and clearance of pathogens in this specific population.

The next step in bacterial and viral defense, T-cell immunity, is impaired in DS as well. Accelerated involution of the thymus, abnormal thymic maturation and low thymic output might explain low absolute numbers of $CD4^+$ and $CD8^+$ T-cells, especially naïve T-cells, decreased cytotoxicity and peripheral proliferation in children with DS. Again, quantitative and qualitative defects in children with DS might result in decreased pathogenic killing and clearance. The increased percentage of activated T-cells described, might not play a role in pathogenic clearance, but rather reflect a chronic or auto-immunologic state in DS.

Besides T-cells, B-cell counts are lower in DS as well. Although IgM antibodies production is normal or decreased in DS, levels of other antibodies are normal or even increased (IgA, IgG). Whether this is caused by an intrinsic functional difference in B-cells or is a reflection of acute or chronic inflammation is unclear. Based on these numbers however, normal


A
Non-specific, not MHC-I restricted killing
Innate cells
NK cells number ↓
NK cell activity ↓
PMN phagocytosis normal or ↓
PMN chemotaxis ↓
PMN apoptosis ↑
Monocytes maturation ↓
Specific, MHC-I restricted killing
CD8
Absolute number ↓
Percentage ↑
Naive ↓
Cytotoxicity ↓
Virus infected cell
ADCC
Cytotoxicity ↓
Thymus
Involution ↑
TCRαβ dim ↑
ICAM-I expression ↑
Incomplete T-cell commitment
T-cell
Percentage activated ↑
TRECs ↓
Proliferation normal or ↓
CD4
Absolute number ↓
Percentage ↓
Naive ↓
B-cell
Counts ↓
IgA normal or ↑
IgG normal or ↑
IgM normal or ↑

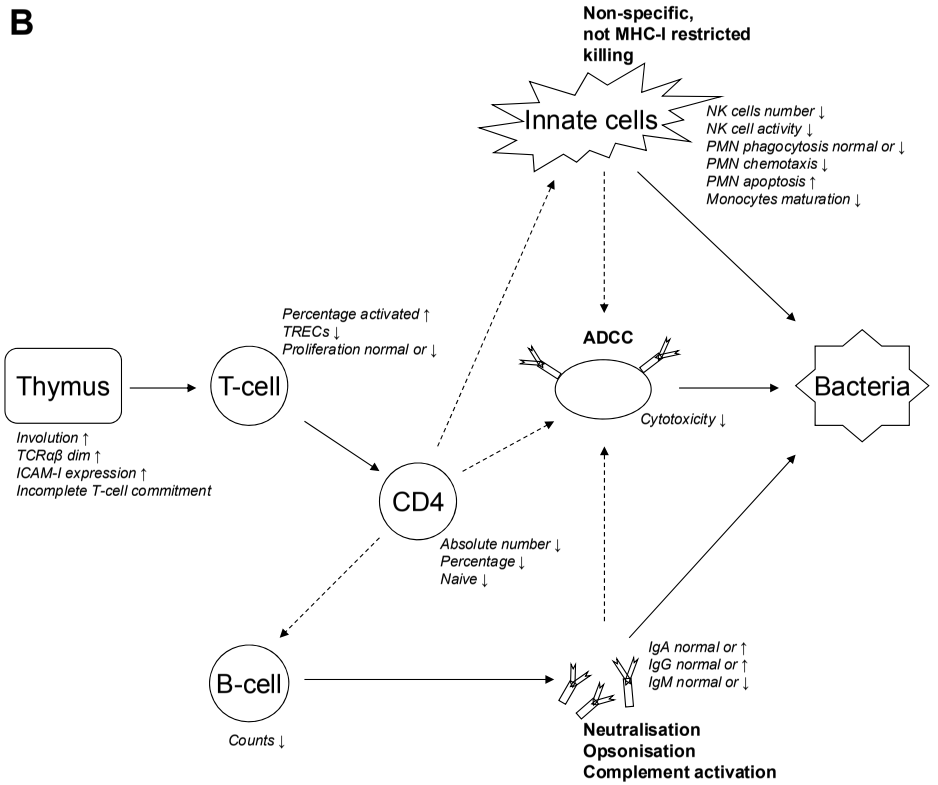


Figure 2.1 The role of immunologic abnormalities in pathogenic clearance in DS.
The role of different immune cells in clearance of virus infected cells is shown in Figure 2.1A. Viral immunity can be roughly divided in non-specific, not MHC-I restricted killing by innate cells (upper part of figure), specific, MHC-1 restricted killing by $CD8^+$ cytotoxic T-cells (middle part of figure) and antibody-dependent cell-mediated cytotoxicity (lower part of figure). Next to each cell type differences in number and function are summarized for children with DS. Figure 2.1B provides a summary of differences in cell types in DS that play a role in bacterial defense: not MHC-I restricted killing by innate cells (upper part of figure), antibody-dependent cell-mediated cytotoxicity (ADCC) (middle part of figure) and neutralisation, opsonisation and complement activation (lower part of figure).

bacterial clearance would be expected in DS. On the other hand, despite even increased levels of IgG, ADCC is decreased in DS. Since ADCC depends not only on the presence of antibodies, but on NK-cell function as well, dysfunction of the latter might be causing the effect measured in DS.

Although increased telomeric loss has been shown in DS, one can question whether this might be the cause or consequence of bacterial and viral infections in DS. Since no evidence can be provided for a direct effect, we would suggest that it has no direct role in the susceptibility to RTIs in children with DS.

7.1. Future topics to be addressed

Although many previously performed studies suggest a role for an abnormal immune system in the pathophysiologic mechanism of increased susceptibility to RTIs in children with DS, definite evidence is still lacking. Future in vitro and in vivo immunologic investigations might give better insight in this issue. Several studies have suggested that low numbers and disturbed function of T-cells in children with DS are caused by abnormal thymic development and function. However, the establishment and maintenance of the naive T-cell pool is a dynamic process, also influenced by cellular lifespan and division. A study on naive T-cell dynamics incorporating thymic output, T-cell proliferation and T-cell loss by antigen driven differentiation or apoptosis would provide a more accurate answer to the role of thymic insufficiency in children with DS. Although multiple immunologic abnormalities have been described in individuals with DS, it is difficult to state whether these abnormalities are the cause or consequence of the high morbidity. Longitudinal studies are needed to determine the etiological relationship between the number of different immune cell types during early childhood in children with DS and the subsequent development of RTIs.

Since an abnormal immune system is suggested to be involved in the increased incidence of RTIs in children with DS, it is of outstanding interest to consider what preventive measures could be taken in this unique population. Unfortunately, the literature is insufficient to point out specific pathogens as major cause of the respiratory morbidity in children with DS. It is unclear what pathogens, either viral or bacterial, have an increased contribution in this matter. Therefore it is difficult to state if antibiotic prophylaxis or specific immunoglobulins would be of use in children with DS. Even if certain pathogens can be defined as a risk factor for the development of severe RTIs, preventive measurements are a matter of debate. For example, the finding of DS as a new risk factor of RSV associated hospitalization has

led to discussions on the role of passive immunization in this specific group of children. Although passive immunization against RSV has been approved for certain risk groups, such as premature born children and children with significant congenital heart disease, it is questionable if it is effective in children with DS as well. And if so, if health gain is achieved at acceptable costs. Based on the long-term implications for children with DS and society, an international multi-centre randomized-controlled clinical trial on the use of passive immunization in children with DS is required before any recommendations can be given.

8. Conclusions

Children with DS have an increased incidence of RTIs which might be associated with congenital heart disease, abnormal airway anatomy and physiology, hypotonia, and aspiration. In addition, patients with DS show multiple abnormalities both in numbers and function of both innate and adaptive immunity. These immunologic abnormalities combined, whether or not directly interacting with each other, strongly suggest diminished viral and bacterial clearance in DS. Although it can be suspected that certain findings reflect a state of inflammation in DS rather than being the cause, we believe that the high incidence of RTIs in children with DS is the consequence of an impaired immune system. Future studies on T-cell dynamics and the etiological relationship between numbers of different immune cell types during early childhood and the subsequent development of RTIs might provide better insight into this hypothesis.

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