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CME REVIEW

Inborn Errors of Adaptive Immunity in Down Syndrome

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Abstract

Down syndrome fits an immunophenotype of combined immunodeficiency with immunodysregulation, manifesting with increased susceptibility to infections, autoimmunity, autoinflammatory diseases, and hematologic malignancies. Qualitative and quantitative alterations in innate and adaptive immunity are found in most individuals with Down syndrome. However, there is substantial heterogeneity and no correlation between immunophenotype and clinical presentation. Previously, it was thought that the immunological changes in Down syndrome were caused by precocious aging. We emphasize in this review that the immune system in Down syndrome is intrinsically different from the very beginning. The overexpression of specific genes located on chromosome 21 contributes to immunodeficiency and immunodysregulation, but gene expression differs between genes located on chromosome 21 and depends on tissue and cell type. In addition, trisomy 21 results in gene dysregulation of the whole genome, reflecting the complex nature of this syndrome in comparison to well-known inborn errors of immunity that result from monogenic germline mutations. In this review, we provide an updated overview focusing on inborn errors of adaptive immunity in Down syndrome.

Keywords Down syndrome · trisomy 21 · immunodeficiency · adaptive immunity · T cells · B cells · immunodysregulation · gene expression dysregulation · immunology

Introduction

Down syndrome (trisomy 21; OMIM 190685) is a multisystem condition associated with mental and motor developmental impairment, facial dysmorphia, and congenital malformations—in particular congenital heart disease. Individuals with Down syndrome have an increased susceptibility to develop infections, hematologic malignancies, autoimmunity, and autoinflammatory diseases, which we have reviewed recently [1].

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Upper and lower respiratory tract infections are frequently reported by parents of children with Down syndrome, but prospective population-based studies to quantify the burden of disease are currently lacking [2, 3]. What we do know is that both viral and bacterial respiratory infections lead to more health care utilization in Down syndrome [4], as well as higher morbidity and mortality. For example, respiratory syncytial virus (RSV) infections lead to more hospital admissions (odds ratio [OR] 8.69, confidence interval [CI] 7.33–10.30), prolonged hospital stays (4.73 days longer, CI 2.12–7.33), and a higher requirement of respiratory support (oxygen requirement: OR 6.53 [CI: 2.22–19.19]; mechanical ventilation: OR 4.56 [CI 2.17–9.58]) [5]. Although infections seem less prevalent in adults with Down syndrome, they still contribute to increased hospital admission rates and remain the most important cause of mortality in Down syndrome in all age groups (standardized mortality ratio 5.58–53.8) [1, 6–8]. These infections and their consequences can at least in part be explained by the numerous anatomical and physiological alterations found in Down syndrome [1].

Hematologic malignancies such as acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) are approximately 20-fold more frequent in children with Down

syndrome, compared to children without Down syndrome [1]. In addition, transient myeloproliferative disorder (TMD) is a unique neoplasia that is specific to neonates with Down syndrome. These hematologic malignancies in Down syndrome show distinct genetic changes. For example, mutations in *Ras* and janus kinase 2 (*JAK2*) are seen in ALL, whereas mutations in transcription factor *GATA1* can be found in TMD [9]. Interestingly, the age-adjusted incidence of lymphoma is the same for individuals with Down syndrome and the general population, while solid malignancies (apart from testicular cancer) are actually less common, resulting in a lower overall risk for malignancies in Down syndrome [10, 11].

The well-known increased incidence of specific autoimmune diseases such as thyroid disorders (affecting 50% of adults), celiac disease (6–10-fold increase), and type I diabetes mellitus (3–4-fold increase)—all characterized by specific autoantibodies—warrants screening strategies [1, 12]. Other inflammatory conditions that are more common in Down syndrome include arthritis (at least 3-fold increase) and hidradenitis suppurativa (5-fold increase) [1, 13–15]. In addition, at least three-quarters of individuals with Down syndrome are affected by early-onset Alzheimer's-like dementia [16–18]. Besides amyloid-β deposition, the role of neuroinflammation has been acknowledged in the development of this condition. Interestingly, the neuroinflammatory phenotype appears to be significantly different in Down syndrome compared to individuals without Down syndrome, which has been identified as a potential target for future treatment [17, 18].

This clinical profile of increased rates of infections, hematologic malignancies, and specific autoimmune and autoinflammatory diseases fits a phenotype of combined immunodeficiency with immune dysregulation. In the last decades, the discovery of many monogenic primary immunodeficiency disorders (PIDs) has improved our understanding of specific immunological pathways and their role in health and disease [19]. This is in high contrast with our understanding of the alterations found in the immune system of individuals with Down syndrome, which are the result of an extra (critical part of) human chromosome 21 (Hsa21). Contrary to common belief, the expression of Hsa21 genes is not always increased by 50%. Instead, each gene on Hsa21 can have increased, decreased, or unchanged expression, as well as show a different expression profile depending on the tissue and cell type [20–22]. Besides, genome-wide expression analysis revealed that trisomy 21 causes extensive dysregulation of the whole genome and modifies the transcriptional and posttranscriptional program of many different genes, and not only those that are located on Hsa21 [23, 24]. Additional complexities may exist due to epigenetic changes. MicroRNA (miRNA) expression is altered, resulting in dysregulation of many specific target proteins involved in immunity [25–28]. Thus, Down syndrome is a complex disorder of gene expression dysregulation and not just a collection of independent single-gene defects.

Over the past decades, the immune system in Down syndrome has been studied extensively, but unraveling the mechanisms behind the immune-mediated conditions in Down syndrome and finding genotype-phenotype correlations remains challenging. Here, we provide an updated overview of adaptive immunity in Down syndrome with parallels drawn between well-known inborn errors of immunity.

Altered Thymic Development with Decreased Thymic Output from Birth

The T cell development and function show many alterations in Down syndrome (see Fig. 1 and Table 1). Individuals with Down syndrome have an abnormal thymus that is intrinsically deficient from the very beginning. The majority of fetuses have a smaller thymus on ultrasound measurements [29]. Thymic investigations in children with Down syndrome undergoing cardiac surgery after birth show significant weight reduction and accelerated thymic involution in the first years of life with altered corticomedullary thymic organization [30–33]. In addition, thymus transcriptome analysis in Down syndrome thymi (< 2 years of age) shows significantly global thymic hypofunction with underexpression of more than 400 genes, predominantly involved in cell division and T cell immunity, such as *IL2RG*, *RAG2*, *CD3D*, *PRDX2*, and *CDK6* [34].

T cell receptor excision circles (TREC) counts as well as recent thymic emigrants can be used to characterize thymic output [35–37]. While both are decreased in children with Down syndrome [33, 38], it is notable that TREC counts are rarely under the cutoff values used in the newborn screening for severe combined immunodeficiency (SCID) [39, 40]. This fits the clinical picture, as newborns with Down syndrome generally do not suffer from opportunistic infections. However, a partial T cell deficiency can contribute to immunological dysregulation and lead to an increased risk of autoimmunity.

Dysregulated AIRE Expression and Increased Autoimmunity

Individuals with Down syndrome have an increased risk to develop autoimmune diseases [41]. Autoimmune regulator (*AIRE*) is located on Hsa21 and plays a pivotal role in negative selection of autoreactive T cells through tissue-restricted antigen expression on medullary thymic epithelial cells (mTECs). Loss-of-function mutations in *AIRE* can cause autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). Patients with Down syndrome and APECED share the presence of autoimmune disease in the context of

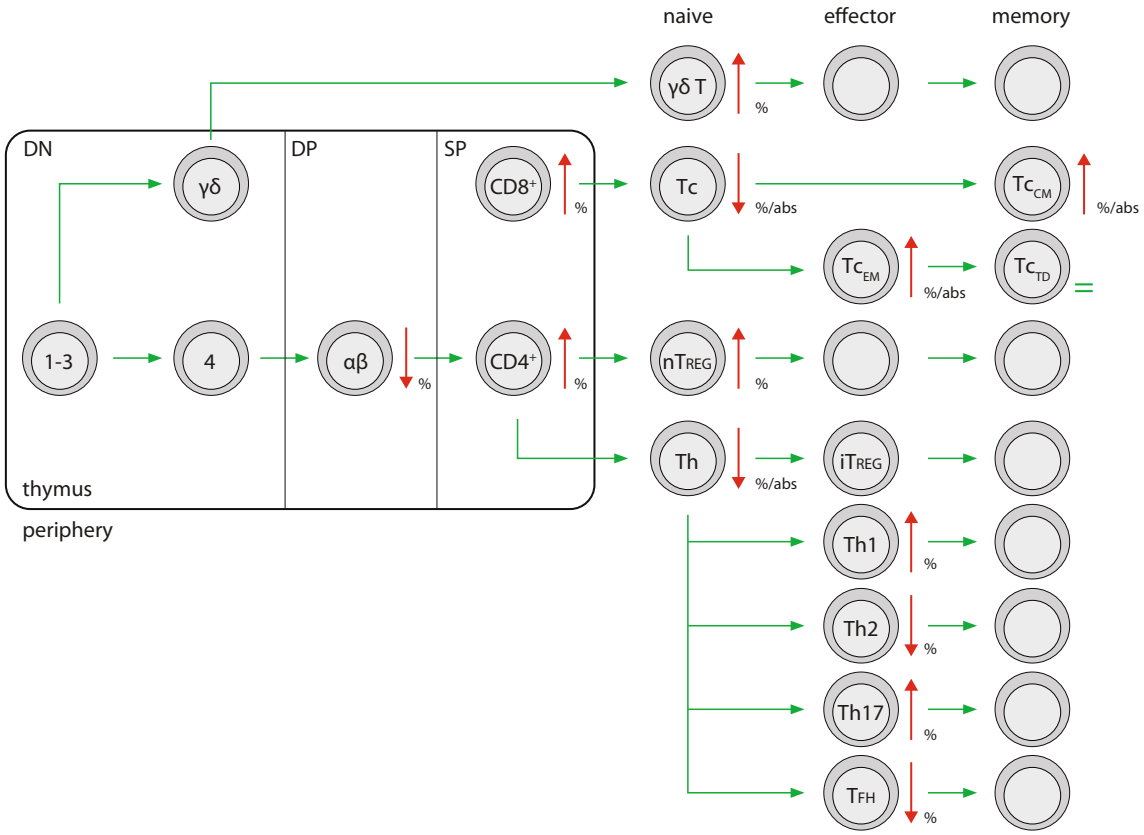

Fig. 1 Schematic overview of T cell development in thymus and periphery. Relative (%) and absolute (Abs) changes in subsets are depicted by red arrows, and normal findings with a green equal (=) symbol. Unknown results not depicted. DN, double negative; DP, double positive; SP, singe positive; Tc, cytotoxic T cell; EM, effector memory; CM, central memory; TD, terminal differentiated; nTreg, natural regulatory T cell; Th, T helper cell; Th1, T helper type 1; Th2, T helper type 2; Th17, T helper type 17; T_{FH}, T follicular helper; iTreg, induced regulatory T cell

specific autoantibodies, although their frequency is lower in Down syndrome [42].

In contrast to APECED, thymic *AIRE* expression is dysregulated rather than absent in Down syndrome. Thymic *AIRE* expression is increased in Down syndrome in the first months of life with more $AIRE^+$ mTECs [33, 43], but its expression is consistently decreased thereafter [33, 34, 43–45]. Children with Down syndrome who have thyroid dysfunction show a significantly lower *AIRE* expression in their thymi compared to those children without thyroid dysfunction, as well as healthy controls [44]. In addition, important *AIRE* partner genes such as *TOP2a*, *LMNB1*, *NUP93*, and *PCNA* are found to be underexpressed in Down syndrome thymi (< 2 years of age) as well [34].

From the first months of life, Down syndrome thymi are smaller and show an increased size of the medulla area with remarkably large Hassall's corpuscles (HC), suggesting early thymic involution [33, 43]. Oxidative stress and mitochondrial dysfunction play an important role in early-onset Alzheimer's-like dementia in Down syndrome [46, 47], but are also thought to contribute to the process of accelerated thymic involution in Down syndrome animal models [48–50]. This is supported by skewed genomic adaptation and miRNA modulation to support stress tolerance in thymic tissue of children with Down syndrome [26]. In addition to accelerated thymic involution, the proportion of $AIRE^+$ cells within the mTEC compartment decreases in children with Down syndrome. This results from a relatively higher number of terminally differentiated (post-AIRE) mTECs that are characterized by *AIRE* downregulation [33, 44]. Thus, the discrepancy of decreased thymic *AIRE* expression despite trisomy 21 and despite initial increased *AIRE* expression can be explained by altered thymic development in general and more specifically by accelerated mTEC maturation with AIRE downregulation.

Dysregulation of thymic AIRE expression and thymic development in Down syndrome may result in abnormal thymic cross-talk and defective T cell education. This would allow for autoreactive T cells to develop and leave the thymus, contributing to increased autoimmunity. AIRE also plays a role in the generation and function of natural regulatory T cells (Treg) [51]. Relative counts of Treg are increased in Down syndrome [45, 52]. However, the suppressive ability of these cells in thymus and peripheral blood is reduced [33, 45], in particular in

Table 1 Summary of immunologic studies in Down syndrome

	Finding	Reference
T cells		
Total T cells	Decreased	52, 66, 68–71
$CD4^+$ T cells	Decreased	
$CD8^+$ T cells	Decreased (child)/normal (adult)	
Naive T cells	Decreased	
Memory T cells	Normal (abs)/increased (%)	
CD4/CD8 ratio	Inverted ratio	
TCR-αβ	Decreased (%)	
TCR-γδ	Increased (%)	
nTreg	Increased (%)/decreased function	33, 45
Th_1/Th_2 ratio	Increased	72, 73
Mitogenic stimulation assay	Decreased/normal (thymidine); increased (Ki-67)	54, 85–88
CD3 stimulation assay	Decreased (thymidine); increased (Ki-67)	54, 86, 89
Antigen-specific stimulation assay	Decreased response tetanus and influenza (thymidine)	91
TREC count	Decreased	33, 38
Thymic anatomy	Accelerated thymic involution, altered corticomedullary thymic organization, enlarged Hassall's corpuscles	30–33
Thymic AIRE expression	Increased early in life/decreased later in life	33, 34
B cells		
Total B cells	Decreased	62, 95, 97, 134, 135
Transitional B cells	Normal	
Naive mature B cells	Decreased	
Natural effector B cells	Decreased	
Memory B cells	Decreased	
Plasma cells	Normal/increased	
$CD21^{low}$ B cells	Increased	
KREC counts	Decreased	40
Immunoglobulins		
IgG	Increased	62, 94, 95, 97, 99, 124, 128, 136–141
IgG_1	Increased	
IgG_2	Decreased	
IgG_3	Increased	
IgG_4	Decreased	
IgA	Normal	
IgM	Decreased	
IgD	Increased	142
Total and specific IgE	Decreased	62, 97, 127, 143, 144

individuals with autoimmune thyroid disease [53]. These findings are not only attributed to AIRE, since various other genes and processes important for Treg differentiation (e.g., autophagy pathways) are found to be dysregulated in thymic transcriptome, gene co-expression network, and miRNA studies in Down syndrome as well [26, 34]. And outside the thymus, repeated peripheral antigen exposure due to increased infection frequency can lead to a chronic inflammatory status especially in Down syndrome, which could cause a decreased sensitivity to Treg-mediated suppression [54]. Furthermore, skewed helper T cell profiles (towards Th_1 and Th_{17} subpopulations) and overproduction of autoimmunity-related cytokines could be important factors contributing to increased autoimmunity in Down syndrome [54].

Thymic research to help understand the underlying mechanisms related to increased autoimmunity in Down syndrome could be focused on more detailed investigations of mTEC subpopulations, including miRNAs and single cell transcriptomics [55–57].

Down Syndrome Is Not Comparable with Other Thymic Defects

Thymic aplasia with severe T cell lymphopenia can be caused by 22q11.2 deletion syndrome. Fortunately, only 0.1% of children with 22q11.2 deletions have a T-negative SCID profile [58]. The majority (~ 80%) of patients have mild to moderate T cell lymphopenia with normal immunoglobulin levels and normal T cell proliferative responses. Overall, T cell numbers improve with age albeit with an increased functional impairment due to T cell exhaustion. A small proportion of children and adults with 22q11.2 deletion syndrome has low IgG levels, presumed to be caused by dysfunctional B cells secondary to impaired T cell help. The clinical phenotype has overlap with Down syndrome, showing an increased incidence and severity of infections, and more autoimmune diseases. As in Down syndrome, numerous anatomical and physiological alterations contribute to increased incidence and persistence of infections as well [59, 60].

However, there are apparent differences between 22q11.2 deletion syndrome and Down syndrome. The most common autoimmune diseases in 22q11.2 deletion syndrome are juvenile idiopathic arthritis and autoimmune cytopenias [61], where individuals with Down syndrome are particularly at risk to develop thyroid disease, celiac disease, and type 1 diabetes. Atopic disease is prevalent in 22q11.2 deletion syndrome and affects up to two-thirds of these children. This is in contrast to children with Down syndrome who have a decreased risk to develop asthma and allergic sensitization [1, 62]. Besides these differences in clinical presentation, the thymic alterations in Down syndrome, including architecture, thymocyte subpopulations, and *AIRE* expression, are different from those found in 22q11.2 deletion syndrome [33]. In addition, Down syndrome has distinct abnormalities in the B cell compartment and humoral immunity which are unlike 22q11.2 deletion syndrome (see below).

About half of all children with Down syndrome suffer from congenital heart disease which frequently requires corrective heart surgery. Partial thymectomy during heart surgery in the first year of life induces thymic hypoplasia and further decreases thymic output [63, 64]. Otherwise healthy children undergoing heart surgery requiring partial thymectomy show a gradual recovery towards normal T cell numbers including naive helper T cells and cytotoxic T cells, as well as TREC counts, in contrast to children with Down syndrome [65].

Lack of Early T Cell Expansion and Imbalanced $CD4^+/CD8^+$ T Cell Ratio

The peripheral T cell compartment of patients with Down syndrome has been studied in more detail over the last decades. Absolute T cell counts, including both cytotoxic and helper T cells, are decreased in all age groups in children with Down syndrome and lack the expansion normally seen in the first years of life [66–68]. Absolute cytotoxic T cell numbers approach age-matched control levels towards adulthood, whereas helper T cells continue to be decreased, causing imbalanced $CD4^+/CD8^+$ T cell ratios in all age groups [66]. We found higher relative counts of TCR-γδ T cells—albeit still low—in all age groups in Down syndrome, as confirmed by others [66, 69, 70].

Differentiation into memory T cells occurs upon antigen-driven activation, and several studies investigating virus-specific T cell formation show that individuals with Down syndrome can mount good effector T cells in response to viruses such as cytomegalovirus (CMV) and varicella zoster virus (VZV) [52, 66].

These studies showed higher numbers of effector T cells in CMV and VZV exposed individuals. It remains the question if this is a requirement to ensure virus control and disease prevention [52, 66], or merely a reflection of T cell dysregulation correlated with interferon hyperactivity (see below) [54]. This might partly explain the skewed distribution seen in the memory cytotoxic T cell compartment, with studies showing increased absolute and relative counts of effector memory [66], central memory [54, 66, 71], and normal [54, 66] to increased [71] terminally differentiated cytotoxic T cells. Relative counts of memory helper T cells are increased as well, but this is mainly due to decreased relative counts of naive helper T cells in all age groups and not due to increasing absolute counts of helper T cells [66–68].

Pro-inflammatory Helper T Cells

The helper T cell compartment in Down syndrome shows a skewed distribution towards increased IFN-γ producing T helper type 1 responses [54], with a significantly higher Th_1/Th_2 ratio suggestive of an imbalance towards pro-inflammatory immune responses [52, 72, 73]. This could potentially play a role in the decreased rate of allergies seen in Down syndrome. More recent studies including Th_{17} show skewed helper T cell distribution towards Th_1 and Th_{17} subpopulations in children with Down syndrome [52]. One study shows a significantly decreased IL-17A expression on helper T cells in children with Down syndrome, suggesting a role in the immune dysregulation [74]. However, another study in adults with Down syndrome states a clear overproduction of IL-17A and IL-22 [54].

T follicular helper cells (T_{FH}) in germinal centers help B cells to generate class-switched immunoglobulins, memory B cells, and long-lived plasma cells [75]. T_{FH} subpopulations show a great degree of plasticity and are able to produce cytokines similar to Th1, Th2, and Th17 cytokine profiles (e.g., T_{FH1}, T_{FH2}, T_{FH17}, respectively) [76]. Dysregulation of T_{FH}

function has been associated with autoimmunity [77, 78]. Overall, children with Down syndrome show smaller sized germinal centers with reduced numbers of T_{FH} cells [79]. Skewed T_{FH} differentiation towards interferon (IFN)-γ T_{FH1} cells is found in blood of children with Down syndrome in one study [80], which was more pronounced in children with Down syndrome-associated arthritis in comparison to children with Down syndrome without arthritis and children with juvenile idiopathic arthritis as well as healthy controls [81].

Chronic Interferon Hyperactivity

Genes coding for 4 of 6 IFN-receptors are Hsa21-derived: type I (*IFNAR1*, *IFNAR2*), type II (*IFNGR2*), and type III (*IL10RB*). These genes are overexpressed in all individuals with Down syndrome, regardless of sex, age, or ethnicity [82]. Mass cytometry of 100 different immune cell types in adults with Down syndrome revealed global immune dysregulation with multi-lineage cell-type-specific hypersensitivity to interferon-α [83]. Genomic and transcriptomic analyses in various Down syndrome cell lines, including T cells, reveal that the interferon signaling cascade is consistently activated in Down syndrome. However, downstream signaling effects on IFN-activated transcription factors (e.g., *IRF3*, *IRF5*, *IRF7*, *STAT1*) and expression of IFN-stimulated genes (e.g., *APP*, *IDO1*) show great inter-individual variation [82], which may contribute to the clinical heterogeneity.

A number of Down syndrome traits overlap with disease pathology seen in the type I interferonopathies. For example, Aicardi-Goutières syndromes (AGS), a disorder caused by a continuous activation of the intracellular DNA/RNA sensoring pathway, is characterized by consistent interferon signaling. Their clinical presentation consists of progressive encephalopathy, intracranial calcification, cerebral atrophy, and thrombocytopenia, as well as systemic lupus erythematosus, chilblains, and spastic paraparesis depending on the different monogenic mutations involved (type 1–7; mutations for example in *ADAR1*, *TREX1*, *RNASEH2A*) [19]. Examples of comorbidities in Down syndrome that at least in part could be related to interferonopathy are transient myeloproliferative disorder, leukemias, autoimmunity, periodontal disease, and various neurological abnormalities [82], although additional factors are likely to contribute to these conditions.

Down syndrome mouse models show that reduction of interferon or interferon receptors improve growth and brain development, but less emphasis has been placed on immune-mediated diseases thus far [84].

Overall, interferon-driven immune dysregulation is likely to contribute to immune-mediated diseases in Down syndrome and warrants more research to improve understanding and investigate potential therapeutic targets.

Inconsistent T Cell Proliferation Data in Down Syndrome

In recent studies, in vitro T cell assays with phytohemagglutinin (PHA) and with cytomegalovirus (CMV) antigen stimulation in children [71], and in vitro anti-CD3/CD28 monoclonal antibody stimulation in adults with Down syndrome [54], show normal [52] to increased production of cytokines as well as increased expression of T cell activation markers and increased relative counts of effector T cells [52, 54, 71]. Furthermore, T cell proliferation in response to PMA/ionomycin—using Ki-67 expression instead of ^{3}H-thymidine incorporation—shows increased proliferation of both cytotoxic and helper T cells in adults with Down syndrome in comparison to healthy controls [54]. These recent data are suggestive of a highly activated T cell repertoire with normal to increased proliferative ability.

This seems to be in sharp contrast with older studies investigating in vitro T cell proliferation using ^{3}H-thymidine incorporation, which show decreased proliferation in children and adults with Down syndrome [85–91], with some exceptions [45, 91]. It is known that there is poor correlation between the Ki-67 expression and ^{3}H-thymidine incorporation techniques [92]. The specific different antigenic or mitogenic stimuli used can further complicate comparison. Furthermore, most older studies did not allow for detailed analysis of specific T cell subpopulations, while recent studies are able to measure extensive cytokine and chemokine profiles as well as expression of various T cell (activation) markers in different T cell subpopulations.

In general, most studies have small study sample size, and age ranges and control groups vary widely between the different studies. Therefore, we would suggest to expand these recent T cell studies to larger cohorts that include different age groups (including neonatal, infant, children) before more final conclusions can be drawn regarding T cell proliferation in Down syndrome. In addition, the study of the in vivo replication history in T cell subsets may be helpful in further understanding T cell proliferation [93].

Impaired Survival of the Naive B Cell Compartment

The peripheral B cell compartment of individuals with Down syndrome shows many changes, which resulted in increased attention over the last decade (see Fig. 2 and Table 1). Absolute B cell counts are decreased in patients with Down syndrome [40, 52, 68, 94–97]. While healthy children show an expansion of their B cell compartment in the first year of life, this phenomenon is lacking in Down syndrome [62, 68, 98]. Only one study reports an inverse correlation between hospitalization rate for infectious diseases and absolute B cell

count [94]; however, this could not be confirmed by others [62, 95, 96, 99].

B cells enter the peripheral blood from the bone marrow as transitional B cells, and shortly thereafter, develop into naive mature cells. Individuals with Down syndrome have normal numbers of transitional B cells, but decreased naive mature B cell numbers [95]. Potential causes for this finding include decreased bone marrow output, impaired B cell homeostasis, and decreased survival.

As of yet, there are no bone marrow studies to characterize the antigen-independent development of B cells in otherwise healthy individuals with Down syndrome. Peripheral blood lymphocytes in Down syndrome are prone to DNA damage in conjunction with impaired DNA repair [100], and it is possible that the B cell development in bone marrow is altered to some degree, analogous to DNA repair disorders such as Nijmegen breakage syndrome (NBS) and ataxia teleangectasia (AT) [101, 102]. This could allow for autoreactive B cells to enter the blood and predispose the development of autoimmune diseases, which is seen in DNA repair disorders as well. In contrast to NBS and AT, homeostatic proliferation of the naive B cell compartment is not increased in Down syndrome, showing that there is no compensatory proliferation as a response to decreased B cells [95, 101, 102].

B cells of individuals with Down syndrome show higher rates of apoptosis [95, 103]. B cell survival depends on B cell-activating factor (BAFF), which has been found in higher levels in Down syndrome, potentially as a compensatory response to the B cell lymphopenia [95, 104]. Extracellular signals can induce apoptosis via interaction with the FAS receptor (CD95). However, the expression of this cell marker is normal in Down syndrome [95]. Another pathway that can induce apoptosis involves the mitochondria. It is known that mitochondrial morphology as well as function are altered in Down syndrome, potentially due to underexpression of > 65 mitochondrial-related genes on the whole genome [105]. This can result in increased oxidative stress and subsequent down-regulation of energy metabolism [106]. In addition, mitochondria in Down syndrome are more susceptible to the effects of damaging agents [107, 108]. Recent studies in healthy controls show a higher dependence of B cells on their mitochondrial energy production compared to T cells [109], which warrants further studies on the mitochondrial functioning in immune cells of individuals with Down syndrome.

B Cell Activation Is Abnormal in the Absence of T Cell Help

Differentiation into memory B cells occurs upon antigen-driven activation of naive mature B cells via the B cell receptor (BCR) complex and co-stimulatory signals. Small expression differences of BCR complex cell markers (i.e., CD19, CD21, and CD81) were found previously in Down syndrome [62, 98], but patients lack the hypogammaglobulinemia that is seen in patients with CD19, CD21, or CD81 deficiency [19].

T cell-independent signaling pathways, including TACI-BAFF/APRIL and pattern recognition molecules (i.e., Toll-like receptors [TLRs]), are important for the formation of two memory B cell subsets: $CD27^{-}IgA^{+}$ and $CD27^{+}IgM^{+}IgD^{+}$ "natural effector" B cells [110]. Both of

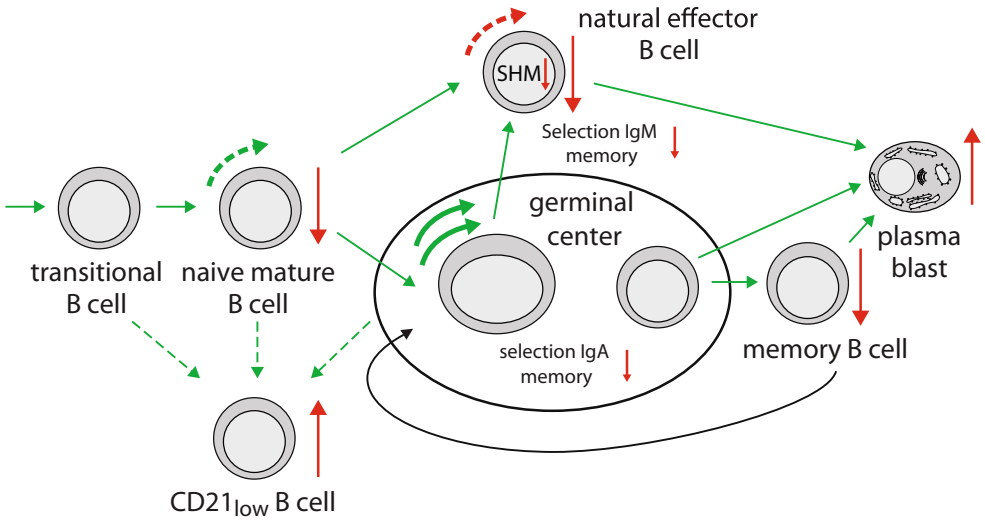

Fig. 2 Alterations of the B cell response in Down syndrome. Individuals with Down syndrome have normal numbers of transitional B cells but decreased naive mature B cells, without increased homeostatic proliferation. The germinal center composition is normal but affinity maturation is impaired in IgA memory. Generation of IgG^{+} memory is normal, but absolute number of memory B cells is decreased. Natural effector B cells show decreased proliferation, somatic hypermutations (SHM). The selection of IgM^{+}/IgA^{+} memory is impaired. Higher numbers of plasma blasts are found in Down syndrome. The aberrant development of $CD21^{low}$ B cells is increased in Down syndrome. All abnormalities are shown in red. Figure reproduced from Verstegen (2014) [152].

these subsets show significant reductions in Down syndrome with $CD27^{-}IgA^{+}$ and natural effector B cell numbers that are ~ 15% and ~ 50% of control values, respectively [95]. Further analysis of natural effector B cells shows a decreased replication history of ~ 5 cell divisions instead of ~ 9–10 cell divisions in controls, as well as decreased somatic hypermutations (SHM) that results from reduced activation-induced cytidine deaminase (AID) activity [95]. However, B cells in Down syndrome show normal TACI expression and, as described before, BAFF levels are slightly increased rather than decreased [95].

TLR activation results in downstream signaling via MyD88-TIRAP-IRAK4, which is required for natural effector B cell homeostasis. Patients with monogenetic PIDs that affect the MyD88-TIRAP-IRAK4 pathway have decreased natural effector B cells similar to numbers found in Down syndrome, but show normal SHM [111, 112]. In contrast to individuals with Down syndrome, patients with IRAK4 or MyD88 deficiency do not develop autoantibodies or autoimmune diseases, despite the crucial role of these pathways in the removal of autoreactive B cells [113].

Individuals with Down syndrome have normal T cell-dependent co-stimulatory signaling. Genetic mutations in *AICDA* that cause reduced activity of activation-induced cytidine deaminase (AID) or mutations that affect signaling through CD40-CD40L, ICOS-ICOSL, and IL21-IL21R result in class switch recombination disorders with altered immunoglobulin levels and SHM [19]. Patients with class switch disorders have absent germinal center configurations and decreased IgG [114]. This is in contrast with individuals with Down syndrome who have germinal centers, although their number and size is decreased [79, 95]. In addition, individuals with Down syndrome actually have increased IgG levels (see below). In Down syndrome, normal SHM in memory B cells as well as unaffected class switch recombination profiles have been found [95]. One study reported normal AID expression in germinal centers, but lower levels of expression in memory B cells, in the context of upregulated *miR-155* expression which is known to suppress AID [79]. Other subsets, including naïve B cells, germinal center B cells, and plasma cells, showed unaffected *miR-155* expression [79].

Reduced Memory B Cell Compartment with Impaired IgA Memory Selection

With the exception of $CD27^{+}IgM^{+}IgD^{-}$ "IgM only" memory B cells, absolute numbers of all memory subsets are decreased in Down syndrome [95, 97]. In addition, they lack the expansion of the memory B cell compartment that normally occurs in the first few years of life [62, 98].

In depth analysis of the memory B cell compartment shows decreased proliferation as well as decreased somatic hypermutations [95]. Whereas IgG^{+} memory B cells show normal positive selection for replacement mutations in complementarity-determining region (CDR), the selection strength was significantly lower in IgA^{+} memory B cells [95]. Other selection mechanisms include *IGHV4-34* usage and *IGH-CDR3* length in memory B cells, compared to naive B cells and centroblasts, respectively. Increased *IGHV4-34* usage and *IGH-CDR3* length are both related to the development of autoimmune diseases and seen in IgG^{+} memory B cells of patients with common variable immunodeficiency (CVID) [115–117]. Besides increased *IGH-CDR3* length in IgA^{+} memory B cells, no significant differences were found in individuals with Down syndrome [95]. As of yet, it is unclear if these—relatively small—differences have any effect on mucosal immunity or the development of autoimmune disease.

Increased Plasma Cells

While the memory B cell compartment shows significant abnormalities compared to healthy controls, it is surprising that individuals with Down syndrome have higher numbers of plasma cells in blood as well as tonsils [95, 97]. Memory B cells in Down syndrome have a decreased tendency to proliferate and differentiate upon stimulation with CpG [79, 95]. Several factors play an important role in proliferation and differentiation of B cells, including memory B and plasma cell formation. As described before, miR-155 expression is increased in memory B cells of individuals with Down syndrome, but not in other subsets [79]. This pattern has also been found in patients with rheumatoid arthritis. In these studies, *miR-155* was suppressed, which resulted in decreased antibody production via PU.1 upregulation [118]. PU.1 maintains the expression of *PAX5*. This results in suppressed *PDRM1* expression, which is a crucial step in plasma cell differentiation [119]. Thus, increased miR-155 expression leads to a predominant shift towards plasma cell differentiation, supporting the higher plasma cell numbers in Down syndrome. Interestingly, in one small study, the expression of *PRDM1*, *BCL-6*, and *PAX5* was studied in naive, germinal center and memory B cell subsets as well as plasma cells, which did not show altered expression compared to controls [79]. It would be of interest to investigate this in more detail and include other plasma cell–related transcription factors such as *IRF-4* and *XBP-1*. Of note, as described before, the abnormalities in TFH could further add to these altered responses.

$CD21^{low}$ B Cells

Patients with autoimmune disease (e.g., systemic lupus erythematosus) as well as CVID have increased numbers of $CD21^{low}$ B cells, which express autoreactive antibodies and

are functionally anergic [120, 121]. More recently, it was shown that CVID patients with $CD21^{low}$ B cells have an IFN-gamma-associated immune dysregulation with increased numbers of T_{FH1} cells [122]. Individuals with Down syndrome have higher percentages of $CD21^{low}$ B cells, increased percentages of T_{FH1} cells, and higher rates of autoimmune disease too [62, 95]. It is unknown how $CD21^{low}$ B cells relate to the development of these conditions, although they could potentially be useful as a marker for increased susceptibility to autoimmunity [122].

Is Down Syndrome a Predominant Antibody Deficiency Syndrome?

It is tempting to compare Down syndrome to patients with CVID since they have significant similarities in their clinical presentation such as high rates of respiratory tract infections as well as features of immune dysregulation (i.e., autoimmune conditions and hematologic malignancies). Although compared to individuals with Down syndrome, patients with CVID have higher rates of lymphoma, solid tumors, and granulomatous diseases [123].

Serum immunoglobulin levels show a distinct pattern in Down syndrome [62, 94, 95, 97, 99, 124]. During the first years of life, total IgG increases and then remains high-normal throughout life (> 10 g/L), in the absence of mono- or oligoclonal M-proteins [62]. IgG_1 and IgG_3 subclasses are generally increased (> 7 g/L and > 0.6 g/L, respectively), whereas IgG_2 is decreased (< 2 g/L). While IgG4 deficiency is common in the general population, more individuals with Down syndrome have very low levels of this immunoglobulin subclass. Furthermore, individuals with Down syndrome have normal IgA levels, decreased IgM levels (< 1 g/L), and low serum IgE levels [62, 97]. Thus, in contrast to many PIDs that are characterized by hypogammaglobulinemia, including CVID, individuals with Down syndrome actually have increased IgG levels.

In contrast to patients with predominant antibody deficiencies, immunization studies in Down syndrome show protective antibody responses to pneumococcal and tetanus immunizations (see Table 2). Nevertheless, it should be noted that overall antibody levels in response to vaccinations are lower and seem to decline faster than in individuals without Down syndrome, which has been studied best in the context of Hepatitis B [125–131]. Repeated (booster) vaccinations, as part of national immunization programs, have shown to improve qualitative and quantitative antibody responses in children with Down syndrome [132, 133]. These findings are independent of the type of vaccine used (e.g., protein, polysaccharide, conjugated), suggesting that both T cell-dependent and T cell-independent responses are affected. It is unclear whether individuals with Down syndrome would benefit from additional immunization boosters in addition to the standard immunization programs.

Down Syndrome Should Be Considered as a Non-monogenic Primary Immunodeficiency Disorder

By comparing the composition and function of the T and B cell compartment in Down syndrome with patients who have a well-defined monogenic PID, we have illustrated that many cellular processes are affected in Down syndrome. However, we also show that their defects are generally milder than those seen in these PIDs. While the question is often asked to identify the one mechanism that can explain the immunologic abnormalities in Down syndrome, the answer is far more complex. First, Down syndrome is a condition that is caused by the additional presence of a large amount of extra genetic material, which has been shown to affect the whole genome. The direct effect of dysregulated gene expression may impact on intracellular pathways and impair common cellular processes such as cell division and mitochondrial function as well those specific for immune cells. Second, since virtually all aspects of the immune system show abnormalities to some degree, impaired cellular communication may contribute to decreased function and dysregulation. Third, the local milieu (i.e., pro-inflammatory cytokine profile, chronic interferon activity, increased oxidative stress conditions) has an important role in the development and function of immune cells. These three mechanisms can affect cellular development as well as impact on their function and survival. As such, the clinical phenotype of Down syndrome results from the compounded effect of a myriad of alterations, small and large.

Individuals with Down syndrome have higher numbers of infections, which are related to significant mortality. The question remains to what extent these are the results of impaired immunity as it is clear that many other factors will contribute to their development, including anatomical variations and impaired physiological function of the respiratory tract. With regard to the development of malignancies, it is unclear if these result from impaired immunosurveillance or from a higher risk of mutagenicity. In contrast, the increased rate of inflammatory conditions—in particular those that are related to specific autoantibody production—is a striking feature consistent with immune dysregulation that affects at least half of the individuals with Down syndrome.

There are parallels in the clinical profile and immunological changes between Down syndrome, aging, and well-known PIDs. However, in each comparison, there are obvious differences. We conclude that individuals with Down syndrome have a pattern of combined immunodeficiency and immune dysregulation, and we suggest that Down syndrome should be considered as a non-monogenic PID, just like 22q11.2 deletion syndrome [19].

Table 2 Immunization responses

Immunization	Vaccine type	Response compared to controls	Comments	Protective (%)	Reference(s)
Tetanus	Protein	Normal/decreased	Normal IgG response Decreased IgG2/IgG4 response and avidity/improves with booster	94%	133, 145
Diphtheria	Protein	Normal	IgG	N/R	145
Polio (oral)	Protein	Normal/decreased	Subtype-dependent IgG response	N/R	146
Pertussis (acellular)	Protein	Decreased	IgG	100%	147
Measles	Protein	Normal	IgG	N/R	145
Mumps	Protein	Normal	IgG	N/R	145
Rubella	Protein	Normal	IgG	N/R	145
Hepatitis A	Protein	Normal	IgG	100%	148
Hepatitis B	Protein	Normal/decreased	Variable conversion rate and IgG titer Earlier decline of titer Impaired response in males and aging	50–100%	125–131
Meningococcal C	Conjugated	Decreased	IgG, IgA, and IgM response	100%	149
Influenza A/B	Protein	Normal/decreased	IgG Switched memory B cells reduced after initial immunization/improves after booster	N/R	124, 145
Pneumococcal	Polysaccharide	Normal/decreased	Subtype-dependent IgG Normal opsonophagocytosis	65–100%	132, 150, 151
Pneumococcal	Conjugated	Normal/decreased	IgG Opsonophagocytosis Switched memory B cells reduced after initial immunization/ improves after booster	61–89%	124, 132

N/R not reported

Future Perspective

In the past decades, enormous progress has been made regarding our knowledge of genomics and immunology. Alongside, our current understanding of immunodeficiency and immune-mediated diseases in Down syndrome has increased by numerous studies. It is clear that individuals with Down syndrome have also profited immensely from the progress made in health care; however, the life expectancy of approximately 65 years of age is still well below that of the general population [6].

Despite the known genetic cause of Down syndrome (trisomy 21), and their effect on the up- and downregulated genes throughout the genome, there is a significant inter-individual variation in downstream signaling effects, which leads to a highly variable morphologic and clinical phenotype. While abnormalities in virtually all components of the immune system in Down syndrome have been identified, no clear correlation with clinical features for individual cases has been established. Some features of Down syndrome are considered disease hallmarks (e.g., specific dysmorphic features, cognitive impairment), other features are highly prevalent (e.g., congenital heart defects, Alzheimer's-like dementia), or specific for Down syndrome but less common (e.g., Down syndrome-associated arthritis, TMD). Clinicians caring for children and adults with Down syndrome are therefore not able to predict who will suffer from immune-mediated disorders such as hematologic malignancies, autoimmunity, and autoinflammatory diseases or identify patients who are more likely to develop severe infections. As a result, there are limited options for preventive strategies, targeted screening programs, or treatment optimization.

With increasing knowledge on the immune system in general, as well as emerging technical opportunities including machine learning, this is the time to start the next level of Down syndrome research to unravel the underlying mechanisms of the immune defects in this condition and connect these findings to the complex genotype and phenotype of these individuals.

Ideally, to understand the immunological alterations and related disorders in Down syndrome, we would need an extensive international collaboration involving patients, clinicians, and researchers, aiming to establish a large prospective Down syndrome birth cohort with structural longitudinal follow-up and standardized data collection, which should include (1) extensive clinical data including laboratory investigations, (2) blood samples to assess innate and adaptive immunity at sequential time points, including "omics" (e.g., genomics, transcriptomics, proteomics, metabolomics), and (3) non-blood samples collected in a biobank, such as bone marrow aspirates, thymic tissue, tonsils, spinal fluid, and synovial fluid for detailed immunologic studies. It will be important to

study the impact of trisomy 21 during all developmental stages of life.

The ultimate goal should be to create a personalized genotype-phenotype fingerprint for individuals with Down syndrome that will predict disease trait variations and support clinical decision-making for individuals, including the application of personalized targeted therapies, as well as to develop new treatment modalities specific for Down syndrome and its related immunological and non-immunological diseases.

CME Questions

1. Which of the following statements about immunizations in Down syndrome is correct?

A. Individuals with Down syndrome have normal seroconversion rates and adequate functional responses to immunizations
B. Individuals with Down syndrome have normal seroconversion rates, but inadequate functional responses to immunizations
C. Individuals with Down syndrome have abnormal seroconversion rates, but adequate functional responses to immunizations
D. Individuals with Down syndrome have abnormal seroconversion rates and inadequate functional responses to immunizations
E. No immunization responses have been tested specifically for individuals with Down syndrome

2. Which of the following immunological finding is *not* associated with normal aging?

A. The absolute B cell count remains stable with aging
B. The T cell repertoire shifts towards memory compartment with aging
C. The B cell repertoire shifts towards memory compartment with aging
D. All immunoglobulin levels decrease with aging, requiring additional immunizations to increase immunoglobulin levels
E. Some immunization seroconversion rates wean with aging, requiring additional booster immunizations to increase antibody responses

3. Which of the following immune-mediated conditions are diagnosed less frequently in Down syndrome?

A. Solid tumors
B. Hematologic malignancies
C. Celiac disease
D. Diabetes mellitus type 1
E. Eczema

4. Which of the following immunologic changes in Down syndrome is *not* related to autoimmunity?

A. Decreased regulatory T cell function
B. Increased Th_2 cytokine production
C. Dysregulated AIRE expression in thymus
D. Increased numbers of $CD21^{low}$ B cells
E. Increased interferon activity

5. Based on the clinical profile and immunological changes described in this article, Down syndrome fits the description of a

A. predominantly antibody deficiency disorder
B. thymic defect disorder
C. immunosenescence
D. type 1 interferonopathy
E. combined immunodeficiency with immunodysregulation

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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