Class-Switch Recombination Defects

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Mirjam van der Burg, Andrew R. Gennery,
and Qiang Pan-Hammarström

15.1 Introduction: Basic Immunology Section Including CSR Mechanism

Class-switch recombination (CSR), also known as isotype switching, is the biological mechanism that changes the isotype of an antibody (immunoglobulin) from one type to the other (i.e., from IgM to IgG, IgA or IgE). During this process, the constant region of the Ig molecule is replaced, while leaving the variable region, which is generated via V(D)J recombination intact. This implies that the antigen specificity of the Ig molecule does not change; however, the effector function and tissue distribution of the Ig molecule change as a result of CSR.

15.1.1 Germinal Center Reaction

CSR takes place in activated B-cells in the germinal centers of the lymph nodes and tonsils [1, 2]. The immune response starts with transport of antigens to the

M. van der Burg (✉)
Department of Pediatrics, Laboratory for Immunology, Leiden University Medical Center, Leiden, The Netherlands
e-mail: m.van_der_burg@lumc.nl

A. R. Gennery
Department of Pediatrics, Institute of Cellular Medicine Paediatric Immunology, Great North Children's Hospital, Newcastle Upon Tyne, UK
e-mail: andrew.gennery@newcastle.ac.uk

Q. Pan-Hammarström
Department of Laboratory Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden
e-mail: Qiang.Pan-Hammarstrom@ki.se

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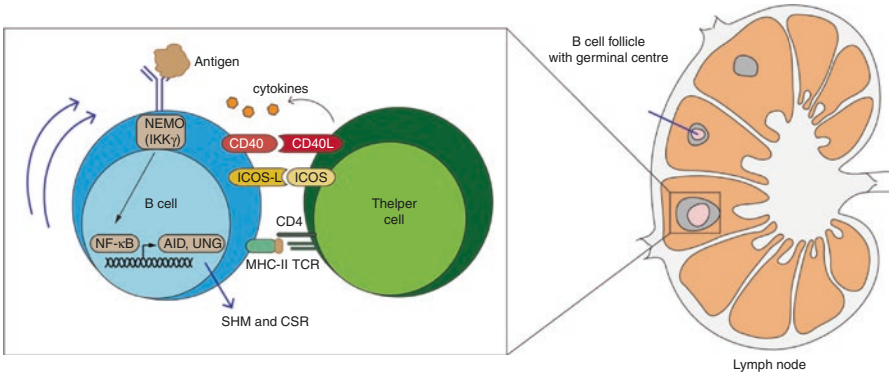

Fig. 15.1 Lymph node with B-cell follicle with germinal center and cognate B-T interaction outlined

spleen and lymph nodes by dendritic cells. After antigen uptake, dendritic cells become activated and express antigen in the context of MHC class II. Together with naïve T-cells, they migrate (in response to CCL19 and CCL21) to the T-cell zone of the lymph nodes. Antigen-specific CD4+ T-cells recognize with their T-cell receptor (TCR) the MHCII-peptide complex (immunological synapse 1) and get activated. This interaction is supported by co-stimulatory molecules and adhesion molecules with its ligands. Activated T-cells express CD40L, produce several cytokines, differentiate into CD4+ effector T-cells (Tfh cells), and migrate to the border of the T-cell zone and the B-cell follicle (see Fig. 15.1 for cognate B-T interaction). B-cells that have recognized antigen followed by uptake and presentation and MHCII-peptide complex also migrate to this border, where they can encounter the activated T-follicular helper (Tfh) cells. The recognition is based on the interaction between the TCR on the Tfh cell and the MHC-II-peptide complex on the B-cell and the co-stimulation via CD40L expressed on the T-cell and CD40 expressed on the B-cell (synapse 2). Both interactions (signals) are required to fully activate the B-cell under influence of cytokines produced by the activated Tfh cells. Subsequently B-cells can proliferate and differentiate in the germinal center. B-cells start proliferating forming a dark zone of centroblasts in the germinal center. During this phase the cells express AID, which is induced upon NFκFB signaling. AID induces somatic hypermutations (SHM) in the variable regions of the Ig molecules thereby changing the affinity of the B-cell receptor. The proliferative phase is followed by differentiation into nonproliferating centrocytes in the light zone of the germinal center. The light zone consists of a large network of follicular dendritic cells (FDCs) that bind long-term antigen-antibody complexes on its cell surface via their Fc receptors, which allow presentation of unprocessed antigen to B-cells. B-cells with BCRs with the highest affinity can best uptake antigen from the FDCs and will express the most MHCII-peptide complex that will lead to the best co-stimulation by the Tfh cells, which are also present in the light zone. Via this mechanism B-cells with the highest affinity have the best survival

(survival of the fittest). Other cells die via apoptosis. Positive selected B-cells undergo class-switch recombination (CSR) and become either memory B-cell and leave the germinal center, recirculate into the dark zone to start another found of proliferation and mutation, or become plasma cell.

15.1.2 SHM and CSR at the Molecular Level

15.1.2.1 Somatic Hypermutation (SHM)

During the germinal center reaction, SHM are induced prior to induction of CSR [3]. The first step in SHM is deamination of dC into dU by AID (i.e., activation-induced cytidine deaminase) in the rearranged V(D)J exons creating U:G mismatches (Fig. 15.2). If replication occurs without repair of these mismatches, transitions occur at C:G pairs. However, these mismatches can also be repaired via base excision repair (BER) or mismatch repair (MMR). During BER, the dU can be recognized and removed by uracil-N-glycosylase (UNG), which creates an abasic site that is recognized by BER proteins. Apyrimidinic endonuclease

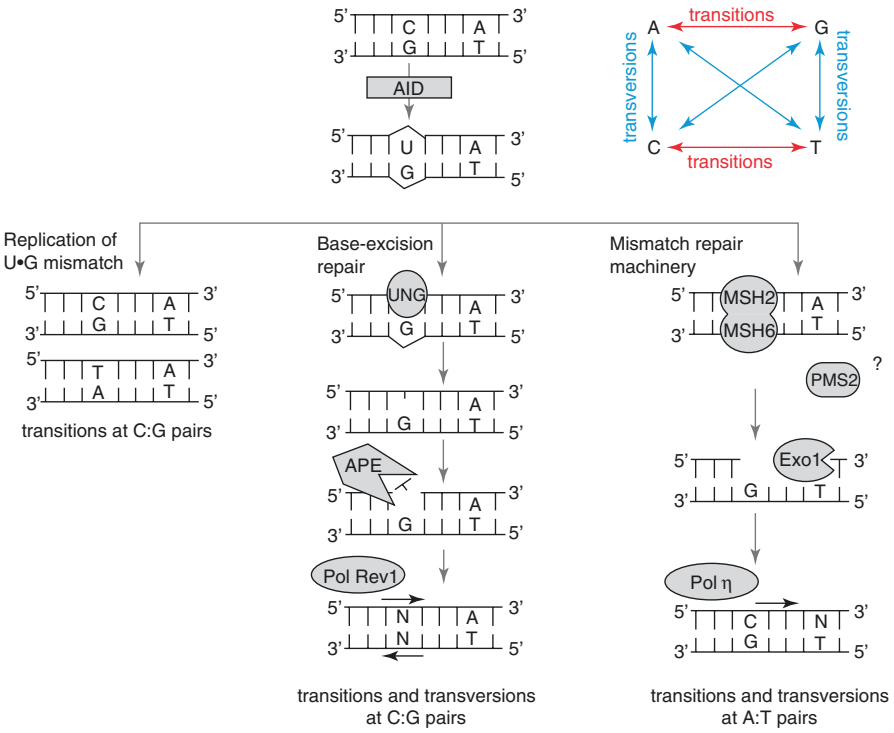

Fig. 15.2 Somatic hypermutations are induced by deamination of dC to dU by AID. The resulting U:G mismatch can be normally replicated resulting in transitions at C:G pairs or repaired via BER or MMR involving error-prone polymerases resulting in transitions and transversions at C:G pair (BER) or A:T pairs (MMR) [3]. Transitions and transversions are indicated in the top right corner

(APE) nicks the phosphate backbone creating a single-strand (ss)DNA gap, which is filled by error-prone polymerases (Pol rev1) resulting in transitions and transversions at C:G pairs. Alternatively, the U:G mismatch is recognized by the MSH2/MSH6 complex, which recruits other MMR proteins, including exonuclease 1 (Exo1), which removes surrounding nucleotides leaving a ssDNA gap. The error-prone polymerase η (Polη) fills this gap and inserts random nucleotides at A:T pairs. Therefore, repair of U:G mismatches via MMR results primarily in transitions and transversions at A:T pairs.

15.1.2.2 Class-Switch Recombination (CSR)

The constant region of the IGH locus consists of several constant regions encoding the different isotypes (Cμ, Cδ, Cγ3, Cγ1, Cα1, Cγ2, Cγ4, Cε, and Cα2), which determine the effector functions of the corresponding antibodies (Fig. 15.3a). Every constant region is preceded by a switch (S) region. S regions are composed of tandem repeats of G-rich sequences (20–80 bp) with a total length of approximately 1–12 kb, which are unique for a giving S region [4]. Only Cδ is not preceded by a switch region, because it formed upon alternative splicing of IGM-IGD transcripts. CSR is initiated by the AID (i.e., activation-induced cytidine deaminase), which deaminates dC to become dU in the top and bottom strands of the S regions, which are transcriptionally active via germline transcription (e.g., Sμ and Sα; see Fig. 15.3b) [5]. Via UNG and APE activity, these AID-initiated ssDNA breaks, which are located in close proximity, are then converted into DNA double-strand breaks (Fig. 15.3c), which can either be repaired by the non-homologous end-joining (NHEJ) pathway or via alternative end joining (aEJ) forming a Sμ-Sα hybrid joint [5]. Via this mechanism the constant region can be replaced with a different isotype leading to a different effector function while leaving the antigen recognition part (i.e., the V(D)J exon) unaffected.

15.2 CSR Deficiencies

Class-switch recombination deficiencies are a heterogeneous group of primary immunodeficiencies characterized by normal or increased levels of serum IgM in combination with reduced or absence of serum IgG, IgA, or IgE. The former name of CSR deficiency was hyper-IgM syndrome. The estimated frequency of CSR defects is around 1:500,000 newborns. There are different underlying genetic causes of CSR deficiencies, and they can be divided into groups with genetic defects hampering the cognate T-B interaction (CD40L, CD40, and NEMO), a group with intrinsic B-cell defects (AID and UNG), and finally a group with DNA repair defects involving the non-homologous end-joining (NHEJ) pathway or the mismatch repair (MMR) pathway [5–7]. The different CSR deficiencies have their specific immunological and clinical characteristics, which also require different treatment strategies. In the next sections, we describe characteristics of the involved genes and the different clinical and pathological findings together with laboratory findings followed by a section about treatment and prognosis.

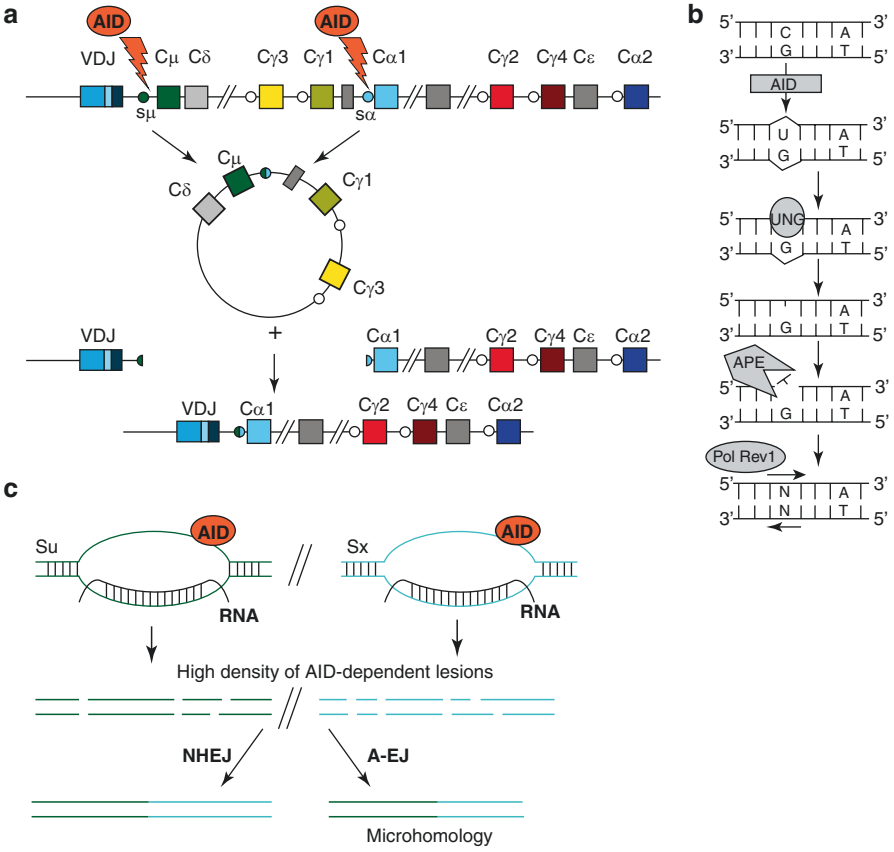

Fig. 15.3 (**a**) Schematic representation of the IGH locus with the different constant regions preceded by Switch regions (S). S regions which are transcriptionally active (germline transcription) are targeted by AID, which induces lesion that result in DNA double-strand breaks. Switch regions are joined forming a switch junction. The intervening part is excised as excision circle. Here switching from IgM to IgA1 is visualized. (**b**) AID deaminates dC into dG which results via UNG and APE activity into a ssDNA gap. (**c**) The high density of AID-dependent lesions in a S region results in the generation of a DNA double-strand break. Two ends of a switch regions (here Sμ and Sα) are repaired via NHEJ or aEJ

15.2.1 Defects in T-B Interaction

15.2.1.1 CD40L Deficiency (OMIM 308230) and CD40 Deficiency (OMIM 606843)

Genes

The X-linked gene CD40LG (*Xq26.3*) codes for CD40 ligand, which is a type II transmembrane belonging to the TNF protein family. It is expressed on activated CD4+ T-helper cells (especially the follicular T-helper cells in the germinal centers) as trimer and interacts with constitutively expressed CD40 on B-cells and other

immune cells. CD40 (20q13.12) is a member of the TNF-receptor superfamily which is constitutively expressed as trimer on B-cells, on dendritic cells, and on monocytes. CD40L-CD40 interaction induces B-cell intracellular signaling, via the NFκB signaling pathway and expression of AID and UNG, the two B-cell-specific proteins that play a key role in CSR and SHM.

Clinical Presentation

X-linked CD40L deficiency is the most common CSR deficiency, whereas autosomal recessive CD40 deficiency is much more rare. Most patients present in early childhood with recurrent upper and lower respiratory tract infections. They have a high susceptibility for *Pneumocystis jirovecii* pneumonia, which is often the first clinical finding [6]. This infection is a sign of impaired cell-mediated immunity due to abnormal T-cell-monocyte interaction. Respiratory infections with CMV, respiratory syncytial virus, *Cryptococcus*, and mycobacteria have also been reported. Protracted diarrhea is another frequently occurring problem, which may require parenteral nutrition. *Giardia lamblia* or *Cryptosporidium* infections are often associated with diarrhea and the latter also with later-onset sclerosing cholangitis, which is a severe and often fatal complication. About 50% of the patients develop neutropenia, which causes oral ulcers. CD40L and CD40 deficiencies are associated with an increased risk of malignancies such as lymphomas, hepato-carcinomas, cholangiocarcinomas, and gastrointestinal and pancreatic tumors. Finally, other less frequent complications are hepatosplenomegaly, lymphadenopathy, and autoimmune manifestations such as hemolytic anemia, thrombocytopenia, and immune-complex (IgM)-mediated nephritis [8].

Laboratory Results

CD40L and CD40 deficiencies have elevated or normal levels of serum IgM with a markedly reduced level of IgG and IgA. A low level of IgM does not exclude the diagnosis of CD40L or CD40 deficiency. Sometimes normal or elevated IgA levels might be observed, which results from the T-cell-independent pathway. In CD40L deficiencies CD40L is not expressed on activated T-cells, which can be demonstrated upon in vitro stimulation with PMA and Ca-ionophore. CD40 expression is absent on B-cells of CD40 deficient patients. The number of B-cells is normal; however, due to impairment of the T-cell-dependent response, switched memory B-cells are absent. However, natural effector/marginal zone B-cells (IgM + IgD + CD27+) are present as well as CD27-IgA+ B-cells, which both arise from the T-cell-independent pathway [9]. The proliferative response to mitogens such as phytohemagglutinin (PHA) is normal, whereas the response to specific antigens is often reduced and there is a low production of TH1 cytokines. The absolute number of CD4+ and CD8+ T-cells is normal.

Treatment and Prognosis

The number of patients with CD40 deficiency is extremely small, and outcome and treatment options are the same as those of patients with CD40L deficiency—they will be therefore considered together. The majority of reported cohorts from

developed countries are historic, and caution should be taken when inferring current outcomes from these historic cohorts, as diagnosis and management has significantly changed. Published data from 79 patients in the US registry in the early 2000s showed an overall mortality in the cohort of 10%, although the majority of patients were <10 years of age and none were >30 years of age. Deaths were from a number of causes including pneumonia, encephalitis, and malignancy, with the median age of death being 14 years (range 9 months–25 years) [10]. Similar causes of death were reported in an historic UK cohort [11]. A more recent report from a Latin American cohort of 35 patients confirms that pneumonia was the most common complication, but encephalitis was also reported, and three of the four deaths (10% of the cohort) were due to central nervous system infections despite immunoglobulin replacement [12]. In a small much younger Indian cohort (seven patients, median age 2.6 years, range 1.6–8 years), pneumonia remains the most common infection, and no encephalitis was reported, although the patients were young [13]. The most recent multicenter international study of patients with CD40L deficiency reported on 176 patients [14]. The median age was 11 years (range, 0.1–60.7 years). Median survival was 25 years, with no statistical difference between those treated conventionally and those receiving hematopoietic stem cell transplantation, although the Karnofsky/Lansky age performance scores were significantly better in the transplanted group. In this cohort, malignancy and hepatic disease were the most common causes of mortality in the non-transplant group, and there was an annual mortality of 2.2%. A multicenter international survey of 93 patients transplanted for CD40L deficiency between 1993 and 2014, from 23 different centers, in 15 countries documented an overall survival of 77.4% [15]. Results were better in transplants performed after the year 2000 (83% overall survival) and in children transplanted when <10 years of age (88.7% overall survival). The median follow-up was 4.3 years (range 0.4–17.1 years). Survival was 96.1% in patients transplanted with no pre-existing chronic lung disease and/or liver disease. While many patients were able to discontinue prophylactic medication, there were patients who required continued immunoglobulin substitution because of poor donor chimerism, and 13% rejected the first transplant, predominantly those who had received reduced intensity conditioning. For patients with severe liver disease, hepatic transplant can be performed before hematopoietic stem cell transplantation [16]. The most concerning feature about these data is the occurrence of potentially fatal complications despite adequate prophylaxis, particularly central nervous system infection and cryptosporidial infection leading to sclerosing cholangitis and hepatocellular carcinoma. Given that most successful transplants occurred in patients <10 years of age, without pre-existing respiratory or hepatic disease, and that the Karnofsky/Lansky age performance scores are better, there is a strong argument to offer stem cell transplant early to these patients, particularly as these will most readily tolerate myeloablative conditioning which is more likely to correct the defect. However, this has to be balanced with the potential long-term toxicities of chemotherapy, particularly on fertility.

Within the field of primary immunodeficiencies, gene therapy in the form of gene addition to defective hematopoietic stem cells using viral vectors is emerging as a potential corrective therapy for some types of severe combined immunodeficiency.

Correction of CD40L deficiency by gene addition in murine models has led to thymic lymphoproliferative disorders, probably because gene expression needs to be tightly controlled [17]. An alternative approach could be to use gene-editing to remove the faulty *CD40LG* gene from peripheral T-cells and replace it with a functional gene, thus restoring function in T-helper cells and enabling T-cell interaction with B-cells and antigen-presenting cells. This method has been shown to be effective in murine models [18], but clinical trials have yet to be developed.

15.2.1.2 NEMO Deficiency (OMIM 300248)

Gene

The IKBKG gene (Xq28) encodes the protein NEMO (NFκB essential modulator), which is the founding member of an evolutionarily conserved family of NEMO-like kinases that function in numerous cell signaling pathways. NEMO/IKKγ is one of the three catalytic subunits of the IKK complex (together with IKKα and IKKβ) [19]. NFκB is a transcription factor sequestered in the cytoplasm of resting cells through binding to inhibitor of NFκB (IκB) proteins. Upon cell stimulation, IκB are phosphorylated by IKK leading to degradation and release of NFκB, which can translocate to the nucleus where it can bind DNA and regulate gene transcription of genes such as AID and UNG.

Clinical Presentation

Hypomorphic mutations in the X-linked NEMO gene result in ectodermal dysplasia, anhidrotic with immunodeficiency (EDA-ID), which is characterized by sparse hair, cone-shaped teeth and hypohidrosis with lack of sweating and a tendency to develop hyperpyrexia [20]. More severe mutations also result in osteopetrosis and lymphedema, but loss-of-function mutations are lethal [21]. Female carriers have signs of incontinentia pigmenti syndrome, characterized by skin abnormalities including blistering rash at birth and in early infancy followed by development of wart-like skin growths [19]. There are signs of hyperpigmentation occurring in a swirled pattern, which fade with time. In adulthood incontinentia pigmenti usually shows lines of hypopigmentation on their arms and legs. Other signs are alopecia and dental and eye abnormalities. NEMO-deficient patients are susceptible to infections with pyogenic bacteria (*S. pneumoniae*, *H. influenzae*, and *S. aureus*) and to infections with mycobacteria (*M. avium* or *M. kansasii*), which causes meningitis, sepsis, arthritis, and osteomyelitis [21, 22]. Opportunistic infections with *Pneumocystis jirovecii* and chronic mucocutaneous candidiasis (CMC) have also been reported in a minority of patients. The overall clinical presentation is heterogeneous and ranges in severity. This might be caused by the nature of the mutation and the level of residual NEMO activity.

Laboratory Findings

NEMO deficiency is immunologically characterized by hypogammaglobulinemia in combination with poor antibody response to polysaccharide antigens. Serum IgM

or IgA can be increased. NK cell function is reduced, and the T-cell responses to mitogens and recall antigens in vitro are variable. T- and B-cell counts in the peripheral blood are normal, although the number of memory B-cells can be reduced. Persistent lymphocytosis in combination with a normal distribution of T, B, and NK cells is also a common finding. In vitro NFκB function can be evaluated by stimulation of peripheral blood mononuclear cells with TLR or IL1R ligands and measurement of IL6 (reduced in NEMO deficiency). Another functional test suitable for evaluation of NEMO variants is measurement of degradation of IκB in patient's fibroblast upon stimulation with IL1β or TNFα [23].

Treatment and Prognosis

While the immunological defects in patients with CD40 or CD40L deficiency can be simply explained by interruption of ligand to receptor binding and signaling in hematopoietically derived cells, and thus restoration of function by replacement of defective hematopoietic stem cells, the solution is not so straightforward for patients with NEMO deficiency. NF-kB and NEMO are widely expressed in many tissues and are involved in many signal transduction pathways, including at least three non-hematopoietic pathways. Therefore, while replacement of defective hematopoietic stem cells may resolve some immunological features, other manifestations, such as lymphedema or ectodermal dysplasia, may remain.

B-cell and antibody deficiencies are the most commonly reported immunological abnormalities reported in a cohort of 72 individuals with NEMO deficiency (median age 4.6 years, range 0–48 years), of which 50% of patients who were alive were receiving immunoglobulin replacement therapy [21]. Twenty-seven patients had died (median age 2.75 years, range 0–48 years), and only 15 were >10 years of age. Serious viral infection occurred in 21% of patients and opportunistic infections occurred in 10%. While most patients do not warrant consideration for hematopoietic stem cell transplantation, one fairly large transplant cohort of 29 patients has been described [24]. Median age at transplantation was 3.4 years, range 0.33–18.8 years. The majority of patients experienced opportunistic infection with mycobacterial or fungal species, and many required nutritional support pre-transplantation. The overall survival was 74% at 108 months after transplantation with a median follow-up of 57 months and an engraftment rate of 93%. Age at transplantation did not influence the survival rate, which was better with matched siblings than unrelated donors. However, patients receiving stem cells from carrier female relatives appeared to have only partial correction of the immunodeficiency. Patients with mycobacterial infection had a worse outcome than those without. Some patients with colitis pre-transplant did not have resolution of symptoms, and two others developed colitis post-transplant, a phenomenon previously reported [25]. These observations suggest that the pathogenesis of NEMO deficiency-related colitis may involve a non-hematopoietic pathway and that transplantation may not correct IBD, possibly reflecting the importance of the NF-κB pathway in intestinal epithelial cells for controlling epithelial gut integrity.

15.2.2 Intrinsic B-Cell Defects

15.2.2.1 AID Deficiency (OMIM 605258)

Gene

Activating-induced cytidine deaminase (AICDA; 12p13.31) is the gene encoding AID, which is exclusively expressed in germinal center B-cells. AID is a DNA-specific cytidine deaminase, which is involved in the induction of CSR and SHM by deamination of cytidine to uracil during transcription of Ig-variable (V) and Ig-switch (S) regions (see Sect. 15.1.2). Mutations located in the C-terminal part of AID result in severe CSR deficiency while SHM is not affected.

Clinical Presentation

AID deficiency is characterized by recurrent bacterial infections of the respiratory tract, mostly due to encapsulated bacteria, which can lead to bronchiectasis [26]. Also, gastrointestinal bacterial infections are a prominent feature, which are sometimes related to persistent *Giardia lamblia* infections. Opportunistic infections and neutropenia, which are characteristic for CD40 and CD40L deficiency, are not observed because in AID deficiency, the T-cell responses are unaffected. Lymphoid hyperplasia is a striking feature affecting mainly cervical lymph nodes and tonsils, which may even require resection. In addition, arthritis and autoimmune features (hemolytic anemia, thrombocytopenia, and autoimmune hepatitis) are frequently found [8].

Laboratory Findings

AID-deficient patients have normal to elevated serum IgM levels and reduced or absent serum IgG and IgA levels. IgM isohemagglutinins and anti-polysaccharide IgM are normally present [6, 26]. The total number of T-cells and B-cells is within the normal range; however, switched memory B-cells are absent. B-cells are able to proliferate in vitro; however, upon stimulation with anti-CD40 and cytokines, they do not undergo CSR, which is characteristic for an intrinsic B-cell defect. The level of SHM is strongly impaired. The lymphoid hyperplasia can be characterized by follicular hyperplasia with giant germinal centers and small mantle zone interfollicular area.

15.2.2.2 UNG Deficiency (OMIM 608106)

Gene

Uracil-DNA glycosylase (UNG; 12q24.11) is expressed in germinal center B-cells in parallel with AID and removes uracil from DNA molecules via cleaving the N-glycosylic bond that has been generated by AID activity. This subsequently induces the error-prone base excision repair (BER) pathway of the SHM process, which generally results in transitions and transversions at C:G pairs. Mutations in the UNG gene result in increased accumulation of genomic uracil [27]. UNG-deficient patients have profound impairment in CSR at a DNA precleavage step and with a partial disturbance of the SHM pattern [28].

Clinical Presentation and Laboratory Findings

Only a few patients with UNG deficiency have been described [28]. They described patients have susceptibility to bacterial infections of the respiratory tract, cervical and mediastinal lymph node hyperplasia, increased serum IgM concentrations, and profoundly decreased serum IgG and IgA concentrations. At the time of diagnosis, antibody titers to pneumococcal and tetanus antigens were reduced. The T-cell counts and functions are normal, including the expression of CD40L on activated T-cells. B-cells also proliferate normally; however, they do not undergo class-switch recombination. The CSR defect occurs between the switch region transcription and induction of DNA double-strand breaks. The frequency of SHM in UNG is unaffected. However, the pattern of SHM is biased toward transitions at G/C pairs (i.e., G > A; C > T), whereas the ratio of transitions and transversions at A/T pairs is normal [28].

Treatment and Prognosis AID and UNG Deficiencies

There are few patients described with deficiency of AID or particularly of UNG, and treatment and prognosis will be considered jointly. Infections in these patients are characteristic of those associated with antibody deficiency, in contrast to patients with CD40, CD40L, or NEMO deficiency who also experience opportunistic infections [12, 26, 28]. Patients with deficient of AID have also been described as having gastrointestinal infections due to giardia. However, in contrast to patients with agammaglobulinemia, these patients appear not to experience enteroviral infections of the central nervous system. Treatment with immunoglobulin replacement should resolve the symptoms. AID-deficient patients may experience extreme lymphoid hyperplasia, for which surgery may provide symptomatic relief. Autoimmunity is also described [29] which may be severe and life-threatening and require treatment with anti-CD20 antibody with or without other immunosuppression.

15.2.3 DNA Repair Defects

15.2.3.1 Ataxia Telangiectasia (OMIM 208900)

Gene

Ataxia telangiectasia mutated (ATM;11q22.3) is a member of the phosphatidylinositol3 kinase family, which includes DNA-PKcs and ATR, which all function in DNA break responses. It exists as an inactive dimer/tetramer that is activated and recruited to sites of double-strand breaks. ATM accumulates at repair foci and regulates binding and activation of double-strand break (DSB) repair proteins and subsequent repair of the DSBs. ATM also initiates a cell-cycle checkpoint until repair is complete [30, 31]. It responses to DNA damage through phosphorylation of essential substrates involved in DSB repair and cell-cycle control. During CSR, ATM organizes the repair complex and might contribute to the correct juxtaposition of DSBs during the long-range interaction required for accurate switch

recombination [32]. However, defective switch to distal constant regions in patients with AT could also (in part) be explained by an impaired ability of B-cells to undergo multiple successful GC responses [33].

Clinical Presentation

AT is characterized by cerebellar ataxia, oculocutaneous telangiectasias, radiosensitivity, chromosomal instability, a propensity for development of (mainly hematologic) malignancies, growth retardation, and endocrine abnormalities. The prevalence of cancer in AT patients is 10–30% [34]. AT patients also have signs of immunodeficiency, predominantly antibody deficiency. However the extent and severity is highly variable. Classical AT refers to patients with an early-onset disease (childhood) in contrast to variant AT, which presents at adulthood. A subset of patients with classical AT has a severe early-onset hypogammaglobulinemia reminiscent of a CSR deficiency [35, 36]. Some patients experience lung infections, chronic lung disease, and recurrent infections, which are associated with immune deficiency. The majority of infections in childhood are caused by *Staphylococcus aureus*, *Haemophilus influenza*, and *Streptococcus pneumoniae*, whereas in older patients *Pseudomonas aeruginosa* is more frequent [37].

Laboratory Findings

The laboratory findings related to CSR deficiency in AT patients are characterized by a frequent reduction in serum IgA and IgG subclass levels. Most patients have disturbed naïve B-cell and T-cell homeostasis, as evidenced by low cell numbers, increased proliferation, a large proportion $CD21^{low}CD38^{low}$ anergic B-cells, and decreased antigen receptor repertoire diversity [33]. AT patients presenting with an early-onset hypogammaglobulinemia have impaired formation of T-cell-dependent memory B-cells. Sμ-Sa junctions in patients with AT showed increased microhomology, whereas the Sμ-Sγ junctions from these patients have severely reduced mutations or insertions, indicating that the predominantly used error-prone NHEJ pathway in CSR is impaired in patients with AT [38] and the proportion of CSR to the distal IGHG2, IGHG4, and IGHA2 constant regions is reduced [33]. However, the frequency of SHM in switched transcripts is not reduced in AT.

Treatment and Prognosis

Ataxia telangiectasia is a chronic, progressive disease, with no curative treatment available and with which patients face a high risk of infection, malignancy, and neurodegeneration. Median survival is into the mid-twenties, with most deaths due to chronic lung disease or malignancy [39]. A multidisciplinary team is best placed to consider the multidimensional aspects to supportive care and management of these patients. The newborn screening test for severe combined immunodeficiency detects T-cell receptor recombination excision circles (TRECs) from infant dried blood spots. Infants with T-cell lymphocytopenia and ataxia telangiectasia have been identified with the SCID newborn screening test in combination with exome sequencing [40]. Early diagnosis enables early family education, genetic counseling, and early, proactive supportive care.

Bacterial infections predominantly affect the sino-pulmonary tract and can be complicated by neuromuscular incoordination leading to aspiration. There is an increased risk of autoimmune or chronic inflammatory disease, including idiopathic thrombocytopenia, arthritis, and vitiligo, and likely related to the immunodeficiency rather than a direct effect of ATM protein dysfunction. There is a relationship between propensity to infection, or to development of lymphoid tumors and breast cancer, and severity of the mutation, with null mutations associated with a greater risk [41, 42]. For patients with antibody deficiency and evidence of recurrent infection, immunoglobulin substitution should be initiated, with or without antibiotic prophylaxis. There should be careful periodic assessment of oro-pharyngeal coordination, to assess swallowing and avoid malnutrition and aspiration. Use of thickened feeds may help, and placement of a gastrostomy may be required in cases of significant neuromuscular dysfunction. Malignancy is particularly challenging in these patients, as tumors are often aggressive, but patients are poorly tolerant of highly cytotoxic chemotherapy and of radiotherapy. Investigation of symptoms can be demanding, as exposure to irradiation should be minimized, alternative imaging modalities are used where possible, and areas not relevant to the diagnosis must be protected from exposure to radiation. The role of hematopoietic stem cell transplantation is controversial, and most results indicate a poor outcome [43]. However, many of these patients are treated for malignancy, and preemptive hematopoietic stem cell transplantation may prevent the development of lympho-reticular malignancy and the need for aggressive conditioning—however, such approaches should only be considered as part of an approved clinical study, with the recognition that successful treatment is unlikely to halt the neurological deterioration. Potential future therapies include the use of antisense morpholino oligonucleotides, to correct aberrant splicing and allow translation of normal ATM protein [44], or the use of aminoglycosides or small molecules for chemical-induced read through of premature stop codons in ATM to produce some functional protein [45]. However, these treatments are some way off clinical use.

Ataxia telangiectasia carriers have one mutated copy of ATM and are healthy. However, they appear to have a reduced lifespan due to breast cancer and gastrointestinal tract carcinoma and of ischemic heart disease [46]. Female carriers have a 2.3-fold increased risk for the development of breast cancer as compared to the general population [47], with the cumulative risk of breast cancer of approximately 6% by age 50 years and approximately 30% by age 80 years [48]. Currently, only standard breast cancer surveillance (monthly breast self-exams and routine mammography at the usual schedule for age) is recommended unless there is family history of breast cancer.

15.2.3.2 Nijmegen Breakage Syndrome (NBS) (OMIM 251260)

Gene

The NBN (Nibrin) gene encoding the NBS1 protein is located at chromosome location 8q21.3. It is a member of the MRE11/RAD50/NBS1 (MRN) complex, which acts as a marker of DNA breaks as it has been shown to accumulate in large nuclear

foci within minutes after DSB formation [49]. It is activated in response to DSBs and keeps the two DNA ends in close proximity [50]. A second function of the MRN complex is ATM activation [51]. MRN recruits ATM to DNA breaks, which results in dissociation of the ATM dimer enabling ATM activity [52]. Furthermore, NBS1 is involved in maintenance of chromosomal integrity, telomere stability, and cell-cycle checkpoint control [53].

Clinical Presentation

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive inherited condition, characterized by microcephaly, dysmorphic "birdlike" face, chromosomal instability, immunodeficiency, and predisposition to malignancy [54]. Recurrent infections are part of the clinical presentation and are mainly found in the upper respiratory tract and lungs but also in the urinary tract. Some patients develop bronchiectasis and chronic lung disease. Opportunistic infections are not part of phenotype. Autoimmune complications were found in a minority of patients and include skin changes, ITP, and AIHA [54].

Laboratory Findings

The immunodeficiency in NBS patients is considerably variable and can affect both humoral and cellular immunity. Reduced serum IgG and/or IgA levels are frequently observed. Sometimes, a normal level of IgG masks an IgG subclass deficiency. IgM concentrations are normal in the majority of cases and sometimes even elevated [54]. NBS patients can have reduced B- and T-cell numbers. In the B-cell compartment, especially the numbers of naïve and memory, B-cells are reduced, whereas the number of natural effector B lymphocytes is increased [55]. The reduction in the number of naïve B-cells can be explained by a loss of juxtaposition of RAG-induced breaks during V(D)J recombination in bone marrow [56]. Regarding the T-cell compartment, the numbers of αβ + T-cells are reduced, but a number of γT+ cells are normal. In addition, T-cells from NBS patients show signs of a senescent phenotype [57]. Analysis of the CSR pathway in NBS patients showed that the NBS1 protein localizes to chromosomal sites of class switching [58] and that the switch junctions show an enhanced presence of microhomology [58, 59].

Treatment and Prognosis

Patients with Nijmegen breakage syndrome are at increased risk of immunodeficiency and malignancy. Overall survival probabilities at 5, 10, 20, and 30 years of age were 95, 85, 50, and 35% in one large cohort study [54]. Recurrent infections tend to be bacterial, affecting the sino-pulmonary tract, and should be treated with appropriate antibiotics—opportunistic infections are generally not a problem. Patients with hypogammaglobulinemia can be treated with immunoglobulin replacement. Autoimmunity can be severe and should be treated with appropriate immunosuppression—anti-CD20 antibody may be required for life-threatening cytopenias.

Malignancy is a leading cause of death [54, 60]. Patients do not tolerate standard chemotherapy protocols well, and regimens need to be adapted—however,

the malignancies are often aggressive [61]. As for patients with ataxia telangiectasia, radiological examination for investigation of malignancy should be judicious and appropriately directed. Hematopoietic stem cell transplantation may have more of a role in the management of these patients [43, 60, 62], with survival of around 75% when reduced intensity conditioning is used. However, whether this approach should be routinely or preemptively offered is yet to be determined.

While heterozygote carriers are unaffected, they do have an increased risk of malignancy, particularly for breast and prostate carcinoma and melanoma [63–65], although there is no consensus on screening for these individuals.

15.2.3.3 DNA Ligase 4 (OMIM 606593) and Cernunnos (OMIM 611291)

Gene

LIG4 located on 13q33.3 encodes the Ligase 4, which is involved the final ligation step of the non-homologous end-joining pathway. The protein forms a complex with XRCC4. NHEJ1 gene (non-homologous end-joining factor 1) also called Cernunnos or XLF (XRCC4-like factor) is located on 2q35. The XLF protein can form a stable complex with XRCC4 where it can bridge DNA ends with its filamentous structure [66]. It binds to DNA and stimulates the XRCC4/ligase IV activity [67]. XRCC4 and Cernunnos are both part of the classical NHEJ pathway, which is primary mechanism for CSR [5]. XRCC4-deficient patients have impaired CSR, however, no clinical signs of immunodeficiency [68–70].

Clinical Presentation

LIG4 deficiency can present with a spectrum of clinical conditions ranging from radiosensitive leukemia [71], radiosensitive T-B-SCID [72] to the LIG4 syndrome characterized by microcephaly and growth retardation [73] and primordial dwarfism [74, 75]. Cernunnos deficiency presents similarly as the LIG4 syndrome with combined immunodeficiency and growth retardation [76]. All patients have recurrent respiratory tract infections caused by *Pneumocystis jirovecii*, CMV, and bacteria [77].

Laboratory Findings

LIG4- and Cernunnos-deficient patients have reduced numbers of peripheral B- and T-cells in combination with a normal number of NK cells. The number of B- and T-cells is dependent on the severity of the mutation. The number of switched memory B-cells is reduced in patients with LIG4 syndrome, and the Sμ-Sα junctions show dramatic shift in using long microhomologies, suggesting an impaired NHEJ during CSR [78]. The Sμ-Sγ junctions however show an increased frequency of insertions but no increase in microhomology [78]. In Cernunnos deficiency, CSR is also affected, and the junctions show similarities with the junctions of LIG4 syndrome patients [79].

Treatment and Prognosis

There are few patients described with deficiency of Cernunnos, and the phenotype is similar to that of patients with DNA ligase 4 syndrome; therefore, treatment and prognosis will be considered jointly. For patients presenting with a severe combined immunodeficiency phenotype, hematopoietic stem cell transplantation should be performed, with best results obtained when reduced intensity conditioning regimens are employed, without radiotherapy [43]. Long-term follow-up of these patients will be required to monitor for late-occurring complications given the systemic nature of the defect, which is uncorrected by transplantation. For patients with less severe immunodeficiencies, treatment with immunoglobulin replacement is recommended, with antibiotic prophylaxis as appropriate. Unfortunately, despite this, there is a high risk of malignancy [80]. The role of preemptive hematopoietic stem cell transplantation is yet to be determined.

15.2.3.4 *PMS2, MHL1, MSH2*, and *MHS6* and Deficiency (OMIM 276300)

Genes

PMS2 (postmeiotic segregation increased, S cerevisiae 2) also called mismatch repair gene PMSL2 is located on 7p22.1 and is a protein required for mismatch repair. MLH1 (MutL homolog 1) is located on 3p22.2. PMS2 and MHL1 can heterodimerize to form MutL alpha, part of the DNA mismatch repair (MMR) system. The exact role of PMS2-MHL1 in CSR remains unclear; however, it has been shown to play a role in inducing DNA double-strand breaks. The other MMR component is MutS homolog consisting of MSH2–6. MutS homology 2 gene (MSH2) is located on 2p21 and forms a complex with MSH6 (2p16.3), which binds AID-induced mismatches in the absence of UNG.

Clinical Presentation

Autosomal recessive mutations in PMS2, MHL1, MSH2, and MHS6 are found in patients with constitutional mismatch repair deficiency syndromes (CMMRD). CMMRD is a rare pediatric autosomal recessive childhood cancer predisposition syndrome with four main tumor types: hematologic malignancies, brain/central nervous system tumors, colorectal tumors and multiple intestinal polyps, and other malignancies including embryonic tumors and rhabdomyosarcoma. Heterozygous germline mutations in *MLH1*, *MSH2*, *MSH6*, and *PMS2* cause Lynch syndrome (LS), an autosomal dominant cancer syndrome associated with hereditary nonpolyposis colorectal cancer (HNPCC), endometrium carcinoma, and other malignancies, occurring on average in the fourth and fifth decades of life.

Many CMMRD patients show signs reminiscent of neurofibromatosis type I, particularly multiple cafe-au-lait macules. It remains unclear whether IgA and/or IgG deficiency is a common feature of CMMRD, because severe bacterial infections do not occur at high frequencies. However, in PMS2 deficiency, defective PMS2 has been shown to be associated with impaired CSR [81]. In these patients CSR was found partially defective in vivo resulting in reduced serum Ig and reduced

numbers of memory B-cells. The SHM frequency was also found to be reduced. The CSR defect is characterized by defective occurrence of double-strand DNA breaks (DSBs) in switch regions and abnormal formation of switch junctions. No detailed immunological laboratory information is available for CMMRD patients.

Treatment and Prognosis

While recurrent bacterial infection may lead to the institution of immunoglobulin replacement therapy, the major issue for these patients is development of malignancy in early childhood, predominantly hematological malignancies, brain tumors, or early-onset gastrointestinal tumors [82]. T-cell lymphomas are usually the first tumors to present, but patients are likely to develop additional tumors. Treatment should be directed at specific tumors, but the prognosis is poor, with few patients reaching adulthood.

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