Inborn errors of immunity caused by defects in the DNA damage response pathways: Importance of minimizing treatment-related genotoxicity

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Abstract
Several primary immunodeficiencies are caused by defects in the general DNA repair machinery as exemplified by the T-B- radiosensitive SCID condition owing to impaired resolution of programmed DNA double-strand breaks introduced by RAG1/2 during V(D)J recombination. The genome instability generally associated with these conditions results in an increased propensity to develop malignancies requiring genotoxic-based anti-cancer treatments. Moreover, the extent of immune deficiency often calls for hematopoietic stem cell transplantation as a definitive treatment, also requiring genotoxic-based conditioning regimen prior to transplantation. In both cases, the underlying general DNA repair defect may result in catastrophic iatrogenic consequences. It is, therefore, of paramount importance to assess the functionality of the DNA repair apparatus prior to any genotoxic treatment when the exact molecular cause of the disease is unknown. For this purpose, two simple assays can be used on patients derived peripheral blood lymphocytes: (1) the PROMIDISα biomarker, based on the next-generation sequencing analysis of the TCRα repertoire, will highlight specific signatures of DNA repair deficiencies; (2) direct analysis of the sensitivity of peripheral lymphocytes to ionizing radiation will formally identify patients at risk to develop toxicity toward genotoxic-based treatments.

KEYWORDS
class switch recombination, DNA damage and repair, genotoxicity, RS-SCID, V(D)J recombination
1 | INTRODUCTION

Living organisms are constantly exposed to genotoxic assaults, whose origin can be either endogenous (biological processes such as cellular respiration), or exogenous (radiation or chemical exposure). Several highly conserved DNA repair mechanisms have been selected during evolution to cope with a large variety of DNA damages in order to maintain genomic integrity. Among DNA lesions, double-strand breaks (DSBs) are considered the most toxic. At least two main DNA repair pathways [homologous recombination (HR) and nonhomologous end joining (NHEJ)] have evolved to operate on DSBs. Nonetheless, the genome, carrier of our genetic information, is not static, but subject to programmed modifications in multiple physiological circumstances. This is, for example, the case during meiosis, when DNA is rearranged through meiotic recombination, an essential molecular process that drives evolution. Likewise, the development and maturation of the adaptive immune system strictly relies on sequential somatic DNA rearrangement and modification steps through V(D)J recombination, class switch recombination (CSR), and the generation of somatic hypermutations (SHM) in immunoglobulin (Ig) genes. Many of these DNA modification processes occur through the introduction of programmed DSBs (prDSBs). The proper repair of these DNA lesions is ensured by ubiquitous DNA repair mechanisms, the defect of which are sources of various conditions in humans and animal models.

2 | DEFECTS IN REPAIRING PROGRAMMED DNA BREAKS IN THE IMMUNE SYSTEM

2.1 | V(D)J recombination and T-B-SCIDs

The adaptive immune system is composed of B and T lymphocytes, which express large arrays of antigen-specific receptors; the Ig or B cell receptor (BCR) on B lymphocytes and the T cell receptor (TCR) on T cells. The genetic elements encoding the variable domains of these receptors are scattered along the chromosomes in distinct Variable (V), Diversity (D), and Joining (J) gene units. A tissue and stage-specific DNA rearrangement process, the V(D)J recombination, results in the physical juxtaposition of one V, D, and J segment, thus forming the variable domain encoding exon. This mechanism is initiated by the recombination-activating genes (RAG)1 and RAG2 lymphoid-specific factors after recognition of recombination signal sequences (RSS) that flank all V, D, and J elements to be rearranged.

Animal models of both RAG1 and RAG2 gene inactivation have unmistakably established the fundamental role of the V(D)J recombination, not only for the production of a diversified adaptive immune repertoire, but primarily for the proper developmental program of B and T lymphocytes. Likewise, human patients harboring biallelic RAG1 or 2 loss of function mutations are entirely devoid of circulating mature B and T cells at birth, resulting in the condition T-B- Severe Combined Immunodeficiency (T-B-SCID) (see Bosticardo et al. for a recent review). Depending on the RAG1/2 mutations, residual activity may concede the emergence of T and B cells with reduced repertoire diversity. V(D)J recombination proceeds through the introduction of prDSBs in immature lymphocytes, pro-B in the bone marrow, and pro-T in the thymus, which are repaired by the NHEJ apparatus, sole mechanism to handle DSBs within G0/G1 arrested cells. Faulty DSB repair caused by impaired NHEJ results in abortive V(D)J recombination, an arrest of B and T cell development, and an increased cellular sensitivity to genotoxic agents causing radiosensitive SCID (RS-SCID) (Table 1). The first instance of RS-SCID was revealed in mice with the spontaneous appearance of the scid mutation, later found to affect the Prkdc gene encoding the DNA-PK catalytic subunit (DNA-PKcs), one of the essential core NHEJ factors. Following was the identification of the gene encoding the Artemis nuclease (DCLRE1C gene), mutated in human RS-SCID. Artemis exerts a distinctive function during V(D)J recombination through its endonuclease activity required for the opening of hairpin-sealed DNA ends specifically generated by RAG1/2. Thus, alike scid mice, patients harboring PRKDC mutations present with RS-SCID. In the last stage of V(D)J recombination, the DNA ends are rejoined by DNA LigaseIV, one of the three eukaryotic DNA ligases, in association with its co-factors XRCC4 and Cernunnos/Xlf. DNA ligase IV or XRCC4 gene inactivation results in late embryonic lethality in mice, caused by apoptosis of post-mitotic neurons, and a RS-SCID phenotype in fetuses owing to an impaired V(D)J recombination. In humans, depending on the underlying DNA ligase IV hypomorphic mutations, the situation is more complex with a large spectrum of clinical presentations within the so-called “Lig4 syndrome,” generally associated with developmental defects such as microcephaly and impaired immunity. Lig4 patients may even remain fully asymptomatic (no microcephaly and no immune deficiency) up to the point when they need to undergo genotoxic treatments for malignancies and develop acute toxicity, thus revealing their general DNA repair defect. The analysis of a series of Lig4 patients without mutations in DNA Ligase IV revealed an additional DNA repair factor encoding gene, Cernunnos, the defect of which causes microcephaly and immune deficiency characterized by a profound B and T cell lymphopenia. Cernunnos was independently identified as XRCC4 like...
TABLE 1  NHEJ factors and the development of the immune system

| Mice                                      | Humans                                      | OMIM    |
|-------------------------------------------|---------------------------------------------|---------|
| Ku70 (XRCC6)                              | RS-SCID                                     | #152690 |
| Ku80 (XRCC5)                              | RS-SCID                                     | #194364 |
| DNA-PKcs (XRCC7, PRKDC)                   | Scid mice                                   | #600899 |
| Artemis (DCLRE1C)                         | RS-SCID                                     | #605988 |
| DNA ligase IV (Lig4)                      | ε lethal                                    | #601837 |
| XRCC4                                     | ε lethal                                    | #616541 |
| Cernunnos/Xlf (NHEJ1)                     | Mild immune defect                          | #611290 |
| PAXX                                      | No immune defect RS-SCID on Xlf KO          | #616315 |

Abbreviation: SSMED, short stature, microcephaly, and endocrine dysfunction.

2.2  Class switch recombination (CSR), somatic hypermutation (SHM), and hyper-IgM (HIGM) syndrome

During the terminal maturation of B lymphocytes, the immunoglobulin genes undergo two additional somatic modifications of their DNA. The class switch recombination (CSR) exchanges the previously Igμ constant region encoding gene unit for a different downstream isotype (α, γ, ε), thereby modifying the effector function of the resulting antibody without altering its antigenic specificity. On the contrary, the process of somatic hypermutation (SHM) introduces mutations at hotspots within Ig variable encoding segments to increase the affinity of the resulting antibody. CSR and SHM are both triggered upon antigen recognition by B lymphocytes within germinal centers of secondary lymphoid organs. They are initiated by the activation-induced cytidine deaminase (AID), identified two decades ago. CSR relies mainly on the canonical NHEJ apparatus for the repair of the AID induced DSBs. However, XRCC4-deficient conditions in mice highlighted the existence of a robust alternative NHEJ pathway (Alt-NHEJ) to cope with DSBs in the absence of the canonical NHEJ pathway during CSR. The Alt-NHEJ during CSR relies on abundant DNA sequence microhomology within switch regions (Sp), thereby leaving a characteristic "footprint" at the CSR junction, which has been extensively studied in various human DNA repair conditions such as ATM deficiency in ataxia telangiectasia (AT) or Cernunnos/Xlf deficiency. Another intriguing discovery in the context of CSR is the dissociation of function within the NHEJ factor 53BP1, which dissociates its DNA repair function from its role during CSR.

Molecular defects in CSR and SHM are responsible for a subset of "predominantly antibody deficiency" syndrome (PAD), previously designated as HIGM syndrome. We will not discuss in depth the molecular basis of PADs since an extensive review has been recently published. According to the authors, about 10% of CSR deficiencies are not yet attributed to a known molecular cause. The extensive use of the in vitro CSR system provided by the IgM+ lymphoma cell line CH12F3 has highlighted the function of several DNA repair factors during CSR. This is, for example, the case of the recently discovered shieldin complex (review in Setiaputra et al. or Fam72a identified through CRISPR/Cas9 genetic screens. Although mutations in these factors have not been associated with PAD until now, they certainly represent serious candidate genes when all other known causes have been eliminated. Likewise, the use of the Human Gene Connectome (HGC), which associates human gene pairs according to several possible biological paths, provides an interesting tool to contribute to the identification of disease-causing mutations in new candidate factors as suggested by Amirifar et al.

factor (Xlf), given its sequence/structure homology with XRCC4. Unexpectedly, despite a severe NHEJ deficiency illustrated by a profound sensitivity to genotoxic agents and an impaired V(D)J recombination in vitro in non-lymphoid cells such as patient fibroblasts or murine MEFs and ES cells, Cernunnos/Xlf-deficient condition is not associated with a major V(D)J recombination defect in vivo as shown by the preserved B and T cell maturation in bone marrow (BM) and thymus both, in humans and mice. Yet, suboptimal V(D)J recombination activity may participate in the phenotype. Likewise, patients with hypomorphic mutations in the XRCC4 gene present short stature, microcephaly, and endocrine dysfunction (SSMED syndrome), but no profound alteration of the adaptive immune system, despite a severe NHEJ defect in vitro. Likewise, although the deficit in the DNA repair factor PAXX has no impact on the development of the immune system in mice and no PAXX-related diseases have been recognized so far in humans, its likely participation during V(D)J recombination was highlighted by the block of B and T cell maturation in PAXX/Xlf doubly deficient mice.

Altogether, V(D)J recombination is essential for the proper development of the adaptive immune system. It proceeds through the introduction of "toxic" prDSBs but has most probably co-opted specific backup systems to mitigate the potential oncogenic outcome of the reaction when not properly controlled. While some patients with general DNA repair defects will develop RS-SCID, other conditions will remain asymptomatic with respect to the immune system. Yet, all these patients present a genuine risk of developing severe adverse effects upon exposure to genotoxic treatments such as anti-cancer radio-chemotherapy or conditioning regimen prior to hematopoietic stem cell transplantation (HSCT), as discussed below.
3 | OTHER DNA REPAIR DEFECTS IN THE HEMATOPOIETIC SYSTEM

In addition to defects in the core factors of the NHEJ apparatus that translate into SCID/CID manifestations described above, overall impairment of the DNA damage response (DDR), notably during the phase of DNA damage signaling, has consequences on the proper function of the hematopoietic system as a whole and the immune system in particular. This is the case of the Nijmegen breakage syndrome (NBS) and ataxia telangiectasia (AT) caused by mutations in the NBN and ATM genes, respectively. These two very similar conditions are characterized by immunodeficiency, genome instability, and cancer predisposition. AT also includes progressive cerebellar degeneration. They are both associated with a clinical radiosensitivity.

4 | TREATMENT OF DNA REPAIR DEFECT-ASSOCIATED IMMUNE DEFICIENCIES

4.1 | Malignancies in PID patients

PIDs carry an increased risk of malignancy, particularly non-Hodgkin lymphoma (NHL) and skin cancers. The risk of developing hematological malignancies is even increased in patients with inherited DNA repair-deficient conditions, given the important role of DSB repair during extensive cell proliferation. Likewise, hematological malignancies in otherwise immunocompetent individuals often harbor somatic mutations in core components of the DNA repair machinery. The mechanisms leading to hematopoietic malignancies in DSB repair deficiency are multiple and intertwined. First, defective immune surveillance resulting from altered T and B cell development, defective lymphocyte proliferation and/or decreased diversity in the B and T cell repertoires may promote tumor escape but also B cell transformation and immortalization by EBV. Moreover, defects of DNA repair per se may induce point mutations, translocations, and even chromothripsis that represent oncogenic driver events. As such, AT and NBS predispose to malignancies, not only because the two defective factors, ATM and NBN, represent critical DDR factors, but also possibly because the resulting immune deficiency being milder, the affected patients do not require early definitive treatment by HSCT. The increased risk of developing malignancies is also shared by LIG4 patients. The influence of Cernunnos deficiency on malignancy is difficult to assess given the rarity of patients, although the occurrence of EBV-negative lymphoma has been reported. Lastly, malignancies have rarely been reported for Artemis-deficient SCID patients, probably because most of them receive HSCT at very early age.

Chemotherapy remains the standard treatment of malignancies but is complicated by severe toxicity due to the importance of the NHEJ pathway in all tissues. Although there are no definitive recommendations, adapted chemotherapy protocols may include an initial reduced dose followed by dose escalation; full doses with increased time lapse between courses and/or replacement of selected cytotoxic agents with less toxic drugs. The incidence of secondary cancers directly due to chemotherapy is difficult to evaluate in these patients, as the prognosis of the first malignancy is often poor. Nevertheless, the high rate of lymphoma as secondary cancer suggests that oncogenesis is driven by the DSB deficiency rather than by chemotherapy complications. Alternative treatments may include immunotherapy (CD30, PD-1, PD-L1 or CD38), especially when lymphoproliferative disease may be difficult to differentiate from overt lymphoma in some PID conditions. Allogeneic CAR-T cells may represent another option in future. HSCT remains the best treatment to prevent secondary malignancies in PID patients.

Numerous examples of extreme and often fatal toxicity of chemotherapy and radiotherapy were described, including veno-occlusive disease and hepatitis, severe hemorrhagic cystitis, mucositis, infection, and pulmonary failure. As a malignant disease may be the first sign of the DSB deficiency, it is of tremendous importance for clinicians to consider these underlying conditions to adjust the treatment.

4.2 | Hematopoietic stem cell transplantation

Despite the large spectrum of disease manifestations in PIDs, allogeneic HSCT often remains the unique curative treatment option for these patients. Although newborn screening programs for SCID are implemented in several countries worldwide, they are not universal. Therefore, a substantial number of SCID patients will continue to be diagnosed upon infectious episode. Genetic testing may not be rapidly available and should not delay the initiation of curative HSCT, particularly in SCID patients with ongoing viral infections. Therefore, HSCT conditioning is often initiated prior to molecular diagnosis. As the DNA repair defect is ubiquitous, all cells are vulnerable to DNA damaging agents. Conditioning-related toxicity is thus a major concern in DNA repair deficiencies contributing not only to transplantation-related toxicity and mortality, but also to poorer post-HSCT outcome. Ideally, patients should be referred to reference centers experienced in these rare conditions. HSCT in these patients is often associated with an increased risk for mucositis, veno-occlusive disease, pulmonary hypertension, thrombotic microangiopathy, as well as other transplant-associated endothelial cell activation syndromes, including capillary leak syndrome, engraftment syndrome, idiopathic pneumonia syndrome, and secondary malignancies. Preexisting infections and chemotherapy-induced tissue damage may also increase the occurrence of graft versus host disease (GVHD). Specific recommendations for the use of appropriate chemotherapeutic protocols have been elaborated in order to overcome these limitations. Of note, total body irradiation should not be used. The use of alkylating agents in Artemis-deficient T-B-NK+ SCID patients was associated with a significantly higher occurrence of poor growth, abnormalities in dental development, and late endocrine effects when compared to RAG1 or 2-deficient T-B-NK+ SCID. The development of specific antibody-drug-conjugates
targeting specifically hematopoietic cells may deplete host stem and immune cells, thereby allowing allo-engraftment without the currently used myeloablative chemotherapy. This is a promising approach to reduce conditioning-related toxicity in future. CD45 is a potential target to target the hematopoietic niche. 68,69

5 FUNCTIONAL AND MOLECULAR DIAGNOSIS OF DNA REPAIR DEFECT-ASSOCIATED IMMUNE DEFICIENCIES

The prototypical example of a fatal adverse effect in the course of genotoxic-based anti-cancer treatment comes from a young patient who died following cranial irradiation as part of his anti-leukemia therapy.20 The cells from this patient were subsequently found to present an increased sensitivity to ionizing radiation (IR), owing to deleterious mutations in the DNA ligase IV gene.58 To avoid possible adverse effects of treatments either during conditioning prior to HSCT or for anti-cancer therapy, it is of paramount importance to identify patients with possible DNA repair deficiencies whenever the underlying molecular cause of the disease remains unknown. Three assays can be used for this purpose.

5.1 TCR-Vα7 and PROMIDISα

The TCRα locus is unique in the sense that it proceeds through organized, sequential VαJα rearrangement waves, starting with the most proximal Vα and Jα segments up to the more distal ones (Figure 1A). Following each wave, the produced TCR is “tested” for its immune relevance and possible T cell positive selection. If not proficient, a new VαJα rearrangement cycle is initiated, using upstream and downstream Vα and Jα, respectively. This process relies on (1) the fitness of thymocytes and (2) the V(D)J recombination efficacy. Circumstances in which one of these two conditions is impaired will result in premature arrest of the recombination process and therefore translate in a bias of the TCRα repertoire in which the most distal Vα and Jα segments are underrepresented as seen in several human conditions such as RORC70 or Cernunnos/Xlf26 deficiency. This TCRα bias can easily be identified by quantifying the frequency of TCR-Vα7 expressing T cells in the blood.71 Indeed, TCR-Vα7 represents the most upstream TCR-Vα segment (TRAV1) and is therefore utilized during the last waves of VJ rearrangements. TCR-Vα7 is expressed by CD161+ mucosal-associated invariant T (MAIT) as well as other conventional CD161−, T cells. Although the frequency of MAIT cells can be highly variable, the frequency of conventional TCR-Vα7+/CD161− T lymphocytes oscillates around 3%–5% in healthy controls. In sharp contrast, patients with V(D)J recombination and/or DNA repair deficiency almost completely lack TCR-Vα7+ T cells, even when hypomorphic mutations spare the development of T cells to some extent as shown for RAG2, Artemis, Lig4, and Cernunnos deficiency in Figure 1B. Bias in TCR Vα and Jα usage caused by V(D)J recombination and/or DNA repair deficiency can also be evaluated using PROMIDISα.71 In this assay (Figure 2) a subset of the TCRα repertoire covering proximal, median, and distal segments is analyzed by multiplexed PCR followed by NGS and statistical analysis. The relative representation of Vα segment and their associated Jα define 9 parameters of the PROMIDISα signatures that are further implemented in a principal component analysis and hierarchical clustering. As shown in Figure 2C, all the V(D)J recombination defective patients (RAG1/2 or RS-SCID) cluster in the same group, away from the collection of healthy controls. AT

![Figure 1](image-url) Determination of TCR-Vα7 expressing T lymphocytes. (A) Organization of the TCRα locus and sequential waves of VαJα rearrangement. (B) TCR-Vα7/CD161 determination by FACS analysis.
patients harboring mutations in the \textit{ATM} gene mostly cluster within a separate group, with very few overlaps with the “VDJ deficient” patients. Interestingly, a couple of patients with NBS syndrome clustered with AT cases. PROMIDIS\textalpha thus provides a very robust tool for the early diagnosis of these particular DNA repair defect conditions and allowed for the functional validation of newly identified variants of unknown significance within the \textit{ATM} gene.

5.2 Radiosensitivity assay on PBMCs

The most straightforward way to diagnose a condition of impaired DSB repair is to directly assess the cellular sensitivity to genotoxic agents such as IR. In the assay presented in Figure 3, PBMC obtained from blood is subjected to increasing doses of IR, followed by activation through the TCR via CD3/CD28 beads. The proliferative response of T cells following 6 days in culture with IL2 is recorded and used as a proxy for their DNA repair capacity at time of IR. While healthy controls maintain an efficient proliferative response (around 70\% relative viability) upon 2 Gy IR, patients with ATM, Lig4, Artemis, and Cernunnos deficiency show a sharp decrease in their relative viability/proliferation (10\% or less), arguing for their underlying DNA repair defect. The assay is particularly robust, rapid, and easy to perform with almost no overlap between controls and patients. Consistent with the recessive mode of inheritance of AT, \textit{ATM}/- obligate carriers do not present an increased radiosensitivity, within the limits of the assay. Nevertheless, when RS is suspected based on clinical presentation, but analysis in PBMCs is normal, one might consider repeating the assay in fibroblasts as the risk for genotoxicity might be overlooked.

6 CONCLUSION

The consequences of DNA repair deficiencies on the hematological system in general and the immune system in particular cover a large spectrum of clinical and biological presentations, from complete alymphocytosis in pediatric cases of RS-SCID to less severe forms of combined immunodeficiency (CID) or autoimmunity, sometimes only revealed in adulthood, and cancer-prone patients without overt...
immunodeficiency. As for other forms of solid tumors, DNA repair deficiency is strongly associated with the onset of hematological malignancies, the treatment of which through genotoxic agents can be accompanied with severe iatrogenic adverse effects. The radiosensitive status of any patient undergoing such treatments should be evaluated as to avoid these pejorative outcomes. Likewise, any patient undergoing HSCT should be evaluated for a possible DNA repair defect as a more suitable conditioning regimen would help in avoiding potential catastrophic post-HSCT.

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CONFLICT OF INTEREST
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