Published Online September 13, 2006

Primary immunodeficiency syndromes associated with defective DNA double-strand break repair

A. R. Gennery

Department of Paediatric Immunology, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, NE4 6BE, UK

Damaging DNA double-strand breaks (DNA-DSBs) following ionizing radiation (IR) exposure, potentially lead to cell death or carcinogenesis. Non-homologous end-joining (NHEJ) is the main repair pathway employed by vertebrate cells to repair such damage. Many repair pathway proteins have been identified. The creation of many diverse lymphocyte receptors to identify potential pathogens has evolved by breaking and randomly re-sorting the gene segments coding for antigen receptors. Subsequent DNA-DSB repair utilizes the NHEJ proteins. Individuals with defective repair pathways are increasingly recognized with radiosensitivity and immunodeficiency. Patients with defects in ataxia-telangiectasia mutated, nibrin, MRE11, Rad50, Artemis, DNA ligase IV and Cernunnos-XRCC4-like factor have been identified. Most exhibit immunodeficiency, with a spectrum of presentation and overlap between conditions. Conventional treatment with immunoglobulin replacement or haematopoietic stem cell transplantation (HSCT) can be effective. A greater understanding of the molecular defect will enable better, tailored therapies to improve survival.

Keywords: Artemis deficiency; ataxia telangiectasia; AT-like disorder; Cernunnos-XLF deficiency; DNA-repair defect; immunodeficiency; ionizing radiation sensitivity; LIG IV syndrome; Nijmegen breakage syndrome; RAD50 deficiency; severe combined immunodeficiency

Accepted: July 7, 2006

Correspondence to: A R Gennery, Department of Paediatric Immunology, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, UK. Tel.: +44 191 2336161; fax: +44 191 2730183; e-mail: a.r.gennery@ncl.ac.uk

Introduction

DNA double-strand breaks (DNA-DSBs) arising after ionizing radiation (IR) exposure, or as intermediates in normal endogenous processes, for example, DNA replication and meiosis, are a serious form of DNA damage, potentially leading to replication errors, loss or rearrangement of genomic material and eventually cell death or carcinogenesis. The induction of DNA-DSBs elicits a cascade of events including breakage sensing, signal transduction and effector functions that leads to cell cycle checkpoint arrest

DOI: 10.1093/bmb/ldl006

and/or apoptosis and can influence DNA repair. These limit generation of harmful mutations and proliferation of damaged cells. In response to DNA-DSBs, cells have developed two general types of repair: homologous recombination (HR) and nonhomologous end-joining (NHEJ) [1]. In mammalian cells, HR is generally limited to late S phase and G2 phase of the cell cycle and uses information from a homologous template to accurately repair breaks, when sister chromatids present readily available templates. NHEJ is the main DNA-repair pathway that mediates the joining of broken regions of DNA that lack extensive homology and is the principle mechanism used in vertebrate cells during the G1 phase of the cell cycle.

DNA-DSB repair proteins

Following DNA-DSBs, the cellular responses are mainly activated by ataxia-telangiectasia mutated (ATM), the phosphatidylinositol 3-kinase-related kinase (PIKK) defective in ataxia telangiectasia (AT). ATM is the central component of the signal-transduction pathway responding to DNA-DSBs, but although ATM activation occurs rapidly after DNA damage, MRE11, RAD50 and NBS1 (the MRN complex)—mutated in patients with Nijmegen breakage syndrome (NBS)—are found at the site of DNA damage immediately after the damage has occurred [2]. The MRN complex is the initial sensor of DNA-DSB damage. Following ATM activation, several DNA-repair and cell cycle checkpoint proteins, including H2AX and NBS1, are activated, leading to cell cycle arrest and DNA repair. NBS1 also acts downstream of ATM by recruiting targets for ATM-mediated phosphorylation.

During cell cycle arrest, error-free repair of DNA-DSBs occurs, mainly via NHEJ. Seven mammalian factors have been identified as the critical NHEJ components [3]. The DNA-binding subunits KU70 and KU80 together with the DNA-dependant protein kinase catalytic subunit (DNA-PKcs—a member of the PIKKs family) form the DNA-PK holoenzyme involved early in the recognition of DNA-DSBs. Activated DNA-PK holoenzyme recruits other DNA-repair proteins involved in NHEJ including Artemis, XRCC4, DNA ligase IV and DNA polymerase-μ to the site of DNA damage (Fig. 1). After phosphorylation by DNA-PKcs, the endonuclease activities of Artemis are activated, leading to resolution of complex DNA ends such as the heterologous loop and stem-loop DNA structures that contain single-stranded DNA adjacent to double-stranded DNA. DNA polymerase-μ is associated with KU and DNA ligase IV and may have a role in gap filling during NHEJ but is not critical for ligation. DNA ligase IV, XRCC4 and the recently identified Cernunnos-XRCC4-like factor (XLF) [4, 5] are also required for the

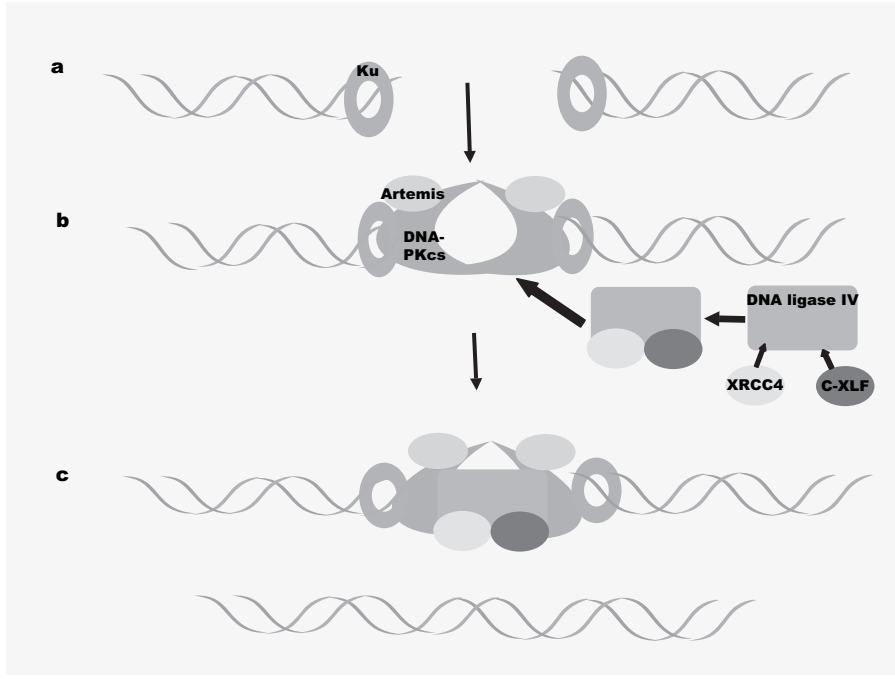

Fig. 1 Non-homologous end-joining in mammalian cells. (**a**) Ku70/Ku80 heterodimers bind the DNA double-strand break (DNA-DSB) ends and recruit DNA-dependant protein kinase catalytic subunit (DNA-PKcs) to form the DNA-PK holoenzyme. (**b**) DNA-PK holoenzyme recruits and phosphorylates Artemis, which processes and resolves complex damaged DNA ends. (**c**) XRCC4, DNA ligase IV and Cernunnos-XLF (C-XLF) co-associate and are recruited to the damaged site. DNA polymerase-μ fills in the DNA gaps (not shown), and the final ligation step of processed DNA ends is performed by the XRCC4/DNA ligase IV/Cernunnos-XLF complex, to leave repaired DNA. (Reproduced with permission of Bubble Foundation, UK.)

ligation reaction that rejoins the DNA-DSBs. The majority of NHEJ functions independently of ATM signalling, although a component dependent upon Artemis requires ATM activity. Thus, although NHEJ is largely ATM-independent, there is some interplay between the signalling and repair machinery.

Role of DNA-DSB-repair proteins in adaptive immunity

Generation of lymphocyte antigen receptors

An effective immune response necessitates recognition of a wide array of foreign antigens, requiring the generation of some 10^{18} genetically diverse cells bearing a unique receptor capable of recognizing a unique antigen/major histocompatibility complex (MHC) combination. In higher organisms, these genetically diverse cells are created by breaking,

randomly re-sorting and joining the DNA sequences coding for antigen receptors [variable, junction and diversity (VDJ) recombination] by adapting the DNA-repair mechanisms that maintain genome stability.

VDJ recombination is a site-specific event occurring at 6 loci: T-cell receptor (TCR) β, γ, α, δ loci, immunoglobulin heavy chain and κ or λ light chain loci. Recombination occurs between component variable (V), junction (J) and in some cases diversity (D) gene segments with fused VJ or VDJ coding sequence subsequently joined to a constant (C) region segment through RNA splicing. Two recombination -activating gene proteins (RAG1/2) initiate this process by introducing site-specific DNA-DSBs at the segments to be rearranged, during the G1 phase of the cell cycle (Fig. 2). Each of the VDJ gene segments is flanked by a

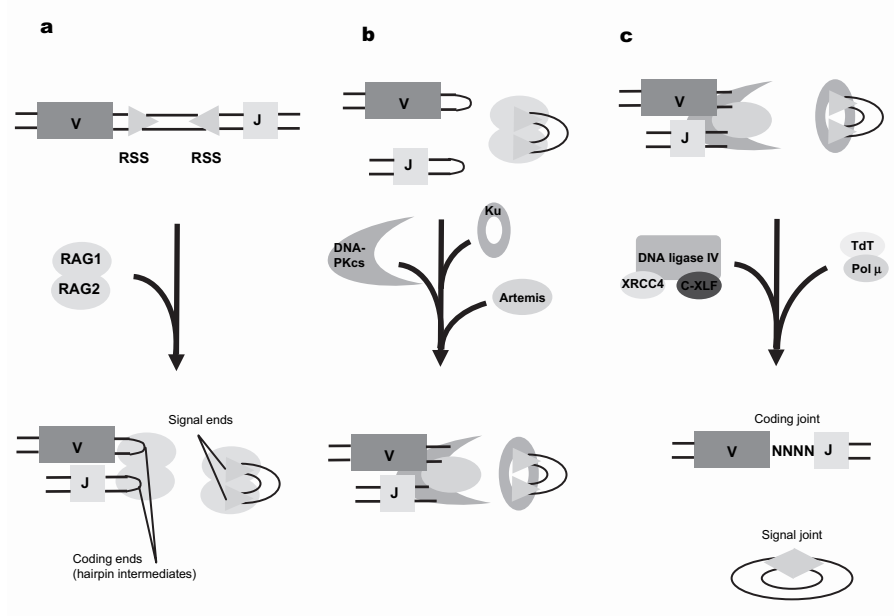

Fig. 2 VDJ recombination. (**a**) The lymphoid-specific recombinase activating gene 1 and 2 (RAG1/2) proteins recognize and bind the recombination-signal sequences (RSS) which flank the VDJ gene segments and introduce site-specific DNA double-strand breaks (DNA-DSBs). The phosphorylated blunt signal ends and the covalently sealed hairpin intermediate of the coding end are held together by the RAG complex. (**b**) Ku70/Ku80 heterodimer binds the coding ends and recruits DNA-dependant protein kinase catalytic subunit (DNA-PKcs) and Artemis, which is required to open the hairpin intermediates. The covalently sealed hairpin intermediate is randomly nicked by the DNA-PKcs/Artemis complex, which generates a single stranded break with 3′ or 5′ overhangs. (**c**) XRCC4, DNA ligase IV and Cernunnos-XLF (C-XLF) co-associate and are recruited to the ends. The signal ends are directly ligated by the XRCC4/DNA-LIG4/C-XLF complex. The opened hairpin intermediate is modified by polymerases, exonucleases and the lymphoid-specific terminal deoxynucleotidyl transferase (TdT), before being repaired and ligated by the XRCC4/DNA-LIG4/C-XLF complex. (Reproduced with permission of Bubble Foundation, UK.)

recombination-signal sequence (RSS) that includes a conserved heptamer and nonamer. The RAG1/2 complex introduces DNA-DSBs precisely at the junction between the coding exons and the RSS.

After the introduction of DNA-DSBs at the coding sequence/RSS junction, two types of DNA ends arise; the ends of coding sequences that reconstitute the immunoglobulin and TCR genes are generated as hairpin intermediates, whereas non-coding signal ends that contain the motifs targeting site-specific cleavage are generated as blunt double-stranded DNA ends. The KU70/KU80 heterodimer binds the DNA ends present at RAG1/2-generated coding ends and recruits DNA-PKcs that phosphorylates and activates Artemis endonuclease activity to process the coding sequence hairpin intermediates. Following cleavage, both coding and signal ends are directly ligated by the XRCC4/DNA ligase IV/Cernunnos-XLF complex. Rejoining of the signal ends does not require DNA-PKcs or Artemis. Furthermore, diversity between coding joints is created by asymmetric nicking of the hairpin bends and by deletions, mutations and addition of non-templated nucleotides to processed coding ends by terminal deoxynucleotidyl transferase (TdT) and DNA polymerase-μ [6].

Artemis-mediated cleavage of coding-joint hairpins does not require ATM or the MRN complex, and ATM is not critical for VDJ recombination. This is in contrast to the role of Artemis in processing IR-induced damaged ends, which occurs via an ATM-dependent process [7].

However, ATM might have important roles in inducing cell cycle arrest during VDJ recombination [8]. ATM, NBS1 and γH2AX are associated with RAG protein-induced DNA-DSBs in developing lymphocytes. The roles of ATM in activating cell cycle checkpoint proteins could contribute to the efficiency of VDJ recombination. In the absence of ATM, lymphocytes with RAG-induced DNA-DSBs may enter the S phase of cell cycle, leading to a reduction in productive VDJ recombination and an increased number of abnormal translocations involving Ig and TCR loci (chromosome 7/14 translocations) [7].

Double-stand break and immunoglobulin class switch recombination and somatic hypermutation

Class switch recombination (CSR) is a somatic DNA arrangement process occurring during B-cell activation, which leads to a switch in the immunoglobulin heavy chain constant (C) region expressed from the region encoded by Cμ to a downstream C region such as that encoded by Cα, Cγ or Cε. CSR is mediated by switch region rearrangement that proceeds each constant gene segment and is the main mechanism that mediates antibody-affinity maturation following repeated challenges

with antigen [9]. Foci of γH2AX, NBS1 and DNA-DSBs can be detected at the switch region during CSR, which suggests that DNA-DSBs are intermediates of CSR [10]. It is probable that activation-induced cytidine deaminase (AID), which is critical for CSR, induces DNA-DSBs, leading to activation of ATM-dependent DNA damage responses, cell cycle arrest and DNA repair. NBS1 and MRE11 are involved in sensing DNA-DSBs introduced into the switch region at the initiation of CSR: ATM is also required for efficient CSR. It is not clear whether CSR is achieved through HR, NHEJ or a distinct mechanism. However, repair of CSR-associated DNA-DSBs requires a subset of NHEJ factors. KU70/80 DNA-PKcs and DNA ligase IV appear to be involved directly or indirectly in CSR [11], although processing of DNA-DSBs by Artemis is not required for CSR.

NBS1 forms foci in an AID-dependent manner at the switch region during CSR, suggesting that NBS1 is involved in sensing DNA-DSBs that are introduced into the switch region at the initiation of CSR [10]. The MRN complex probably has an important role in mediating ATM function in VDJ recombination as NBS1 hypomorphic mice have impaired lymphocyte development with extensive chromosomal translocations in their lymphocytes.

Somatic hypermutation

The mechanism of somatic hypermutation introduces mutations in the structure of the B-cell receptor, resulting in minor conformational changes. DNA-DSBs are a necessary intermediate for CSR, but their role in somatic hypermutation is not clear [12]. NBS1 over expression increases DNA-DSBs in the V gene segment of the rearranged immunoglobulin A (IgA) locus, indicating that the MRN complex is involved in DNA cleavage at AID-induced abasic sites or in repair of the cleaved sites during somatic hypermutation. The mechanisms creating variability require helicases, polymerases and DNA ligases, all proteins that operate during DNA-repair process; thus, potentially, defects in a range of proteins could confer overlapping deficiencies in DNA repair and immune responsiveness.

Primary immunodeficiency syndromes associated with sensitivity to ionizing radiation

Many genetic defects in the DNA-DSB repair machinery have now been identified in humans, providing insight into clinical consequences of genomic instability on the immune system. Increasingly immunodeficiency is recognized as a feature of these syndromes (Table 1).

Table 1 Proteins associated with human primary immunodeficiency and DNA double-strand break repair defects

Gene	Disease	Affect on lymphocyte development	Microcephaly	Lymphoid tumours	Immunodeficiency
ATM	Ataxia telangiectasia	CSR	No	Common	Hypogammaglobulinaemia (IgA, IgG), SPAD, lymphopenia
NBS1	Nijmegen breakage syndrome	CSR	Yes	Common	Hypogammaglobulinaemia (IgA, IgG), hyper IgM, SPAD, lymphopenia
hMRE11	Ataxia telangiectasia-like disorder	CSR	Some patients	Not reported	SPAD, lymphopenia not reported
Rad50	Nijmegen breakage-like syndrome	Not reported	Yes	Not reported	Nil reported
Artemis	RS-SCID, CID	CJ formation	No	EBV-associated B cell lymphoma	Agammaglobulinaemia, hypogammaglobulinaemia, lymphopenia
DNA ligase IV	RS-SCID, CID	CJ fidelity, SJ fidelity, CSR	Some patients	EBV-associated lymphoma, T cell ALL	Hypogammaglobulinaemia (IgA, IgG), SPAD, lymphopenia
Cernunnos-XLF	RS-SCID	CJ fidelity, SJ fidelity,? CSR	Some patients	Not reported	Hypogammaglobulinaemia (IgA, IgG), hyper IgM, Lymphopenia

ALL, acute lymphoblastic leukaemia; CID, combined immunodeficiency; CJ, coding joint; CSR, class switch recombination; EBV, Epstein-Barr virus; RS-SCID, radiosensitive severe combined immunodeficiency; SJ, signal joint; SPAD, specific pneumococcal polysaccharide antibody deficiency.

Genetic defects critical for lymphocyte development

Artemis deficiency

Artemis is critical for VDJ recombination, and null mutations in the *Artemis* gene give rise to a T-B-NK+ severe combined immunodeficiency (SCID) phenotype with absent immunoglobulins [13], originally described in Athabascan-speaking native Americans [14]. Patients classically present in early infancy with viral or pneumocystis pneumonitis, persistent viral diarrhoea and growth failure, as with other forms of SCID. Marrow cells and fibroblasts from these patients exhibit increased cellular sensitivity to IR (radiosensitive, RS-SCID) [15].

A further two clinical phenotypes are described because of hypomorphic mutations in *Artemis*. First, infants may present with lymphadenopathy, hepatosplenomegaly, erythroderma, alopecia, agammaglobulinaemia apart from a raised IgE, T lymphocytosis and absent B cells with accompanying respiratory and gastrointestinal symptoms and failure to thrive [16]. This collection of symptoms, known as Omenn syndrome, is analogous to the clinical presentation because of hypomorphic RAG mutations. T cells are activated and show a restricted VB repertoire. Biopsies of affected skin demonstrate a histopathological pattern, consistent with graft-versus-host disease, although T cells are autologous.

Second, patients may present with a progressive combined immunodeficiency (CID) from later infancy, characterized by recurrent sinopulmonary or gastrointestinal infection, T and B lymphopenia, hypogammaglobulinaemia and autoimmune cytopenias [17, 18]. Some show susceptibility to Epstein-Barr virus (EBV)-associated B-cell lymphomas [17]. Interestingly, chromosome 7:14 inversions and translocations have been described in these patients. No specific dysmorphic features of microcephaly have been noted. Artemis is not essential for viability, as many patients with RS-SCID have complete loss of functional alleles. In humans, *Artemis* defects specifically affect the formation of coding joints; signal joints are spared. Interestingly, whilst Artemis knockout mice demonstrate a defect in coding-joint formation, some are able to generate lymphocytes containing virtually normal coding joints, which suggests that additional factors can function in opening hairpin intermediates.

DNA ligase IV deficiency

The first patient described with a DNA ligase IV defect was normal until T-cell acute lymphoblastic leukaemia developed. Treatment with the MRC-UKALL X protocol resulted in disproportionately severe cytopenia, because of which standard chemotherapy consolidation therapy was omitted, but cranial irradiation prophylaxis was substituted. However, an extreme reaction to radiotherapy resulted, including marked and prolonged cytopenia, a severe desquamative reaction, and death from radiation-induced encephalopathy 8 months following treatment [19]. Subsequently, four patients with microcephaly, developmental delay, growth failure, lymphopenia, hypogammaglobulinaemia, and recurrent infection were described [20]. A further three patients with T-B-NK+ RS-SCID have been reported, all with microcephaly and growth delay [21, 22]. Finally, three patients with microcephaly and a CID phenotype have been reported, of whom one developed an EBV-associated diffuse large cell non-Hodgkin lymphoma, and one developed T-cell acute lymphoblastic leukaemia [23, 24]. Chromosome 7:14 inversions and translocations have not been described in these patients to date. The phenotypic spectrum ranges from normal individuals with extreme sensitivity to IR through combined immune deficiency with microcephaly and mental retardation, analogous to patients with NBS, to RS T-B-NK+ SCID. Additional clinical features overlap with those observed in AT including photosensitivity. Absolute deficiency of DNA ligase IV leads to embryonic lethality in mice demonstrating that DNA ligase IV is essential for viability, and in humans, T-B-SCID has not been associated with complete loss of gene function.

Moderate impairment of VDJ recombination is seen in LIG4-deficient fibroblast VDJ recombination assays: an almost normal frequency of

coding- and signal-joint formation is observed, but fidelity of both coding- and signal-joint formation is impaired, with marked infidelity in coding-end rejoining [20, 22]. These *in vitro* findings are less severe than the clinical immunodeficiency, suggesting that DNA ligase IV is required during lymphocyte development or homeostasis at stages beyond VDJ recombination, possibly to repair DNA damage that may occur during lymphocyte proliferation. Similar observations have been identified in patients with hypomorphic *Artemis* mutations [18]. Patients with LIG4 defects also have altered resolution of CSR junctions [11].

Cernunnos-XLF deficiency

A third gene critical for lymphocyte development has recently been described [4, 5]. The description of a patient with T-B-NK+ RS-SCID in whom no defect in the known proteins involved in DNA-DSB repair could be found suggested that another factor was required for NHEJ and VDJ recombination [25]. The new factor, Cernunnos-XLF, interacts with the XRCC4/DNA ligase IV complex; defects are described in seven patients. The first two presented with low, although not absent, T- and B-cell numbers, with a normal number of NK cells [4, 25]. Subsequently, 5 patients with CID have been described [5]. All had a similar lymphocyte phenotype to the original kindred, but additionally had low IgA and IgG. Two had raised IgM, suggesting a role for Cernunnos-XLF in CSR. Some patients described were microcephalic, two exhibited autoimmune cytopenia, and all suffered from recurrent bacterial and opportunistic infection. Two demonstrated several chromosomal alterations, although chromosome 7:14 translocations were not described. Lymphomas have not been described to date.

In vitro coding- and signal-joint formation was reduced in patients compared with controls, with an increase in nucleotide loss in coding joints. Interestingly, the fidelity of signal joints was severely impaired in patient cells, with possible use of microhomology during joining [5], features previously described in LIG4 patients [19, 21]. VDJ deficiency in these patients is less severe than in the Artemis-deficient RS-SCID and probably accounts for the low numbers of T and B cells found in the patients. As in patients with *LIG4* deficiency, the *in vitro* findings are less severe than the clinical immunodeficiency, suggesting that Cernunnos-XLF may also be required during lymphocyte development at stages beyond VDJ recombination. Other clinical abnormalities include bone malformations (low implantation of the thumb, hypoplasia of the middle phalanx of the fifth finger) and nephroptosis, and one patient demonstrated mental retardation, features that overlap with those described in DNA ligase IV deficiency.

Other DNA-repair proteins associated with human immunodeficiency

Ataxia telangiectasia

AT is a rare systemic autosomal recessive disorder caused by mutations in *ATM* [26]. Clinical manifestations include progressive cerebellar ataxia, oculocutaneous telangiectasia, gonadal sterility, general growth retardation and a high incidence of mainly lymphoid tumours. Immunodeficiency causes frequent sinopulmonary infections that combined with recurrent aspiration, lead to chronic lung disease [27]. Lymphocytic interstitial pneumonitis has been described in one patient. The incidence of infections in AT patients is variable with some individuals having no higher incidence than unaffected siblings, whereas other succumbs to progressive respiratory infection, usually because of severe defects in both humoral and cellular immunity. Immunological responses to bacterial antigens are generally reduced, particularly to polysaccharide antigen [28]. The AT is characterized by thymic hypoplasia or absence probably because of a developmental defect. Thymic output, evaluated by measuring TCR excision circle-positive T cells, is low; the peripheral T-cell population is characterized by a bias towards terminally differentiated effector cells reflected by a low ratio of naïve to memory T cells and a skewed T cell receptor beta variable (TRBV) region repertoire of peripheral lymphocytes marked by oligoclonal expansions and a restricted TRBV-chain repertoire [29]. This suggests that the few ATM-deficient thymocytes migrating to the periphery undergo homeostatic expansion. The number of peripheral ATM-deficient T cells could be limited by a survival defect during the proliferative burst associated with homeostatic expansion rather than solely by low thymic output. ATM deficiency does not result in a profound block in lymphocyte development, but fidelity rather than completion of VDJ recombination may be affected in the absence of ATM, which may be required to monitor recombination intermediates. VDJ coding joints are normal. B-cell repertoire is similarly restricted and skewed by diffused oligoclonal expansions with normal VDJ joints. IgA, IgE and IgG subtypes are reduced or absent in AT patients. B cells from AT patients have an intrinsic defect in maturation from IgM to other classes, because of a defect in CSR to the most distant loci, reflecting the requirement of ATM for efficient recombination between immunoglobulin switch regions [30].

Nijmegen breakage syndrome

NBS is recognized by characteristic facial appearances with receding forehead, receding mandible and prominent midface. Additional features include epicanthal folds, large ears and sparse hair with microcephaly and mild mental retardation. Patients are susceptible to lymphoma, particularly B-cell lymphoma and are prone to infection,

particularly of the respiratory tract. Cellular immunity is consistently deficient in NBS patients with reduced T-lymphocyte proliferation. Lymphopenia is common, and whilst CD8+ cells are generally of normal number, there are reduced proportions of CD3+ and CD4+ T cells [31]. Agammaglobulinemia is reported in about a third of NBS patients, whereas in others the humoural immune deficiency is more variable. Deficiencies of IgA or IgG4 alone or in combination are common. Few patients (10%) have normal immunoglobulins. The immunodeficiency may result from reduced fidelity of VDJ recombination, as NBS1 is involved with ATM in inducing cell cycle arrest during this process [8]. Frequency of VDJ recombination in NBS patients is normal however with normal immunoglobulin heavy chain rearrangement. The deficiency of serum IgG and IgA with normal or raised IgM is probably because of altered CSR in B lymphocytes. Chromosomal inversions and translocations, particularly chromosome 7:14 translocations are characteristic of NBS.

Interestingly, NBS and Fanconi anaemia (FA) share overlapping features. FA is an autosomal recessive chromosomal instability disorder characterized by developmental defects, progressive bone marrow failure and cancer susceptibility; patients develop primarily marrow failure and haematological malignancies but also squamous cell carcinomas. Twelve genetic groups have been defined [32]. FA cells are sensitive to DNA cross-linking agents such as mitomycin C (MMC) and diepoxybutane. Patients with FANCD2 subtype are sensitive to both MMC and IR. When normal cells are exposed to IR, ATM phosphorylates FANCD2 activating the IR-inducible S-phase checkpoint response. IR-activated phosphorylation of FANCD2 is distinct and independent of MMC-activated monoubiquitination. NBS1 and FANCD2 proteins can interact and NBS cells are also sensitive to cross-linking agents. NBS cells show reduced monoubiquitylation of FANCD2. FANCD2 therefore functions at the intersection of two signalling pathways, one involving IR activation by ATM, the other involving MMC activation by the FA complex [33]. The enzyme/substrate interaction of ATM and FANCD2 accounts at least in part for the common clinical and cellular phenotypes of AT and some FA patients. $MRE11^{-/-}$ cells also show MMC sensitivity, and NBS1 binds MRE11 and co-localizes with activated FANCD2 to confer MMC resistance. Patients with certain *NBS1* mutations have features similar to FA [33, 34], although immunodeficiency is more pronounced, presumably because of the other actions of *NBS1* in VDJ recombination and CSR.

Ataxia telangiectasia-like disorder

The AT-like disorder is extremely rare with <20 patients reported worldwide. Clinical features are similar to those in patients with AT

with progressive cerebellar ataxia that is of later onset and slower progression than of those with AT [35]. There is absence of telangiectasia. Immunoglobulin levels seem normal, although deficiency in specific function antibodies has been reported, particularly to pneumococcal polysaccharide antigen [36]. Defective CSR has been reported, however [36]. Lymphoid tumours have not been reported. Some patients are microcephalic, although intelligence is normal. The gene defect in *hMRE11* encodes for a protein that associates in the MRN complex, and patients have features overlapping with both AT and NBS. The defect is probably to be hypomorphic—the MRE11 knockout mouse exhibits embryonic lethality. It is interesting that there is no associated recurrent pulmonary infection because of immunodeficiency. Whether this simply reflects clinical variability as seen in AT but restricted to a small number of patients, or whether more generalized immunodeficiency is unrelated to the disorder is not yet clear.

RAD50 deficiency

Rad50 is a component of the MRN complex. One patient with two germline mutations in *RAD50* has been identified [37]. The phenotype is similar to that of NBS patients, with cellular radiosensitivity and microcephaly, although the patient has no apparent immunodeficiency Clinical and cellular features of this patient are presently being investigated in more detail.

Undefined defects

A number of patients with radiosensitivity or with NBS-like features but no defect demonstrated in the known DNA-DSB repair proteins have been reported, suggesting that other proteins involved in DNA-DSB repair and lymphocyte generation may exist. Furthermore, patients with defects in known proteins involved in DNA-DSB repair, including Ku70/80, DNA-PK and XRCC4, have yet to be identified.

Treatment

For patients presenting with a SCID or CID phenotype, haematopoietic stem cell transplantation (HSCT) is curative and has been described for patients with Artemis, DNA ligase IV and Cernunnos-XLF defects [5, 16, 19]. In most cases, the preparative chemotherapy conditioning does not include irradiation, and successful outcomes have been achieved. Such treatment is more controversial for patients with NBS. Two patients have been successfully transplanted, although only partial T-cell chimerism was achieved [38]. This corrected the associated immunodeficiency, but it is not clear whether the lymphoma risk has decreased. The

risk of lymphoma-inducing chromosomal translocation should be reduced as the donor lymphoid progenitors will not exhibit genomic instability, and tumour surveillance may be improved. There may be a long-term risk of other non-lymphoid tumours arising; so, careful follow-up is required.

For the majority of NBS patients who have not been transplanted, prophylactic antibiotics, and in some cases immunoglobulin substitution therapy may prevent recurrent sinopulmonary infection. The risk of lymphoid malignancy is high (>50%) and may develop rapidly; high clinical suspicion of malignancy is required for patients with NBS in long-term follow-up. Treatment of malignancy may be difficult, balancing the risk of adequate doses of chemotherapy to effectively treat the tumour against toxicity. At present, the treatment for patients with AT is more conservative. Prophylactic antibiotics and immunoglobulin substitution therapy may prevent recurrent sinopulmonary infection, but measures to prevent gastro-oesophageal reflux, which may lead to interstitial lung disease, are also important. The incidence of malignancy, particularly lymphoid malignancy, is high, and as in patients with NBS, treatment can be difficult, balancing efficacy against the unique toxicity profile that AT patients have. The IR and radiomimetic agents should be avoided, but significant toxicity is also associated with cyclophosphamide, methotrexate, vincristine and etoposide. HSCT in *ATM*-deficient mice restored immunity and prevented the development of lymphoma [39]. However, at present, HSCT is not recommended for patients with AT, first because of the high risk of side effects from the preparative chemotherapy. Second, the neurological deterioration is progressive and not halted by HSCT. The correction of premature termination codon (PTC)-induced *ATM* dysfunction by aminoglycoside-induced PTC read through does hold some promise for the 14% of AT patients with single-nucleotide changes that result in PTC [40] and may slow neurological deterioration in such patients and possibly reduce tumour development and immune deficient-related damage.

Conclusion

Our understanding of the role of DNA-DSB-repair proteins in lymphogenesis has been enhanced by the detailed clinical description and biochemical analysis of immunodeficient patients. Conversely, our search for the underlying genetic defects in such patients has been aided by our understanding of the defects in lymphocyte development in patients with known defects in DNA-DSB-repair proteins. Although animal and yeast models may give useful insights into these processes, careful study of human disease is critical, as many human diseases present with hypomorphic rather

than null mutations, and so may portray a leaky phenotype, with a variable spectrum of disease presentation. A greater understanding of the molecular defects in these patients will allow targeted modifications in therapy to aid treatment, for instance modified chemotherapy regimens for HSCT or lymphoid malignancy treatment, and enable development of novel therapies.

References

1 O'Driscoll M, Jeggo PA. The role of double-strand break repair – insights from human genetics. *Nat Rev Genet* 2006;**7**:45–54.
2 Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J* 2003;**22**:5612–21.
3 Xu Y. DNA damage: a trigger of innate immunity but a requirement for adaptive immune homeostasis. *Nat Rev Immunol* 2006;**6**:261–70.
4 Ahnesorg P, Smith P, Jackson SP. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* 2006;**124**:301–13.
5 Buck D, Malivert L, de Chasseval R *et al*. Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell* 2006;**124**:287–99.
6 Lieber MR, Ma Y, Pannicke U, Schwarz K. The mechanism of vertebrate nonhomologous DNA end joining and its role in V(D)J recombination. *DNA Repair (Amst)* 2004;**3**:817–26.
7 Riballo E, Kuhne M, Rief N *et al*. A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to gamma-H2AX foci. *Mol Cell* 2004;**16**:715–24.
8 Xu Y. ATM in lymphoid development and tumorigenesis. *Adv Immunol* 1999;**72**:179–89.
9 Kenter AL. Class switch recombination: an emerging mechanism. *Curr Top Microbiol Immunol* 2005;**290**:171–99.
10 Petersen S, Casellas R, Reina-San-Martin B *et al*. AID is required to initiate Nbs1/gamma-H2AX focus formation and mutations at sites of class switching. *Nature* 2001;**414**:660–5.
11 Pan-Hammarstrom Q, Jones AM, Lahdesmaki A *et al*. Impact of DNA ligase IV on nonhomologous end joining pathways during class switch recombination in human cells. *J Exp Med* 2005;**201**:189–94.
12 Bemark M, Sale JE, Kim HJ, Berek C, Cosgrove RA, Neuberger MS. Somatic hypermutation in the absence of DNA-dependent protein kinase catalytic subunit (DNA-PK(cs)) or recombination-activating gene (RAG)1 activity. *J Exp Med* 2000;**192**:1509–14.
13 Moshous D, Callebaut I, de Chasseval R *et al*. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 2001;**105**:177–86.
14 Jones JF, Ritenbaugh CK, Spence MA, Hayward A. Severe combined immunodeficiency among the Navajo. I. Characterization of phenotypes, epidemiology, and population genetics. *Hum Biol* 1991;**63**:669–82.
15 Cavazzana-Calvo M, Le Deist F, de Saint Basile G, Papadopoulo D, de Villartay JP, Fischer A. Increased radiosensitivity of granulocyte macrophage colony-forming units and skin fibroblasts in human autosomal recessive severe combined immunodeficiency. *J Clin Invest* 1993;**91**:1214–8.
16 Ege M, Ma Y, Manfras B *et al*. Omenn syndrome due to ARTEMIS mutations. *Blood* 2005;**105**:4179–86.
17 Moshous D, Pannetier C, de Chasseval R *et al*. Partial T and B lymphocyte immunodeficiency and predisposition to lymphoma in patients with hypomorphic mutations in Artemis. *J Clin Invest* 2003;**111**:381–7.
18 Evans PM, Woodbine L, Riballo E, Gennery AR, Hubank M, Jeggo PA. Radiation-induced delayed cell death in a hypomorphic Artemis cell line. *Hum Mol Genet* 2006;**15**:1303–11.
19 Riballo E, Critchlow SE, Teo SH *et al*. Identification of a defect in DNA ligase IV in a radiosensitive leukaemia patient. *Curr Biol* 1999;**9**:699–702.

20 O'Driscoll M, Cerosaletti KM, Girard PM *et al*. DNA ligase IV mutations identified in patients exhibiting developmental delay and immunodeficiency. *Mol Cell* 2001;**8**:1175–85.
21 van der Burg M, van Veelen LR, Verkaik NS *et al*. A new type of radiosensitive T-B-NK+ severe combined immunodeficiency caused by a LIG4 mutation. *J Clin Invest* 2006;**116**:137–45.
22 Buck D, Moshous D, de Chasseval R *et al*. Severe combined immunodeficiency and microcephaly in siblings with hypomorphic mutations in DNA ligase IV. *Eur J Immunol* 2006;**36**:224–35.
23 Ben-Omran TI, Cerosaletti K, Concannon P, Weitzman S, Nezarati MM. A patient with mutations in DNA Ligase IV: clinical features and overlap with Nijmegen breakage syndrome. *Am J Med Genet A* 2005;**137**:283–7.
24 Enders A, Fisch P, Schwarz K *et al*. A severe form of human combined immunodeficiency due to mutations in DNA ligase IV. *J Immunol* 2006;**176**:5060–8.
25 Dai Y, Kysela B, Hanakahi LA *et al*. Nonhomologous end joining and V(D)J recombination require an additional factor. *Proc Natl Acad Sci USA* 2003;**100**:2462–7.
26 Chun HH, Gatti RA. Ataxia-telangiectasia, an evolving phenotype. *DNA Repair (Amst)* 2004;**3**:1187–96.
27 Lefton-Greif MA, Crawford TO, Winkelstein JA *et al*. Oropharyngeal dysphagia and aspiration in patients with ataxia-telangiectasia. *J Pediatr* 2000;**136**:225–31.
28 Sanal O, Ersoy F, Yel L *et al*. Impaired IgG antibody production to pneumococcal polysaccharides in patients with ataxia-telangiectasia. *J Clin Immunol* 1999;**19**:326–34.
29 Giovannetti A, Mazzetta F, Caprini E *et al*. Skewed T-cell receptor repertoire, decreased thymic output, and predominance of terminally differentiated T cells in ataxia telangiectasia. *Blood* 2002;**100**:4082–9.
30 Reina-San-Martin B, Chen HT, Nussenzweig A, Nussenzweig MC. ATM is required for efficient recombination between immunoglobulin switch regions. *J Exp Med* 2004;**200**:1103–10.
31 Digweed M, Sperling K. Nijmegen breakage syndrome: clinical manifestation of defective response to DNA double-strand breaks. *DNA Repair (Amst)* 2004;**3**:1207–17.
32 Taniguchi T, D'Andrea AD. Molecular pathogenesis of Fanconi anemia: recent progress. *Blood* 2006;**107**:4223–33.
33 Nakanishi K, Taniguchi T, Ranganathan V *et al*. Interaction of FANCD2 and NBS1 in the DNA damage response. *Nat Cell Biol* 2002;**4**:913–20.
34 Gennery AR, Slatter MA, Bhattacharya A *et al*. The clinical and biological overlap between Nijmegen breakage syndrome and Fanconi anemia. *Clin Immunol* 2004;**113**:214–9.
35 Taylor AM, Groom A, Byrd PJ. Ataxia-telangiectasia-like disorder (ATLD)-its clinical presentation and molecular basis. *DNA Repair (Amst)* 2004;**3**:1219–25.
36 Lahdesmaki A, Taylor AM, Chrzanowska KH, Pan-Hammarstrom Q. Delineation of the role of the Mre11 complex in class switch recombination. *J Biol Chem* 2004;**279**:16479–87.
37 Bendix-Waltes R, Kalb R, Stumm M *et al*. Rad50 deficiency causes a variant form of Nijmegen breakage syndrome. *Eur J Hum Genet* 2005;**13**(Suppl. 1):63.
38 Gennery AR, Slatter MA, Bhattacharya A *et al*. Bone marrow transplantation for Nijmegen breakage syndrome. *J Pediatr Hematol Oncol* 2005;**27**:239.
39 Bagley J, Cortes ML, Breakefield XO, Iacomini J. Bone marrow transplantation restores immune system function and prevents lymphoma in Atm-deficient mice. *Blood* 2004;**104**:572–8.
40 Lai CH, Chun HH, Nahas SA *et al*. Correction of ATM gene function by aminoglycoside-induced read-through of premature termination codons. *Proc Natl Acad Sci USA* 2004;**101**:15676–81.