

# A Human Severe Combined Immunodeficiency (SCID) Condition with Increased Sensitivity to Ionizing Radiations and Impaired V(D)J Rearrangements Defines a New DNA Recombination/Repair Deficiency

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## Summary

The products of recombination activating gene (RAG)1 and RAG2 initiate the lymphoid-specific phase of the V(D)J recombination by creating a DNA double-strand break (dsb), leaving hairpin-sealed coding ends. The next step uses the general DNA repair machinery of the cells to resolve this dsb. Several genes involved in both V(D)J recombination and DNA repair have been identified through the analysis of in vitro mutants (Chinese hamster ovary cells) and in vivo situations of murine and equine severe combined immunodeficiency (scid). These studies lead to the description of the Ku-DNA-dependent protein kinase complex and the XRCC4 factor. A human SCID condition is characterized by an absence of B and T lymphocytes. One subset of these patients also demonstrates an increased sensitivity to the ionizing radiation of their fibroblasts and bone marrow precursor cells. This phenotype is accompanied by a profound defect in V(D)J recombination with a lack of coding joint formation, whereas signal joints are normal. Functional and genetic analyses distinguish these patients from the other recombination/repair mutants, and thus define a new group of mutants whose affected gene(s) is involved in sensitivity to ionizing radiation and V(D)J recombination.

Key words: human • immunodeficiency • V(D)J recombination • DNA repair • severe combined immunodeficiency

Immunoglobulin and TCR genes are composed of variable (V),<sup>1</sup> diversity (D) and joining (J) segments scattered along the chromosome that require somatic rearrangement before their expression (1). The V(D)J recombination process is common to both B and T cells and can be divided into three steps. The initiation of the V(D)J recombination involves the recognition of recombination-specific sequences (RSS) flanking the V, D, and J segments by the lymphoid specific recombination activating gene (RAG)1 and RAG2 proteins. RAG1 and RAG2 then introduce a DNA double-strand break (dsb) at the site of the RSS, leaving covalently sealed (hairpin) coding ends (2). The critical role

for the RAG proteins in V(D)J recombination has been demonstrated via targeting experiments in mice (3, 4), and more recently through the description of human Rag mutants suffering from SCID (reference 5 and our unpublished observations). An intermediate phase in the V(D)J recombination process is characterized by the recruitment of the DNA-dependent protein kinase (DNA-PK) complex at the site of the DNA dsb (6). DNA-PK is composed of a 450-kD catalytic subunit (DNA-PKcs) and a DNA targeting subunit called Ku. Ku itself is a heterodimer of the 70- and 80-kD proteins (Ku70 and Ku80, respectively), and displays DNA end-binding activity in vitro (7). The whole DNA-PK complex is essential for the V(D)J recombination/DNA repair processes as illustrated by the analysis of (a) the murine and equine scid models (8–10), (b) several Chinese hamster ovary cell (CHO) mutants (11–14), (c) Ku80 knockout mice (15, 16), and (d) Ku70 knockout experiments (17–19). More specifically, nonsense and frame-shift

<sup>1</sup>Abbreviations used in this paper: CHO, Chinese hamster ovary; cs, catalytic subunit; D, diversity; dsb, double-strand break; J, joining; PK, protein kinase; RAG, recombination activating gene; RSS, recombination-specific sequences; T-B<sup>-</sup>, absence of circulating T and B lymphocytes; V, variable; XRCC, x-ray cross-complementing.

mutations in the DNA-PKcs encoding gene are responsible for the murine and equine scid conditions, respectively (20, 21), whereas point mutations in the Ku80 gene have been found in the x-ray cross-complementing (XRCC)5 complementation group of CHO mutants (22, 23). Alteration of either one of the DNA-PK complex partners results in a defect in both V(D)J recombination and DNA repair, identified by an increased sensitivity to any DNA dsb-causing agent. The product of the XRCC4 gene (24) is another important effector, as a defect in this gene is responsible for the impairment of V(D)J recombination and DNA repair in the XR1-CHO mutant (11, 12). The final step in V(D)J recombination results in the rejoining of the coding ends by a molecular mechanism that is largely unknown thus far, although several recent studies have suggested the possible involvement of DNA ligase I and/or DNA ligase IV in this process (25–29).

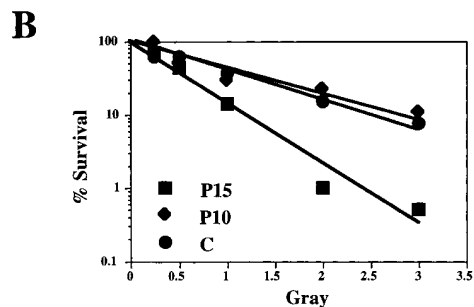
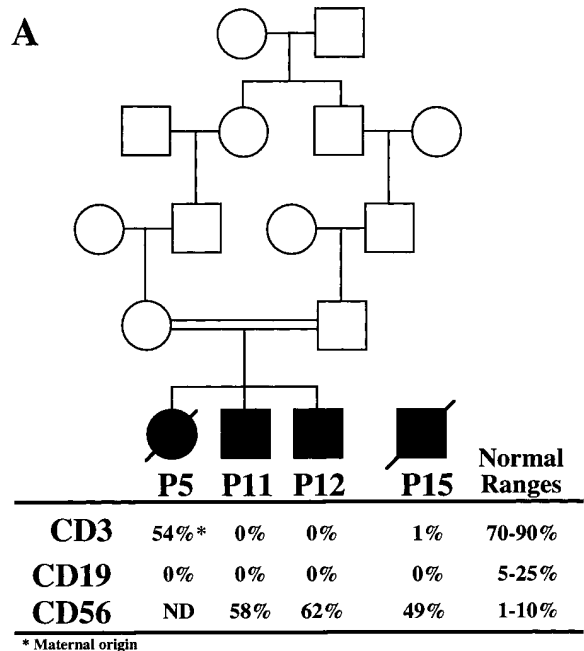
A human SCID condition of autosomal recessive inheritance is characterized by an absence of circulating T and B lymphocytes ( $T^-B^-$ ), whereas NK cells are present (30). Absent or aberrant pattern of DQ52-Jh rearrangement of the immunoglobulin heavy chain locus were demonstrated in some of the patients with this condition (31), whereas bone marrow-derived, EBV-transformed B cell lines obtained in some cases displayed germline status of Ig genes (32). We demonstrated that marrow cells and fibroblasts from one subset of  $T^-B^-$  SCID patients exhibited increased radiosensitivity (33). In normally radiosensitive  $T^-B^-$  SCID, mutations in the RAG1 or RAG2 genes were recently described (reference 5 and our unpublished results). Abnormally radiosensitive human SCID resembles the murine scid condition, as patients are devoid of mature lymphocytes and display ubiquitous increased sensitivity to ionizing radiation (33). However, normal kinetics of DNA repair after a 50-Gray  $\gamma$ -radiation, as judged by pulse-field gel electrophoresis, together with normal DNA-PK activity *in vitro*, have cast doubt on the hypothesis that this condition truly corresponds to a V(D)J recombination/DNA repair defect group of mutants (34). To address this question more directly, we analyzed the V(D)J recombination activity in RAG1/2-transfected fibroblasts from  $T^-B^-$  SCID patients using extrachromosomal recombination substrates. Moreover, we took advantage of a consanguineous family with three affected children to perform genetic studies using polymorphic markers flanking genes involved in V(D)J recombination/DNA repair processes.

## Materials and Methods

**Patients and Cells.** Patients P1, P2, P5, P6, P10, P11, P12, and P15 were characterized by an absence of peripheral CD3<sup>+</sup> T cells and CD19<sup>+</sup> B cells (34). For all  $T^-B^-$  SCID patients except P11 and P12 radiosensitivity status was determined on marrow cells (33, 34). P11 and P12 are the brother and sister of P5 and were born to related parents (see Fig. 1 A). P10 is a  $T^-B^-$  SCID patient caused by mutation in the RAG2 gene (our unpublished observation) with no increased cell radiosensitivity. Primary skin fibroblast cell lines were obtained as previously described (34) and

maintained in complete culture medium consisting of RPMI 1640 (Glutamax; GIBCO BRL, Gaithersburg, MD) supplemented with 15% FCS. SV40-transformed fibroblast lines were obtained by transfection of the SV40 large T antigen encoding plasmid pLAS-wt (35) and by overgrowth of transformed cells over nontransformed fibroblasts without other means of selection.

**DNA End-binding and DNA-PK Activity Assays.** DNA-PK activity was determined in fibroblast cell extracts as previously described (34). In brief, 5  $\mu$ g of cellular extract were incubated with a p53 peptide (Promega, Charbonnieres, France) with or without 200 ng of linear DNA as activator. Incorporation of  $\gamma$ [<sup>32</sup>P] into the peptide was monitored. The MO59J and MO59K human cell lines (36) were used as negative and positive controls, respectively. For the DNA end-binding assay, 1  $\mu$ g whole cell extracts prepared in DNA-PK lysis buffer were incubated with 0.5 ng of a <sup>32</sup>P-labeled



**Figure 1.** (A) Pedigree and biological status of P5, P11, P12, and P15. Peripheral blood counts of mature T, B, and NK cells were determined by FACS<sup>®</sup> immunostaining with anti-CD3, anti-CD19, and anti-CD56 mAbs, respectively. (B) Sensitivity to ionizing radiation in CFU-GM from P10 and P15. Plating efficiency of marrow cell cultures was determined after increasing doses (0.5–3 Gray) of  $\gamma$ -radiation. Results are expressed as percentage of survival relative to unirradiated cells. C represents the survival curve obtained with cells from an age-matched control. P10 shows normal radiosensitivity, whereas the survival curve from P15 cells is representative of the group of patients showing increased cell radiosensitivity.

double-strand oligonucleotide probe (5'-CGCGTGGTACGTAG-GTCACTCTC-3') in 1XGR buffer (20 mM Tris, pH 7.5, 2 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 0.25 mM dithiothreitol, and 5% glycerol) with 800 ng of circular plasmid DNA. After 15 min incubation at 22°C the reactions were analyzed by 4% PAGE in 1× TGE buffer (50 mM Tris, pH 8.5, 380 mM glycine, and 2 mM EDTA). For supershift experiments, whole cell extracts were preincubated (30 min at 4°C) with 500 ng anti-Ku70/80 (clone 162, Ab-3 NeoMarkers) mAb or anti-Rag1 (clone G189-1417; PharMingen, San Francisco, CA) as control before the addition of the oligonucleotide probe.

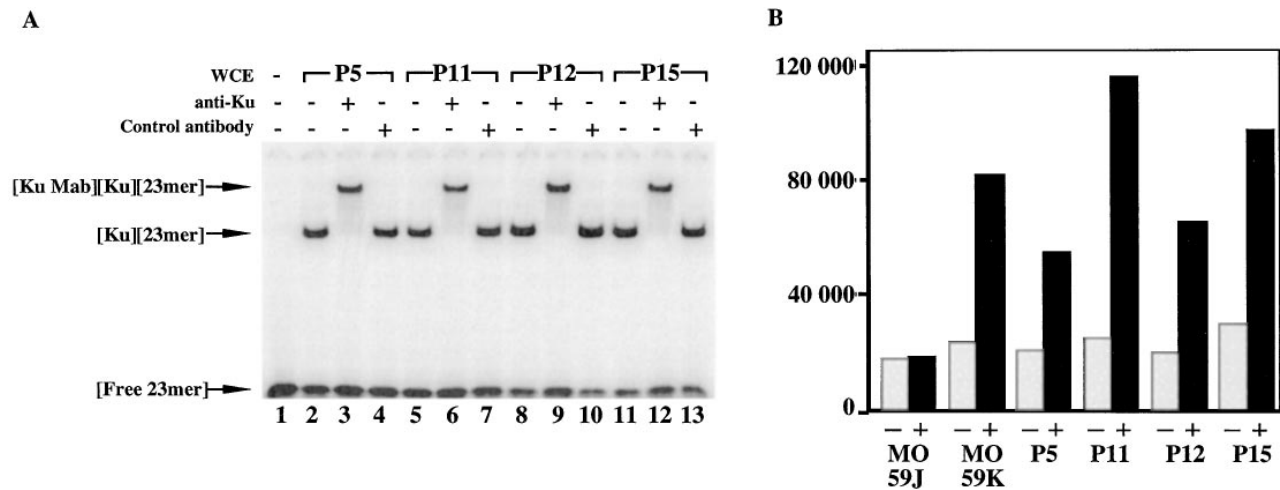
**Transient Transfection Assay for V(D)J Recombination.** V(D)J recombination assay was performed on SV40-transformed fibroblasts using the V(D)J extrachromosomal substrates pHRecCJ and pHRecSJ (see Fig. 3 A). pHRecCJ was constructed by replacing the polyoma early region (large T antigen and origin of replication) from pBlueRec (37) with analogous sequences from SV40 to allow for replication in human cells (38). The recombination of the pHRecCJ plasmid leads to the formation of a coding joint upon deletion of the two RSS sites. For signal joint formation analysis, the two RSS sites were inverted in the pHRecSJ plasmid. Recombination of the pHRecSJ plasmid leads to the fusion of the two RSS (signal-joint) creating a diagnostic ApalI restriction site. 5 × 10<sup>6</sup> exponentially growing SV40-transformed fibroblasts were electroporated (Easyject, Eurogentec, Angers, France; setting: 250 V, 1,500 μF, ∞ Ω) in 400 μl of complete culture medium with 2.5 μg of either pHRecCJ or pHRecSJ along with 6 μg of RAG1, and 4.8 μg of RAG2-encoding genes in eukaryotic expression vector (gift of M. Oettinger, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; reference 39). 48 h after transfection, extrachromosomal DNA was prepared by rapid alkaline lysis (40), digested with DpnI to eliminate nonreplicated plasmids, and used for transfection of bacteria or PCR analysis using T3 and T7 primers. The percentage of recombination was determined by transforming DH10B bacteria in the presence of Xgal (5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside) and IPTG (isopropyl-β-thiogalactopyranoside) and counting the blue colonies. Integrity of the recombined substrates following V(D)J recombination was analyzed by DNA sequencing using T3 primer.

**XRCC4 cDNA Sequence Analysis.** Total RNA was extracted from fibroblasts and reverse transcribed using random hexamers and Moloney murine leukemia virus reverse transcriptase without RNase H according to manufacturer's recommendations (GIBCO BRL). XRCC4 cDNA was PCR-amplified using XRF1 5' GGGCTGCCTCTTTAAATAAC and XRR1 5' ATGATG-TTTTCTTTGTCCCTGTTTT primers and TaqAdvantage (Clontech Labs., Inc., Palo Alto, CA) enzyme. Cycling parameters were 30 s of denaturation at 94°C, 30 s of annealing at 50°C, and 2.5 min of extension at 72°C for 30 cycles. PCR products were cloned into pGemT (Promega) and sequenced using the DYEnamic cycle sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ) and the Dye terminator cycle sequencing kit (Perkin-Elmer Corp., Norwalk, CT) on an ABI377 sequencer (Perkin-Elmer).

**Genotype Analysis.** DNA from P5, P11, P12, and their parents were genotyped using 12 polymorphic microsatellite PCR markers referenced at Généthon (Evry, France; reference 41): D2S137 and D2S301 for Ku80; D22S276 and D22S537 for Ku70; D8S519 and D8S1745 for the DNA-PKcs; D5S2029 and D5S428 for XRCC4; D13S173 and D13S285 for DNA ligase IV; and D19S412 and D19S866 for DNA ligase I. The forward primers of each pair were end-labeled with γ-[<sup>32</sup>P]ATP using polynucleotide kinase. The amplifications were performed in a final reaction volume of 17.5 μl containing 100 ng template DNA, 0.2 mM deoxynucleotides, 3 pmol unlabeled reverse primer, 1.5 pmol unlabeled forward primer, 1.5 pmol end-labeled forward primer, and 0.07 U Taq polymerase (ATGC Biotechnologie, Noisy-le-Grand, France). PCR conditions consisted of 30 cycles, each consisting of 45 s at 94°C, 1 min at the annealing temperature specific for each pair of primers, and 45 s at 72°C. The PCR products were analyzed by electrophoresis on 5% denaturing polyacrylamide gels.

## Results

**Increased Radiosensitivity in Human T<sup>-</sup>B<sup>-</sup> SCID with Normal DNA-PK Activity.** We previously reported on the occurrence of human immune deficiency characterized by

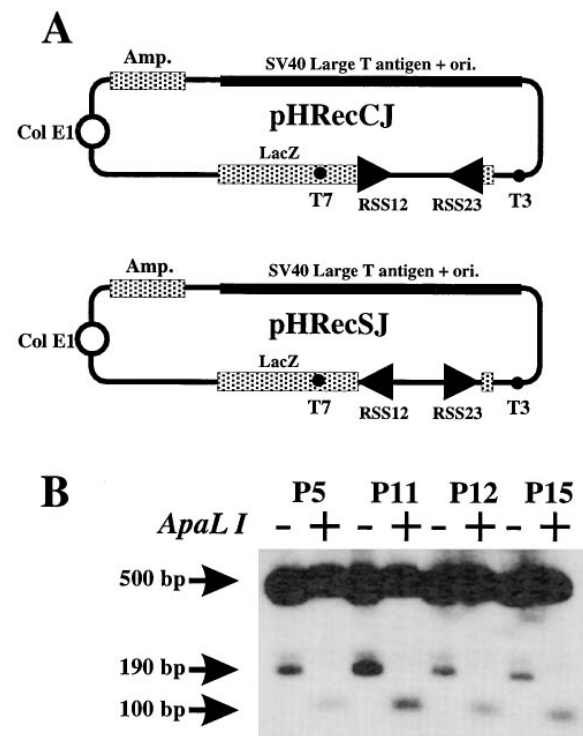


**Figure 2.** (A) DNA end-binding assay using cellular extracts from T<sup>-</sup>B<sup>-</sup> SCID patients P5, P11, P12, and P15. Whole cell extracts were preincubated for 30 min at 4°C or not with specific mAb for Ku70/80 (lanes 3, 6, 9, and 12) or Rag1 (lanes 4, 7, 10, and 13) as control. (B) DNA-PK activity in cellular extracts from P5, P11, P12, and P15. MO59J and MO59K cell lines (36) lacking and containing active DNA-PKcs, respectively, were used as controls. DNA-PK activity was determined in the absence (-) or presence (+) of linear DNA as specific activator.

a lack of both B and T lymphocytes, accompanied by an increase in radiosensitivity of bone marrow precursor cells and fibroblasts (33, 34). We here extended our series of patients with three new cases (P11, P12, and P15). P11 and P12 are two brothers of the previously described patient, P5, and were born to related parents (Fig. 1 A). These three patients were devoid of both mature T and B lymphocytes (Fig. 1 A). Bone marrow cells from P5 were previously shown to be abnormally radiosensitive and cells from P15 showed the same extent of increased sensitivity to  $\gamma$ -rays (Fig. 1 B). The radiosensitivity status of marrow cells from P11 and P12 was not assessed as they are brothers of P5, but according to the following results (V(D)J recombination assay) one can assume that they would display increased radiosensitivity as well. One striking finding in our previous report was the normal DNA-PK activity in cellular extracts from T<sup>-</sup>B<sup>-</sup> SCID with increased cell radiosensitivity (34). DNA-PK activity was also normal in cellular extracts from the four patients described here (Fig. 2 B), in contrast to the absence of activity in the DNA-PK negative MO59J cell line. Given the normal DNA-PK activity in these patients, it was not surprising to find a functional Ku70/Ku80 complex in these cellular extracts as tested by the DNA end-binding assay (Fig. 2 A). The real nature of the Ku-DNA complex was further proven by supershift experiments using anti-Ku70/80 mAb, whereas the anti-Rag1 control antibody did not result in a shift of this complex. Indeed, any defect in this complex would have severely compromised the activation of the DNA-PKcs and ultimately resulted in the absence of kinase activity as is the case in various CHO mutants (for review see reference 42). Altogether, the findings concerning the three newly studied patients have confirmed and extended the results of our previous studies.

**Defective V(D)J Recombination in T<sup>-</sup>B<sup>-</sup> SCID Patients with Increased Cell Radiosensitivity.** The "DNA-repair" phase of the V(D)J recombination can be analyzed by transient transfection of RAG1, RAG2, and an extrachromosomal substrate into fibroblasts. The two recombination substrates used here to analyze both coding and signal joint formation are depicted in Fig. 3 A. These plasmids were derived from pBlueRec (37), in which the polyoma large T antigen and replication origin sequences were replaced by those of SV40 to allow for replication in human cells (38). In pHRecCJ, the LacZ gene is interrupted by a DNA stuffer flanked by two RSS that will be deleted upon coding joint formation. In pHRecSJ, the two RSS sequences were inverted so that the fusion of the two RSS (signal joint) remains on the plasmid after recombination. A functional LacZ gene is reconstituted upon recombination of the two plasmids that lead to the formation of blue colonies when introduced into bacteria and plated on Xgal- and IPTG-containing medium. The ratio of blue colonies to white colonies is therefore indicative of the capacity of the cells to fully accomplish V(D)J recombination. In the case of the coding joint formation, only one-third of the recombined substrates will lead to blue colonies because of random nibbling of the coding ends, leading to shifts in the LacZ gene

open reading frame (37). In contrast, the signal joint is perfect without deletion of nucleotides, so that the number of blue colonies represent the total number of recombined substrates. SV40-transformed fibroblast lines from T<sup>-</sup>B<sup>-</sup> radiosensitive SCID patients and two control cell lines (P10 and Hela) were transiently transfected with RAG1 and RAG2 expression plasmids together with either pHRecCJ or pHRecSJ. 48 h later the reporter constructs were recovered, digested with DpnI to eliminate plasmids that did not replicate in the fibroblasts and reintroduced into bacteria, and the ratio of blue to white colonies was determined (Table 1). The recombination frequency ranged from  $3.86 \times 10^{-3}$  to  $11.06 \times 10^{-3}$  in P10 and Hela cells, respectively, using the coding joint-specific plasmid pHRecCJ. Sequence analysis of these recombination products revealed that they were bona fide coding joints with limited deletions at the junction (data not shown). P10 is a T<sup>-</sup>B<sup>-</sup> SCID patient with mutations in the RAG2 gene (our unpublished observation). P10 belongs to a different group of patients whose



**Figure 3.** (A) Structure of the V(D)J recombination extrachromosomal substrates. pHRecCJ and pHRecSJ constructs were adapted from pBlueRec (37). They contain the SV40 large T coding sequence and the SV40-Ori for autonomous replication in human cells, and a LacZ gene interrupted by a 310-bp DNA stuffer flanked by RSS on both sides. pHRecCJ is used for studying the coding joint formation. In the pHRecSJ plasmid, the two RSS were inverted to allow for the analysis of signal joint formation. (B) Analysis of signal joints after pHRecSJ recombination. The recovered pHRecSJ reporter plasmids were PCR-amplified with T3 and T7 primers, digested (+) or not (-) by ApalI, and run on a 2% agarose gel. The 500-bp band represents the PCR band obtained from unrecombined pHRecSJ. The 190-bp band represents the fusion of the RSS in the recombined plasmid in the absence of treatment with ApalI. This band is further digested to a 100-bp fragment with ApalI.

**Table 1.** *V(D)J Recombination Assay in Human T<sup>-</sup>B<sup>-</sup> SCID Fibroblasts*

		pHRecCJ coding joints			pHRecSJ signal joints		
		Blue	Total	R ( $\times 10^{-3}$ )*	Blue	Total	R ( $\times 10^{-3}$ )‡
Hela	Exp. 1	38	10,300	11.06	48	11,200	4.28
P10	Exp. 1	209	96,000	6.53	–	–	–
	Exp. 2	38	29,500	3.86	–	–	–
P1	Exp. 1	2	109,000	0.05	–	–	–
P2	Exp. 1	0	54,000	<0.02	–	–	–
P5	Exp. 1	8	1,250,000	0.02	24	18,600	1.29
	Exp. 2	4	390,000	0.03	15	2,410	6.20
P6	Exp. 1	3	217,000	0.04	11	6,600	1.66
	Exp. 2	2	560,000	0.01	243	420,000	0.58
P11	Exp. 1	3	242,000	0.04	8	6,500	1.23
	Exp. 2	3	730,000	0.01	49	41,000	1.19
P12	Exp. 1	7	370,000	0.05	14	5,000	2.80
	Exp. 2	3	790,000	0.01	196	370,000	0.53
P15	Exp. 1	2	72,000	0.08	23	8,100	2.84
	Exp. 2	6	500,000	0.03	36	5,000	7.20

\*R (coding joints) =  $3 \times (\text{Blue col.}) / (\text{Total col.}) \times 1,000$ .

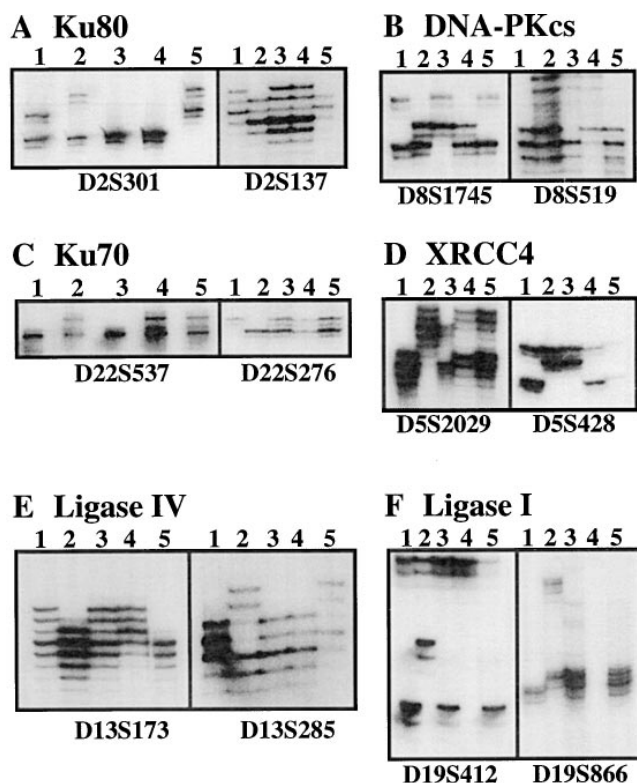
‡R (signal joints) =  $(\text{Blue col.}) / (\text{Total col.}) \times 1,000$ .

DNA repair machinery is not affected and whose marrow cells do not display increased radiosensitivity (Fig. 1 B). Analysis of signal joint formation in HeLa cells using the pHRecSJ plasmid leads to a recombination frequency of  $4.28 \times 10^{-3}$ . The V(D)J recombination was assessed in fibroblast lines from seven T<sup>-</sup>B<sup>-</sup> radiosensitive SCID patients and the coding joint formation was found to be profoundly affected in all cases with recombination frequencies  $<0.08 \times 10^{-3}$  (Table 1). Interestingly, the frequency of signal joint formation, determined with the pHRecSJ construct, was not significantly different in these patients compared with the HeLa control (mean:  $2.55 \times 10^{-3}$ ). The perfect fusion of the heptamer sequences at the signal joint causes the formation of an ApalI site. This ApalI diagnostic site was used to check for the integrity of the signal joints formed in T<sup>-</sup>B<sup>-</sup> SCID cells (Fig. 3 B). The pHRecSJ plasmid was PCR-amplified using T3 and T7 primers after transfection into fibroblasts. The signal joints resulted in a 190-bp PCR fragment that could always be digested into a 100-bp ApalI fragment, indicating the integrity of these joints in SCID cells. Altogether, these results clearly establish that the DNA repair machinery necessary for the completion of the V(D)J recombination is profoundly impaired in T<sup>-</sup>B<sup>-</sup> SCID with increased cell radiosensitivity.

*The Ku80-DNA-PK Complex Is Not Affected in T<sup>-</sup>B<sup>-</sup> SCID Patients with Increased Cell Radiosensitivity.* In light of the molecular defects responsible for the various CHO mutants and the murine and equine scid conditions (8–12, 14, 43), the functional assay for DNA-PK and Ku activity in human T<sup>-</sup>B<sup>-</sup> SCID (Fig. 2) implies that the Ku70 and Ku80 pro-

teins are present and capable of binding DNA and recruiting the DNA-PKcs in our series of patients. This also indicates that the DNA-PKcs is expressed with a functional kinase domain. However, this in vitro assay does not rule out the possibility of mutations in other, as yet unknown, domains of these three proteins involved in V(D)J recombination and DNA repair. This limitation is of particular importance when considering the size (450 kD) of the DNA-PKcs protein. P5, P11, and P12 are three siblings from a consanguineous family. In a consanguineous family it can be assumed that a mutated allele for a given gene will be inherited as a homozygous trait in affected children and will be heterozygous in both parents and in healthy siblings. The same holds true for nearby flanking markers. Therefore, we looked for homozygosity of highly polymorphic markers flanking the Ku-DNA-PK complex encoding genes in P5 family members. Results of this analysis (Fig. 4, A–C) showed independent segregation patterns between these markers and the disease locus in the different affected children. These results therefore irrevocably exclude the Ku70, Ku80, and DNA-PKcs genes in the T<sup>-</sup>B<sup>-</sup> SCID condition, at least in this family.

*XRCC4, Ligase I, and Ligase IV Are Not Involved in T<sup>-</sup>B<sup>-</sup> SCID.* Among the various CHO mutants that were shown to be defective in V(D)J recombination and DNA repair (for review see reference 44), the XR1 cell line is characterized by defects in both coding and signal joint formation, but does not show any defect in DNA end-binding and DNA-PK activities (8, 45, 46). A human cDNA, called XRCC4, that complements the XR1 defect has



**Figure 4.** Genotype analysis of P5 family members with highly polymorphic microsatellite markers. Lane 1, father; lane 2, mother; lane 3, P5; lane 4, P11; lane 5, P12. For each gene upstream and downstream markers were used. None of the markers that are heterozygous in the parents are found homozygous in the three affected children.

been cloned (24). Although cells from human radiosensitive T<sup>-</sup>B<sup>-</sup> SCID show normal signal joint formation (Table 1 and Fig. 3 B), the possible involvement of the XRCC4 gene in the pathology was assessed by cDNA sequence analysis in four unrelated patients (P1, P2, P6, and P11) and by genotype analysis of flanking polymorphic markers in P5, P11, and P12 (Fig. 4 D) and in P15 (data not shown). Normal cDNA sequences (data not shown) and the absence of cosegregation between flanking markers and disease inheritance excluded an XRCC4 gene defect as being responsible for this condition in all patients tested. Recently, the XRCC4 protein has been found to interact with DNA ligase IV in mammalian cells and stimulate its activity in vitro (26, 27). Moreover, the yeast counterpart of DNA ligase IV plays a critical role in DNA dsb repair by nonhomologous end joining as demonstrated by the increase of radiosensitivity of yeast strains carrying DNA ligase IV mutations in a Rad52 defective background (25, 28, 47). Altogether, these reports make ligase IV an attractive candidate for the religation process during V(D)J recombination. Genotype analysis of DNA ligase IV gene flanking markers in P5, P11, and P12 excluded this gene as being implicated in the T<sup>-</sup>B<sup>-</sup> SCID condition (Fig. 4 E). Moreover, cDNA sequence analysis of ligase IV in P15 did not reveal any mutation in the coding region (data not

shown). This result was perhaps not surprising provided that the signal joint formation, which can be considered as a nonhomologous end joining event, was not affected in these patients (Table 1 and Fig. 3 B). Lastly, another DNA ligase (ligase I) has been shown to enhance cell-free V(D)J recombination in vitro (29) and represents another potential player of the V(D)J recombination reaction. Again, genotype analysis excluded this gene as being defective in P5, P11, and P12 (Fig. 4 F). Moreover, an impaired DNA ligase I activity does not result in a T<sup>-</sup>B<sup>-</sup> SCID phenotype (48–50) nor does it lead to faulty V(D)J recombination activity in vitro (51, 52).

## Discussion

Several in vivo conditions are characterized by an absence of B and T lymphocyte development associated with a defect in DNA dsb repair. In the murine and equine SCID conditions a lack of DNA-PK activity caused by mutations in the DNA-PKcs coding gene are responsible for the lack of V(D)J recombination (21, 53, 54). Specific gene targeting of the Ku80 gene also results in a T<sup>-</sup>B<sup>-</sup> phenotype in mice (15, 16), in accord with the V(D)J defect first noted in CHO cells harboring Ku80 mutations (for review see reference 42). Finally, mice with a targeted deletion of the Ku70 gene (18, 19) harbor a somewhat surprising phenotype: an absence of B cells but an almost normal number of mature T lymphocytes. This last result contrasts with the profound defect of V(D)J recombination noted in vitro in embryonic stem cells homozygous for a Ku70 gene deletion (17) and suggests the existence of T cell lineage specific factors that complemented the defect in vivo. The human T<sup>-</sup>B<sup>-</sup> SCID condition we describe is yet another setting in which early arrest in T and B lymphocyte development is most probably caused by a primary defect in the DNA repair/recombination machinery. However, the factor(s) involved in this pathology are clearly different from the Ku70–80–DNA-PK complex and from XRCC4, another factor found to be critical for both V(D)J recombination and DNA repair in vitro in CHO-XR1 cell line but for which no in vivo model are yet available (24). Human RAG1<sup>+</sup>RAG2<sup>+</sup> T<sup>-</sup>B<sup>-</sup> SCID patients therefore define a new complementation group of mutants whose affected gene(s) are involved in sensitivity to ionizing radiation and V(D)J recombination. The finding of a normal activity of signal joint formation in fibroblasts from SCID patients in vitro indicates that the ultimate ligation step of the broken ends is probably not affected in these patients. The exclusion of DNA ligase I and DNA ligase IV as candidate genes in three patients certainly strengthens this assumption. The defect in V(D)J recombination in these patients appears to be specific of the coding joint formation and may therefore involve enzymatic activities acting around the resolution of the hairpin-sealed coding ends. A more general survey of which kind of broken ends cells from these patients are able or not to repair should help clarifying the role of the defective molecule(s).

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