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Review

The SCID Mouse: Relevance as an Animal Model System for Studying Human Disease

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The simultaneous description some 5 years ago of two methods for the partial reconstitution of a human immune system in severe combined immune-deficient (SCID) mice (collectively, human:SCID mice) was met with great enthusiasm. At the time, it was hoped that human:SCID mice would provide experimental animal model systems for studying human disease and the human immune system. Many of these hopes have been borne out. Importantly, the experimental results obtained from these chimeric human/animal studies appear to be relevant to human disease and immune function. In spite of these glowing achievements, the SCID mouse may not represent the optimal experimental system with which to address these questions. The incomplete penetrance ("leakiness") of the scid mutation and the recent discovery that the mutation is not lymphoid specific, but rather affects a general DNA repair pathway, will only serve to complicate the interpretation of already complex biological interactions. Recently other immune-deficient mice have been described that appear to overcome one or both of these problems and thus these mice could represent improved hosts for the adaptive transfer of a human immune system. The current status of the SCID mouse in light of these new findings is discussed. (Am J Pathol 1993, 143:1511–1522)

Homozygous mutation of the *scid* (severe combined immune-deficient) locus in the mouse (SCID mice) disrupts both B- and T-cell lymphoid development, resulting in low serum immunoglobulin (Ig) levels and the lack of functional T cells.[1] The intrinsic ability of SCID mice to support lymphoid development, however, is not impaired. Immune function in SCID mice was fully restored after reconstitution with normal mouse bone marrow.[2] This observation lead several investigators to attempt the xenogeneic engraftment of human lymphoid cells into SCID mice.[3,4] The success of these experiments and similar schemes[5,6] has enticed a large number of investigators to use SCID mice as a model system to study many different aspects of human disease. Phenomenon as diverse as T-cell ontogeny,[7] human cancer (eg, multiple myeloma,[8,9] leukemia,[10,11] and lymphoma[12,13]), immunotherapy,[14,15] and viral infections (e.g., Epstein–Barr virus,[16] human cytomegalovirus,[17] and human immunodeficiency virus[18–21]) have been investigated using these systems.

This review will attempt to 1) examine in depth the molecular basis of the *scid* mutation and describe how two peculiar aspects of the mutation may limit the usefulness of the human:SCID models, and 2) discuss new immune deficient murine strains which may be better suited to xenogeneic studies. This review will focus predominantly on experiments carried out in the last 2 years, and the reader is referred to an excellent review of the SCID mouse by Bosma and Carroll (1991)[22] for comprehensive background information. It should be emphasized that this review does not attempt to critique the relevance of either system as model systems for studying human disease. For this information, the reader is directed to recent reviews and studies on either SCID-hu[23,24] or hu-PBL-SCID[25–27] mice. In addition, other immune-deficient mice, such as nude (*nu/nu*)[28,29] or BNX

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(*beige/nu/xid*)[30–32] mice, which have been utilized for human disease studies, but which have not gained nearly as much acceptance as the human:SCID systems, will not be discussed here. The reader is referred to current articles in which the utility of these two strains is compared with the SCID systems.[29,31–33]

Lymphoid V(D)J Recombination

Wild-Type V(D)J Recombination Mechanism

To appreciate the complexities of the *scid* mutation as it relates to using SCID mice as animal models for studying human disease, it is essential to understand the mechanistic basis of lymphoid V(D)J recombination. Functional Ig and T cell receptor (TCR) genes are assembled from separate gene elements via somatic gene rearrangement—V(D)J recombination—during lymphoid differentiation (for recent and more thorough reviews see Alt et al.,[34] Gellert,[35] and Schatz et al.[36]). The gene elements (V, variable; D, diversity; and J, joining) targeted for rearrangement are flanked by conserved signal sequences which mediate the rearrangement event (Figure 1). V(D)J recombination is initiated by the recognition of two signal sequences by the recombination machinery. Protein:protein interaction, rather than DNA:DNA pairing, are then hypothesized to bring the two signal sequence elements into close association resulting in a protein:DNA synaptic complex (step 1). Site-specific cleavage, presumably staggered or blunt double-strand breaks (DSBs), at the junction of one of the coding elements and its signal sequence then occurs (step 2).[37] Normally, the proposed reaction proceeds with cleavage at the second signal sequence to generate an intermediate with four free DNA ends retained in close proximity to each other (step 3).[38] DNA rearrangement occurs with the resorting of the four ends and the formation of two new junctions: the signal junction (steps 4 and 7) and the coding junction (steps 5, 8, 9, and 10). The DNA sequences formed at these two junctions indicate that they are asymmetrically processed before being sealed. The coding junctions usually have processed ends (ie, nucleotides added or deleted; step 9) and this junction is modified by proteins involved in general DNA repair and recombination.[39–42] Signal junctions on the other hand, rarely show evidence of processing and so these ends may be joined by a V(D)J-mediated process (step 7). This model of V(D)J recombination also accounts for the existence of hairpins on coding junction ends (steps 2 and 3), random (N nucleotides[43]; step 9) and non-random (P nucleotides[44–46]; steps 8 and 9) nucleotides at coding joints (for a review see Lieber[47]) as well as two alternative products of the V(D)J joining process: hybrid junctions (steps 6 and 11), where a signal end is incorrectly joined to a coding joint end instead of another signal end[48,49] and "open/shut" junctions (the reverse of steps 2 and 3) where initiation of recombination, junctional diversification and resealing of the signal sequence-coding element juncture occurs without gene rearrangement.[38,48]

Wild-Type V(D)J Recombination Gene Products

In addition to SCID, 14 genes have been implicated as being involved in V(D)J recombination. V-3,[50] XR-V9B,[51] *xrs*,[52] XR-1,[53] *sexi*-1, *sexi*-2, *sexi*-3, and *sexi*-4 (Lee *et al.*, unpublished results) are eight mammalian cell mutants originally isolated based upon their increased sensitivity to x-irradiation.They were subsequently shown to be defective for DNA DSB repair and recently were shown to be impaired in both coding and signal junction formation during V(D)J recombination[41–42] and Zahalsky *et al.* unpublished results. Thus, the V-3, XR-V9B, XRS, XR-1, and SEXI-1 through SEXI-4 gene products appear to play very important, though as yet largely ill-defined, roles in V(D)J recombination. These gene products are hypothesized to act at later steps in the rearrangement pathway (Figure 1, steps 4 through 11). A ninth gene that has been implicated in lymphoid gene rearrangement is TdT (terminal deoxynucleotidyl transferase). This enzyme is thought to be responsible for the random addition of nucleotides (N regions) often found at coding junctions.[43] Three other gene products (heptamer binding protein, HBP; nonamer binding protein, NBP; and recombination signal sequence protein, RSSP[54–56] have been circumstantially implicated in V(D)J recombination based upon their ability to bind to part or all of the V(D)J signal sequence. There is no direct experimental evidence as yet to show that any of these three genes are in fact involved in lymphoid gene rearrangement.

For the purpose of this review, the two most important genes to consider are the recombination activating genes, RAG-1 and RAG-2 (for a review, see Oettinger[57]). These genes were originally isolated based upon their ability to activate V(D)J recombination in fibroblast cells that do not otherwise un-

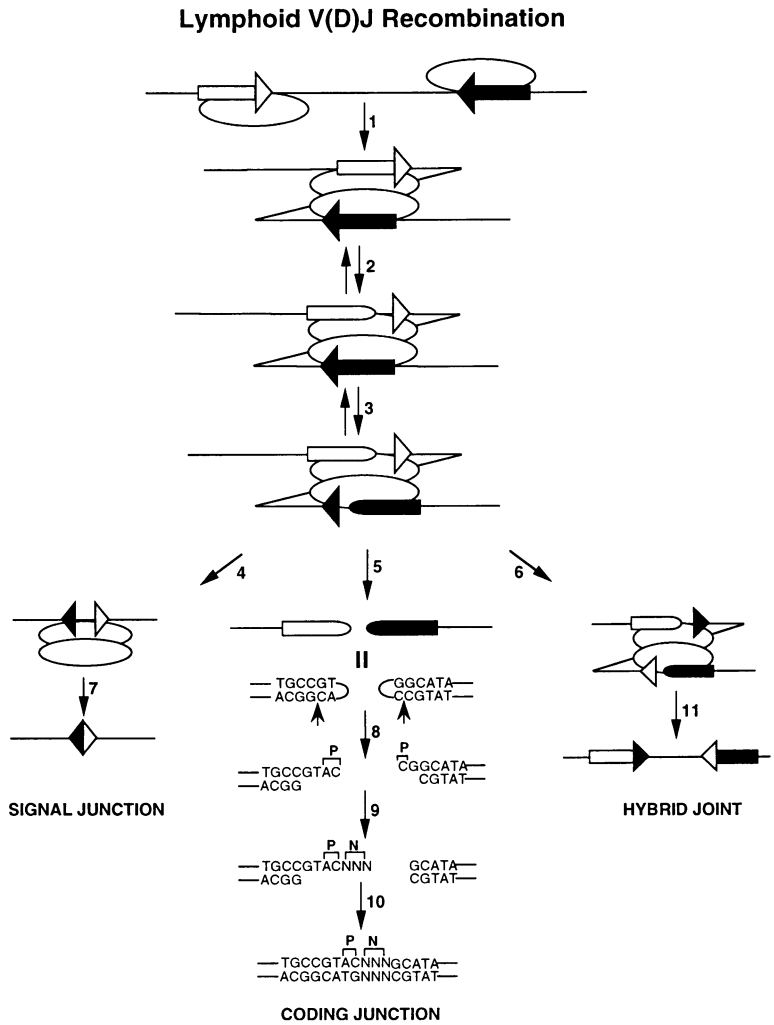

Figure 1. *Model for V(D)J recombination. The rectangles represent gene coding elements and the triangles represent their corresponding signal sequences. The egg-shaped circles represent the V(D)J recombinase complex that probably consists of multiple proteins. After recombinase complexes recognize the two gene elements to be recombined, they are brought together in a synaptic complex (step 1). DSBs are then introduced independently at each of the signal sequence:coding element junctures and hairpinned ends are subsequently formed on the coding strands (steps 2 and 3). The reversibility of steps 2 and 3 accounts for the formation of "open and shut" recombination products. The resolution of the synaptic complex containing two coding ends and two signal sequence ends can proceed in several ways. Occasionally, the signal sequence ends are simply inverted with respect to one another (step 6). Subsequent ligation to the coding strands (step 11) results in a hybrid joint. This recombination reaction is nonproductive and its role (if any) in "legitimate" V(D)J recombination is not understood. Normally, the signal sequence ends are brought together (step 4) and ligated to form a signal junction (step 11). This step in the reaction generally proceeds without the loss or addition of nucleotides to either signal end. The resolution of the coding ends requires the opening of the hairpins (step 8) which is probably accomplished by introducing single-strand nicks (barbed arrows). Current data are compatible with the sequence of the coding ends and the position of the nicks being completely random. Thus, the DNA sequence shown at the coding ends and the location of the nicks is arbitrary and is meant only to serve as an illustrative purpose. The opening of the hairpin results in the formation of small palindromes (P nucleotides) at the ends of the coding strands (step 8). These coding ends may undergo nucleotide addition (N nucleotides) or nucleotide loss (step 9). DNA polymerization and DNA ligation are then required to complete the formation of the coding junction (step 10). The SCID gene product is postulated to participate principally in step 5, 8, or 10.*

dergo lymphoid gene rearrangement.[58,59] The expression of these genes is lymphoid-specific and limited to the stages of differentiation where V(D)J rearrangement normally occurs. Although the expression of these genes in isolated cell lines is not always equivalent,[60,61] the evidence is compelling that co-expression of both genes is necessary, though perhaps not sufficient[62] for their recombination activating function.[59] The characterization of the RAG genes has proved to be extremely frustrating and the exact biochemical activity of either gene is still unknown. RAG-1 has limited topoisomerase homology.[63] However, when mutations were made in this region of RAG-1 the gene was still functionally active, casting doubt onto the significance of the homology.[64] Indeed, it is as yet un-

clear whether these genes act as transcriptional inducers of the genes required for recombination or whether they themselves participate enzymatically in the rearrangement process. However, transgenic "knockout" mice with either disrupted RAG-1 or RAG-2 genes have been generated and both of these animals are completely deficient in V(D)J recombination establishing the essential nature of these gene products in rearrangement.[65,66]

scid V(D)J Recombination

Mice homozygous for the *scid* (severe combined immune-deficient) mutation lack detectable mature B and T lymphocytes due to a V(D)J recombination defect (reviewed by Bosma and Carroll[22]). Our analysis of Abelson murine leukemia virus (A-MuLV)-transformed *scid* pre-B cells,[38,67,68] as well as work from other laboratories,[69–76] showed that almost all V(D)J rearrangement events in *scid* mice were aberrant. Examination of the recombinational junctions indicated that the correct gene elements (V, D, or J) were utilized; however, all rearrangement events were accompanied by deletions of varying sizes. These deletions removed all or most of the coding sequences and resulted in lymphoid cells incapable of synthesizing Ig heavy chain, light chain, or TCR proteins. The break points of the rearrangement events did not correspond to the utilization of novel recombination signal sequences, but probably arose by nonspecific deletion from distal coding strands in the process of recombination. Interestingly, signal junction formation appears to proceed in a reasonably normal fashion in SCID mice, suggesting that the resolution of coding junction strands and signal junction strands proceeds by independent mechanisms.[68,69,75,77] Recently, the coding ends of V(D)J recombination intermediates in *scid* thymocytes have been shown to exist as hairpinned molecules (Figure 1).[78] It is currently believed that such hairpinned coding ends are a normal product of the rearrangement pathway and that the *scid* mutation renders lymphoid cells incapable of resolving those hairpins.[47,79] It was originally postulated that the SCID gene product may encode the DNA nicking activity, which opens the hairpinned ends back up (Figure 1, Step 8) and makes them available for further modification before ligation.[47] This now seems unlikely, as recent experiments have shown that DNA molecules with hairpins are resolved as efficiently in *scid* as in wild-type cells (S. Lewis, personal communication). Alternatively, then, I suggest that the SCID gene may encode a DNA binding protein which holds the two hairpinned coding ends in close proximity so that the recombination machinery can resolve and religate them. Regardless of its exact biochemical function, the SCID gene product unequivocally plays a crucial role in coding joint formation during V(D)J recombination.

Additional SCID Phenotypes

The scid "Leaky" Phenotype

In the original description of SCID mice in 1983 it was reported that homozygous SCID animals had few if any lymphocytes.[1] Indeed, in many research articles in which SCID mice are used for reconstituting some aspect of the human immune system, statements are still frequently found emphasizing the fact that SCID mice are completely deficient in B and T cells. However, it has been known since 1988 that the *scid* mutation is probably only partially penetrant and that homozygous SCID mice can be detected with measurable levels of serum Ig or T cells ("leaky" SCID mice).[80] Initially, it was observed that only a small percentage (2 to 23%) of SCID animals contained low levels of lymphoid cells. However, with improved detection methods and by observing that the leaky phenotype increased with age and antigen exposure it was subsequently reported that virtually all SCID mice have detectable B and T cells at 1 year of age.[32,81,82] In addition, the transfer of neonatal thymocytes to SCID mice resulted in all of the transplanted animals producing serum Ig of the SCID allotype.[83,84] These experiments suggest that all SCID animals have some residual functional B cells that can be induced to proliferate in the presence of T cells and/or antigen.

The residual immune system in leaky SCID mice may affect the function of a transplanted human immune system. Leaky SCID lymphocytes can respond to mitogens, are capable of producing cytokines and serum Ig, and may develop reactions to allogeneic tissue.[80,81,85] Indeed, because of the potential interference with graft acceptance (graft versus host disease), leaky SCID mice are generally screened for and actively excluded from most studies in which the transfer of human tissue is attempted.[25] However, because the number of SCID mice that produce functional lymphocytes increases with age, it is probably very difficult to recognize and completely eliminate leaky SCID mice from such transfer studies.

The molecular basis of the leakiness is not known, although two theories have been put for-

ward.[68,85,86] Historically, leaky mutations are mutations that 1) revert at a detectable frequency or 2) are non-null ("incompletely penetrant") mutations. These explanations are not mutually exclusive. Indeed, in the case of *scid*, evidence for both of these phenomenon has been obtained. Petrini et al[86] identified multiple wild-type endogenous TCR gene rearrangements within single alloreactive T cell clones. This is what would be expected if these cells had reverted their *scid* phenotype. In addition, a homozygous *scid* Abelson-virus-transformed pre-B cell line has recently been described that exhibits many of the characteristics of a true revertant.[87] Hendrickson et al,[68] however, were able to identify pre-B cell clones that produced predominately but not completely *scid*-like rearrangements, consistent more with the partial penetrance of the *scid* mutation. The correct explanation of the leaky phenotype awaits the identification of the SCID gene and a molecular characterization of the *scid* mutation.

The scid X-Ray-Sensitive Phenotype

In terms of studying human immune function after transfer of human tissues or cells into SCID mice, the leaky phenotype of the *scid* mutation, as noted above, can be partially overcome by screening for and excluding leaky SCID animals from study. Unfortunately, a recently discovered additional pleiotropic effect of the *scid* mutation is not as likely to be overcome. Animals homozygous for the *scid* mutation, in addition to being immune deficient, are also profoundly sensitive to x-irradiation.[88] This observation was originally made by investigators performing murine bone marrow transplants into SCID mice. The SCID mice were routinely x-irradiated with 4 Gy before transplant to suppress residual immune function and to subsequently reduce the likelihood of graft versus host disease.[89] A large fraction of the SCID animals did not survive the irradiation regimen. Indeed, the x-irradiation LD_{50} for SCID mice is only 3 Gy, which is less than half the dose required for an LD_{50} in wild-type mice.[40,90] Importantly, this x-ray sensitivity is not confined to the lymphoid compartment, but affects all cells of the mouse that have so far been analyzed, including a wide variety of normal diploid and transformed fibroblasts as well as tumor tissues.[39,40,88,90–93] The x-ray-sensitive phenotype of *scid* cells appears to be cell intrinsic. Bone marrow derived from wild-type mice and transplanted into SCID mice still retains its wild-type x-ray-resistant profile.[88]

The scid DNA DSB Repair-Defective Phenotype

Further insight into the nature of the x-ray sensitivity came when it was determined that the sensitivity correlated with a defect in DNA DSB repair.[39,40] In retrospect, this observation was not surprising, since it has long been known that x-irradiation can induce DSBs which can stimulate chromosomal deletions as well as aberrant rearrangements and are lethal if not repaired.[94] Interestingly, the DSB repair defect is much more pronounced after higher x-ray doses,[39,92] suggesting that there is a threshold level of DNA damage that *scid* cells can adequately repair. This is consistent with the partially penetrant V(D)J recombinational activity described under "The scid 'Leaky' Phenotype." A diminished, but non-null, level of *scid* gene activity could potentially carry out limited V(D)J rearrangements and cope with a modicum of DNA damage. The overlap between recombination and DSB repair pathways is presumed to result from the postulated role of double-stranded ends as structural intermediates in many types of recombination and repair.[95]

SCID cells are not solely sensitive to x-ray-induced DSBs. The integration of linear DNA into *scid* fibroblasts is reduced 11- to 75-fold in comparison to wild-type fibroblasts.[96] Although the mechanism by which exogenous DNA integrates into a chromosome is unknown,[97] it presumably requires, at least transiently, a chromosomal DSB. Thus, the reduction in DNA integration is consistent with a defect in DNA DSB repair. The integration of retroviral DNA into *scid* fibroblasts and lymphoid cells, however, does not seem to be noticably affected.[38,68,69] The integration of retroviral DNA, however, is mediated in part by viral, rather than cellular, proteins and appears to proceed in a highly concerted fashion and thus may represent an exception to, or special case of, general DNA DSB repair.[98]

Interestingly, there appears to be a difference in the processing of chromosomal versus extrachromosomal DSBs in *scid* fibroblasts. Using an extrachromosomal plasmid assay, Harrington et al[96] could not detect any significant effect of the *scid* mutation in intra- or intermolecular end-joining reactions. This was true no matter whether the ends were compatible, non-compatible, or even blocked by dideoxynucleotides.[96] Chang et al[92] obtained virtually identical results using a similar assay system. In sharp contrast, though, these same authors

using the direct electroporation of restriction enzymes into cells were able to show that the restriction enzymes were much more cytotoxic to *scid* than wild-type cells.[92] Presumably, the restriction enzymes are introducing DSBs into the chromosomes which cannot be repaired and these events are ultimately lethal.

As noted above, a possible, but unlikely, role for the SCID gene product could be in resolving hairpinned DNA structures. While it is certainly possible that the integration of linear DNA and the repair of restriction-enzyme-digested chromosomes proceed through hairpinned intermediates, there is to date no empirical evidence to support such models. Thus, it is tempting to speculate that the function of the SCID gene product may be to align or hold together the broken ends of chromosomes while they are being repaired. Such an activity is clearly essential for the formation and resolution of V(D)J synaptic complexes and might explain the lymphoid gene rearrangement defect. To account for the result that the *scid* mutation does not appear to effect plasmid end-joining reactions, the SCID chromosome binding activity would have to be redundant with other factors, or dispensible for, extrachromosomal DNA DSB repair. In this light it is interesting to note that researchers using the extrachromosomal assay for V(D)J recombination,[99] which in general accurately recapitulates the *scid* defect,[76] have obtained differences, particularly in signal junction formation,[77] from investigators examining chromosomal rearrangements.[68,69,75] These differences, which have never been satisfactorily explained, could be resolved if the SCID gene product is preferentially involved in the resolution of chromosomal broken ends.

The SCID Locus

The resolution of many of the questions concerning the function of the SCID gene and its roles in V(D)J recombination and DNA DSB repair will probably be answered with the molecular cloning of the SCID gene. Unfortunately, intense effort from a number of laboratories using a variety of approaches has to date proved futile. The most fruitful current attempts involve positional cloning and functional complementation of the x-ray-sensitive defect. The murine SCID gene was originally mapped to chromosome 16 between the Igλ light chain locus and the mahoganoid (a recessive coat color marker) locus, an interval of some 10 to 15 cM.[22] Recently, using more backcrosses and probes from chromosome 16, Miller et al[100] have shown that the SCID gene is tightly linked to the λ5 and VpreB loci, which are colocated approximately 1 cM centromeric from the Igλ locus. Since both λ5 and VpreB genes have been cloned, these may provide molecular tools for identifying cosmid or YAC clones that span this region. Interestingly, these authors speculated that the human SCID gene may be located on either of the regions of human chromosomes 16 or 22, which are syntenic with mouse chromosome 16.[100] This is in disagreement, however, with a very recent report using radiation-reduced human:murine hybrids in which human chromosome 8p12-q22 was shown to functionally complement the *scid* x-ray-sensitive defect.[101] In addition, it has recently been shown that human chromosome 8 will also complement the *scid* V(D)J defect (D. Weaver, personal communication), a prediction that should be fulfilled by the authentic human SCID gene. Surprisingly though, there is no other known synteny between mouse chromosome 16 and human chromosome 8, and there are no known human DNA repair or immune deficiency diseases that map to this region.

The SCID Mutation and Implications for Human:SCID Models

The above observations demonstrate that the SCID gene, which was originally believed to be lymphoid restricted and limited to a role in V(D)J recombination encodes, in fact, a ubiquitously expressed protein whose primary function is probably generalized DNA DSB repair. These observations may severely compromise some of the uses planned for the human:SCID model systems. The "holy grail" for these systems would be the complete reconstitution of a human immune system in SCID mice so that processes such as human tumorogenesis or human immunodeficiency virus infection could be studied. One major goal of such studies would be the isolation of pharmacological agents that would slow or abrogate tumor growth or viral infection. Unfortunately, many anticancer and antiviral reagents are either DNA-damaging agents or compounds which affect DNA metabolism. For example, bleomycin is a potent antineoplastic compound that has been used to treat human tumors.[102] The *scid* mutation, however, renders the SCID mouse extremely sensitive to bleomycin.[39,40] Obviously, determining the efficacy of bleomycin as an antiviral or antineoplastic agent in human:SCID mice would be exceptionally difficult. Similar difficulties are likely to be encountered with other pharmacological agents.

New Immune-Deficient Murine Strains

The above sections have attempted to demonstrate that the leaky phenotype and DNA repair defects associated with the *scid* mutation may limit the usefulness of the SCID mouse as an experimental animal model system to study human disease. Recently, novel murine strains have been described in which either one or both of these problems have been solved. These strains may represent improved hosts for human immune system studies.

Non-Leaky Strains of SCID Mice: SCID.BG and C3H SCID

Very recently, the *scid* mutation, which occurred spontaneously in the BALB/c C.B-17 strain,[1] has been bred into two additional murine strains. SCID mice were crossed with mice homozygous for the *beige* mutation (SCID.BG mice).[82] The *beige* mutation is known to suppress natural killer activity.[103] The intent of performing this construction was to reduce natural killer activity, which is normal in SCID mice.[2] Surprisingly, however, the introduction of the *beige* mutation to the SCID strain also severely depressed the leakiness of the *scid* mutation.[82] Virtually 100% of older SCID mice are leaky[32,80,81] as defined by serum Ig concentrations of more than 5 μg/ml, whereas by the same criteria only 3% of SCID.BG animals could be considered leaky. In addition, 100% of SCID mice produced B cells and showed a 10- to 100-fold increase in serum IgM when transplanted with neonatal T cells.[83,84] In contrast, only 1 in 10 of SCID.BG animals showed any increase in serum IgM after transplantation.

Similarly, the *scid* mutation has been introduced into the C3H strain (C3H SCID) mice by extensive backcrossing.[32] The C3H SCID animals are commercially available from the Jackson Laboratories (Bar Harbor, ME). Whereas 79% of 3-month-old SCID mice showed detectable and sometimes high levels of serum Ig, only 15% of C3H SCID mice had serum Ig and only at low levels (>10 μg/ml).[32] Importantly, the number of "leaky" C3H SCID animals did not increase with age and these animals showed no or very small increases in serum Ig upon the transfer of neonatal T cells.

Since SCID, SCID.BG and C3H SCID mice are all homozygous for the *scid* mutation, these mice clearly demonstrate another hitherto unknown quality about the *scid* mutation; namely, that other mutations and strain-specific genes can significantly affect the penetrance of the *scid* mutation. The fact that all F1 progeny of a SCID × C3H SCID cross (all animals of which are necessarily homozygous for the *scid* mutation, but heterozygous for all other all alleles) have detectable levels of serum Ig powerfully demonstrates this point.[32] In summary, SCID.BG and C3H SCID mice represent two new mouse strains in which the leaky phenotype of the *scid* mutation appears to be suppressed and as such, these animals may be better suited for cell transplant experiments.

RAG-1 and RAG-2 Knockout Mice: The Perfect Cell Transfer Recipients?

The SCID.BG and C3H SCID strains of mice described above significantly, but not completely, suppress the leaky phenotype of standard SCID mice. However, neither of these strains is likely to have any impact on difficulties presented by the *scid* DNA repair defects. As discussed under "Lymphoid V(*D*)J Recombination," two genes, RAG-1 and RAG-2, have been identified as being necessary for V(D)J recombination.[59] These genes have been cloned and recently two strains of mice containing homozygous germline gene disruptions have been generated (RAG-1 and RAG-2 knockout mice, respectively).[65,66] These animals, while fertile and apparently normal in all other aspects, completely lack functional B and T cells. The absolute numbers of cells in the spleen and thymus of either knockout animal are 10 to 100 times lower than that seen in a normal mouse. In addition, serum Ig could not be detected, even using sensitive enzyme-linked immunosorbent assays, in any RAG knockout animal. FACS analysis demonstrated that these animals have no detectable mature B or T cells. Impressively, when lymphoid tissue from these animals was examined, all Ig and TCR loci were in germline configuration, demonstrating that the process of V(D)J recombination was not initiated in these animals.

Although there have been suggestions of the involvement of RAG-1 and RAG-2 in other biological functions besides V(D)J recombination,[60,104] the RAG knockout animals, with the exception of the profound immune deficiency, appear to be completely normal. While neither of these strains is as yet commercially available and further studies on the immune capacity of these animals and their ability to accept or reject transplanted human tissue is clearly required,[105] the existing data strongly suggests that both RAG-1 and RAG-2 knockout mice will make superior recipients for human immune system transfer experiments. It will also be interest-

ing to breed these genetically engineered immune-deficient strains with naturally occurring mutant strains such as *scid*, *beige*, or *nude* in the hopes of generating even better recipient animals.

Summary

In the 10 years since the isolation and description of the SCID mouse, it has proved to be a scientific "gold mine" by providing basic scientists with a plethora of information on the mechanisms of V(D)J recombination and DNA repair. In addition, in clinically oriented studies, it has proved to be an adequate recipient and model system for the transfer and study of human immune components. However, the leaky phenotype and DNA repair effects intrinsic to the mutation may ultimately limit the usefulness of the human:SCID mouse models. New immune-deficient strains of mice have recently been described that appear to represent superior hosts for such human transfer experiments. Clearly, the information, animal husbandry, and techniques developed during the course of work on the human:SCID model systems should greatly expedite the introduction of the new immune-deficient animals into the clinical setting.

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