Perspective

Reconstruction of an Immune System

GEORGE D. YANCOPOULOS AND FREDERICK W. ALT

THE MAMMALIAN IMMUNE SYSTEM FUNCTIONS THROUGH complex interactions between various cells and their products. Antigen-specific responses by the immune system are effected by B and T lymphocytes; T cells mediate cellular immunity (for example, graft rejection) and B cells mediate humoral immunity (antibody production). Other cell types such as macrophages and natural killers act nonspecifically to eliminate foreign elements. A variety of heritable immune deficiency states have been described; these can involve almost any aspect of the immune system. Severe combined immunodeficiency (SCID) refers to a heterogeneous (and often devastating) set of disease states initially described in humans; in a SCID disease both B and T lymphocyte functions are impaired, resulting in the loss of antigen-specific immunity. Several years ago Bosma and colleagues described a mouse model for SCID that resulted from a spontaneous autosomal recessive mutation (*scid*); mice homozygous for this mutation (SCID mice, *scid/scid*) lacked detectable B and T cells or lymphocyte function (*1*). However, SCID mice could be cured of immunodeficiency by grafts of normal murine bone marrow or cultures containing murine lymphoid stem cells (*2*). Thus, the SCID mouse presented an ideal model system for studying lymphoid deficiency and for elucidating the differentiation of the lymphoid system after reconstitution with appropriate normal murine precursor cells. As reported on page 1632, McCune, Weissman, and their co-workers now exploit the characteristics of the SCID defect to undertake the reconstruction of a human immune system within the body of a mouse (*3*). This mouse-human chimera raises fascinating possibilities for analyses of the development, function, and diseases of the human immune system within the convenient context of a laboratory mouse.

B and T cell differentiation can be divided into two phases: primary (antigen-independent) and secondary (antigen-dependent). During primary differentiation, stem cells proceed through a series of differentiation events that generate a multitude of B or T lymphocyte clones, each of which expresses on its surface an antigen receptor with a novel set of specificities. For B cells, these events take place in the fetal liver or adult bone marrow; for T cells, the events may be initiated in these same primary organs, but are concluded in the T cell–specific primary differentiation organ, the thymus. Unlike the antibodies produced by B cells, the T cell receptor evolved to recognize foreign antigens in the context of self-molecules encoded by the major histocompatibility complex—the thymus plays a major role in educating the T cell population with respect to self–non-self specificities. Primary B or T cells move into peripheral lymphoid organs such as the spleen or lymph nodes. The peripheral immune organs evolved to allow interaction of primary lymphocytes with antigens; upon interaction with antigens that specifically bind to their receptor, lymphocytes undergo secondary differentiation into effector cells. For B cells, this step involves differentiation into a cell that secretes specific antibody into the blood stream; for T cells, the step involves activation into one of several effector cell types—for example, cytotoxic cells that can kill cells bearing foreign antigens.

The antigen-binding proteins of B cells (antibody or immunoglobulin) and T cells (T cell receptor or TCR) are composed of evolutionarily related, but quite distinct, sets of heterodimeric polypeptide chains. There are three families of immunogloblin (Ig) chains and four known families of TCR chains—each encoded in a distinct chromosomal location. The amino-terminal portion of these chains has an amino acid sequence that varies even between chains that are members of the same family; the variable regions of the chains comprising a given heterodimer associate to form the specific antigen-binding site. The mammalian immune system generates a nearly limitless diversity of variable region sequences (antigen-binding specificities), the number far exceeding the coding potential of the germ-line genome. The ability to produce this large repertoire is due, in large part, to the fact that the genes encoding Ig and TCR variable regions are assembled from germ-line gene segments, the variable region of a given type of chain being encoded by either two (V and J) or three (V, D, and J) gene segments (*4*). For each Ig or TCR family, there are multiple, different copies of the V, (D), and J segments in the genome; the combinatorial assortment of these segments generates far more coding information than that specifying the individual segments. Each clonally related set of B or T cells shares its own, specifically assembled variable region genes and, as a result, expresses a novel set of antigen-binding specificities.

The murine SCID defect is of great interest because, as described below, it affects the molecular processes that allow differentiating B and T cells to construct genes that encode antigen receptors. Ig and TCR variable region genes are assembled, respectively, during the primary differentiation of B and T cells (*5*). Expression of Ig or TCR chains from the assembled genes is necessary for differentiating B or T cells to continue progression through their differentiation pathway. Individual Ig and TCR variable region gene segments are directly flanked by conserved signal sequences (Fig. 1A); various lines of evidence suggest that the signal sequences target the activity of a lymphocyte-specific recombination system (VDJ recombinase). Although this recombinase is expressed constitutively during the early phases of lymphocyte differentiation, its activity is directed in a tissue- and stage-specific fashion by modulating accessibility of the various sets of substrate V gene segments; this level of control, for example, allows specific assembly of Ig variable region genes in B cells and TCR variable region genes in T cells. The general mechanism by which this system functions is known, at least in outline (*5*). The process involves the recognition of the signal sequences followed by endonucleolytic scission directly at the border of the signal sequences and adjacent coding sequence (Fig. 1B). Subsequently, the signal sequences and coding sequences are joined in separate events (Fig. 1C); the signal sequences are precisely joined, whereas nucleotides may be added or deleted from the coding sequences (generating further diversity).

The complete absence of B or T lymphocytes in SCID mice suggested a defect in some element common to these pathways. Among possibilities considered was a defect in the common VDJ recombinase; if variable region genes could not be assembled (and as a result expressed), then mature lymphocytes could not be generat-

G. D. Yancopoulos is a Lucille P. Markey Scholar and F. W. Alt is a professor of biochemistry and microbiology and investigator of the Howard Hughes Medical Institute at Columbia University, 630 West 168 Street, New York, NY 10032.

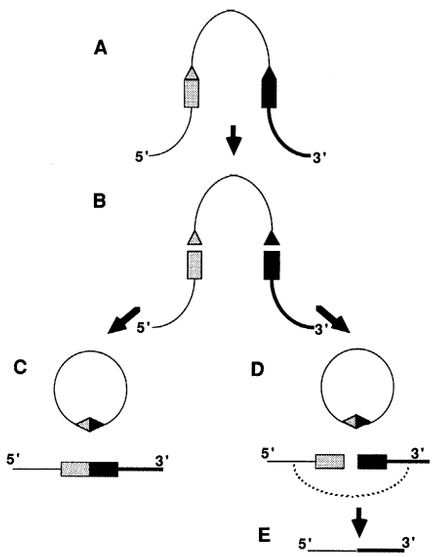

Fig. 1. The SCID mutation affects antigen receptor variable region gene assembly. A germline variable region locus is represented by a V segment (light box) flanked by a 3′ signal sequence (light triangle), and a J segment (dark box) flanked by a 5′ signal sequence (dark triangle). A proposed normal joining mechanism is shown in panels A, B, and C. In SCID mice, specific joining of the chromosomal segments that contain the V and J coding sequences is impaired (panel D); intact chromosomes are proposed to be generated only through illegitimate recombination events that result in deletion of either one (not shown) or both participating coding segments as depicted in panel E (*7*). [Adapted from Malynn *et al.* and Blackwell *et al.* (*7*)]

ed. To test this hypothesis, precursor B cell lines were made by infecting bone marrow cells of SCID mice with the Abelson murine leukemia virus (A-MulV); infection of normal murine marrow by A-MuLV transforms some of the earliest precursor B cells into permanent lines which can assemble complete Ig variable region genes during growth in culture. Consistent with the defective recombinase hypothesis, A-MuLV–transformed pre-B cells from SCID mice did not correctly assemble Ig variable region gene segments, but rather made large deletions within the variable region locus that often extended beyond the gene segments that should be joined (*6*). Similar deletions were found within the TCR variable region gene loci in certain T cell tumors that arise in older SCID mice. Together, these findings strongly supported the notion that the SCID defect affects the common VDJ recombinase (*6*). More detailed recent analyses have supported this idea and further clarified the nature of the lesion. Briefly, the VDJ recombinase system in SCID mice, as in normal mice, recognizes appropriate gene segments to be joined, makes proper endonucleolytic scissions between the targeted signal and coding sequences, and ligates the two signal sequences; but, joining of the coding sequences is impaired (Fig. 1, B and D) (*7, 8*). The exact nature of this impairment is unknown. However, in SCID pre-B cell lines that do ligate "coding segment strands," the ligation point for one or both partners is always a substantial distance from the actual coding segment (therefore not generating a functional variable region gene) and probably occurs by a nonspecific (illegitimate) recombination mechanism (Fig. 1E) (*7*).

The most dramatic demonstration of defective lymphoid immunity in SCID mice was the inability to recognize self from non-self—SCID mice did not reject skin grafts from histoincompatible donors (*1*). Other hematopoietic lineages and all nonlymphocytic components of the immune system in SCID mice appear normal, perhaps explaining the ability of these mice to survive non–germ-free (albeit clean) environments for long periods of time (*9*). Furthermore, long-term functional reconstitution of SCID mice with the use of syngeneic (genetically identical) bone marrow cells verified that the various microenvironments in SCID bone marrow, thymus, and peripheral lymphoid organs function normally and allow the introduced normal lymphoid precursors to correctly mature and differentiate, ultimately regenerating a functional immune system (*2*). These findings underscore the utility of the SCID system for reconstitution experiments aimed at understanding the function of various lymphoid precursors or subsets. Thus, purified lymphocyte populations or subpopulations can be isolated from normal mice and introduced into SCID mice to examine the function of their progeny. For such reconstitution experiments, the SCID system has obvious advantages over other systems such as lethally irradiated mice (radiation chimeras). In the latter, the entire hematopoetic system is destroyed and must be restored; in the SCID, only B and T cells are missing allowing selective reconstitution with just these cells or their subsets. Alternatively, transgeneic mouse technology is now being used to create "clonal" B or T cell subsets within a SCID mouse. These experiments once again are based on the observation that the SCID defect involves only the inability to assemble Ig and TCR variable region genes. To exploit this system, completely assembled Ig or TCR genes have been introduced into the germ line of normal mice and, through selective backcrossing, are now being introduced into the germ line of SCID mice. For example, by supplying in the germ line the assembled Ig genes necessary to encode a complete Ig molecule, it should be possible to produce an SCID mouse in which every differentiating precursor B cell now produces an identical Ig molecule; production of these molecules should allow the precursor cells to mature and basically generate a mouse with a monoclonal (expressing a single Ig) B cell population. The ability to produce mice expressing only a single or limited set of Ig's or TCR's will be invaluable for determining the function of different classes of these molecules and how expression of a particular type of Ig or TCR in appropriate precursors affects development of cellular subsets within those lineages. Appropriately constructed animals also should be useful for studying many fundamental immunological processes such as the mutational mechanisms that generate diversity within completely assembled Ig genes and the various cell and molecular interactions that are thought to mold the immune system and regulate its action.

The Weissman group now reports remarkable experiments that appear to push this system to its incredible but logical extreme—SCID mice were reconstituted, not with murine lymphoid cells, but rather with the corresponding cells from humans (*3*). To accomplish this reconstruction, these workers took advantage of two guiding principles that appear to be verified by their results—first that the absence of lymphocytes that led to the inability of SCID mice to reject allogeneic (genetically dissimilar) murine grafts would also facilitate engraftment of human tissues, and second that, because of its constant and early exposure to murine tissues, the developing fetal human immune system should develop tolerance to (that is, not recognize as foreign) the murine host. The cross-species reconstitution could not be accomplished by engraftment of human lymphoid stem cells alone; it required the engraftment of human fetal liver, thymus, and lymph node into the SCID mouse. Thus, the human cells appear to require human microenvironments to differentiate. In fact, the developing human immune system in SCID mice seems almost independent of the mouse immune system; the human lymphoid cells do not seem to fill any niches in mouse primary or secondary immune organs. Whether the inability of the human lymphoid cells to repopulate murine organs is due to species-specific barriers (for example, lack of appropriate receptors) or other barriers remains to be determined. In the latter context, it has been noted that effective reconstitution of SCID mice with murine lymphoid stem cells is more effective in mice that have been sublethally irradiated; such irradiation presumably reduces the number of endogenous pre-lymphocyte precursors allowing these niches to be filled by transferred cells (*2*).

By developing a mouse model that allows for direct analysis of the human immune system, McCune *et al.* (*3*) also have implicated the

SCID mouse as a potential host for the direct study of the physiology and development of any engraftable human tissue. The studies of SCID-human (SCID-hu) chimera are still in their early stages; exciting results can be expected as potentially important aspects of this system continue to be verified or optimized (or both). In particular, it will be important to understand why the presence of the human immune system appears transient in these mice, to determine if this transiency is a limitation to experimentation, and, if so, to develop methods to extend its presence. B cell differentiation and function in the chimera will be explored by attempting to elicit a humoral response to introduced antigens by the human B cells. If such attempts are successful, the SCID-hu chimera may be an ideal source of human monoclonal antibodies. The aspect of the current study most suggestive of functional human immune cells in the SCID-hu mouse is the possibility that the chimeras are protected against the opportunistic infections to which SCID mice normally succumb. A further test of T cell function in these chimeras will center on how the chimeras respond to grafts from both compatible and incompatible mouse and human donors. Finally, it seems likely that the lymphoid system of the chimera will be susceptible to pathogens, such as the human immunodeficiency virus (HIV), that have thus far attacked lymphocytes only in humans. A most exciting possibility is that HIV will not only infect the human T cells of the chimera, but that this infection will reverse the apparent protective functions bestowed by these cells, returning the chimera to an immunodeficient state. The production of an acquired immunodeficiency disease in a mouse due to infection of introduced human T cells by the precise virus that causes such a disease in humans could present an unprecendented opportunity to understand the causative mechanisms of this disease and to initiate interventions aimed at prevention and cure.

REFERENCES AND NOTES

1. G. C. Bosma, R. P. Custer, M. J. Bosma, *Nature* **301**, 527 (1983).
2. G. M. Fulop and R. A. Phillips, *J. Immunol.* **136**, 4438 (1986).
3. J. M McCune, R. Namikawa, H. Kanishima, L. D. Schultz, M. Lieberman, I. L. Weissman, *Science* **241**, 1632 (1988).
4. S. Tonegawa, *Nature* **302**, 575 (1983).
5. The molecular aspects of lymphocyte differentiation and the mechanism of variable region gene assembly have been reviewed [F. W. Alt, T. K. Blackwell, G. D. Yancopoulos, *Science* **238**, 1079 (1987)].
6. W. Schuler *et al.*, *Cell* **46**, 963 (1986).
7. B. A. Malynn *et al. ibid.* **54**, 453 (1988); T. K. Blackwell *et al.*, in preparation.
8. E. A. Hendrickson, D. G. Schatz, D. T. Weaver, *Genes Dev.* **2**, 817 (1988).
9. K. Dorshkind *et al.*, *J. Immunol.* **132**, 1804, (1984); A. A. Czitrom *et al.*, *ibid.* **134**, 2276 (1985); K. Dorshkind, S. B. Pollack, M. J. Bosma, R. A. Phillips, *ibid.*, p. 3798.

24 August 1988; accepted 31 August 1988