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# SCID Mice and the Study of Parasitic Disease

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## INTRODUCTION

Parasitic diseases remain a scourge of humans. Malaria alone causes more than a million deaths yearly, while helminths such as hookworm and ascaris infect more than a billion people worldwide. Diseases such as amebiasis and schistosomiasis remain significant public health problems in developing countries, while cryptosporidiosis and giardiasis are continuing threats in the industrialized world. The problem of parasitic diseases goes beyond human infection, because parasitic infection of domesticated animals has a tremendous impact on productivity and health worldwide.

A key to understanding these diseases is the study of the interactions between the host and the parasite. This is a complex relationship that has evolved over millennia and centers on the host immune response to infection. This complexity is underlined by the finding that while some host defense mechanisms may protect against infection, other responses paradoxically exacerbate disease. This is dramatically shown in diseases such as schistosomiasis, in which most of the pathology is secondary to the host immune response to parasite antigens. These considerations are critical when one considers vaccine development for any parasitic disease, and they underlie the need for models that allow us to identify which components, if any, of the host immune response are protective against infection. The challenge then becomes one of finding ways to manipulate the host response to the parasite such that protective responses but not pathological pathways are stimulated.

Animal models of infections have been the central tool in studying the immunopathogenesis of parasitic diseases. Recently, mice with severe combined immunodeficiency (SCID) have been used to study a variety of parasitic diseases. In this review, we will provide an introduction to the biology of the SCID mouse and provide some specific examples of how studies in these mice are generating new insights into the host-parasite interaction.

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## BIOLOGY OF SCID

SCID was first recognized as a rare primary immunodeficiency of humans that is defined by a congenital absence of functional B and T cells (33). Because of the protection afforded by maternally transmitted antibodies, children affected with this disease generally appear healthy during the first several months of life, becoming ill with a variety of bacterial, viral, and opportunistic pathogens upon the disappearance of these antibodies (reviewed in reference 75). In 1983, Bosma et al. described a mouse showing the SCID phenotype (14). Like their human counterparts, these mice show severe lymphopenia and agammaglobulinemia and are susceptible to a variety of infections. Only when SCID mice are isolated in specific-pathogen-free barriers can they be maintained and kept from succumbing to fatal infections. The propagation of these mice has led to their use as an experimental tool in studies of normal immune system components as well as various host-pathogen interactions.

The human SCID phenotype is caused by a variety of genetic defects, with both X-linked and autosomal recessive patterns of inheritance (reviewed in references 75 and 76). Reported causes of SCID in humans include the absence of enzymes in the purine nucleotide cycle (30, 31), failure to express major histocompatibility protein II on the surface of lymphocytes (55, 87), defects in signal transduction pathways (21), and, in the case of X-linked disease, a mutated form of a chain shared by a family of cytokine receptors (53, 67).

The *scid* defect in mice appeared spontaneously in the inbred mouse strain C.B-17, which is congenic with the BALB/c mouse strain, differing only at the immunoglobulin heavy-chain locus (14). SCID mice have both low levels of circulating lymphocytes and hypoplastic peripheral lymphoid tissues, and they lack circulating immunoglobulins. The genetic defect in SCID

mice does not appear to correlate with any of the defects seen in human SCID. Instead, the defect in SCID mice is associated with the recombinase activity responsible for the generation of B- and T-cell antigen receptors (80). Both B and T cells utilize a gene rearrangement strategy to generate high levels of receptor diversity without using a large percentage of the germ line (reviewed in reference 54). In this process, the variable (V), diversity (D), and joining (J) regions targeted for rearrangement are flanked by conserved signal sequences. Protein-protein interactions involving these signal sequences bring the targeted areas of the DNA into close proximity, and blunt-end double-stranded breaks are then formed in a site-specific manner. A re-sorting of the ensuing four free DNA ends occurs, and the formation of two new junctions results in proper rearrangement and coding for a functional receptor. Failure of proper joining of these ends results in the failure to produce the antigen-specific receptors of the B and T cells. As the surface expression of these receptors is an important checkpoint in the development of these cells, the cells fail to mature beyond this initial stage.

The *scid* gene is 1 of 15 genes that have been implicated in this rearrangement process. A clue to the function that the *scid* gene product may have in this process is that SCID mice not only have defective T and B cells but also show an inability to repair double-stranded DNA breaks after exposure to irradiation or chemical agents (27). The common link between these two defects is the recombination of the four single-stranded DNA ends into properly joined DNA segments. Further speculation about role of the *scid* gene product can be derived from recent experiments in which the treatment of newborn SCID mice with low-dose irradiation or a chemical capable of inducing double-stranded DNA breaks restored normal V(D)J rearrangement in T cells but had no effect on the restoration of DNA rearrangement in B cells (24). These results are intriguing because they point to a difference in the gene rearrangement machinery, or the regulation of that machinery, between B and T cells. They also are consistent with the *scid* gene playing a role in the regulation of a protein important in gene rearrangement rather than actually encoding the protein itself. Thus, the introduction of double-strand breaks, either chemically or with the use of irradiation, would bypass the *scid* regulation defect and turn on the necessary downstream double-strand break repair mechanism. Alternatively, the induction of double-strand breaks induces another break repair mechanism that is present in T cells and not B cells, which is capable of complementing the *scid* defect but is distinct from the pathway of the *scid* gene product. Genetic mapping has localized the *scid* defect to chromosome 16 of the mouse and human chromosome 8 (15, 48, 49, 52).

Recent findings have shown that reconstitution of radiation-sensitive SCID cells correlates with the presence of a component of a DNA-dependent serine/threonine kinase that is encoded on human chromosome 8 (47). Furthermore, the investigators have found that SCID mice show greatly reduced levels of this kinase when compared with wild-type mice. The role that the kinase plays in the repair of double-stranded DNA breaks remains to be clarified; possibilities include (i) an initial signal to the DNA damage repair machinery on DNA damage and (ii) alteration of the chromatin structure around a DNA lesion, allowing for access of the DNA repair machinery.

The defect in SCID mice affects only the B and T lymphocytes that constitute the specific immune system and does not alter what has been termed the innate immune system, consisting primarily of macrophages, natural killer cells, and neutrophils. The SCID mouse has provided an excellent tool for the study of this arm of the host immune response in the absence of lymphocyte-based specific immunity. Studies of SCID mice infected with the intracellular pathogen *Listeria monocytogenes* led to the delineation of a previously undefined pathway for macrophage activation (9; reviewed in reference 10). In this pathway, cytokine cascades involving only the macrophage and the natural killer cell are capable of activating macrophages to heightened levels of phagocytic and bactericidal activity (11, 88, 89). Further studies of *Listeria*-infected SCID mice have elucidated the role of each of the three major cell types of the innate immune system in the host response to infection (8, 23, 73, 96). Thus, the use of SCID mice as a system devoid of specific immunity has led to the elucidation of many of the pathways of innate immunity in the mouse.

One potential problem in using the SCID mouse as an immunologic tool is that the *scid* defect is not absolute. Some animals are capable of carrying out functional V(D)J and VJ rearrangements and generating functional T and B cells (16, 18). These animals are termed leaky; they can be detected by monitoring SCID mice for the presence of immunoglobulin G in serum. The leaky phenotype increases with age and antigen exposure and can be minimized by housing animals in as clean an environment as possible and by using young SCID mice. The functional significance of the leaky phenotype is unclear, because it has been shown that the B and T cells present in leaky SCID mice are pauciclonal, with a limited receptor diversity. Many B cells cloned from leaky mice had identical V(D)J rearrangements and identical specificity (32, 44).

SCID mice have now been used to study a wide variety of disease states, including the host response to viral, bacterial, fungal, and parasitic pathogens. We can define three general ways in which SCID mice have been used to study parasitic diseases (Fig. 1). First, for several parasitic diseases for which a murine or small-animal model was lacking, infection has been successfully established in SCID mice. In such instances, the successful infection of the SCID mouse has served to greatly advance the ease with which the parasitic disease can be studied. Second, establishment of a parasitic infection in SCID mice provides the opportunity to examine the role of individual components of the specific immune response in disease. Because SCID mice lack B and T cells, they can be reconstituted with defined populations of naive or immune lymphocytes which will all be of donor origin (Fig. 2). This is a powerful tool in the mouse, in which the availability of specific antibodies against multiple lymphocyte markers allows for the reconstitution of SCID mice with lymphocyte subsets, such as $CD4^+$ T cells. Finally, because SCID mice lack lymphocyte-based immunity, they can accept xenografts from other species, including humans. If infection can be established in the human-derived xenograft, a parasite that naturally infects only humans can be studied in the mouse. This can be an extraordinarily useful system for studying pathogenesis or initiating therapeutic trials.

## USE OF SCID MICE TO ESTABLISH ANIMAL MODELS OF PARASITIC DISEASES

### Filariasis

Human filariasis, caused by nematode worms, is a major public health problem in many developing countries. Lymphatic forms of the disease caused by *Wuchereria bancrofti* and *Brugia malayi* infect 100 million people worldwide, while *Onchocerca volvulus* infects as many as 20 million people and is the major cause of blindness in areas where the infection is endemic (4, 5). The life cycle of these filarial parasites is complex and involves arthropod vectors, multiple larval stages, and

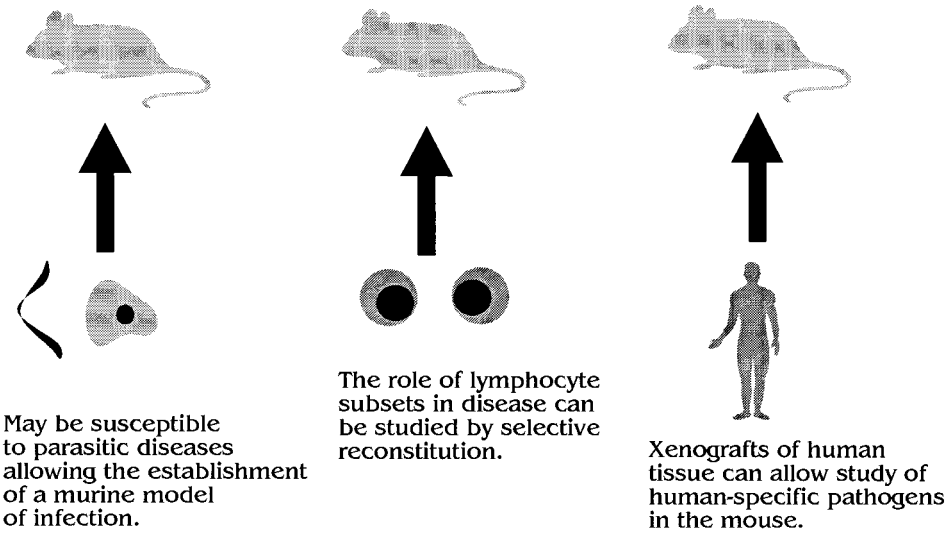


FIG. 1. Role of SCID mice in the study of parasitic diseases.

the presence of both adult worms and microfilarial stages in humans. Studies of the role of the immune system in the pathogenesis of filarial diseases have been plagued by two factors, the lack of a suitable small-animal model and the inability to propagate the organism outside the host. Small-animal models of the infection caused by *B. malayi* were limited to the Mongolian jird and the ferret, neither of which accurately mimics the pathology seen in humans (6, 39). Further limitations to these models are that the immune systems of the jird and the ferret are not well defined and that specific immunologic reagents for these animals are not available. The small-animal model in which the immune system is best defined, the mouse, is resistant to infection with *B. malayi.*

In contrast, SCID mice proved to be susceptible to infection by *B. malayi* (66). *B. malayi* L3 larvae introduced intraperitoneally or subsequently by subcutaneous infection (to mimic the bite of an infected mosquito) into SCID mice developed into adult worms in 90% of the animals. Furthermore, the anatomic localization of the developing worms accurately mimicked that seen in the human disease, because worms were found in the afferent lymphatics and in the perilymphatic sinus of the lymph nodes. There was also inflammation associated with the developing worms, leading to lymphatic dilatation and retention of lymph. Reconstitution of SCID mice with spleen cells from the congenic C.B-17 strain rendered the animals resistant to *B. malayi* infection (66). If infection was allowed to become patent in SCID animals prior to reconstitution, however, the course of infection was not altered, suggesting that lymphocyte-based immunity is critical in preventing the initiation of filarial infection but is ineffective against established disease.

A limiting factor in the study of *O. volvulus* has been the inability to maintain the parasite outside of primates. *O. volvulus* will grow only in the natural human host and a select group of nonhuman primates (1). Recently, in an attempt to establish infection in a murine system, human onchocercomata (skin nodules containing adult worms) were subcutaneously implanted into SCID mice (70). After up to 20 weeks in the mouse, onchocercomata contained both viable adult worms and microfilaria. This model is therefore the first to successfully culture *O. volvulus* outside a primate, and it could lead to an improved method for obtaining viable worms to study.

Both the *B. malayi* and the *O. volvulus* models of infection in the SCID mouse have provided new avenues for research on these parasites. The *B. malayi* model has a number of similarities with human disease and is being used to further define the necessary components of the immune system for protection against infection. The *O. volvulus* model, although not mimicking the disease in humans, also constitutes a significant advance in the field by providing a more convenient and feasible method for cultivating the parasite and a potentially useful tool for the evaluation of therapeutic agents capable of eliminating or altering the development of *O. volvulus.*

## Amebiasis

The protozoan parasite *Entamoeba histolytica* causes amebic dysentery and amebic liver abscess, both major causes of morbidity and mortality worldwide (95). Despite the medical importance of this organism, little is known about the nature of the host response to amebic infection. While experimental models of amebic liver abscess have been successfully established in the jird and hamster, the lack of specific immunologic reagents in each of these systems has hampered progress in this area. In response to the need for a reliable murine model of infection, attempts were made to establish amebic liver abscesses (22) and intestinal amebiasis in SCID mice. Direct inoculation of virulent amebic trophozoites into the liver of SCID mice resulted in the development of large amebic liver abscesses (occupying more than 25% of the liver) with histopathologic findings that resemble those of amebic liver abscesses in humans (22). The congenic C.B-17 mice have proved much more resistant to infection, although amebic liver abscesses do develop in some C.B-17 mice. Recently, in a closed-loop model of infection (3, 83), intestinal amebiasis has been produced in SCID mice, with both mucosal and submucosal invasion by *E. histolytica* trophozoites (98).

Studies with the SCID mouse model of amebic liver abscess have provided insights into amebic pathogenesis and the nature of protective immunity to amebiasis. In vitro studies have suggested that a 27-kDa cysteine proteinase produced by *E. histolytica* may play a critical role in amebic invasion of host tissues by lysing host proteins, such as laminin, fibronectin, and collagen (45, 84, 86). To test the role of the cysteine proteinase in vivo, SCID mice were challenged intrahepatically with vir-

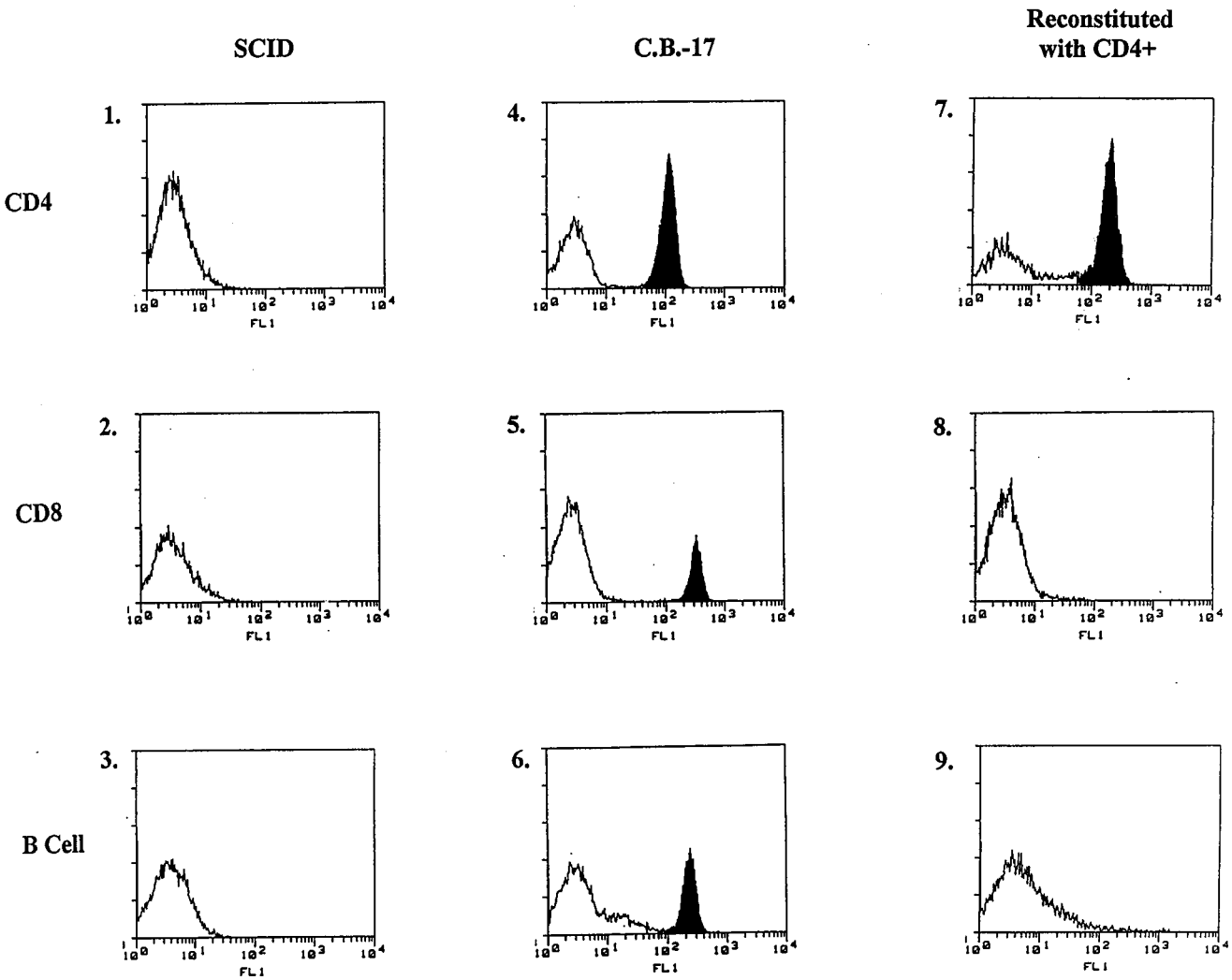


FIG. 2. SCID mice have no detectable $CD4^+$ or $CD8^+$ T cells or B cells in their lymph nodes (panels 1 to 3). In contrast, C.B.-17 mice possess all three of these subsets (panels 4 to 6; the positive cell population is shown by the shaded peak). SCID mice that are reconstituted with $CD4^+$ T cells show $CD4^+$ in their peripheral lymph nodes 10 days postreconstitution (panel 7) but fail to show any $CD8^+$ T cells or B cells (panels 8 and 9).

ulent amebic trophozoites in the presence and absence of the specific cysteine proteinase inhibitor L-*trans*-epoxysuccinyl-leucylamido(4-guanidino)butane (E-64). After 48 h, all control SCID mice had amebic liver abscesses with a mean size of 36% ± 6% of the liver, while among 10 SCID mice challenged with amebae coincubated with E-64, 2 had no liver abscesses and the remaining 8 had amebic liver abscesses occupying only 6% ± 2% of the liver (85). This study strongly suggests that the amebic cysteine proteinase plays an important role in liver abscess formation.

The amebic liver abscess model in SCID mice has also provided new information about the role of antibody in protective immunity to amebiasis. Because patients with amebic liver abscess develop high titers of anti-amebic antibodies, which appear to have no influence on the course of infection, a protective role for antibody in amebiasis has been considered unlikely. However, the role of antibody in preventing the initiation of amebic infection had not been extensively studied. Passive immunization of SCID mice with polyclonal antibodies to amebic antigens or with monospecific antiserum to a protective antigen of amebae, the serine-rich *E. histolytica* protein, before intrahepatic challenge with amebae has provided nearly complete protection of SCID mice from amebic liver abscess (22, 97). This protection is mediated by immunoglobulin G and may work by directly blocking amebic adherence to host tissue. Studies are now under way to further explore the components of protective immunity in both amebic liver abscess and intestinal disease in SCID mice.

## USE OF SCID MICE IN SELECTIVE RECONSTITUTION EXPERIMENTS TO ELUCIDATE THE ROLE OF LYMPHOCYTE-BASED IMMUNITY IN PARASITIC DISEASES

### Leishmaniasis

Leishmaniasis is a disease caused by a variety of dimorphic protozoan parasites of the genus *Leishmania*. In its promastigote form, the parasite is introduced into the host via the bite of an infected sandfly. The promastigote is then internalized specifically by the host macrophage by receptor-mediated endocytosis. Within the phagolysosome of the macrophage, the

parasite differentiates into the amastigote form, which can proliferate and spread to neighboring cells. Leishmanial infection in humans covers a spectrum of clinical outcomes ranging from spontaneous self-healing skin lesions to fatal systemic visceral disease. The fact that different inbred mouse strains also show this wide range of disease severity has led to the elucidation of many of the components of the immune response responsible for susceptibility and resistance to *Leishmania* spp. Although the SCID mouse did not play an integral role in the initial characterization of these responses, it has proven to be an elegant model for the confirmation of a number of these findings.

Most mouse strains, including C57BL/6, display self-healing lesions when cutaneously challenged with *Leishmania major*. The BALB/c strain, however, presents with a progressive disease that eventually leads to a fatal outcome. This dichotomy in disease progression appears to be the result of a difference in cytokine responses between the mouse strains (38, 82), which has been attributed to differences in the response to infection by the two major helper T-cell subsets (81, 94). $CD4^+$ helper T-cell (Th) lymphocytes can be divided into a Th1 subset that produces predominantly gamma interferon (IFN-γ) and interleukin-2 (IL-2) and a Th2 subset that produces predominantly IL-4, IL-5, and IL-6 (65). Because of mutual downregulation of the opposing response or a lack of capability to synthesize both sets of cytokines simultaneously, the immune response to a particular antigen is polarized toward either a Th1- or Th2-dominated response (41). The Th1 cytokine profile is associated with cell-mediated immune responses, characterized by the delayed-type hypersensitivity response, whereas the Th2 cell is more proficient at promoting antibody production from B cells. Thus, C57BL/6 mice, which mount a predominantly Th1 response, cure themselves after leishmania challenge, whereas BALB/c mice, which characteristically respond with a Th2-type response, develop progressive infection. This difference in clinical outcome is thought to be due to the production of IFN-γ by the Th1 cell type, leading to activation of the parasitized macrophage to a state of increased parasiticidal activity (71; reviewed in reference 56). Experiments in which administration of a IFN-γ-neutralizing antibody has been shown to change the healing phenotype to that of a nonhealer are compatible with this view (13).

The SCID mouse has proved to be an ideal system for confirming the critical role of the helper T-cell response in the outcome of leishmanial infection. *Leishmania*-specific T cells of either the Th1 or Th2 phenotype (confirmed in vitro by cytokine profile analysis) were injected into SCID mice, and the recipient mice were subsequently challenged with *L. major* (40). The animals reconstituted with the Th1 clone were able to control the infection, displaying the healer phenotype, while the animals receiving the Th2 clones developed progressive disease. This clearly demonstrated that both the healing and nonhealing phenotypes could be transferred solely by the helper T cell. Thus, the ability to reconstitute SCID mice with defined populations of T cells provided strong evidence that the differing clinical outcomes in experimental leishmaniasis can be directly attributed to the nature of the helper T-cell response.

## Cryptosporidiosis

*Cryptosporidium parvum* is a coccidian protozoan parasite that can infect the intestinal epithelial cells of most mammalian species. In an immunocompetent host, the illness is self-limiting, with the host experiencing 1 to 3 weeks of abdominal discomfort and diarrhea. In the immunocompromised host, such as individuals with AIDS, the infection may become chronic and severe. In these individuals, infection can spread to anatomic sites outside the intestinal tract, including the epithelium of the gallbladder, pancreas, or biliary duct. There is currently no effective treatment for the disease; consequently, a large percentage of AIDS patients acquiring *C. parvum* suffer from relapsing, chronic infection (reviewed in reference 29).

The experimental infection of mice with *C. parvum* mimics the situation in humans. Immunocompetent mice develop a self-limiting acute infection, while SCID mice develop a chronic, sometimes lethal disease (60). With a high initial infective dose of cryptosporidial oocysts, extraintestinal manifestations, including primarily hepatobiliary disease, can be seen in SCID mice (62). Furthermore, this extraintestinal hepatic disease is associated with a rise in alkaline phosphatase levels in serum, thus providing a noninvasive method to monitor these complications. This model of *C. parvum* infection may prove very valuable for the testing of therapeutic agents against either intestinal or extraintestinal cryptosporidiosis. Indeed, the SCID mouse model has been used to show that orally administered polyclonal or monoclonal antibodies to *C. parvum* are capable of decreasing oocyst shedding and intestinal epithelial infection in chronically infected mice (68, 72). These interventions fail to decrease the parasite load in the hepatobiliary system, suggesting that alternative methods of delivery or alternative modes of therapy will have to be explored to deal adequately with elimination of infection of these sites.

The clear-cut dichotomy of clinical outcome between immunocompromised and immunocompetent mice in this model lends itself well to the further dissection of the immune system components necessary for protective immunity. SCID mice have been used to study the role of natural killer cells, IFN-γ, nitric oxide, and various T-cell subsets in the control of cryptosporidial infection (36, 51, 61, 74). The role of lymphocyte-based immunity has been dramatically illustrated by studies in which reconstitution of SCID mice with naive spleen cells from C.B-17 mice resulted in a significant drop in oocyst shedding and intestinal infection in chronically infected animals (69). This protection appears to be mediated by the $CD4^+$ subset of T cells, rather than $CD8^+$ cells, because mice treated with antibodies against CD4 or IFN-γ failed to be protected as well as animals treated with anti-CD8 or anti-natural killer cell antibodies (20).

## Toxoplasmosis

*Toxoplasma gondii* is an intracellular protozoan parasite that is often acquired through the ingestion of encysted organisms in undercooked meat. In immunocompetent individuals, disease is often asymptomatic or mild and limited. In infected individuals, however, the parasite persists in an encysted form in muscle or brain. Reactivation of the dormant organism can occur in individuals who become immunocompromised, such as those developing AIDS. In these individuals, the ensuing toxoplasmic encephalitis is a life-threatening infection. This encephalitis can be treated, but the drugs commonly used for therapy fail to act against the encysted form (29). Therefore, life-long therapy is required to prevent subsequent excystation.

The SCID mouse has been used as a model for both the acute and chronic relapsing stages of toxoplasma infection. Infection of SCID mice with virulent strains of *T. gondii* leads to a rapid and fatal necrotizing toxoplasmosis (12). However, the study of infection of SCID mice with less virulent strains of *T. gondii* has established the importance of the natural killer cell in the immune response against *T. gondii* (25, 28, 46, 79).

The release of the cytokine IL-12 by macrophages infected with *T. gondii* causes natural killer cells to secrete IFN-γ, a cytokine that stimulates macrophage phagocytosis and killing of the parasite. To study the chronic relapsing stage of the disease, investigators have used the finding that SCID animals treated with sulfadiazine survive the acute infection, and enter a chronic carrier state similar to that seen in immunocompetent humans (12, 43). An added feature of this model is that subsequent removal of the sulfadiazine treatment will result in parasite reactivation and fulminant disease. Thus, this model allows the establishment of a chronic infection that can be reactivated at will. The SCID mouse model of chronic toxoplasmosis has been used to investigate the role of immune cells in preventing reactivation (12). In SCID mice after withdrawal of sulfadiazine therapy, reconstitution with spleen cells from an immune syngeneic mouse prevented the ensuing death from encephalitis. When either $CD4^+$ or $CD8^+$ cells were depleted from the donor splenic cells prior to transfer, mortality was still prevented, although depletion of $CD4^+$ cells did lead to histologic evidence of reactivation whereas depletion of $CD8^+$ cells did not. Thus, T cells of either subset can suppress the reactivation of chronic toxoplasmosis. This model has significant potential for the study of the pathogenesis of reactivation and for investigation of therapies designed to prevent reactivation in the immunocompromised host.

### Malaria

The nature of protective immunity to *Plasmodium* spp. has been studied in mice by using the murine malarial parasites, *Plasmodium chabaudi* and *Plasmodium yoelii*. Mice that have been depleted of B cells by lifelong treatment with anti-immunoglobulin M antibodies recover from acute *P. chabaudi adami* malaria but go on to develop chronic low-grade parasitemia, showing the need for B cells for sterilization of infection (34). Adoptive transfer studies in nude mice, which fail to clear primary infection, had implicated $CD4^+$ T cells in protective immunity to *P. chabaudi adami* infection (19, 34). However, because nude mice retain a B-cell compartment and the B-cell defect in anti-immunoglobulin M-treated mice may be incomplete, the mechanism of protection by $CD4^+$ cells remained unclear. Recent studies with a mouse genetically engineered to be deficient in B cells have resolved this issue (92). Acute *P. chabaudi adami* malaria is cleared in these mice, indicating that the resolution of this infection is not dependent on the presence of B cells. These mice do, however, maintain a long-term infection after clearance of the acute infection, thus indicating that B cells are necessary for sterilizing immunity.

The selective reconstitution of SCID mice infected with another subspecies of *P. chabaudi*, *P. chabaudi chabaudi*, produced slightly different results. SCID mice will succumb to an injection of *P. chabaudi chabaudi*-infected erythrocytes, whereas normal immunocompetent mice clear the parasitemia and survive. When SCID mice were reconstituted with only the $CD4^+$ subset of T cells from previously infected mice, the parasitemia persisted, although more than 70% of the animals survived (63). When the SCID mice were reconstituted with B cells from immune animals in addition to the $CD4^+$ cells, all mice survived and cleared their parasitemia. These SCID mice also showed significant levels of circulating antimalarial antibodies. Therefore, it appears that although $CD4^+$ cells alone are capable of providing some level of protection from acute *P. chabaudi chabaudi* infection, an antibody response is necessary for sterilizing immunity.

The SCID mouse model has also been used to study the role of αβ and γδ T cells in the response to infection with *P. chabaudi adami* (93). Most T cells express a T-cell receptor that utilizes an α chain and a β chain, but a smaller subset of T cells express a receptor composed of a γ chain and a δ chain. There may be functional differences between these two subsets of T cells (37). In these studies, SCID mice were selectively reconstituted with $CD4^+$ T cells and then infected with *P. chabaudi*. The number of αβ-expressing T cells increased during the development of parasitemia, whereas the number of γδ T cells increased dramatically during the resolution of the parasitemia. In further studies, $CD4^+$ αβ or γδ T cells were selectively depleted with a monoclonal antibody and the relationship between the γδ response and clearance of parasitemia was confirmed (91).

Studies with yet another *Plasmodium* species, *P. yoelii*, have defined a non-T-, non-B-cell pathway in the protection against malarial challenge. In these studies, SCID animals treated with a single injection of IL-12 are capable of clearing acute malarial infection (81a). The authors postulate that this protection is mediated through the cytokine cascade that was originally defined in the *Listeria* system, in which the secretion of IFN-γ by natural killer cells leads to the activation of macrophages (9, 10). The IL-12 functions in this pathway via activation of natural killer cells to secrete IFN-γ. The investigators went on to show that this protection was mediated through an IFN-γ- and nitric oxide-dependent pathway.

### Schistosomiasis

In the case of infection with the helminth *Schistosoma mansoni*, selective reconstitution of SCID mice with a cytokine rather than a cell type has led to elucidation of the mechanism of hepatic pathology in this disease. The major symptom seen in immunocompetent mice that are infected with *S. mansoni* is a granulomatous fibrosis around schistosome eggs in the portal venules of the liver. This pathology is not seen upon infection of SCID mice, thus implicating an immune response against the parasite rather than the parasite itself in disease development. Upon addition of whole spleen cells or upon injection with a single dose of tumor necrosis factor alpha, this granuloma formation is restored (2). A further finding that has arisen from these studies is the permissive quality of tumor necrosis factor alpha to egg laying and egg excretion by *S. mansoni*. These functions are decreased in SCID mice but are restored upon addition of tumor necrosis factor alpha. Therefore, it appears that the pathogen has so adapted to the host immune response that it uses a part of that immune response as a signal to increase its own survival functions.

## USE OF SCID MICE AS XENOGRAFT RECIPIENTS

### Theileriosis

The intraerythrocytic protozoan *Theileria sergenti* is an etiologic agent of theileriosis, a major infectious cause of anemia in cattle. Study of this disease has been severely hampered by the lack of a suitable small-animal model. The inability of the SCID mouse to reject transplants from other species has recently led to the development of such a model. Weekly injections of bovine erythrocytes into the SCID mouse result in long-term bovine erythrocyte survival in the recipient animals (90). When mice are splenectomized prior to infusion with erythrocytes, more than 70% of their circulating erythrocytes are bovine derived, and with weekly infusions, this state has been maintained for more than 60 days (35). To study *T. sergenti*, the investigators infused erythrocytes from infected cattle into SCID mice. They found that when an initial infusion

of cells, 1.9% of which were infected, was followed by infusion of unparasitized cells, a long-term parasitemia was established. The animals maintained a parasite load of greater than 10% of the transplanted bovine erythrocytes for up to 30 days. Thus, as the parasite load increases in the presence of the infusion of unparasitized cells, this model allows for the active spread of the parasite rather than merely the survival of parasitized erythrocytes. Further investigation has revealed that this active spread of parasites can be seen upon injection of the cells by either the intraperitoneal, intravenous, or subcutaneous routes, thus eliminating the possibility that the parasite was replicating in the peritoneal cavity in the initial studies (35). This model appears to be accurate in mimicking the natural course of the disease, a blood-borne spread of the parasite. The development of this model should provide a powerful tool for studies on the chemotherapy of and immune response to *T. sergenti* infection.

### Malaria

The hepatic, or exoerythrocytic, stage of *Plasmodium falciparum* (the major cause of fatal malaria worldwide and a human-specific parasite) has been difficult to study because of the lack of any small-animal model. The ability of SCID mice to accept human xenografts has been used to establish a suitable small-animal model for the exoerythrocytic stage (77). Human adult liver tissue has been successfully engrafted under the kidney capsule of SCID mice. Subsequent intravenous injection of *P. falciparum* sporozoites results in selective infection of the human liver tissue. Immunofluorescence studies have demonstrated that 9 days following infection, the *P. falciparum* sporozoites develop normally and produce numerous merozoites. The potential applications of this model to the study of therapeutic agents, to the characterization of exoerythrocytic stage-specific antigens, and to the understanding of the immune response to the exoerythrocytic stages are numerous. Indeed, Sacci et al. showed that passive immunization of the SCID mice with an antibody reactive to the circumsporozoite protein dramatically decreased infection in this model (77). Other groups, although able to replicate studies of the transplantation of liver cells into the SCID mice, have been unable to replicate the infection of these cells with sporozoites (7). The one barrier that remains between the current model and the completion of the parasite life cycle in the SCID mouse is the establishment of erythrocytic infection. In initial attempts in this area, SCID mice failed to support the survival of human erythrocytes (17). Another group has used slightly different immunodeficient mice, the SCID/nonobese diabetic (SCID/NOD) mice, which have multiple immunodeficiencies in addition to the SCID phenotype (64). This group has recently managed to maintain *P. falciparum*-infected human erythrocytes in vivo for up to 7 days (64). In splenectomized mice, the survival of the erythrocytes increased to 14 days. Furthermore, when anopheline mosquitoes were allowed to feed on the infected mice, the gametocytes were ingested and developed into oocytes in the midgut of the mosquitoes.

### Schistosomiasis

One of the initial hopes for the SCID system was for the complete engraftment of the human immune system into the SCID mouse. The engraftment of a human lymphoid system into the SCID mouse would ideally result in an immunological environment in the mouse that is similar to that of the human. This SCID-Hu chimera could then be utilized in a variety of ways, including the evaluation of human vaccines. While successful engraftment of human peripheral blood cells (PBLs) into SCID mice (Hu-PBL-SCID) has been achieved, full reconstitution of a functional human immune system has proven difficult (59). Initial problems have included poor engraftment of human cells and a failure of engrafted cells to produce primary immune responses unless the cells were primed in vitro prior to their transfer to SCID mice (57). In more recent studies, increasing the number of human donor cells, treating SCID mice with antibodies to deplete natural killer cells, and low-dose irradiation have led to increased levels of human cell engraftment (78). Under these conditions, primary immune responses have been produced in Hu-PBL-SCID mice, but a fatal graft-versus-host disease developed in animals within 21 to 28 days of engraftment (78). Others have found that a primary immune response can be generated in Hu-PBL-SCID mice only when the primary immunogen is conjugated to a protein that is capable of generating a T-cell memory response (42). An unexpected problem that has recently been noted in the Hu-PBL-SCID system is reversion of the SCID phenotype. When large doses of human PBLs are introduced into SCID mice, the human graft appears to complement the mouse defect and antibodies of murine origin are produced (26).

Despite these problems, Hu-PBL-SCID mice have been used successfully in studies of the parasitic helminth *Schistosoma mansoni*. In one study, Hu-PBL-SCID mice produced primary B-cell responses (human antibody) against antigens derived from adult *S. mansoni* worms (58). Although the antibody response was transient, this finding suggests that this model may be useful in studying the human immune response to schistosome antigens. In an intriguing study, PBLs from individuals infected with *Schistosoma japonica* were introduced into SCID mice (50). When the recipient Hu-PBL-SCID mice were immunized with antigens derived from killed *S. japonica*, they developed significant hepatic pathology that resembled the findings seen in human disease. This result is consistent with other data that suggest that the liver damage seen in schistosomiasis is not due to direct damage by the worms but, instead, is due to the immune response against parasite antigens. Why the liver appears to be specifically targeted for damage in what should be a systemic immune response to the schistosome antigens in this system remains unclear. This model should prove highly useful in the further elucidation of the causes of liver pathology in schistosomiasis and should provide a system for evaluating methods for preventing hepatic damage.

## CONCLUSIONS

The SCID mouse has proven to be a valuable tool in furthering our understanding of the host-parasite interaction and in elucidating parasite pathogenic mechanisms. While the SCID mouse model has some limitations, including the leaky phenotype, reversion under certain reconstitution conditions, and resistance to complete human immune system reconstitution, it has become a powerful system for establishing murine models of parasitic infection, studying the role of different lymphocyte subsets in parasitic diseases, and examining human-specific parasitic infections in the mouse. Improvements in reconstitution and xenograft techniques, the continued growth in knowledge about the biology of the SCID defect, and genetic manipulation of SCID mice should broaden the potential applications of these animals in the study of parasitic diseases and provide new tools for understanding the host-parasite interaction.

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