The Immunodeficient *scid* Mouse as a Model for Human Lymphatic Filariasis

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Summary

The C.B.-17-*scid/scid* mouse (hereafter referred to as the *scid* mouse) is homozygous for a recessive mutation at a locus that influences the assembly of intact immunoglobulin and T cell receptor genes. Therefore, *scid* mice cannot generate functional B or T lymphocytes, are profoundly immunodeficient, and have been reported to be receptive to reconstitution with human immune cells. In the present study, we injected *scid* mice with infective larvae of the human filarial parasite *Brugia malayi*. Within 6–10 wk after subcutaneous injection of infective L3 larvae, both male and female worms were observed in various stages of development in 90% of the mice. In animals tested 8 weeks or more after infection, microfilariae were detected in the blood or peritoneal cavity of 52% of the mice examined. Adult worms were observed in the lymphatics of the infected *scid* mice, where their presence was associated with lymphangitis and lymphangiectasia. These results suggest that the *scid* mouse model of lymphatic filariasis may be important in investigation of the interaction of the murine, and possibly the human, immune system with the lymphatic filarial parasite.

Filariasis is a group of diseases caused by nematode worms. Human filariasis is a major public health problem in a number of developing countries, where there are an estimated 100 million cases of the lymphatic form, caused by the organisms *Wuchereria bancrofti* and *Brugia malayi* (1), and about 20 million cases of the ocular form caused by the organism *Onchocerca volvulus* (2). The sexes are dioecious and dimorphic and, after mating, the females liberate larvae called microfilariae (mf)[1]. Transmission from one vertebrate animal to the next occurs via a blood-sucking arthropod vector, which ingests the mf when they feed on infected individuals. Vector passage lasts 14 d and is obligate for the parasite to become infective to man. Within the vector, the mf larvae undergo defined molts to become the infective larvae (also called the L3 larvae).

Numerous investigators have postulated that host immune effectors have a major role in the development of overt symptomatology in filariasis (3) and that the determination of the immunopathogenesis is central to our understanding of the disease. Whereas work on animal model systems has greatly assisted research into the basic pathophysiology of a number of human diseases, filariasis suffers from the lack of an optimal animal model. The most widely investigated animal model is that of Ash and Riley (4) who demonstrated that a substrain of *B. malayi* would grow in a rodent called the Mongolian jird (*Meriones unguiculatus*). The major deficiency of this animal model is that the infection in the jird does not mimic the human disease in the anatomic localization of the adult worms, in the symptomatology, or in the immune effector mechanisms that may be involved. Furthermore, reagents for identification and characterization of immunoglobulins or lymphoid cell subsets in the jird are not readily available. Another animal model, using the ferret (5), suffers from similar handicaps.

Given the extensive knowledge of murine immunogenetics, the mouse would be the ideal animal for any investigation of the role of the immune system in the immunopathogenesis of a disease. Such studies have been hampered by the fact that normal, immunocompetent mice are nonpermissive for infection with human filarial parasites. Over the past decade, Sodeman, Vickery, and collaborators (6–11) have performed elegant studies on the T lymphocyte immunodeficient mutant mouse called *nude* and have shown that it can serve as a host for Brugian filarial parasites. These studies prompted us to examine whether the T and B cell immunodeficient *scid* mouse would similarly permit the growth and maturation of filarial parasites.

In the present study, we report the establishment of a new animal model of human filariasis using the severe combined immunodeficiency (*scid/scid*) mouse. The *scid* mouse, which lacks functional T and B lymphocytes (12–14), readily allows

[1] *Abbreviation used in this paper:* mf, microfilariae.

the growth and differentiation of infective *B. malayi* larvae into mature adults, which mate and produce mf. This model should allow the future investigation into the interaction of the murine and human (15, 16) immune system with the lymphatic filarial parasite.

Materials and Methods

Mice. C.B.-17-*scid*/*scid* mice were obtained from the breeding colony of Dr. Leonard Shultz at The Jackson Laboratory, Bar Harbor, ME. Because of their severe immunodeficiency, these animals were maintained prophylactically on a combination of sulfamethoxazole and trimethoprim (15), which prevents them from succumbing to *Pneumocystis carinii* pneumonia.

Antibodies. The anti-CD3 mAb (2C11) was the kind gift of Dr. J. Bluestone, Chicago, IL, and was developed for immunofluorescence with mouse anti-hamster Ig-FITC. Surface Ig^+ (B) cells were detected with an FITC goat anti-mouse IgG (heavy and light chain specific). The percent positive cells were determined by flow cytometry as previously described (17).

ELISA. The amount of Ig present in the serum of mice was determined by ELISA, as previously described by Bosma et al. (12).

Recovery of B. malayi L3 Larvae. Infective *B. malayi* L3 larvae were obtained from mosquitos using procedures described by Ash and Riley (4). The larvae were counted and aliquoted by hand, and injected intraperitoneally or subcutaneously into mice using 21-gauge needles.

Histology. Tissues from necropsied mice were fixed immediately in Bouin's fixative (75 ml of 12% picric acid, 25 ml formalin, 5 ml acetic acid). 24 h after Bouin's fixation, they were washed in water and placed in 10% PBS-buffered formaldehyde solution. The tissues were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopic examination.

Results and Discussion

Fate of Infective Larvae Injected Intraperitoneally. To examine the fate of *B. malayi* L3 larvae in the *scid* mouse, seven mice were injected intraperitoneally with 50 infective L3 larvae each. Six mice were examined by necropsy 6 wk later. Adult worms and mf were present in the peritoneal cavities of all six mice. The yield of adult worms ranged from 6 to 12 per mouse, which compares favorably with the recovery of adult worms in previously described animal models of lymphatic filariasis (4–11). The remaining mouse was examined 4 mo after L3 injection, and adult worms and mf were present in the peritoneal cavity.

Fate of Infective Larvae Injected Subcutaneously. The peritoneal infection of mice with *B. malayi*, which results predominantly in peritoneal localization of adult worms, does not resemble closely the human infection. The anatomical localization of the worms is different from that in man, and the worms in the peritoneal cavity do not cause lymphatic histopathology. Therefore, we sought to define whether subcutaneous injection (the physiological mechanism of entry via the mosquito vector of infective larvae into the mammalian host) of L3 larvae would allow the adult worms to preferentially home to the lymphatics as in humans. 37 *scid* mice were each injected subcutaneously with 50–100 *B. malayi* L3 larvae in the left inguinal area and necropsied periodically to determine: (*a*) the anatomical localization of the injected worms; and (*b*) the histopathology induced in the host.

Of special interest were the histological results. No histopathology was observed in the liver, spleen, or testis of the subcutaneously injected mice. Numerous adult worms, L4 larvae, and mf were observed in the heart and lungs, but histopathology in these organs has not yet been systematically examined. The most consistent histopathological changes were found in and around the lymphatics. Representative tissue sections are demonstrated in Fig. 1. As early as 3 wk after subcutaneous injection of larvae, we were able to demonstrate developing worms in the afferent lymphatics and in the perilymphatic sinus of the lymph node (Fig. 1, *A* and *B*). In some cases, there were significant inflammatory changes and retention of lymph fluid containing proteinaceous material (Fig. 1, *C* and *D*) associated with the presence of the worms. In addition, inflammatory cells were seen accumulating both within lymphatics (*E*) and in the perilymphatic adipose tissue (*F*). Since *scid* mice lack functional T and B cells (12–14) and since eosinophilia is dependent on cytokines produced by T cells, two very striking features of this inflammatory infiltrate are the absence of lymphocytes and eosinophils.

Immune Reconstitution Does Not Clear an Established B. malayi Infection. Immunocompetent (C.B.-17-+/+) mice were resistant to infection with *B. malayi* L3 larvae (Table 1). This led us to examine whether an established infection could be eliminated by immunocompetent spleen cells. A group of seven *scid* mice were each given three subcutaneous injections of 50 infective stage L3 larvae at ~1-mo intervals. The worms were allowed 11 wk to grow to maturity. The animals were reconstituted with 2.5×10^7 naive C.B.-17-+/+ spleen cells intraperitoneally. After 4 wk, the reconstituted mice were necropsied and worm burdens determined. Although T lymphocytes, B lymphocytes, and circulating Ig were present in the reconstituted *scid* mice, adult worms were present at levels similar to that of nonreconstituted *scid* mice (Table 1). The data suggest that an established *B. malayi* infection will not be cleared by reconstitution with naive immune cells.

These observations suggest that the *scid* mouse is an excellent model for human lymphatic filariasis. Subcutaneous injection of *B. malayi* L3 larvae into *scid* mice recapitulates some aspects of the human infection, in that; (*a*) some fraction of the parasites home to lymphatics where they cause lymphatic dilation, retention of lymph, and inflammatory changes, including lymphangitis and lymphadenitis; and (*b*) immunological reconstitution of *scid* mice before injection of infective larvae prevents the establishment of infection, whereas a normal, functional immune system is unable to cure established infection. Since the only difference between *scid* mice (permissive for infection) and C.B.-17-+/+ mice (nonpermissive for infection) is the absence or presence of an antigen-specific immune system, these data indicate that a functional immune system is critical for the prevention of the establishment of infection in mice. It is not surprising that a rare (1/4) C.B.-17-+/+ mouse examined 19 d after L3 injection harbored viable L4 larvae. Vincent et al. (10) have previously observed viable worms in euthymic mice up to 27 d after injection of infective larvae, that with time, are effectively

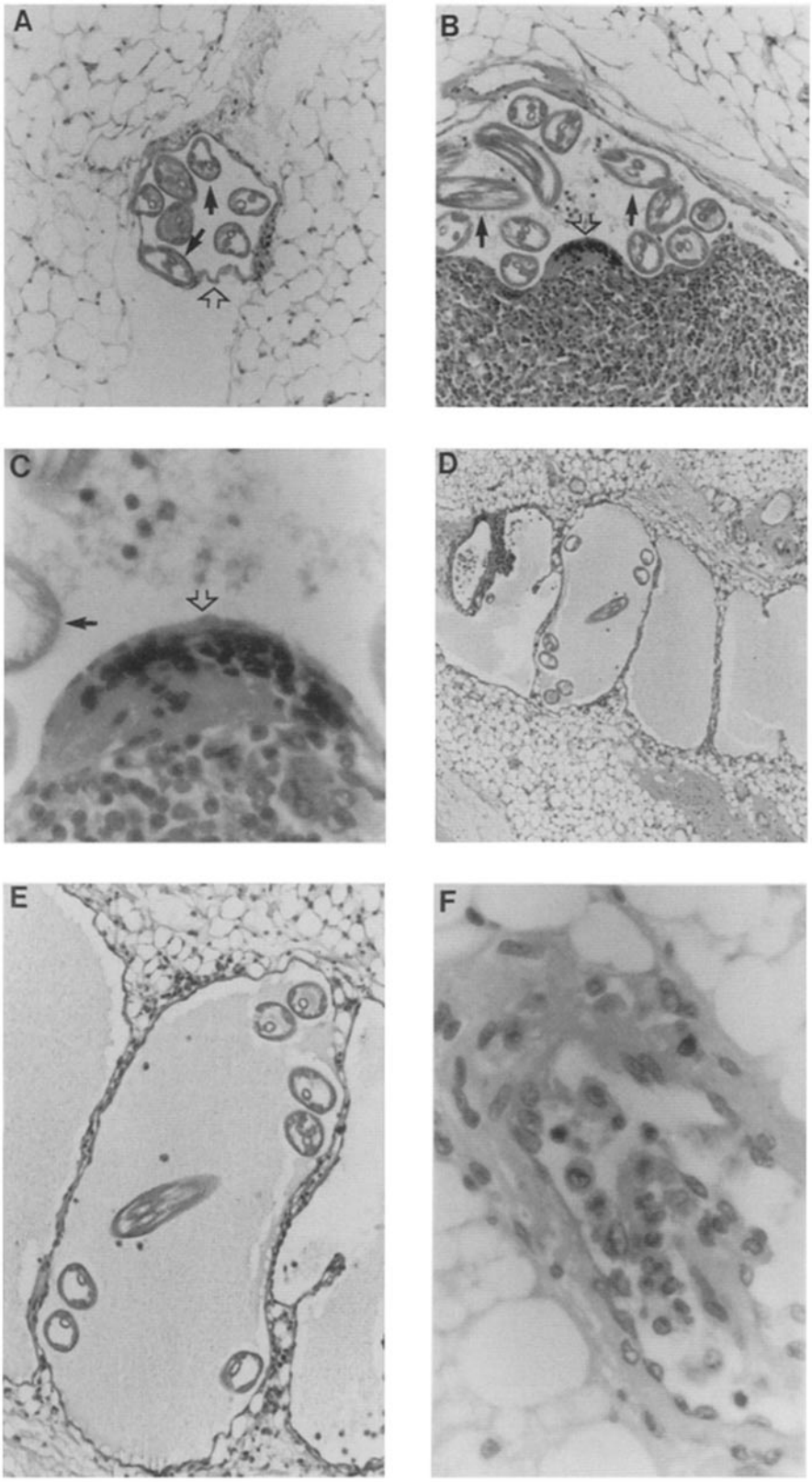

Figure 1. (*A*) A lymphatic (*open arrow*) from a *scid* mouse injected with L3 larvae 3 wk before necropsy. There is no perilymphatic or intralymphatic inflammatory reaction. While numerous cross-sections of nematode are visible (*filled arrows*), they may represent a single worm cross-cut multiple times or several worms, each cut once. (*B* and *C*) Micrographs of the pericapsular lymphatic sinus of the same lymph node at different magnifications. The filled arrows (*B*) point to cross-sections of worm(s). Note the multinucleated giant cell response to the presence of the worms in the pericapsular lymphatic sinus (*open arrow*, *B* and *C*). As in *A*, we are uncertain whether the numerous cross-sections of worms seen in this view represent a single worm or several. (*D* and *E*) Views of a single, highly dilated and tortuous lymphatic containing worms suspended in proteinaceous fluid, at different magnifications. By comparison with the size of cross-section of the worm, we are able to estimate that the lymphatic has been dilated to ~500 μm. (*F*) A lymphatic cut at some distance from the location of the worm(s) shown in *D* and *E*. The lymphatic is dilated and contains inflammatory cells. These cells are predominantly polymorphonuclear cells and monocytes. Notable is the lack of lymphocytes or eosinophils.

cleared in vivo. Furthermore, since *scid* mice lack only functional T and B lymphocytes, while normal natural killer and macrophage activity can be detected (18, 19), these results further suggest that the protective mechanism is T and/or B lymphocyte dependent. This is an important conclusion since it suggests that an appropriate antigen(s) may be

Table 1. *Infection and Immune Status of B. malayi–infected Mice*

Host	Reconstitution	Infection status			Immune status			
						Spleen lymphocyte subsets*		
		Infected‡	Worm burden§	Microfilariae‖	Cells/spleen	CD3	sIg	Serum Ig
					$\times 10^{-6}$			*mg/ml*
scid	–	26/29 (90%)	6.6 ± 6.4	15/29 (52%)	5.7 ± 5.1	<2.0	<2.0	<0.1
+/+	–	1/4 (25%)	4¶	N/A	39.0 ± 11.0	28.3 ± 3.2	59.6 ± 4.3	6.7 ± 0.5
scid	Spleen**	7/7 (100%)	3,9 ± 2.7	3/7 (43%)	10.0 ± 9.3	29.6 ± 12.7	19.4 ± 12.6	9.8 ± 2.9

* The percentage of spleen lymphocyte subsets was determined by flow cytometry as previously described (17). Results represent the mean ± SD of three or more mice.

‡ Mice were necropsied 8 or more wk after subcutaneous injection of 50–100 infective *B. malayi* L3 larvae. The frequency of infection was determined by gross examination of inguinal and mesenteric lymphatics, heart, lungs, liver, spleen, scrotal area, and peritoneum as described in Materials and Methods. This value represents a minimal estimate of worm burden since not all tissues and organs were examined. Two mice that failed to demonstrate adult worms upon gross examination contained mf.

§ Worm burden was determined as above and the value represents the mean ± SD only of mice that contained worms.

‖ mf counts were determined only from blood and peritoneal lavage fluid. Only those mice that were allowed to survive at least 8 wk post-infection are included. The N/A across from the C.B.-17-+/+ mice stands for not applicable, since these mice were necropsied before patent infection would develop. The percentage of mice demonstrating mf represent a minimal estimate since we find that the proportion of mice with mf increases with time after infection.

¶ One C.B.-17-+/+ mouse had four L4 larvae when examined 19 d after infection.

** Mice were injected with naive C.B.-17-+/+ spleen cells 11 wk after infection with L3 larvae.

identified for vaccination of individuals in endemic areas to prevent infection.

The *scid* model we have described will hopefully complement the studies of Vickery et al. and Vincent et al. (6–11) on the *nude* model and further emphasize the importance of an antigen–specific immune response in the defense against filarial parasites. Given the extensive knowledge of murine immunogenetics, it should be possible, using these two models, to approach aspects of the role of the immune system in pathology and resistance to infection in a manner that would not be feasible with other animal models. In addition, the *scid* model could permit two types of studies that may not be possible in the *nude* system: it will be possible to reconstitute the *scid* mouse with purified T lymphocytes or thymocytes to generate a mouse that possesses only T cells, in the absence of B cells. Such a reconstitution in the *nude* mouse will generate an essentially normal mouse, since there is no endogenous defect in its B cell lineage. Such a T cell reconstitution in the *scid* mouse will allow us to determine whether T cells alone, in the absence of any humoral antibody response, can prevent the establishment of filarial infection. Finally, it has been reported that there is the prospect for the establishment of a functioning human immune system in the *scid* mouse. This exciting but developing technology may allow us, in a system unique to the *scid* mouse, to determine the interactions between the human immune system and a human parasite in a manner that may not be possible in any other model.

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