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Role of Gamma Interferon and Interleukin-4 in Host Defense against the Human Filarial Parasite *Brugia malayi*

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We have investigated the roles of gamma interferon (IFN-γ) and interleukin-4 (IL-4) in host defense against *Brugia malayi*. Our data suggest that the lack of either IFN-γ or IL-4 prolongs the time required to achieve sterile immunity, suggesting that both canonical type 1 and type 2 responses are involved in the clearance of infection.

Lymphatic filariasis afflicts over 120 million people worldwide (2). Despite decades of work, many details of host-parasite interactions in this disease remain obscure. Mice bearing targeted mutations in various genes that encode immunologically relevant proteins are powerful tools used to analyze these interactions. Many of these mutations were initially engineered in embryonic stem cells of the 129/SvJ strain and bred into the segregating (C57BL/6J × 129/SvJ [hereafter, B6,129S]) background. In order to study the effect of background genes on parasite resistance in these mice, we examined *Brugia malayi* larval development in B6,129S and C57BL/6J mice in the absence of T and B cells. Our results prompted us to examine the impact of critical cytokines, gamma interferon (IFN-γ) and interleukin-4 (IL-4), on the growth of *B. malayi* using BALB background mice bearing homozygous disruptions of the genes encoding these two cytokines.

B6,129S-Rag1^{tm1Mom} (hereafter, B6,129S-Rag1), C57BL/6J-Rag1^{tm1Mom} (hereafter, B6-Rag1), BALB/cByJ $^{+/+}$ (hereafter, +/+), BALB/c-*Prkdc*^{scid}/*Prkdc*^{scid} (hereafter, SCID), BALB/c-I14^{tm2Nmt} (hereafter, IL-4 KO), and BALB/c-Ifng^{tmITS} (hereafter, GKO) mice were obtained from Leonard Shultz (The Jackson Laboratory, Bar Harbor, Maine) and housed under specific-pathogen-free conditions in micro-isolator cages. All of the mice used in this study were males between 4 and 8 weeks of age. L3 larvae of *B. malayi* were obtained from the insectarium of Thomas Klei (Louisiana State University, Baton Rouge) and injected at a dose of 50 L3 larvae per mouse intraperitoneally (4). Mice were necropsied at the different time points indicated. Peritoneal lavages from individual mice were examined under a dissecting microscope to quantitate adult worms and to determine the presence of microfilariae (MF). Mouse carcasses were further soaked with their peritoneal cavities open in Tris-buffered saline for collection and counting of the remaining worms.

We infected B6,129S-Rag1 and B6-Rag1 mice with *B. malayi* L3 larvae and examined L4 larval yields 2 weeks following infection. B6,129S-Rag1 mice exhibited consistently smaller larval burdens than B6-Rag1 mice (Table 1), ranging from 25 to 50% of those of B6-Rag1 mice (Table 1). These data suggest that the segregating B6,129S background is poorly permissive for *B. malayi*, even in the complete absence of adaptive immunity, and may therefore be an inappropriate background in which to study the impact of various components of adaptive immunity on host resistance to *B. malayi*. In view of these results, we decided to examine the role of IFN-γ and IL-4 in resistance to infection using knockout mice in the more permissive BALB background.

To examine the role of IFN-γ in host resistance, we infected cohorts of +/+, SCID, and GKO mice with 50 *B. malayi* L3 larvae. Cohorts of mice from each group were necropsied at various time points following infection, and worm burdens were determined (Table 2). This allowed us to examine both the yields of adult worms and the development of patent infection (i.e., the generation of MF). SCID mice had greater worm burdens than +/+ mice at all time points. GKO mice exhibited significantly greater worm burdens than +/+ mice at the early phase ($P < 0.05$). At later time points, they appeared not to differ from wild-type controls by statistical evaluation.

To determine the importance of IL-4, we infected +/+, SCID, and IL-4 KO mice with *B. malayi* (Table 3). IL-4 KO and SCID mice had significantly greater worm burdens than +/+ mice at all of the time points examined. In addition, IL-4 KO and SCID mice were positive for MF at 10 and 12 weeks.

Our results suggest that both IL-4 and IFN-γ play some role in host resistance to *B. malayi*. Unlike situations wherein Th1 and Th2 responses yield polar results (3), the complete clearance of *B. malayi* is delayed in the absence of either pathway. The effects of the lack of IL-4 are more profound in that patent infection develops in its absence. However, it is important to note that even in the absence of IL-4, worm burdens were smaller than in SCID mice at all of the times examined. Thus,

TABLE 1. Growth of *B. malayi* L4 larvae in B6-Rag1 and B6,129S-Rag1 mice

Expt	Mutant strain (no. of mice)	Mean % larval recovery ± SD[a]
1	B6-Rag1 (5)	71.6 ± 9.4
	B6,129S-Rag1 (5)	16 ± 8.3
2	B6-Rag1 (7)	50.9 ± 6.2
	B6,129S-Rag1 (6)	22.7 ± 25.9

[a] Larval yield is expressed as a percentage of the number of L3 larvae injected. The mean larval yields from B6-Rag1 mice are significantly different from those from B6,129S-Rag1 mice in both experiments at week 2 postinfection ($P < 0.05$).

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TABLE 2. *B. malayi* infection in BALB/c-SCID, GKO, and +/+ mice

Expt	Genotype (no. of mice)	Mean % worm recovery[a] ± SD at:				Peritoneal MF at wk 10
		Wk 4	Wk 6	Wk 7	Wk 10	
1	SCID (5)		35 ± 9		47.5 ± 11	Present
	GKO (5)		14 ± 11		2.6 ± 4	Absent
	+/+ (5)		0.4 ± 0.8		0 ± 0	Absent
2	SCID (5)	58 ± 25		56 ± 13		
	GKO (5)	30.8 ± 7.8		10.8 ± 11		
	+/+ (5)	13.75 ± 6.2		2.8 ± 1.8		

[a] Worm recovery is expressed as a percentage of the number of L3 larvae injected. The yield of adult worms in GKO mice was significantly different from that in +/+ mice ($P < 0.05$) at 4 and 6 weeks but not at 7 and 10 weeks ($P > 0.05$) and from that in SCID mice at both time points.

while IL-4 is important in preventing the development of patent infection, there must be other host protective responses that gradually reduce worm burdens in these mice. While these results may seem to be in conflict with the earlier report of Lawrence et al. (1), it is important to emphasize that those studies were conducted in the B6,129S background, which appears to eliminate a large percentage of the worm burden even in the absence of any component of adaptive immunity.

In contrast to the clear-cut data from the IL-4 KO mice, results from GKO mice are subtler but nonetheless real. At most of the time points that we have examined, GKO mice had greater worm burdens than intact mice. It is also clear that the development of sterile immunity was significantly delayed and even at 10 weeks postinfection some worms remained viable. This is seldom, if ever, seen in intact mice. However, unlike the situation with IL-4 KO mice, mice with GKO disruptions do not become patently infected and do not develop microfilariae.

The mechanism(s) by which the lack of IL-4 or IFN-γ delays parasite clearance is unclear. However, our demonstration of a model in which the host becomes permissive for patent infection after disruption of IL-4 provides us with a model system in which further (mechanistic) studies can be undertaken.

TABLE 3. *B. malayi* infection in SCID, IL-4 KO, and +/+ mice

Expt	Mouse strain	Mean % worm recovery ± SD[a] at:			Peritoneal MF[b]
		Wk 6	Wk 10	Wk 12	
1	SCID	36 ± 14		28 ± 10	Present
	IL-4 KO	27 ± 14		18 ± 11	Present
	+/+	0 ± 0		0 ± 0	Absent
2	SCID	35.3 ± 8.5	40.5 ± 10.4		Present
	IL-4 KO	30.3 ± 23.8	22.3 ± 11.4		Present

[a] Worm recovery is expressed as a percentage of the number of L3 larvae injected. The yield of adult worms in IL-4 KO mice was significantly different from that in +/+ mice ($P < 0.05$) at 6 and 12 weeks and not significantly different from that in SCID mice at all time points ($P > 0.05$).

[b] The presence or absence of MF in the peritoneal cavity at 10 or 12 weeks is shown.

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