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A Comprehensive, Model-Based Review of Vaccine and Repeat Infection Trials for Filariasis

C. Paul Morris,[a,b] Holly Evans,[a] Sasha E. Larsen,[a] Edward Mitre[a]

Department of Microbiology and Immunology, F. Edward Hébert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA[a]; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA[b]

SUMMARY

Filarial worms cause highly morbid diseases such as elephantiasis and river blindness. Since the 1940s, researchers have conducted vaccine trials in 27 different animal models of filariasis. Although no vaccine trial in a permissive model of filariasis has provided sterilizing immunity, great strides have been made toward developing vaccines that could block transmission, decrease pathological sequelae, or decrease susceptibility to infection. In this review, we have organized, to the best of our ability, all published filaria vaccine trials and reviewed them in the context of the animal models used. Additionally, we pro-

Address correspondence to Edward Mitre, edward.mitre@usuhs.edu.

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vide information on the life cycle, disease phenotype, concomitant immunity, and natural immunity during primary and secondary infections for 24 different filaria models.

INTRODUCTION

Filariae are tissue-invasive, vector-borne parasitic nematodes that cause tremendous morbidity worldwide (Table 1). The causative agents of lymphatic filariasis (*Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*) infect over 120 million people. These agents cause genital diseases (typically hydrocele) in approximately 25 million and lymphedema/elephantiasis in approximately 15 million people (1). *Onchocerca volvulus*, which causes river blindness and skin disease, is estimated to infect 37 million people and is responsible for blindness or visual disturbance in approximately half a million people (2, 3). Other filarial infections that cause disease in humans include *Loa loa*, certain *Mansonella* species, and, occasionally, *Dirofilaria* (4). Over 1 billion individuals live in areas where filarial worms are endemic.

Current efforts to control or potentially eradicate filarial diseases include the Global Program To Eliminate Lymphatic Filariasis, the Onchocerciasis Elimination Program of the Americas, and the African Programme for Onchocerciasis Control. These programs function primarily through repeated mass drug administration (MDA) of antifilarial medications to populations in countries where filarial worms are endemic and at times also incorporate strategies of vector control. Vaccines against filarial diseases would provide an important tool for these control efforts (5).

Animal studies evaluating vaccine approaches for filariasis have been conducted since the 1940s. An understanding of the lessons learned from prior vaccine studies, however, is challenging, as the work has been conducted using a large variety of filariasis models. Since the different animal models of filariasis have distinct life cycles and various degrees of permissiveness, it is difficult to understand the implications of specific vaccine trials without an in-depth knowledge of the models used.

In this review, we have attempted, to the best of our ability, to gather all filarial vaccine trials and to understand them within the context of the models in which they were carried out. Filarial vaccine articles were obtained first by conducting numerous PubMed searches and then by checking reference sections of investigational and review papers. Articles published up until May 2012 were included for review. The reviewed studies have utilized nine different filarial species (*Acanthocheilonema viteae*, *Brugia pahangi*, *B. malayi*, *Dirofilaria immitis*, *Litomosoides sigmodontis*, *L. loa*, *Onchocerca ochengi*, *Onchocerca lienalis*, and *O. volvulus*) and 13 different mammals (mice, rats, hamsters, jirds, *Mastomys coucha*, *Mastomys natalensis*, dogs, cats, ferrets, mandrills, chimpanzees, rhesus monkeys, and cattle) in various combinations for a startling total of 27 different filaria models.

Since every combination of filarial parasite and animal has its own strengths and limitations, this review is organized by animal model. Vaccine trials for all 27 models have been included in summary tables. Due to limited information for some models, only 24 models are discussed in the text. Furthermore, in three cases (*B. pahangi*/*B. malayi* in mice, *B. malayi* in *Mastomys natalensis*/*Mastomys coucha*, and *O. volvulus*/*O. lienalis* in mice), two models are discussed in the same section because the models are extremely similar and because the literature occasionally referred to the similar models interchangeably or in an unclear fashion. Thus, there

TABLE 1 Human diseases caused by filarial worms

Disease	Organism(s)	Prevalence	Vector	Acute symptom(s)	Chronic symptoms	Notes
Lymphatic filariasis	*W. bancrofti*, *B. malayi*, *B. timori*	120 million	Mosquito (*Culex*, *Aedes*, *Anopheles*)	Fever, lymphangitis, lymphadenitis	Lymphedema, elephantiasis, hydrocele	Risk of posttreatment lymphangitis
Onchocerciasis	*O. volvulus*	37 million	Black fly (*Simulium*)	Onchodermatitis	Blindness, dermatitis, hanging groin, hydrocele	Risk of posttreatment eye and skin inflammation
Loiasis	*L. loa*	12 million	Mango and deer fly (*Chrysops*)		Urticaria, calabar swelling, glomerulopathy	Risk of posttreatment cerebritis and death
Dirofilariasis	*D. immitis*, *D. repens*	Unknown; some areas of the world show 25–50% seropositivity	Mosquito (*Culex*, *Aedes*, *Anopheles*)		Pneumonitis, cough, coin lesion	Very expensive workup, increasingly recognized, possible emerging zoonosis

are 21 different chapters where models are discussed, with sections for each summarizing what is known regarding life cycle, disease phenotype, natural immunity during primary and secondary infections, concomitant immunity, and vaccine trials. At the end of the review, we provide a few conclusions that we have come to after reviewing the filaria vaccine literature and make suggestions for possible future directions in the field. We hope that this work will serve as a useful reference for clinicians, microbiologists, and immunologists when interpreting work done in the field of filaria vaccinology.

MODELS OF FILARIASIS

Acanthocheilonema viteae

For *Acanthocheilonema viteae*, the natural hosts are gerbils (including jirds and the great gerbil *Rhombomys opimus*) (6). The experimental hosts are hamsters, jirds (*Meriones unguiculatus*, also known as Mongolian gerbils), rats, and *Mastomys* species. The vector is the soft tick, *Ornithodoros moubata* (*Ornithodoros tartaskovskyi*) (7) (Table 2).

***A. viteae* in hamsters. (i) Permissiveness.** Hamsters are permissive to infection, with transient microfilaremia. Male hamsters are more susceptible to infection than females, possibly due to a protective effect imparted to females by 17-β-estradiol and progesterone (8). Infection of hamsters by subcutaneous injection of 100 L3-stage larvae obtained from tick dissections results in the development of 26 to 52 worms per animal, depending on the hamster strain (9). While microfilaremia is typically transient (details below), some inbred hamster strains develop stable microfilaremia (9).

(ii) Life cycle. Except for transient microfilaremia, the life cycle is assumed to be similar to that observed in jirds (Fig. 1), with adults residing in deep subcutaneous tissues and microfilariae (MF) circulating in the blood (see "*A. viteae* in jirds"). Patency commences at 6 to 8 weeks postinfection (p.i.), peaks at approximately 11 weeks p.i., and declines to undetectable levels by 19 weeks p.i. (9–11). After this time, hamsters are considered "latently" infected, meaning that they still harbor adult worms despite being amicrofilaremic. Latent infections can continue until at least 200 days p.i. in hamsters (11).

(iii) Disease. Glomerular basement thickening, glomerulonephritis, hepatitis, amyloidosis, and cellular infiltrates of the lung have all been observed in infected hamsters (12, 13). Hamsters infected with 1,000 larvae over the course of a year have more pronounced signs of disease than animals infected once with 500 larvae and develop subcutaneous abscesses containing live or dead worm material (12).

(iv) Natural immunity. Research on natural immunity in this model has been focused on protection against microfilaremia in latently infected hamsters. Transfer and immunosuppressive studies suggest that adult worms in latently infected hosts are still capable of producing MF and that latency is most likely due to IgM antibodies that induce antibody-dependent cellular cytotoxicity against MF (11, 14–16). Latency in this model may be associated with an inability of *A. viteae* to immunomodulate hamster antibody production (17). Indeed, chronic infection in rats and jirds is associated with decreasing worm-specific IgG and IgM antibody titers, which does not occur in infected hamsters (17).

(v) Immunity in the setting of repeated parasite exposures. Studies in this model show, at most, the development of only a small degree of concomitant immunity (10, 18). Protection acquired from repeated infections is best obtained from many low doses of L3s and results in an immune response that may arrest development of newly invading L3s (18). This phenomenon is not present when hamsters are infected with higher doses for fewer repetitions. At high doses of L3s, worm burdens continue to increase, and there appears to be no arrest in worm development (18). Superinfection with high doses does, however, result in increased numbers of subcutaneous nodules containing pus and adult worm fragments (10, 18).

Implantation of female worms, but not implantation of male worms or injection of male excretory/secretory (ES) products, prior to infection with L3s, has been shown to induce a protective immune response when microfilaremia is used as a marker of susceptibility (14). Due to the endpoint used, it is not clear whether this protective immune response is against MF or infective larvae.

(vi) Vaccine studies. Vaccination with irradiated L3s results in antibody responses to 68- and 205-kDa L3 antigens and moderate protection against development of L3 larvae to adult worms (59.4%). However, this does not significantly alter the number of circulating MF (19). Vaccination with adult extracts or crushed MF results in decreased duration of patency and, in the case of female extract, decreased adult worm burdens (11).

(vii) Lessons learned and clinical significance. This is a permissive model that exhibits transient microfilaremia and weak concomitant immunity. *A. viteae* shares some antigens and similarities in life cycle with *Onchocerca volvulus*. While this is not a model of any of the hallmark diseases associated with filariasis, infected hamsters develop glomerular disease, a finding that can occur with all of the major filarial pathogens of humans. Hamsters infected with *A. viteae* exhibit stage-specific immunity, wherein the microfilarial stage is killed but adult worms continue to survive. This state appears to be due to IgM antibodies against the MF and can be induced by implantation of adult female worms or vaccination with parasite extract. Despite the high level of protection against MF, this model system exhibits only weak concomitant immunity against infectious L3 larvae.

***A. viteae* in jirds. (i) Permissiveness.** Jirds are permissive to infection. All jirds infected with 20 L3s by subcutaneous (s.c.) injection develop stable microfilaremia for more than 18 months (20).

(ii) Life cycle. The majority of L3s move into host muscles within 24 h p.i. and then begin migrating through the musculature and subcutaneous tissues (6, 21). Larvae molt at 7 days p.i. and again at 23 days p.i., after which the adult worms remain in the subcutaneous tissue or muscular fasciae (6, 22). Patency commences at 50 to 72 days p.i. (6, 20) and remains stable for a period of 2 years, provided that the jirds are not overwhelmed with a large infection (20).

(iii) Disease. Our search yielded no mention of pathology other than the finding that parasite burdens of 10 or more mating pairs result in high levels of microfilaremia and can lead to host death (20).

(iv) Immunity in the setting of repeated parasite exposures. Jirds infected with *A. viteae* exhibit concomitant immunity, a phenomenon in which active infection with living adult worms protects against additional infections by L3 larvae. When jirds are repeatedly infected with L3s, parasite burdens eventually plateau to a steady state, and further injection of L3s does not result in

TABLE 2 Vaccine and repeat infection trials using *A. viteae*[a]

Host	Immunization category	Immunization	Adjuvant(s)	Dose	Protection (%)[b]		Reference(s); note(s)
					L3/adult	MF	
Hamster (transiently permissive)	Live worms	Live adult females (CI)		5 1×		75	14
		Live adult males (CI)		5 1×		↑ susceptibility	14; had the same effect even if males were transplanted on day 26 p.i.
		Repeat L3 infection (CI)		50–300, varied	0–46		10, 18; protection obtained only with many repeated low-dose infections
	Irradiated larvae	Irradiated L3		50 L3s 1×	59		19; 35 kilorads
	Homogenates or fractions	Crushed MF		Varied	0	Faster clearance	11; no protection when extract of MF was given 6× prior to infection
		Adult female extract		1 female	49	Faster clearance	11
		Adult male extract		2 males	0	Faster clearance	11
	ES products	Male ES products		3×		↑ susceptibility	14; similar results are obtained if administered 40 and 45 days p.i. with L3s
Jird (permissive)	Live worms	Repeat L3 infection (CI)		5–60 L3s 1–18×	75–95		21, 23–26; killing happened approximately 10 days p.i.
		Live adult female (CI)		1 1×	43–69		25; protection within 2 wk postimplantation
	Irradiated larvae	Irradiated L3s		1–3×, varied	61–100		7, 19, 24, 32, 33; killing 2–5 days p.i.; micropore chamber provides much lower protection
	ES product	L3 culture supernatant	STP	500 μl 3×	68		7
	Muscular protein	Tropomyosin	STP	25 μg 1×	29–35		31; worm derived or recombinant; no protection if alum is used as an adjuvant
		pcDNA/AvTropomyosin	Alum	25 μg 1×	41		31; less protection without adjuvant
		rMOv14-MBP	CFA, IFA, PBS	30 μg 3×	46	89	29; 136-residue portion of *O. volvulus* tropomyosin
	Other	OvB20	CFA, IFA, PBS	30 μg 3×	49–60	97	28; L2–L4 larva-specific protein in the hypodermis, cuticle, and ES product
		MBP-MOv2	CFA	30 μg 3×	36–55	89	30; present in all stages, is secreted, is in cuticle of L3s and uterine wall of adult females
M. coucha (permissive)	Irradiated larvae	Irradiated L3		50 L3s 1×	55		19; 35 kilorads

[a] All repeat infection studies are shaded. Repeat infection studies that clearly tested for the presence of concomitant immunity by giving a challenge infection in the setting of an ongoing active infection are labeled CI. ES, excretory-secretory products; IFA, incomplete Freund's adjuvant; PBS, phosphate-buffered saline; MBP, maltose binding protein; AvTropomyosin, *A. viteae* tropomyosin.

[b] Challenge was done by inoculation with L3s unless otherwise stated.

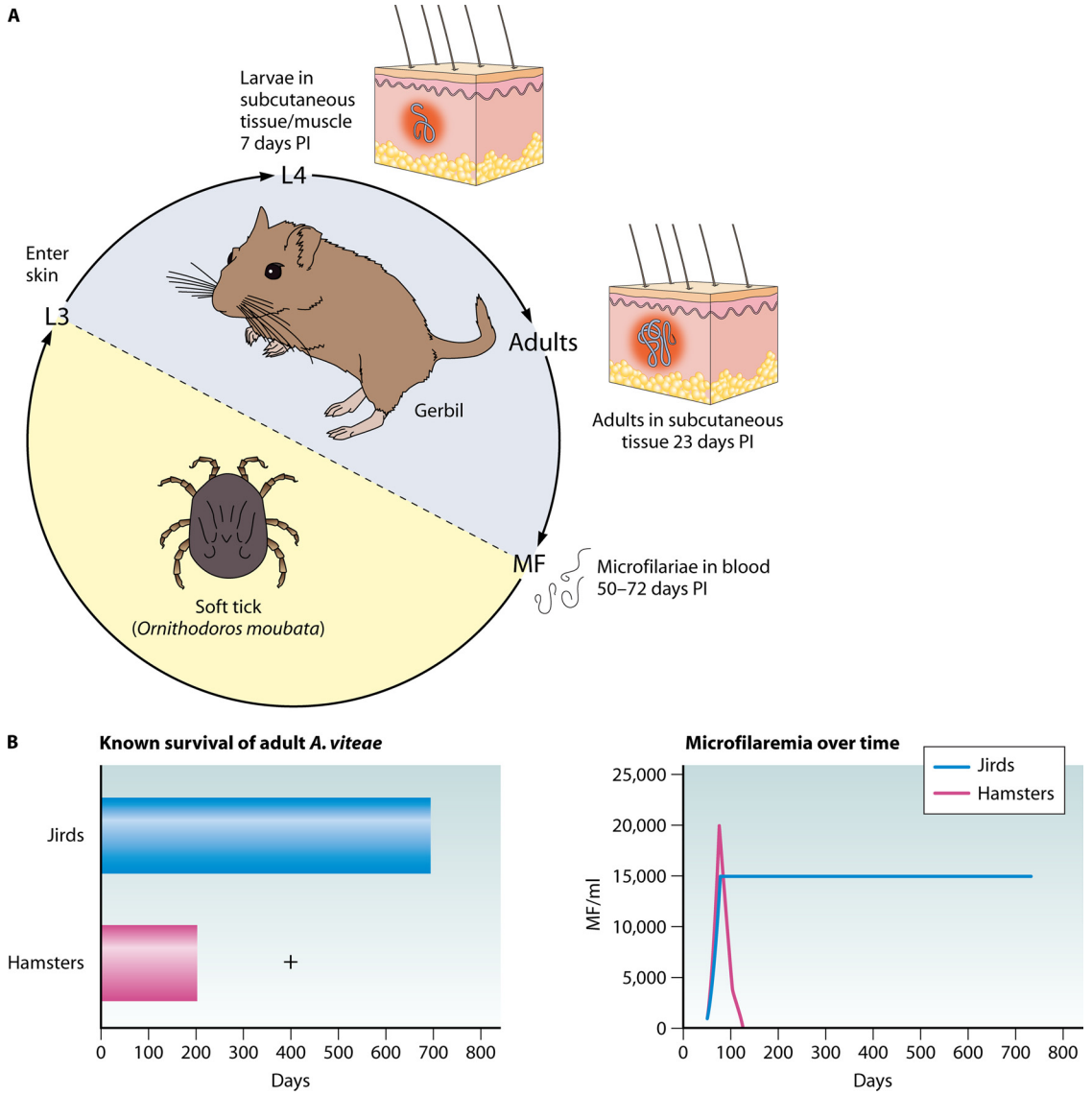

FIG 1 (A) Life cycle of *A. viteae* within its natural host, the gerbil. (B, left) Known survival of worms after infection in various hosts. + indicates that the host most likely lives longer, but no published reports have specifically shown longer survival. (Right) Rough outline of the course of microfilaremia over time after infection with 20 L3s in jirds (Mongolian gerbils) (20) or 160 L3s in hamsters (14).

higher parasite burdens (23). Interestingly, this steady state is variable depending on the infectious dose used for repeated infections but not on the number of times that the jird is infected (23, 24).

The development of effective concomitant immunity in this model is not dependent on the host encountering L3s, as it can be induced by implantation of a single adult female worm (25). The mechanism of this protection is not completely clear but may be associated with decreased migration or death of larvae within 5 days after superinfection (25). However, other studies using implantation chambers suggested that killing of the superinfecting larvae happens between 7 and 14 days p.i. (26). Regardless of the exact time frame, histological analyses show eosinophil-rich granuloma formation around larvae after superinfection (25, 26). Protective immune responses in this model may be due to IgG antibodies directed against the L3 cuticle (24). Partial resistance to superinfection has been correlated to IgG levels (24), and *in vitro* studies support the importance of the host antibody response, as heat-labile factors in serum of immune jirds have larvicidal effects (27).

(v) Vaccine studies. Several *Onchocerca* antigens have been evaluated in this model, including two that can be detected in *A. viteae* ES products (OvB20, and MOv2) and one that is a subunit of *O. volvulus* tropomyosin (MOv14) (28–30). All of these antigens induced moderate protection (36 to 60%) against adult worm burdens and exceptional protection against microfilaremia (89 to 97%).

However, when jirds are vaccinated with recombinant *A. viteae* tropomyosin, protection against L3 challenge is slightly lower (29 to 35%) (31). Interestingly, this vaccination protocol is protective when given with STP (squalane, Tween, and Pluronic), a type 1-inducing adjuvant, but not alum, a type 2-inducing adjuvant.

Irradiated L3s provide the greatest protection in this model (7,

TABLE 3 Vaccine and repeat infection trials using *B. malayi*[a]

Host	Immunization category	Immunization	Adjuvant	Dose	Protection (%)[b]		Reference(s); note(s)
					L3/adult	MF	
Cat (permissive)	Irradiated larvae	Irradiated L3		100 2×	0	0	87; 10–40 kilorads
Ferret (transiently permissive)	Live or dead worms	Repeat infection with MF after L3 infection		50 1×		Faster clearance	38; challenge with MF was conducted after development of amicrofilaremic state
		Living or dead MF		Varied	0–76	Faster clearance	38, 40; greatest protection was obtained by intradermal injection of MF (L3 or MF challenge)
Jird (permissive)	Irradiated larvae	Irradiated L3		75–100 1-3×	56–91	100	44; 10 kilorads not protective
	Fractions or homogenates	BmAg	CFA	100 μg 3×	25	50	47
		Freeze-thawed MF	CFA	500,000 1×	25		46
		Soluble MF antigen		10 μg 2×	48	~75	45; jirds given MF antigen at 10 and 12 wk p.i. showed some delayed patency and a trend downward in adult worm burden
		F6	CFA	10, 5, 5 μg 3×	42	Yes	62
		CFA_2-6	CFA	25 μg 5×	84	Less patent jirds	49; only 1/9 vaccinated jirds had patent infection
		CFA_2-6	CFA	25 μg 5×		87	49; MF challenge
	Muscular proteins	rMyosin	CFA	25 μg 3×	64	76	47; recombinant myosin heavy chain
		BM5 MBP	CFA	30 μg 2×	43		108, 259; *Brugia malayi* paramyosin and maltose binding protein
		D. immitis paramyosin	CFA	30 μg 2×	Insig.		259
		BM5/pjw4303kc (DNA) (paramyosin)		3×	Insig.		260; paramyosin
	Abundant larval transcripts	pVBmALTII (DNA)	None	100 μg 3×	57		261; 64% protection when third injection was 25 μg protein in alum
		BmALTII	Alum	25 μg 3–5×	69		53, 261
		BmALT1	CFA	75 μg 4×	76		52; L3-specific abundant larval transcript
	Cuticle remodeling	Recombinant chitinase	CFA	5 μg 3×	Insig.	33	262; MF-specific antigen found by screening sera from amicrofilaremic patients
		F7R2 chitinase fragment	CFA	5 μg 3×	Insig.	55	262; carboxy-terminal fragment of chitinase
		175-kDa collagenase	CFA	20 μg 4×	76		51
		BmTGA	Alum	25 μg 5×	30		53; transglutaminase
	Antioxidant	ScGST	CFA	15 μg 4×	83		50; glutathione *S*-transferase purified from *Setaria cervi*
		EC-SOD	Alum	25 μg 5×	39		263; superoxide dismutase
		EC-SOD	Alum	25 μg 5×		30	263
		rWbGST	Alum	15 μg 3×	61		264; glutathione *S*-transferase from *Wuchereria*—predominantly L3 expressed
		BmTPX	Alum	25 μg 5×	43		53; thioredoxin peroxidase
	Mixed	BmTGA + BmALT2	Alum	25 μg 5×	47		53
		BmTGA+ BmTPX	Alum	25 μg 5×	74		53
		BmALTII + BmVah				78	265
	Other	SXP1	CFA	5 μg 3×	Insig.	77	262; present in multiple worm stages
		BmA-2	CFA	25 μg 4×	88		48; 120-kDa SDS-soluble *B. malayi* adult worm antigen
		rBm-SL3	CFA	25 μg 4×	64		266; found by screening sera from healthy population in area of endemicity against a cDNA library of *B. malayi* L3s (micropore challenge)
		rBm-SL3	CFA	25 μg 4×		67	266; MF in micropore challenge
M. coucha (permissive)	Live worms	CAI		100 L3s			61; no protection with DEC; albendazole is possibly different, but control animals did not become infected
	Fractions and homogenates	BmAFII	CFA	50 μg 3×	76	85	267; see the text
		BmAFI		50 μg 3×	↑ susceptibility		55; allows for survival in the peritoneal cavity
		F5	CFA	10, 5, 5 μg 3×	0		62; 68- to 84-kDa fraction of adult worms
		F6	CFA	10, 5, 5 μg 3×	64	Yes	62; 72% protection when challenged with adult implantation
		F10	CFA	10, 5, 5 μg 3×	0		62; 38- to 42-kDa fraction of adult worms
		F14	CFA	10, 5, 5 μg 3×	0		62; 66% protection when challenged with adult implantation

	MT	CFA	10 μg 3×	66	67		268
	NR	CFA	10 μg 2×	52	62		268
	Antioxidant	rBmTRX	Alum	50 μg	63		63; epitope from thioredoxin peroxidase (micropore challenge)
	Abundant larval transcript	BmALTII	+/− alum	50 μg 4×	71–74		269
	Mixed	rBmTRX p1-TRXp2	Microsphere	4×	75		63; micropore challenge
	Other	BmT5	CFA	50 μg 3×	67	69	64; 34-kDa protein from adult worms
M. natalensis (permissive)	Fraction	BmA-2	CFA	25 μg 4×		91	48; MF challenge
Mice (BALB/c) (nonpermissive)	Live or dead worms	Repeat L3 infections		2–100 L3s 1×	45–100		110, 117; primary infection was performed i.m., i.v., s.c., and i.p., and all were protective; *B. pahangi* followed by *B. malayi* was also protective; implantation of adults or L4s prior to L3 infection was also protective
		Live MF		1×		Faster clearance	132; transfer of sera was able to protect naive mice; a monoclonal antibody was able to mimic this effect (MF challenge)
		Live MF		5,000 1×	52–74		117
		Freeze-killed larvae		50 1×	34–46		117
		Freeze-thawed MF	CFA	20,000–500,000 1×	89–98		46; protective in C3H/HeJm, C3H, and HeN but not C3H/HeJ mice
	Irradiated larvae	Irradiated L3s		30–100	95–100		46, 114, 129, 131; transfer of splenocytes (specifically nonadherent) or sera to naive mice was protective, but splenocytes performed better than sera
	Fractions and homogenates	Soluble fraction of larvae		50 1×	42		117; increased to 49–76% with 2 injections using CFA as an adjuvant
		Insoluble fraction of larvae		50 1×	0		117
		PBS extract of MF	CFA	100,000 MF 2×	85		46
		SDS extract of MF	CFA	100,000 MF 2×	89		46
		Soluble MF antigen	Pluronic 121, Squalane, Tween	5 μg 1–3×		0–79	133; 1× dose was ineffective and associated with a type 1 response, and 3× vaccination gave a type 2 response and was associated with faster clearance (MF challenge)
		SDS extract of adult	CFA	0.1 ml 2×	86		134; protective fractions were >200 kDa, 170–200 kDa, 40–44 kDa, 33–36 kDa, 23–28 kDa, 17–19 kDa, and 20–22 kDa; other fractions were not protective
		SDS extract of MAb OVH affinity column eluate	CFA	0.1 ml 2×	83		134; several fractions of this were tried, and none of these fractions performed nearly as well as the whole extract; transfer of nonadherent splenocytes from mice vaccinated with the 26- to 29-kDa fraction was protective
	Abundant larval transcript	PVAX-ALT2		100 μg 2×		34	135; abundant larval transcript (MF challenge)
	Antioxidant	PVAX-TPX		100 μg 2×		37	135; thioredoxin peroxidase (MF challenge)
	Cuticle remodeling	BmTGA (DNA)		100 μg 5×		21	137; transglutaminase (MF challenge)
	Heat shock protein	HSP12.6αc	Saponin	100 μg DNA 2×, 15 μg protein 2×	83		139; performed best when given as a prime-boost (2× DNA vaccine followed by 2× protein); this subunit performed better than vaccination with the full protein
	Mixed	92-kDa fusion protein (TrpE + 62-kDa MF antigen)		1–3 μg 2×		42	270; MF challenge
		PVAX-ALT-2 + TPX		100 μg 2×		78	135; MF challenge
		BmVAL-1/BmALT-2 (DNA and protein)	Alum	150 μg protein, 100 μg DNA 4×	84		136; protein or DNA alone of either antigen performed worse than the combination
Mice (CF1) (nonpermissive)	Muscular protein	Bm97		2 μg 2×	40–60		138; serum from these animals reacted with paramyosin from *S. mansoni*
		B. malayi paramyosin		5 μg 2×	42–60		138

(Continued on following page)

TABLE 3 (Continued)

Host	Immunization category	Immunization	Adjuvant	Dose	Protection (%)[b]		Reference(s); note(s)
					L3/adult	MF	
		Caenorhabditis elegans paramyosin		5 μg 2×	22–56		138
Rhesus monkey (permissive)	Live worms	Repeat L3 infections		Varied		Lower MF counts after 2 inoculations	66
	Irradiated larvae	L3 cultured with immune sera		100–400 worms 2×		0	65
		Irradiated larvae		100–400 (10–40 kilorads) 2×	75	73–100	65; only 2 monkeys autopsied for adult worms; best results in the 20-kilorad group regardless of no. of worms; however, if 400 worms were inoculated, the 10- and 20-kilorad groups also had 100% protection
	ES products	L3 ES products		100–400 worms 2×		74	65

[a] All repeat infection studies are shaded. Repeat infection studies that tested for protective immunity after chemical abrogation of the primary infection are labeled CAI. BmAg, *B. malayi* antigen; Insig., insignificant; EC-SOD, *E. coli*-expressed superoxide dismutase; MT, mitochondrion-rich fraction; NR, nucleus-rich fraction; rWbGST, recombinant *Wuchereria bancrofti* glutathione *S*-transferase; MAb OVH, monoclonal antibody OVH; PVAX, a commercially available plasmid.

[b] Challenge was done by inoculation with L3s unless otherwise stated.

19, 24, 32, 33). Jirds vaccinated once with 5 irradiated L3s displayed a 61% reduction in worm burden (7), and vaccination with 50 irradiated L3s resulted in 90% protection (7). Vaccination with irradiated larvae results in clearance of infectious L3 larvae at between 2 and 5 days after challenge with eosinophils, macrophages, and neutrophils surrounding, trapping, and rupturing larvae (32).

(vi) Lessons learned and clinical significance. While this model does not appear to mimic any human disease caused by filarial infection, the permissive nature of jirds for *A. viteae* makes this an excellent small-rodent model for filaria vaccine studies. The primary limitation of this model for vaccine studies is the practical difficulty of enumerating adult worm burdens. As the worms reside in the deep subcutaneous spaces, counting adult worms requires whole-animal dissection, a process that is both time-consuming and technically challenging. The successful use of *Onchocerca* antigens to induce protection in this model is promising. Successful vaccination with tropomyosin and STP, but not alum, suggests that adjuvants that induce type 1 responses may be preferable to those which induce the type 2 responses observed in natural infection. In contrast to the findings in this model, in the *Onchocerca*-mouse model, mice vaccinated with OvB8, Ov64, or Ov7 were protected when the antigen was adsorbed to alum but not when given with complete Freund's adjuvant (CFA) (34). The results suggest that the most effective immune response will vary depending on the vaccine candidate used.

There is a high degree of concomitant immunity observed in this model. It is interesting that the adult worm burden needs to be carefully controlled in this model in order for the host, and subsequently the parasite, to survive. As such, this model appears to be an excellent one for investigating the mechanisms of concomitant immunity.

Brugia malayi

For *Brugia malayi*, the vectors are mosquitoes (*Mansonia*, *Anopheles*, and *Aedes*). The natural hosts are humans, cats (35), and monkeys. The experimental hosts are jirds, *Mastomys*, monkeys, mice, and ferrets (Table 3).

***Brugia malayi* in ferrets. (i) Permissiveness.** Ferrets are permissive to infection, with transient microfilaremia.

(ii) Life cycle. Our search yielded little information on early parasite development in this model, yet it is clear that *B. malayi* carries out its entire life cycle in the ferret. Five to eight months after s.c. inoculation of L3s, adults can be found mainly in the lymphatics but also in the skin and heart (36, 37). Patency develops at 3 months p.i. and lasts until 6 to 8 months p.i. in 85 to 90% of infected ferrets (Fig. 2) (36, 37). Some studies utilize intravenous (i.v.) injection of MF into naive ferrets, which results in constant microfilaremia for 3 to 4 weeks, followed by a gradual decline to zero microfilaremia over 4 months (38).

(iii) Disease. Ferrets display many of the pathological sequelae exhibited by humans infected with *B. malayi.* These include lymphatic changes such as lymphangiectasia, lymphadenopathy, lymphatic obstruction, and subsequent formation of collateral lymphatic channels (36, 37). Although single infections induce only transient episodes of lymphedema, ferrets that are repeatedly infected with *B. malayi* develop chronic leg edema (36). Ferrets also develop eosinophilic granulomas in the liver, lungs, and lymph nodes that mimic lesions seen in human tropical pulmonary eosinophilia (TPE) (36, 39). Simi-

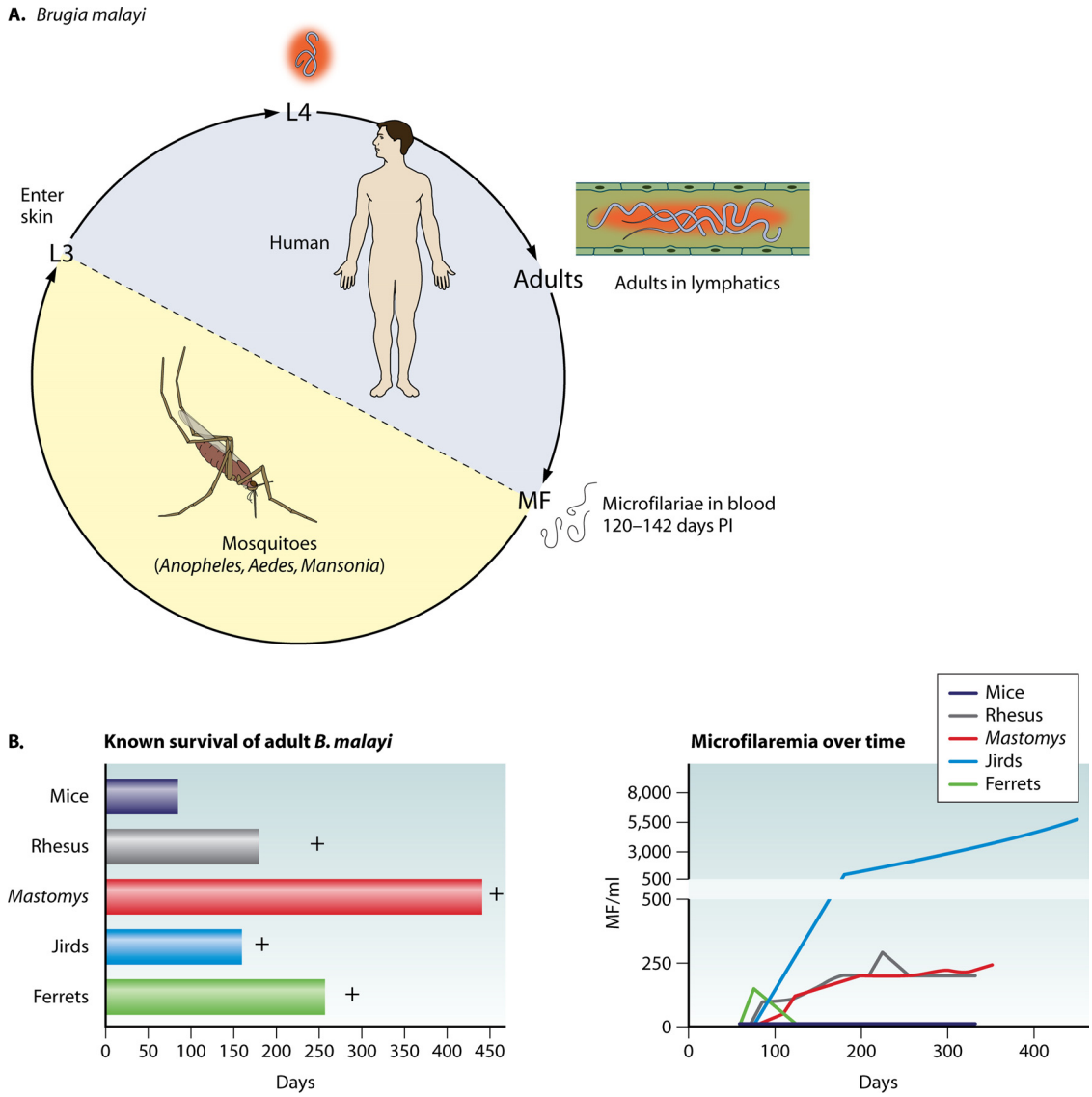

FIG 2 (A) Life cycle of *B. malayi* in its natural host, humans. (B, left) Known survival of worms after infection in various hosts. + indicates that the host most likely lives longer, but no published reports have specifically shown longer survival. (Right) Rough outline of the course of microfilaremia over time after subcutaneous inoculation of 35 to 100 L3s into jirds (92), 150 to 200 L3s s.c. into ferrets (37), 50 L3s s.c. into *Mastomys* rodents (54), and 100 L3s into rhesus monkeys (66).

larly, ferrets develop a transient pneumonia while clearing microfilariae from the bloodstream (36).

(iv) Natural immunity. Nearly all ferrets are susceptible to infection. Necropsies performed at 5 to 8 months p.i. found at least 1 adult worm in each of 9 inoculated ferrets (37). Although there was a large variance in the number of worms found per animal in this study (1 to 25 worms), it is unclear if there is an immunological mechanism responsible for partial resistance to adult worms in this model. There is, however, evidence that the development of an amicrofilaremic state is due to an adaptive immune response. Serum transfer studies suggested that infected ferrets develop a sheath-reactive IgG antibody that is sufficient to protect against microfilaremia (38).

(v) Immunity in the setting of repeated parasite exposures. Ferrets that have achieved amicrofilaremia and are subsequently injected with MF intravenously are able to clear those MF faster than do naive ferrets (38).

(vi) Vaccine studies. i.v. as well as intradermal injection with live or dead MF results in >50% protection against subsequent challenge with L3s injected s.c. (40). Additionally, prior i.v. inoculation with microfilarial worms causes substantially accelerated clearance of MF after subsequent i.v. injection of MFs (38, 40).

(vii) Disease after treatment or vaccination. Ferrets that have been vaccinated with MF and subsequently infected a single time with L3s developed pathological sequelae similar to those seen in repeatedly infected ferrets (40). In one study, 8 out of 13 vaccinated and infected ferrets developed gross lymphedema, whereas none of the nonvaccinated ferrets developed this symptom. The factors responsible for this increased pathology are not known, but this increased pathology was postulated by Crandall et al. to be

associated with increased reactivity to the lymphatic stages of this parasite (40). Despite this association, passive immunization with sheath-reactive IgG appears to be associated with smaller numbers of immune lesions (38).

(viii) Lessons learned and clinical significance. This is a small-mammal model of elephantiasis. Like humans with elephantiasis, who are often amicrofilaremic, clinical lymphedema in ferrets develops only after an effective immune response against microfilariae develops. It is interesting to note that prior exposure or vaccination in this model results in increased pathological sequelae postchallenge. These findings suggest that vaccine strategies will need to be rigorously tested for safety before human trials are conducted. Because ferrets can develop clinical lymphedema, infection of ferrets with *B. malayi* may be an excellent model for testing vaccine safety.

***Brugia malayi* in jirds. (i) Permissiveness.** Jirds are permissive to infection. Male jirds are more susceptible to infection, with up to 70% of male jirds developing patent infections (41, 42).

(ii) Life cycle. L3s injected subcutaneously remain primarily in the subcutaneous tissues or enter the viscera through the first molt, which occurs at 7 to 10 days p.i. Afterwards, they begin to localize to the testes, heart, and lungs and undergo the L4-to-adult molt at 29 to 35 days p.i. (42). Adults are located most often in the spermatic cord and lymphatic vessels, including those of the testes, heart, and lungs (41, 43). Patency occurs at 79 to 116 days p.i. and can last for at least 26 weeks (41). Jirds have been inoculated with infective larvae intraperitoneally (i.p.) for some vaccine studies. Larvae inoculated into the peritoneal cavity remain and develop within this anatomic location, allowing for easy worm enumeration.

(iii) Disease. Disease is similar to that caused by *B. pahangi* in the jird, with lymphatic vessel dilation and development of intralymphatic thrombi at 150 days p.i. (43).

(iv) Vaccine studies. Many vaccine candidates have been studied in this model, yet no protocol has achieved better protection than vaccination with irradiated larvae, which provides high levels of protection against s.c. or i.p. L3 challenge (56 to 91%) and complete protection against microfilaremia (44). Vaccination with irradiated larvae elicits the production of antibodies that bind to the surface of L3s and causes larvae to become encased in eosinophil-rich granulomas (44). Optimal protection with this approach was achieved with a single inoculation of 100 L3s irradiated with 15 kilorads (44).

Of the crude vaccine preparations, the soluble portion of MF performs best, providing 75% protection against future microfilaremia and 48% protection against incoming larvae when jirds are challenged by s.c. inoculation of L3s (45). Vaccination with soluble MF antigens did not require any adjuvant and induced substantially better protection than vaccination with dead MF and CFA (46). Soluble adult antigen administered with CFA in this model provides minor (25%) but significant protection against future infection (47).

Of the many different specific antigens or purified fractions of antigens tried in this model, those that provide 70% or greater protection against future infection are a 120-kDa SDS-soluble adult worm antigen (BmA2) (88%) (48), a 43-kDa antigen isolated from *W. bancrofti* MF (CFA_2-6) (84%) (49), glutathione *S*-transferase purified from *Setaria cervi* (ScGST) (83%) (50), a 175-kDa collagenase purified from *Setaria cervi* (76%) (51), *B. malayi* abundant larval transcript I (BmALTI) (76%), and BmALTII (70%) (52).

A relatively recent trend in filaria vaccine research is to utilize combinations of specific protective antigens to boost vaccine efficacy. In this model, the combination of *B. malayi* transglutaminase (BmTGA) and *B. malayi* thioredoxin peroxidase (BmTPX) achieves 74% protection, compared with protection of less than 50% with either single antigen (53). A challenge associated with the development of combination vaccines is that some combination vaccines perform worse than either antigen alone (53). Thus, each combination needs to be tested.

Transfer studies to determine mechanisms of protection have been performed for CFA_2-6 and BmA2. For both of these vaccines, antibodies from vaccinated mice were sufficient to protect against challenge infection in naive jirds (48, 49).

(v) Lessons learned and clinical significance. As jirds are permissive for *B. malayi* and develop only very low levels of protection after exposure to adult worm antigens, this appears to be a good model for early screening of filariasis vaccine candidates. Because this model uses a parasite that commonly infects humans, it can be used to test vaccine efficacy of antigens recognized by antibodies from protected (putatively immune) humans. The drawbacks to this model are a lack of information on concomitant immunity and a lack of clinical disease markers. However, while jirds do not develop clinical lymphedema in this model, some information may be able to be gleaned from histological changes in the lymphatics.

Although there have been very promising candidates found with this model, no vaccine protocol has yet induced sterilizing immunity. In some cases, single antigens can be combined to improve protection (53), and single antigens from each mammalian stage of the parasitic life cycle have been used to elicit very high levels of protection in this model. As this model has shown protection using antigens from worms other than *B. malayi*, it suggests that there is the potential to one day produce a vaccine that could induce broad protection against multiple species of filarial worms.

***Brugia malayi* in *Mastomys* (multimammate rodents).** Note that *M. coucha* and *M. natalensis* have both been used. The life cycle of *B. malayi* appears similar in both *M. coucha* and *M. natalensis*. Since the literature commonly confuses these two very similar rodent species, their information will be combined.

(i) Permissiveness. *M. coucha* and *M. natalensis* are permissive, with 11 to 21% of worms surviving 153 to 442 days p.i. after initial s.c. inoculation as L3-stage larvae. Up to 90% of *M. natalensis* rodents infected by subcutaneous injection of L3 larvae into the neck develop microfilaremia (54), with the vast majority maintaining stable microfilaremia (54). Successful patent infections are markedly reduced (to around 66%) when larvae are injected into the groin (54). Approximately 20% of injected worms survive as living adult worms in tissues throughout the body 6 months after infection (54). Despite this, naive *Mastomys* rodents are resistant to i.p. infection (55, 56). Studies with the GRA strain of *M. natalensis* suggest that males are more susceptible to infection than females (57).

(ii) Life cycle. The majority of worms localize to the heart, lungs, and lymphatics of the testes after subcutaneous inoculation (54, 58). Inoculation into the groin as opposed to the neck yields a higher percentage of worms in the testes and lymphatics. Patency is dependent on the colocalization of at least one mating pair, and

MF densities correlate with adult female burden (58). The length of the prepatent period is variable but typically lasts between 97 and 142 days after subcutaneous infection and longer after natural infection or inoculation into the groin (54, 57, 59). Patency persistence is variable, lasting between 168 days and more than 350 days in *M. natalensis* (54, 57).

(iii) Natural immunity. As with other models, there are various levels of susceptibility within the host population. Infected *Mastomys* rodents display one of three courses of infection: chronic microfilaremia, transient microfilaremia, and amicrofilaremia. Amicrofilaremic *Mastomys* rodents have lower adult parasite burdens than microfilaremic animals (60), yet it is unclear as to whether this is immune mediated. Transient microfilaremia, however, is not associated with lower adult parasite burdens and is thus perhaps due to an MF-specific immune response (60). In all infected *Mastomys* rodents, some adult worms can be found encapsulated as early as 190 days p.i., suggesting the development of immune responses to adult worms. Despite some worms being encapsulated and dying as early as 190 days p.i., infected *Mastomys* rodents are unable to clear all adult worms within 435 days p.i. (60).

Surprisingly, in this model, i.p. injection yields a very different response. *Mastomys* rodents infected i.p. do not develop a chronic infection, and dying larvae can be found encased by host cells as early as 7 days p.i. (56).

(iv) Immunity after prior exposure. Infections have been chemically abbreviated by using either albendazole or diethylcarbamazine (DEC) (61). Abbreviation of infection with DEC does not elicit any significant protection against future infection (61). *Mastomys* rodents that were cleared of infection by using albendazole displayed resistance to subsequent infection. However, this might not be due to the chemically abbreviated infection, as the control group, which received only albendazole, was also resistant to infection (61).

(v) Vaccine studies. Vaccine research has focused largely on the use of fractions of adult worms separated primarily by size. It has been shown that the 54- to 68-kDa fraction (fraction 6 [F6]) and the 20- to 28-kDa fraction (F14) contain vaccine candidates that may be effective at eliminating adult worms (62), although only F6 has been shown to be protective against challenge with infective larvae (62). Other promising vaccine candidates from adult antigens include BmA-2, which is a 120-kDa antigen, BmT5, which is 34 kDa, and a single epitope from thioredoxin peroxidase (48, 63, 64). A fractionation study that used Sephadex G200 for separation yielded three main groups of antigens, named BmAFI to BmAFIII. The BmAFI fraction induces production of interleukin-10 (IL-10) in the host. Interestingly, sensitization with this fraction makes *Mastomys* more permissive to the intraperitoneal route of *Brugia* infection (55).

(vi) Lessons learned and clinical relevance. The clinical relevance of this model in terms of disease and concomitant immunity is largely unknown. However, the variable course of microfilaremia with this model is an area that could be investigated to increase our understanding of the factors important for altering microfilaria levels in humans. One drawback to this model is that the broad tissue range of adult worms in this model makes it somewhat challenging to obtain accurate adult worm counts after vaccination trials.

This model is interesting in that *Mastomys* rodents are resistant to i.p. infection and that this resistance can also be abrogated by a vaccination protocol that induces IL-10 production. These findings suggest the presence of an innate immune response element in the peritoneal cavity that can eradicate worms given by this route. Also, these results suggest that worm immunoregulatory factors can in some situations prevent this response. It would be useful to identify the specific antigen or antigens within the BmAFI fraction responsible for inducing IL-10 production and a permissive state for i.p. challenge infections.

***Brugia malayi* in rhesus monkeys. (i) Permissiveness.** Rhesus monkeys are permissive to infection (65, 66). However, susceptibility to microfilaremia is highly variable (66, 67).

(ii) Life cycle. Patency generally commences 10 to 12 weeks after subcutaneous injection of L3 larvae but may occur as late as 39 weeks p.i. (66, 67). As many as half of infected monkeys remain microfilaremic at 1 year p.i. (65).

(iii) Disease. Infected animals commonly exhibit disruption of lymphatic flow, dependent edema, and grossly enlarged lymph nodes (66–68). Microscopically, there is evidence of hyperplasia and eosinophilic lymphadenitis (67). Similar to humans, rhesus monkeys that develop lymphedema tend to be amicrofilaremic and exhibit strong immune responses to filarial antigens (68).

(iv) Natural immunity. The existence of one monkey that never developed infection despite 20 repeated subcutaneous inoculations of 20 L3s suggests that there are naturally resistant animals in this model (65). While the correlates of protection have not been elucidated, serum transfer studies suggest that antibodies are not sufficient to induce protection (65).

Latency in this model is correlated with the presence of specific antimicrofilaria IgG sheath antibodies, which have been shown to promote cytoadherence to MF (69, 70). Surprisingly, *in vitro* studies suggest that sera from postmicrofilaremic monkeys alone are sufficient to cause degradation of MF (70).

(v) Immunity in the setting of repeated parasite exposures. Monkeys that receive more than two infections display higher eosinophilia and lower microfilaremia levels than monkeys that receive one or two infections (66).

(vi) Vaccine studies. Vaccination with irradiated larvae has provided the best results in this model, reducing adult burdens by 75%. Furthermore, vaccination decreases both the percentage of microfilaremic monkeys and the duration of microfilaremia (65). Vaccination produces a protective immune response that lasts for at least 12 months postvaccination (65). As in other models, the dose of radiation given to the parasites is important in inducing a protective immune response. The best results were obtained by vaccination with larvae that had received 20 kilorads (65).

Vaccination with the ES products obtained from L3s may have a protective effect in this model, but it is not clear if this is a real effect (65). This is partially because adult worms were not quantified in this experiment, and only one control animal was used to determine microfilaremia.

(vii) Lessons learned and clinical relevance. Despite being an expensive and difficult model, this model is perhaps the model most relevant to human lymphatic filariasis. This is a permissive monkey model in which lymphedema develops in response to a human pathogen of lymphatic filariasis. Moreover, this model shows a highly differential outcome for both infection status and disease. As such, putatively immune monkeys could be tested for antibody and cytokine responses in an effort to determine optimal vaccine approaches. Furthermore, this would be an ideal model for end-stage testing of vaccine candidates for safety and efficacy

after they have shown promise in other models. The vaccine study using irradiated larvae in this model is the only vaccine study to show protection in a nonhuman primate model of filariasis.

Brugia pahangi

For *B. pahangi*, the vectors are mosquitoes (*Aedes aegypti* and *Armigeres obturbans*) (71). The natural hosts are cats and dogs (71). The experimental hosts are jirds and mice (Table 4).

***Brugia pahangi* in cats. (i) Permissiveness.** Cats are permissive to infection. Ninety-six percent of cats experimentally infected with *B. pahangi* become microfilaremic (72). Male and female cats are equally susceptible to infection, with an average of 8 to 25% of injected larvae surviving to adulthood (73–75).

(ii) Life cycle. When L3s are injected into the footpad, approximately one-half of infective larvae penetrate the lymphatics within 3 h (74) and subsequently travel to the popliteal lymph node, where they molt to stage 4 larvae at approximately 7 days p.i. (Fig. 3) (74, 76). At 20 days p.i., larvae migrate down the afferent lymphatic, where they undergo their final molt at 24 to 33 days p.i. (73, 74, 76). Patency commences at 53 to 94 days p.i., increases over the first year, and plateaus at 1 year p.i. in repeatedly infected cats (72, 73). While MF levels in repeatedly infected cats are variable, ranging from 50 to 40,000 MF/ml, almost all infected cats develop microfilaremia (73). Transfusion of MF into naive cats demonstrated that MF live for a median of 46 days (range, 2 to 136 days) (77).

Parasite survival undergoes two major declines. About one-half of invading larvae are cleared within the first hours after infection, and one-half of the remaining worms then die at 25 days p.i., around the time of the final molt (74).

(iii) Disease. This model involves a pathological response similar to that seen in humans. Infection in the foot results in an enlarged popliteal lymph node and dilated, swollen, varicosed lymphatic vessels (74, 78). Although lymph nodes and vessels become fibrotic over time in infected cats, collateral channels form in response to fibrosis, and only a minority of cats develop frank lymphedema of infected limbs (72, 79).

(iv) Natural immunity. While most infected cats maintain their microfilaremic status for years, there are some cats that never develop microfilaremia (naturally resistant) and others that spontaneously become amicrofilaremic after a period of patency (transiently microfilaremic) (74, 80, 81). Cats naturally resistant to microfilaremia are somewhat rare (~5%), do not develop microfilaremia after repeated injections of L3s, and quickly clear MF after i.v. injection (74, 77, 80).

It is not clear how often cats develop resistance after a period of patency. Reports in the literature stated that 2.2 to 75% of infected cats will spontaneously clear microfilaremia (74, 81). This end of patency occurs between 15 and 45 weeks p.i. and is associated with the development of specific antibodies against MF, adults, and L3s (81). Transfer studies suggested that IgG antibodies, most notably IgG1, are important in resistance to MF via antibody-dependent cellular cytotoxicity (82).

(v) Immunity in the setting of repeated parasite exposures. A number of studies have demonstrated development of concomitant immunity in this model after multiple inoculations with infective larvae. When cats are repeatedly infected with infectious L3 larvae every 10 days, there is no significant reduction in percent yield of adult worms until the cats have been reinfected approximately 12 times, at which point yields start to drop dramatically.

TABLE 4 Vaccine and repeat infection trials using *Brugia pahangi*[a]

Host	Immunization category	Immunization	Adjuvant	Dose	Protection (%)[b]		Reference(s); note(s)
					L3/adult	MF	
Cats (permissive)	Live worms	Repeat L3 infections (CI)		50–200 L3s 1–67×	0–95	0–100	73, 80; % recovery similar until 12 repeated infections
		CAI		6×	Insig.	Insig.	78; chemically abbreviated infection
	Irradiated larvae	Irradiated L3		300 1×	72		85
Jirds (permissive)	Live worms	Repeat L3 infections (CI)		Varied, 1–20×	0–39	None	88, 98–100, 271, 272
		CAI		50–100 1-5×	0–77		101, 102, 273; timing of treatment altered protection significantly
		MF	CFA	5,000 3×	Insig.	66	95
	Irradiated larvae	Irradiated L3s		50 3–5×	39–76	Yes	106; best results with 90 kilorads of irradiation × 5 vaccinations
	Fractions or homogenates	Adult soluble antigen	CFA	150 μg 3×	Insig.	42	95; no better than CFA alone
Mice (nonpermissive)	Live worms	Repeat L3 infections		25 L3s 1×	Faster clearance		110, 117–119, 128
	Irradiated larvae	Irradiated L3s		50–100 2-3×	79–100		129, 130
	ES products	L3 ES product		25 worms 1×	70		128; this effect was specific to L3 ES products; ES from adults; MFs and L4s were not protective
		Abundant larval transcript	IE9 Ab	1×	58		128
		Other	Cuticles (L3-to-L4 molt)	25 worms 1×	97		128
		BmTCTP	IE9 Ab	1×	Insig.		128; IE9 monoclonal antibody was used to increase time to which the mouse was exposed to antigen

[a] All repeat infection studies are shaded. Repeat infection studies that clearly tested for the presence of concomitant immunity by giving a challenge infection in the setting of an ongoing active infection are labeled CI, and repeat infection studies that tested for protective immunity after chemical abrogation of the primary infection are labeled CAI; all other repeat infection studies either tested for protective immunity after natural clearance of a primary infection (especially in nonpermissive models) or did not explicitly state the status of the first infection at the time of secondary challenge. Ab, antibody; BmTCTP, *Brugia malayi* translationally controlled tumor protein.

[b] Challenge was done by inoculation with L3s unless otherwise stated.

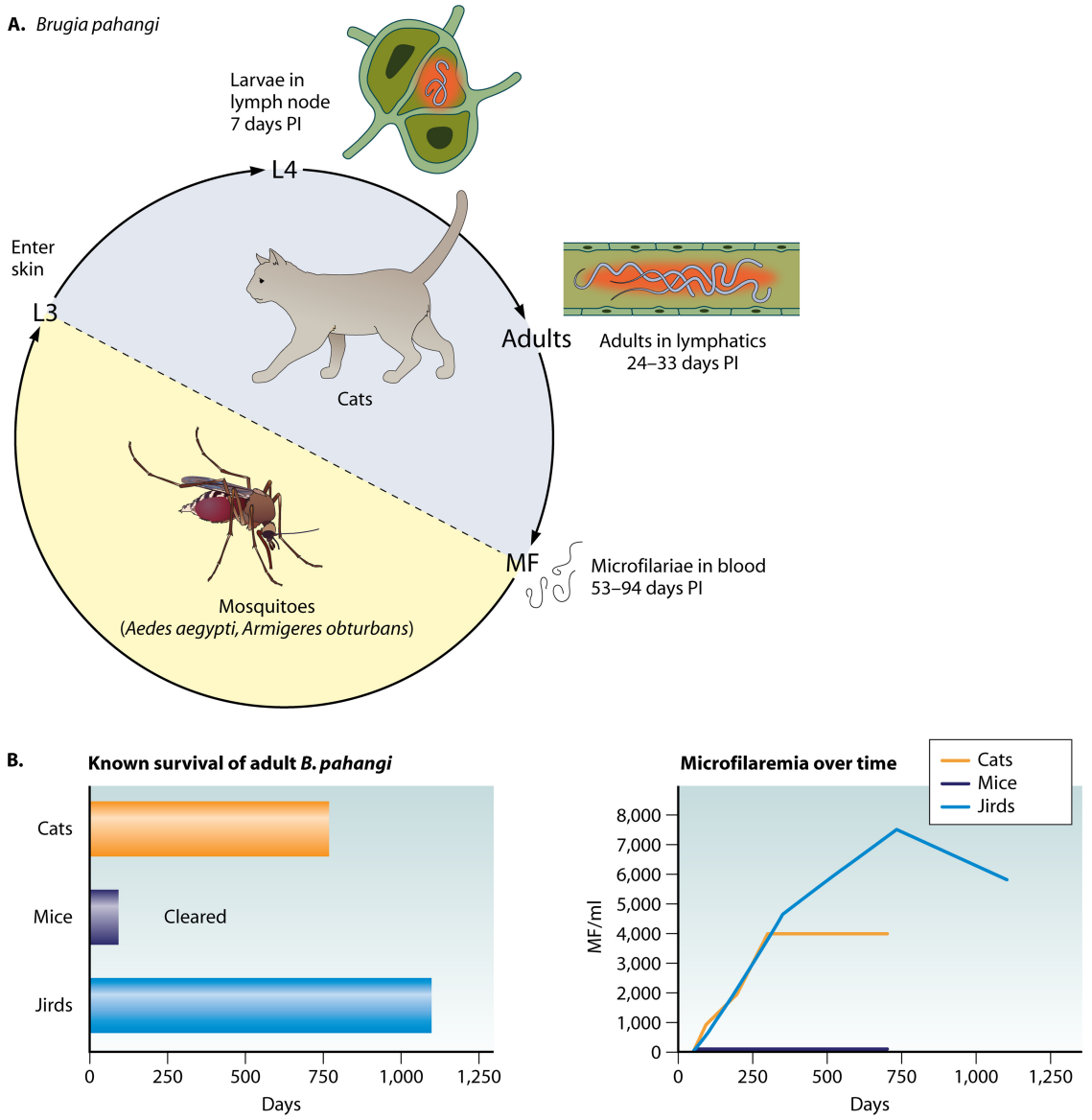

FIG 3 (A) Life cycle of *Brugia pahangi* within its natural host, the cat. (B, left) Survival of worms after infection in various hosts. Cleared indicates that the host is specifically known to clear infection at that time. (Right) Rough outline of the course of microfilaremia over time after infection with 100 L3s s.c. in cats (72) and 30 to 100 L3s s.c. in jirds (92) and mice (110).

After 20 repeat infections, burdens of adult worms do not increase further (73). Despite development of resistance to additional infections, the majority of cats (70 to 75%) remain microfilaremic for years after repeated inoculations with L3 larvae (73, 83). When cats do become amicrofilaremic, it can occur in three patterns: clearance of adult worms followed by gradual decline in microfilaria levels, dramatic drop in microfilaremia followed by slow decline in adult worm burden, and rapid clearance of MF with persistence of gravid adult females (83). Interestingly, worm-specific IgE is more commonly detected in cats that have cleared adult worms than in those with persistent infection (84).

(vi) Immunity after prior exposure. Other than a brief mention in a review article, there are no published studies of immunity to *B. pahangi* in cats following chemically abbreviated infection. In a review article in 1977, Denham and McGreevy mentioned that cats that have been repeatedly infected and treated with anthelmintics after the onset of patency continue to develop the same levels of microfilaremia, suggesting the absence of a protective immune response (78).

(vii) Vaccine studies. Cats vaccinated on 10 occasions with 300 irradiated L3 larvae showed an 80% reduction in adult worm burden after challenge, an increased time to patency, and a decreased chance of becoming microfilaremic (50%) (85).

(viii) Lessons learned and clinical relevance. This may be one of the better models in terms of relevance to humans. Infection dynamics, rare natural immunity, and the lack of protection after chemically abbreviated infection suggest that, immunologically, this model closely mimics human filariasis. Furthermore, this model exhibits disease symptoms of gross lymphedema.

The protection observed by a study that tested vaccination with

irradiated larvae in this model is promising for future vaccine research (86). However, the vaccine protocol utilized in this study has a number of limitations. First, the large number of vaccinations given (10 vaccinations) is not practical. It is not clear if the authors of this paper felt that this was necessary because of the failed trial using *B. malayi* in cats (87) or if unpublished trials with fewer vaccinations were not protective. Additionally, in this study, irradiated larvae were given a dose of only 10 kilorads, which sterilizes worms but does not prevent molting or pathology (86). These could both be prevented by giving a higher dose of radiation (86). In other models, the best results have been obtained when L3s were irradiated with a dose high enough to prevent molting.

***Brugia pahangi* in jirds. (i) Permissiveness.** Jirds are permissive to infection. While male and female jirds display no difference in susceptibility to infection from the L3 to the adult stage (88), more than 80% of male jirds develop stable microfilaremia, compared to less than one-half of female jirds (42, 89, 90).

(ii) Life cycle. The time course of infection is similar to that found in cats, with the L3-to-L4 molt taking place at about 7 to 9 days and the final molt at 18 to 24 days. Prepatent periods range from 57 to 118 days (43, 90, 91), and infections can last longer than 18 months (92).

Three methods of effectively infecting jirds with *B. pahangi* L3 larvae are used experimentally: i.p. injection, s.c. injection, and ocular inoculation. Each method results in some differences in localization of parasites. After s.c. injection, the majority of larvae are found in the skin near the injection site within the first 4 days and later found primarily in the lymphatics (90). About 25% of larvae develop into mature adult worms, which reside in the lymphatics, heart, lungs, and pleural cavity (88, 90). Worms infecting female jirds are more likely to infect the lymphatics than those infecting male jirds (88). In males, the majority of worms are found in the lymphatics that drain the testes, whereas in females, most are found in the lymphatics that drain the lower extremities (90).

i.p. injection of larvae allows for greater worm recoveries, with up to 50% of injected larvae developing to the adult stage (88, 90). i.p. injection enables easier collection and counting of worms, as over 90% of surviving larvae remain and develop within the peritoneal cavity (88, 90, 93). Despite being a more effective route for establishment of adult worm infections, worm localization and pathological sequelae after i.p. injection are different than those of natural infection, potentially limiting the utility of experiments conducted with i.p. inoculation.

Ocular inoculation is performed by dropping L3s onto the cornea of jirds. L3s penetrate the cornea, and within 5 min, some can be found in the pleural cavity, where the majority of worms will eventually localize (93). This induces a relatively low-level microfilaremia, which may be due to the necessity of MF to penetrate capillaries before entering the circulation (93).

(iii) Disease. While infection of jirds with *B. pahangi* does not cause gross lymphedema, subcutaneous inoculation does result in substantial inflammatory changes in lymphatic vessels (90). These changes begin at approximately 1 month p.i., become maximal by 4 months after infection (94), and are present in the setting of both living and dead adult worms (90, 94). Grossly, vessels become uniformly or irregularly dilated, which can give the vessel a beaded appearance. Histological changes include enlargement of regional lymph nodes; lymphatic vessel dilation; fibrosis of vessel walls; endothelial hyperplasia; intraluminal white and yellow lymph thrombi, which may occlude the vessels; and perilymphatic cellular infiltrates with neutrophils, macrophages, eosinophils, plasma cells, and lymphocytes (90, 94, 95). However, inflammatory changes are not limited to the lymphatic vessels. Granulomatous lesions will form around dying worms both in the lymphatics and in the peritoneum (96), and patent jirds also develop microfilaria-associated granulomas. Approximately one-half of infected jirds develop pulmonary congestion (90).

(iv) Natural immunity. All jirds appear to be susceptible to *B. pahangi*. Even though some jirds develop only transient microfilaremia, the exact reason for this is unknown. Jirds that develop only transient microfilaremia are not protected against future infection with L3s (97).

(v) Immunity in the setting of repeated parasite exposures. Despite some discrepancy in the literature regarding the development of protective immune responses from repeat infections, it is clear that if any concomitant immunity exists, it is relatively minor. The highest level of protection shown from repeat infections was reported by Kowalski and Ash, who found a 37% decrease in worm yields after 4 inoculations of 75 larvae (98). The work of Denham et al. showed a similar decreased yield after 5 to 20 repeat infections compared to a single infection (88). However, even after 20 repeat infections, jirds continued to accrue new parasites. Furthermore, the work of Klei et al., which had control groups for each set of infective larvae, suggests that there is either increased susceptibility or no protection derived from repeated infections even after 8 inoculations of 50 L3s (99, 100).

(vi) Immunity after prior exposure. Chemical abbreviation of active infections results in partial protection against subsequent infections, although this phenomenon is dependent on the timing of anthelmintic administration (101, 102). Treatment with flubendazole prior to inoculation with L3 larvae results in 40% protection against L3 larvae administered over 100 days later (103). Horii and colleagues conducted a fascinating study in which they observed that administration of mebendazole during the late prepatent period of infection (7 to 9 weeks p.i.) provided 77% protection against future infection with L3 larvae, whereas mebendazole given after patency provided no protection (101, 104). In addition to protecting against development of adult worms after L3 inoculation, chemical abbreviation during the prepatent period also induced marked protection against intravenous microfilaria challenge (101).

While not a study of direct prior exposure, evaluation of infection rates in progeny of infected mothers showed that *in utero* exposure conferred neither protection nor increased susceptibility and was associated with a decreased IgG response to *B. pahangi* antigens (105).

(vii) Vaccine studies. The irradiated larval vaccine is effective in this model (106). Larvae irradiated with 25, 45, and 90 kilorads have been used to vaccinate jirds, with up to 75% protection being obtained with 3 to 5 injections of L3s irradiated with 90 kilorads (106). Similar results were obtained by Storey and Al-Mukhtar, using irradiated L3s from *L. sigmodontis* followed by a heterologous challenge with *B. pahangi* (107). While the mechanism by which the irradiated larval vaccine confers protection in this model has not been elucidated, there is some evidence that there is a differential pattern of antigen recognition after vaccination with irradiated larvae compared to infection (108). Interestingly, vaccination with irradiated larvae elicits protection against an i.p. challenge equivalent to that against s.c. challenge (106).

Of note, intravenous inoculation with frozen MF and intramuscular (i.m.) and subcutaneous administration of adult soluble antigen in CFA produced no significant reduction in adult worm burdens after L3 challenge, although MF-vaccinated animals displayed a modest decrease in levels of circulating MF (95).

(viii) Lessons learned and clinical relevance. Subcutaneous inoculation of *B. pahangi* L3 larvae into jirds appears to be a good rodent model for studying potential vaccines for lymphatic filariasis. The worms localize to the lymphatic system, microscopic pathological changes are consistent with those found in humans, and protection is not obtained through infection alone.

As with other models, vaccination with irradiated larvae confers substantial, but not sterilizing, protection against infection. However, there is some evidence to suggest that there may be different mechanisms of protection after vaccination with irradiated larvae depending on the model used. For the *Litomosoides sigmodontis*-BALB/c model of filariasis vaccination, it was suggested that parasite clearance happens early and is the result of rapid clearance of invading L3 larvae before entrance into small-vessel lymphatics (109). However, the protection seen after i.p. challenge in the jird model suggests that mechanisms other than prevention of entry into small-vessel lymphatics are at play (106).

This model has also provided great insight into the protective effect of chemically abbreviated infections. Protective responses elicited by chemically abbreviated infections have been highly variable, ranging from increasing susceptibility of the host to providing over 90% protection. While these inconsistencies may simply be due to differences in each model, the study by Horii et al. implicates the timing of anthelmintic administration as another possible source of these differences (101).

***Brugia* in mice.** Note that *B. pahangi* and *B. malayi* infections in mice exhibit similar life cycles and correlates of natural immunity. In some of the studies using *Brugia* infections in mice, both *B. pahangi* and *B. malayi* were studied interchangeably, and it was difficult to determine exactly which model was used for each experiment. For these reasons, we are combining the information on these two very similar models.

(i) Permissiveness. Immunologically competent mice are nonpermissive hosts for *B. pahangi* and *B. malayi*, meaning that they do not develop microfilaremia after L3 inoculation despite occasionally harboring adult worms (110). C57BL/6 mice clear their infections by 6 weeks p.i., whereas BALB/c mice can harbor worms for up to 12 weeks p.i. The inability of *B. malayi* and *B. pahangi* to cause patent infections in mice appears to be due to the host immune response against the worms, as *Brugia* infections result in microfilaremia in both nude mice, which are deficient in T cells, and SCID mice, which lack both B and T cells (111, 112).

(ii) Life cycle. Mice can be infected with *Brugia* L3 larvae by i.p., i.m., i.v., and s.c. injections. s.c. inoculation is most similar to natural infection, as it results in worms residing in the lymphatics and heart (113). However, quantifying worms at the end of a vaccine experiment is difficult after s.c. inoculation, so some experiments utilize i.p. inoculation of L3s (after which worms remain primarily in the peritoneum), whereas others surgically implant distribution chambers containing L3s (114). The L3-to-L4 molt occurs at 7 to 10 days p.i., and the L4-to-adult molt occurs at around 30 days p.i. Microfilaremia develops by 8 weeks p.i. in immunodeficient mice (112).

(iii) Disease. s.c. inoculation of *Brugia* larvae into immunocompetent mice results in lymphatic inflammation but not in gross elephantiasis (110, 115). Changes include granulomatous inflammation around degenerating worms or cast cuticles, lymphangitis, mild lymphatic vessel dilation, and some lymphatic fibrosis. In immunocompetent mice, lymphangitis becomes maximal at 2 weeks p.i., after which the worms begin to be cleared by the host (115). Granulomas in immunocompetent mice are comprised of epithelioid and giant cells, lymphocytes, eosinophils, and fibroblasts (115) and can be seen around dying worms after i.p. injection as well (110). Interestingly, nude and SCID mice, which are unable to rapidly clear adult worms, develop frank lymphedema when infected with *Brugia* worms s.c. (112, 116). Histological changes include lymphatic dilation, lymphatic fibrosis, lymphangiectasia, and perilymphatic inflammatory infiltrates comprised mostly of neutrophils and monocytes but a lack of well-formed granulomas (112, 115). Interestingly, nude mice will develop an elephantoid syndrome when infected with *B. malayi* but not when infected with *B. pahangi* (116).

(iv) Natural immunity. While there is some strain variation in the time frame of events that transpire in mice infected with *Brugia*, infected mice develop an effective granulomatous immune response that clears the infection. C57BL/6 mice are more resistant to infection than BALB/c mice due partially to innate immune factors, as SCID mice in the C57BL/6 background are still more resistant to infection than SCID mice in the BALB/c background (110). In the intraperitoneal C57BL/6 model, parasite burdens stay steady for approximately 7 days p.i. and then decline rapidly at the same time as the L3-to-L4 molt. Worms that survive to 14 days p.i. are cleared at a decreased rate, but all worms are cleared by 4 to 6 weeks p.i. (110). Rather than exhibiting a rapid decline in parasite burden at the 7-day time point, worms inoculated by i.p. injection into BALB/c mice undergo a gradual decline commencing at 14 days p.i., with only 5% of the worms surviving by 4 to 6 weeks p.i. (110). Time course studies of *Brugia* worms injected i.p. into BALB/c mice by Carlow and Philipp demonstrated somewhat different survival kinetics, with a rapid decline in worm numbers from the time of inoculation to day 12 followed by a gradual decrease until elimination of all worms at 30 days p.i. (117).

Many immunological mechanisms underlying protection in this nonpermissive model have been elucidated through an elegant series of studies conducted by the laboratory of T. V. Rajan (118–125). Studies using depletion strategies and numerous strains of knockout mice demonstrated that T cells (120), B cells (121), IL-4 (124), IL-5 (119), gamma interferon (IFN-γ) (124), B1 B cells (122), IgE (126), and IgM (125) all play a part in protection. While both T cell and B cell responses likely contribute to immunity, B cell deficiency enhances permissiveness far more than T cell deficiency (127), suggesting that antibody responses are a major factor responsible for inherent resistance. Clearance of adult worms is related to the development of granulomas, which can encircle the body of parasites. Granulomas appear at 2 weeks p.i. in C57BL/6 mice and at 4 to 6 weeks p.i. in BALB/c strains (110) and consist of macrophages, eosinophils, and multinucleated giant cells (110). Consistent with the finding that B cells may play a major role in parasite elimination, there are data demonstrating that IgM produced from B1 B cells initiates the development of these granulomas, as mice that are unable to secrete IgM are deficient in cytoadherence to L3s (125).

Eosinophils have an important role in protection, as their absence is associated with increased permissiveness (118), and they

have been shown to penetrate into and under the cuticle of worms that otherwise appear healthy (110, 123). T cell-deficient mice are inept at recruiting immune cells to the infection site (125). However, the role of T cells in this immune response may be to simply activate B cells, which, once primed, are sufficient to provide protection to T cell-deficient mice (121). Eosinophil recruitment appears to be dependent in some way on antibody production, as JH mice, which have no mature B cells, show no increase in peritoneal eosinophil numbers after intraperitoneal infection.

Of note, the effective immune response which occurs in this model is dependent on infection with infective larvae, as implantation of adults into the peritoneum of outbred mice results in a patent infection that can last longer than 6 months (111).

(v) Immunity in the setting of repeated parasite exposures. Repeated infections shorten the clearance time in immunocompetent mice (110, 117–119, 128). As this is a nonpermissive model, repeat infections are not a direct measure of concomitant immunity.

(vi) Immunity in the setting of prior exposure. Previous infection is protective regardless of the method of inoculation (117), and as few as 2 worms can mediate this effect (117). The transfer of CD4 and CD8 T cells from previously infected mice to naive mice is sufficient to induce an accelerated immune response (128).

(vii) Vaccine studies. Since this is a nonpermissive model, the percent protection obtained by each vaccine study is dependent on the timing of the study endpoint, because no worms survive to patency. While Tables 3 and 4 contain percent protection reported by different studies using this model, it is important to note that these percentages cannot be directly compared because they were obtained at different time points.

Irradiated larvae (129, 130), L3 cuticles (128), BmALTII (128), and L3 ES products (128) have all been shown to be effective vaccines in the *B. pahangi*-mouse model, showing accelerated clearance after challenge infections. Efficacy of the L3 ES vaccine is mediated at least partially by antibodies, as the transfer of sera from vaccinated to naive mice is sufficient to induce a protective immune response (128). ES products from other stages of worm development (MF, L4, or adult) were not effective vaccine candidates (128).

Far more vaccine work has been done with *B. malayi* in mice. In contrast to work done with other filaria models, almost every reported vaccine trial using *B. malayi* in mice has demonstrated efficacy. Irradiated larvae (46, 114, 129, 131), dead larvae (117), the soluble fraction of larvae (117), live MF (117, 132), killed MF (46), MF antigen (46, 133), and SDS extracts of adults (134) were all protective against L3 infection, even though many of these antigen preparations contained no adjuvant. Specific antigens that have been tried in this model include abundant larval transcript (135, 136), thioredoxin peroxidase (135), transglutaminase (137), Bm97 (138), HSP12.6αc (139), and paramyosin (138), all of which have been shown to be at least partially protective.

Some work has been done to understand the protective immune response from the irradiated larval vaccine in this model. Transfer studies have shown that the transfer of T cells to naive mice is more efficient at eliciting a protective immune response than the transfer of sera (46, 131). Surprisingly, while most studies of irradiated L3 treatment have shown protection rates greater than 90% in this model, experiments evaluating L3 survival in implanted diffusion chambers found reductions of only 34% in the number of larvae that survived for 3 weeks. However, none of the larvae in vaccinated mice had molted to the L4 stage, whereas 96% had molted in the nonvaccinated mice (114).

Protection from MF appears to be mediated via antibodies, as a monoclonal antibody was found to promote faster clearance in mice (132). A strong type 2 response has also been associated with protection from microfilaremia (133).

(viii) Lessons learned and clinical relevance. Due to the high degree of resistance in this model, the most relevant attainable information lies in evaluating natural protective immunity in filariasis. Studies using this model suggest that effective immune responses depend on contributions from both B and T cells and culminate in a granulomatous reaction that kills the infective larvae. Furthermore, studies using this model suggest that the adaptive immune response helps to prevent disease development as nude mice develop lymphedema.

The major disadvantage to conducting vaccine studies with this model is that protective results may not accurately predict efficacy in humans. Because immunocompetent mice are naturally resistant to infection, experiments can demonstrate only whether clearance of parasites can be accelerated. The factors that help a resistant animal eliminate an infection more rapidly, however, may not necessarily induce protection in a permissive host. In contrast to vaccine studies in permissive models, almost every vaccine trial reported for *Brugia* infection of mice has shown some protection.

Dirofilaria immitis

For *D. immitis*, the vectors are *Anopheles*, *Culex*, and *Aedes* (reviewed in reference 140). The natural hosts are dogs, cats, wolves, coyotes, foxes, and ferrets (reviewed in references 140 and 141). The experimental hosts are mouse and Lewis rat (Table 5).

***D. immitis* in dogs. (i) Permissiveness.** Dogs are permissive to infection. When dogs are experimentally infected, one-half of infective larvae survive to the adult stage. Essentially all dogs experimentally inoculated subcutaneously with L3s will develop a chronic infection (4, 141), and at least 80% of naturally infected dogs develop microfilaremia (142).

(ii) Life cycle. The life cycle of *D. immitis* was reviewed by McCall et al. in 2008 (4). In brief, infective larvae molt to the L4 stage at 3 to 12 days p.i. and to the adult stage at 50 to 70 days p.i. During the early time course of the infection, the majority of larvae remain within the subcutaneous tissue. By 21 days p.i., most larvae have migrated to the abdomen, and by 41 days p.i., some have started to invade the thorax (reviewed in reference 4). Adults begin to penetrate the heart and lungs at 70 days p.i., where they reside preferentially in the right ventricle and pulmonary arteries (Fig. 4) (143, 144). Autopsy studies 136 days after subcutaneous inoculation demonstrated that 25 to 50% of L3 larvae administered by subcutaneous inoculation develop into adult worms (145). Dogs develop chronic microfilaremia starting at 6 to 7 months p.i. (4, 141), with adult worms surviving 5 to 7 years (4).

i.v. transplantation of adult worms is another method of experimental infection that can be carried out to immediately establish adult worm infection (4).

(iii) Disease. Sequelae of infection in this model can be both extensive and dire. The obstructive presence of adult worms combined with the inflammatory milieu leads to substantial vascular changes, endarteritis, arterial muscular hypertrophy, pulmonary hypertension, pleural effusions, and sometimes death resulting from respiratory distress or cachexia (4, 146, 147). Other possible

complications include eosinophilic pneumonitis, anemia, caval syndrome, and diverse kidney pathology (147). Pathological conditions of the kidney are discussed by Paes-de-Almeida et al. and may be the result of immune complex formation (148).

(iv) Natural immunity. Latent infections, in which adults survive without circulating MF, are rare but occur occasionally. This phenomenon is potentially the result of an immune response in which antibodies to MF ES products cause agglutination of MF and their subsequent destruction by eosinophils, neutrophils, and lymphocytes (4). Natural protective immunity to adult worms is not known to occur, although extensive studies have been conducted only on beagles.

(v) Immunity after prior exposure. Chemically abbreviated infections provide moderate to excellent protection (47 to 98%) in this model, depending on the protocol used (143, 149). The best results of 98% protection were obtained with three abbreviated infections of 400, 150, and 300 larvae 532, 420, and 329 days prior to challenge infection, followed by ivermectin 2 months after each priming infection (149). Interestingly, in one study, inoculation of animals with Freund's complete adjuvant, which skews the immune system toward a type 1 phenotype instead of the type 2 phenotype (which typically occurs in response to helminths), increased protective immunity obtained by chemical abbreviation of infection from 50% to 72% (143).

(vi) Vaccine studies. Vaccination with irradiated *D. immitis* L3 larvae provides 45 to 88% protection. The best results are obtained when the vaccine is separated from infection by at least 3 months (145). The protective effect appears to be mediated within the first 41 days of challenge infection (145) and is associated with immune responses to specific worm antigens (150, 151).

(vii) Disease after treatment or vaccination. Larvae irradiated with 20 kilorads and subsequently inoculated do not survive beyond 66 days p.i., are not found in the heart or lungs after infection, and do not induce patent infections (145).

Treatment of chronically infected dogs with anthelmintics can result in severe complications. Death of adult worms and subsequent worm degeneration provide a milieu where worm detritus can get trapped in small blood vessels and lead to impaired blood flow and thromboembolism in the host (reviewed in reference 152).

(viii) Lessons learned and clinical relevance. Despite the fact that dogs are extremely susceptible in this model, both chemically abbreviated infections and irradiated larval vaccines are protective against future infection. As the pathological consequences of infection are caused mainly by the presence and number of adult worms, sterilizing protection is not necessary to gain a benefit from vaccination. An immune response that helps to minimize the number of adult worms that invade the pulmonary arteries and heart can be beneficial to the host. The increased protective effect seen when there is a prolonged period between vaccination and challenge is interesting and has not been noted in other models. Indeed, when using *Litomosoides* in mice, protection begins to decline 6 months after vaccination with irradiated L3 larvae (153).

While it is possible that work has been done in the private sector toward producing a *Dirofilaria* vaccine, it is surprising that more work has not been done in this model in the public setting. The finding of a vaccine that can induce substantial immunity in this model not only would aid in human filarial research but also could be immensely beneficial for the protection of dogs from this

TABLE 5 Vaccine and repeat infection trials using *D. immitis*[a]

Host	Immunization category	Immunization	Adjuvant	Dose	Protection (%)[b]		Reference(s); note(s)
					L3/adult	MF	
Dogs (permissive)	Live worms	CAI	+/− CFA	Varied	47–98		143, 149
	Irradiated larvae	Irradiated L3		150–1,000 1–3×	42–88		143, 145, 150
Ferrets (transiently permissive)	Live worms	CAI	None	30 L3s 2×	92		155; many of the ferrets died from unknown causes in this study
Lewis rat (nonpermissive)	Dead worms	Dead MF	CFA	10^5 MF		Faster clearance	274; MF challenge
	Fraction or homogenate	MF extract	CFA	50–100 μg 4×		Faster clearance	274; MF challenge
Mice (nonpermissive)	Live or dead worms	Repeat infection	None	30–300 1–5×	25–39		159
		Freeze-killed larvae		75 3×	↑ survival		159
		Irradiated L3s		75 3×	45		159
	Irradiated larvae	Insoluble fraction of larvae	CFA	56 μg 2×	18		162
	Fractions or homogenates	Soluble fraction of intestinal antigen	CFA	11 μg 2×	51		162; other fractions were not protective

[a] All repeat infection studies are shaded. Repeat infection studies that tested for protective immunity after chemical abrogation of the primary infection are labeled CAI.
[b] Challenge was done by inoculation with L3s unless otherwise stated.

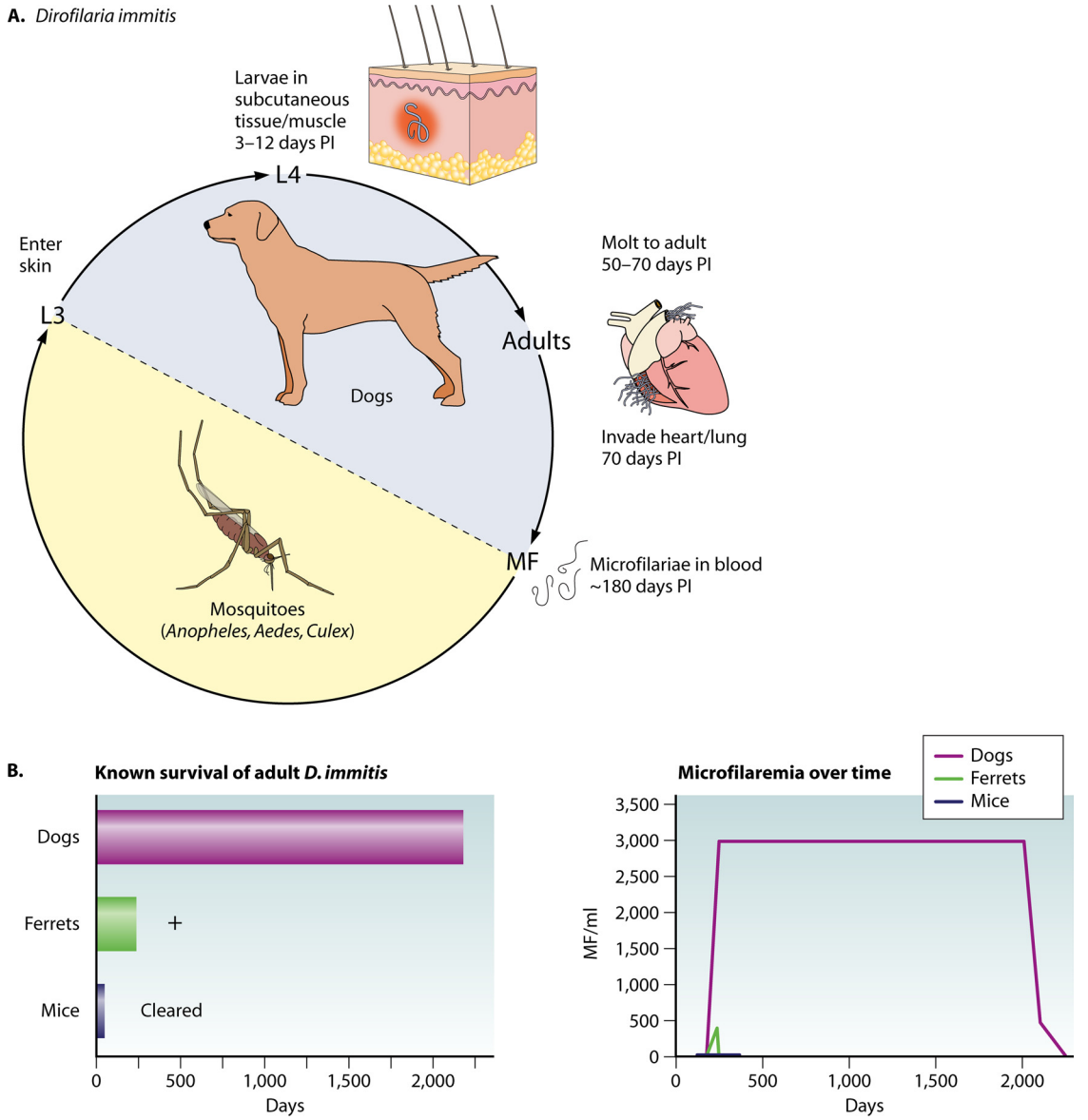

FIG 4 (A) Life cycle of *D. immitis* in its natural host, dogs. (B, left) Survival of *D. immitis* after infection in various hosts. Cleared indicates that there is evidence that infection has been cleared by that time point; + indicates that the host most likely lives longer, but no published reports have specifically shown longer survival. (Right) Rough outline of the possible course of microfilaremia over time after infection in dogs based on stable microfilaremia and long survival and in mice based on lack of patency. The ferret curve was based on s.c. inoculation with 14 L3s (158) and on statements that microfilaremia is transient and at low levels (4).

dreadful disease. Furthermore, the advent of a vaccine against *Dirofilaria* in dogs would presumably also decrease the transmission of this disease to humans.

***D. immitis* in ferrets. (i) Permissiveness.** Ferrets are permissive, with transient microfilaremia. Both male and female ferrets inoculated with L3s develop chronic infection with adult worms. Although the worms develop to sexual maturity and produce MF by 7 months p.i., the duration of microfilaremia is short (141). Pet ferrets are often naturally infected with *D. immitis* (141).

(ii) Life cycle. When larvae are inoculated subcutaneously, most either remain in the subcutaneous tissue or migrate into the muscles for the first 90 days p.i. (154). Both molts occur in these locations, with the L3-to-L4 molt starting at day 3 and the L4-to-adult molt starting at day 56. Adult worms begin to invade the right heart chambers at 70 days p.i. (141), and the majority of adult worms end up in the cranial and caudal vena cava (4). When it occurs, microfilaremia usually starts at 7 months p.i. Although *D. immitis* worms have been known to survive for at least 262 days p.i. in ferrets (155), the full life span has not been studied with this model (4).

(iii) Disease. *D. immitis* causes the same clinical features in ferrets as in dogs, although severe complications develop much sooner in ferrets after infection (4). Sequelae include anemia, anorexia, dyspnea, right-sided heart enlargement, heart murmur, cyanosis, pleural effusion, caval syndrome, heart failure, and sudden death, usually from pulmonary embolism (156; reviewed in

references 4 and 141). Infection is fatal when ferrets are infected with more than a few worms (155).

(iv) Natural immunity. Virtually all ferrets experimentally infected with L3 larvae develop chronic infections with adult worms, with average recoveries of 50 to 60% of infected larvae, suggesting no substantial natural immunity to primary infection (reviewed in reference 141). It is not known whether the transient nature of the microfilaremia is due to protective immune responses from the host against MF or due to other factors.

(v) Immunity after prior exposure. Challenge infection after chemical abbreviation of infection has been examined in only one study, which found that treatment of two infections during the prepatent period with ivermectin provided exceptional (92%) protection against future challenge infection (155).

(vi) Disease after treatment or vaccination. Ivermectin has been shown to clear parasites from ferrets when given within 30 days p.i. (157). Under these circumstances, all worms end up dying before patency would commence. While the chemically abbreviated infection proved to be very effective at preventing future infection, many of the animals in this study died from unknown causes prior to the challenge worms reaching the heart and lungs (155). The timing of death may suggest that something other than the vaccination/infection protocol was responsible for the ferrets' deaths, but this is not clear, as a high inoculum can kill ferrets as quickly as 16 days p.i. (158). Since chemotherapeutic treatment of filarial infections in humans does not seem to predispose to increased pathology during subsequent natural infections, it is not clear whether this finding is relevant for studies evaluating the safety of human filarial vaccines.

(vii) Lessons learned and clinical relevance. While this model does not mirror the pathology of any major human filarial infection, it could be an important model in its own right. If a serious attempt at a vaccine against *D. immitis* was made for animal companions, it would be best if the vaccine were effective in dogs, cats, and ferrets, as they are all susceptible to infection. Due to cost and size of housing, this model could be used as a first step for screening of *D. immitis* vaccine candidates for safety and efficacy.

***D. immitis* in mice. (i) Permissiveness.** Mice are nonpermissive to infection.

(ii) Life cycle. As all vaccine trials have been performed by using distribution chambers in the mouse model, we will focus on this method of inoculation. Worms are placed inside Lucite rings with either a 3- or 5-μm-pore-size membrane and then implanted into a subcutaneous pocket lateral to the spine. More than 80% of larvae survive and continue to grow in chambers for at least 2 weeks (159). Other methods of study include intravenous inoculation of mice with MF and transplantation of adult worms into the peritoneal cavity (160, 161).

(iii) Disease. Implantation of *D. immitis* worms in chambers does not cause significant disease in mice. Tissue encapsulation of chambers throughout these experiments is minor or nonexistent (159).

(iv) Immunity in the setting of repeated parasite exposures. Repeated inoculations of 30 to 300 larvae up to 5 times prior to implantation of *D. immitis* L3s in chambers results in 25 to 39% decreased survival of implanted larvae (159). In this study, protection against *D. immitis* was correlated with increased antibody titers to the soluble fraction of L3 (159).

(v) Immunity after prior exposure. Mice administered live L3s by subcutaneous injection exhibit modest protection (~25%) when subsequently challenged with L3 larvae in an implantation chamber (159).

(vi) Vaccine studies. Subcutaneous injection of irradiated larvae confers moderate (45%) protection when L3s are attenuated but not killed by radiation (159). The highest levels of protection in this model have been obtained by vaccination with the soluble fraction of intestines obtained from adult worms, using CFA as an adjuvant (162). Antigens from the intestinal tract of *Dirofilaria* were considered "hidden" by the authors of this study because dogs infected with *Dirofilaria* produced little to no antibody response to these antigens. Mice vaccinated with the soluble fraction of *D. immitis* intestine, however, produced antibodies that could bind to the intestinal tract of the parasite. The mechanism of protection was postulated to be due to activation of complement within the digestive tract of the worm or blockade of absorption and/or digestive enzyme function within the intestine of the worm (162).

(vii) Lessons learned and clinical relevance. This model does not show any relevance to human disease and is resistant to infection. Despite the high level of resistance in this model, worm clearance from implanted chambers is not markedly accelerated after repeated infections or vaccination with irradiated larvae. It is unclear whether the low level of protection garnered by vaccination with irradiated larvae in this model is from a lack of contact of challenge larvae with host tissues or from relatively weak host immune responses.

It is interesting that the highest level of protection in this model has been achieved with intestinal antigens, which may be somewhat hidden from the immune response during natural infection. Helminths are astounding in their ability to survive in susceptible hosts for years despite the presence of many parasite-specific antibodies. The presence of potentially protective antigens to which the host does not naturally respond is intriguing for future research.

Litomosoides sigmodontis

The vector of *Litomosoides sigmodontis* is the mite (*Ornithonyssus bacoti*). The natural host is the cotton rat. The experimental hosts are jirds, mice, albino rats, and *Mastomys* (Table 6).

***L. sigmodontis* in albino rats. (i) Permissiveness.** Albino rats are permissive to infection, with transient microfilaremia.

(ii) Life cycle. Infective larvae migrate through the lymphatics to the pleural cavity and preferentially infect the right pleural cavity (163). Patency commences at 57 to 77 days p.i. (164). MF counts peak 4 weeks after patency develops, remain at high levels for another 4 to 6 weeks, and then drop until the rats enter a latent state, with no microfilaremia despite the presence of adult worms (Fig. 5) (163, 164).

(iii) Disease. Infection results in pathological changes in the lungs, splenomegaly, and decreased function of the liver and spleen (165, 166).

(iv) Natural immunity. Latency has been shown in this model to be dependent on cell-mediated immune factors that hamper MF from penetrating the pleural capillaries (167). Additionally, IgE production is temporally associated with clearance of microfilaremia and has been shown to cause adhesion of macrophages and neutrophils to MF (168). However, the latent state achieved in this model is not solely antibody dependent, as the transfer of serum from a latent rat to a newly infected rat does not impart protection in this model. In latently infected rats, the transfer of

TABLE 6 Vaccine and repeat infection trials using *L. sigmodontis*[a]

Host	Immunization category	Immunization	Adjuvant	Dose	Protection (%)[b]		Reference(s); note(s)
					L3/adult	MF	
Albino rats (transiently permissive)	Live and dead worms	i.t. implantation of male and female adult worms				Yes	167; challenge by i.t. implantation of female worms
		i.t. implantation of adult males				No	167; challenge by i.t. implantation of female worms
		i.t. implantation of adult females				Yes	167; challenge by i.t. implantation of female worms
		i.t. implantation of killed worms				No	167; challenge by i.t. implantation of female worms
	Irradiated larvae	Irradiated L3s		45–55 L3s 2×	91	Yes	171; irradiated L3s lived a maximum of 25 days with no molting (40 kilorads)
	Fractions or homogenates	Sonicated MF	CFA	50,000 2×	94	100	172
		Sonicated L3s	CFA	120 2×	95	100	172
		Soluble adult antigen	CFA	0.75 mg 2×	Insig.	88	172
		Adult worm homogenate	CFA	0.75 mg 2×	64	99	172
		Adult male worm homogenate	CFA	0.75 mg 2×	Insig.	Insig.	172
Cotton rat (permissive)	Live worms	MF given s.c.		5×	None	88	183; similar results were found when challenge was by i.v. MF or natural infection; repeated i.v. injections of MF caused faster clearance of MF
		Repeat L3 infections (after clearance of initial infection)			None		181; stunted size of worms
		Repeat L3 infections (CI)		Varied	0–20		181, 182; infective larvae displayed retarded growth and delayed molting; no clear decreases in worm yield even after 5 L3 challenges
	Irradiated larvae	Irradiated L3s		50 3×	↑ Survival		180; challenge was performed by i.p. implantation of adults
	Fractions or homogenates	Ground antigen			None		181, unpublished data
		D. immitis adult antigen		20 mg/kg of body wt 2–9×	0		275; similar results if given during or prior to infection
Jird (permissive)	Live worms	Repeat L3 infections (CI)		30 L3s 1×	33–48	Yes	186, 187
	Irradiated larvae	Irradiated L3s		50–100 3×	98	Yes	107
M. natalensis (permissive)	Live worms	MF		500,000 MF 6×	Insig.	+/−	193; protection only when challenge was MF by i.v. route but not when challenge was L3s by s.c. route
		Repeat infection at time of latency		Natural infection	None	95–99	188; see text for details
		CAI		Natural infection	33	40–60	188; treated with either amoscanate or furazolidone-DEC
		Intrauterine forms		6×10^6/kg	None	Yes	192; boosted with i.v. and i.p. injections of MF
Mice (transiently permissive)	Dead worms	MF (amotile)	Alum	100,000 3×	0–75	70–100	219; vaccinated and nonvaccinated mice showed similar worm burdens until ~70 days p.i.
	Irradiated larvae	Irradiated L3s		25 L3s 3×	49–99		109, 153, 214, 216, 217, 276, 277; abrogated by IL-5 deficiency in C57BL/6 mice and in BALB/c mice treated with IL-5 neutralizing antibody
	Wolbachia protein	*Wolbachia* surface protein	Alum/CFA	50 μg 3×	Insig.		220; increased worm burden when given with CFA

[a] All repeat infection studies are shaded. Repeat infection studies that clearly tested for the presence of concomitant immunity by giving a challenge infection in the setting of an ongoing active infection are labeled CI, and repeat infection studies that tested for protective immunity after chemical abrogation of the primary infection are labeled CAI; all other repeat infection studies either tested for protective immunity after natural clearance of a primary infection or did not explicitly state the status of the first infection at the time of secondary challenge.

[b] Challenge was done by inoculation with L3s unless otherwise stated.

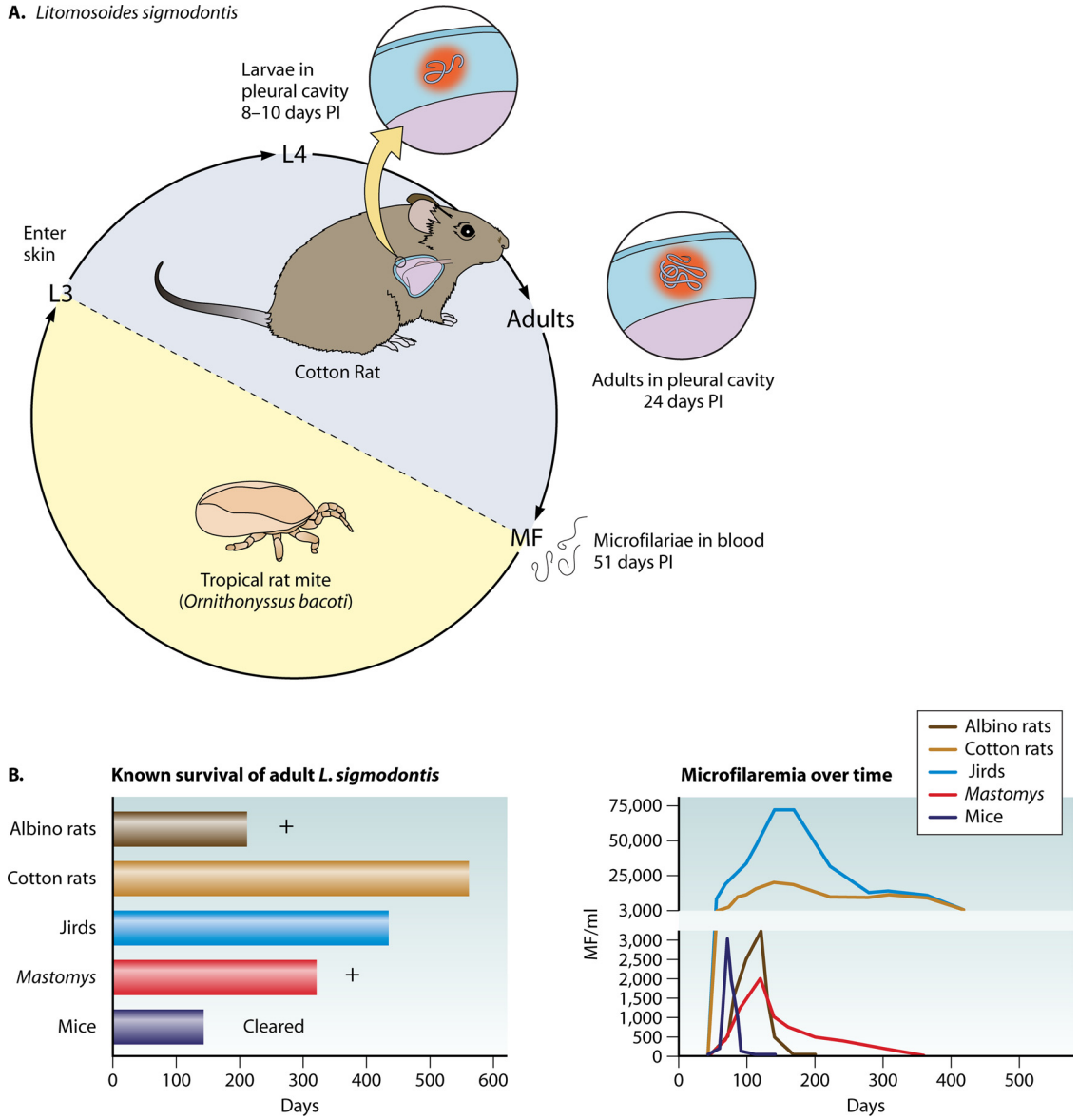

FIG 5 (A) Life cycle of *Litomosoides sigmodontis* in its natural host, the cotton rat. (B, left) Survival of worms after infection in various hosts. + indicates that the host most likely lives longer, but no published reports have specifically shown longer survival; Cleared indicates that there is evidence that infection has been cleared by that time point. (Right) Rough outline of the course of microfilaremia over time after natural infection in albino rats (163), intradermal injection of 100 L3s into cotton rats (176), intradermal injection of 100 L3s into jirds (176), injection of 60 L3s into *Mastomys* rodents (192), and natural infection of mice (219).

adult worms from the pleural cavity to the peritoneal cavity circumvents protection from microfilaremia, suggesting that the location of adult worms is important in this latent state (163).

(v) Vaccine studies. *(a) Protection against microfilaremia.* Vaccines containing MF antigen are effective at preventing microfilaremia in this model. Vaccination with MF, irradiated MF, sonicated MF, and adult worm homogenate containing MF and intrathoracic (i.t.) implantation of adults harboring MF have all been shown to impart protection against developing microfilaremia in this model (167, 169). In contrast, soluble adult worm extract and the presence of adult male or female worms without MF do not substantially alter microfilaremia. Vaccination with ES products from MF produced IgG antibodies in albino rats that were able to clear circulating MF in *M. natalensis* (170).

(b) Protection against infective larvae. Partial protection against infection with infectious L3s in this model has been achieved with sonicated L3s in CFA, adult worm homogenate with CFA, sonicated MF in CFA, and irradiated L3s (171, 172). Protection achieved with adult worm homogenate may be due to the presence of MF in adult worm homogenate, as adult male homogenate fails to impart significant protection (172).

Vaccination with irradiated larvae provides striking (91%) protection against future infection with infective-stage larvae and is associated with IgG production that promotes cytoadherence to both MF and L3s (171). Larvae require 40 kilorads or more of irradiation to induce a protective immune response.

(vi) Lessons learned and clinical relevance. Although infected rats show some pathological changes after infection, this model is

not a model of human disease. It does, however, appear to be a reasonable model in which to study filarial latency. This model exhibits a latent infection mediated by a mechanism that is distinct from those of other models. A study by Bagai and Subrahmanyam in 1968 showed that transplantation of adult worms from the pleural cavity to the abdominal cavity of the same animals resulted in redevelopment of microfilaremia in animals that had previously been amicrofilaremic, suggesting that protection against microfilaremia may be due to local (in tissue) immune responses, as opposed to anti-MF IgM or IgG antibodies (163). The subsequent finding of Mehta et al. that IgE is critical for antibody cytotoxicity to MFs during natural infection suggests that cells which are activated by binding antigen-specific IgE on their surfaces (such as basophils, mast cells, and eosinophils) may be critical effector cells in this process (168).

In terms of vaccine development, as with other models, the finding that MF-containing vaccines protect against microfilaremia suggests that this stage may be amenable to vaccine-mediated protection for the purpose of preventing spread of infection and that various immune mechanisms can be utilized to this end.

***L. sigmodontis* in cotton rats. (i) Permissiveness.** Cotton rats are permissive to infection. Inoculations as low as 5 L3s are sufficient to establish patent infection (173). When exposed to numerous L3 larvae, cotton rats can harbor up to 1,000 *L. sigmodontis* adults (174, 175). Experimental infections suggest that approximately 21% of inoculated larvae survive to adulthood (176).

(ii) Life cycle. Bertram's paper from 1966 provides a very extensive review on the life cycle and effects of repeated infections of *L. sigmodontis* in cotton rats. After introduction by the bite of a mite, L3s travel preferentially to the pleural and pericardial cavities of the cotton rat (175). When parasite burdens reach approximately 400 worms, the peritoneal cavity of the cotton rat will also become parasitized (175). However, adults can sometimes be found in the peritoneum of rats containing fewer than 400 worms (174). Larvae molt to the L4 stage at 8 to 10 days p.i. and to the adult stage at 24 days p.i. Microfilaremia develops on day 51 p.i. (177) and increases steadily until 10 weeks p.i. At this time, there is a dichotomy regarding the course of microfilaremia. MF levels start to decline at week 10 p.i. in half of cotton rats and then disappear 3 months later (173). This truncated course of microfilaremia is not associated with death or a change in fertility of adult worms (173). In the other half of cotton rats, MF levels continue to increase until between weeks 20 and 24 p.i. and then undergo a slow decline (176).

(iii) Disease. Infected cotton rats can display hepatosplenomegaly, lymphadenopathy, pleural hypertrophy, pleural papillary nodules, pleural edema, lymphatic dilatation, alveolar thickening, histological changes in the bronchioles consistent with asthmatic changes, and scattered myocarditis (178). The severity of pathological change is often proportional to worm burden. Entrapment of MF in the capillaries of the lung can result in reactive tissue eosinophilia and lymphocytosis (178). Death sometimes occurs at 24 to 32 weeks p.i. in cotton rats, after a period of wasting (176).

(iv) Natural immunity. Adult worms start to become encapsulated and die at around 1 year p.i. Concurrently, MF levels gradually decrease until the host becomes amicrofilaremic (175). The truncated microfilaremia that is exhibited by some cotton rats is associated with an inability of microfilaria to enter the circulation from the pleural cavity (173).

The life cycle of the parasite plays an important role in inducing an immunologic milieu necessary for adult survival. When adult worms are surgically implanted into the thorax or peritoneal cavity of a naive cotton rat, most worms are quickly encapsulated and die within 10 days posttransplantation (174, 179). This effect is not seen in splenectomized cotton rats with a nonfunctioning reticular endothelial system, infected cotton rats, or cotton rats that have been vaccinated with irradiated larvae or *D. immitis* antigen (174, 180).

(v) Immunity in the setting of repeated parasite exposures. In this model, there is very little acquired resistance that develops from natural infection. L3 larvae administered subcutaneously into cotton rats with active infections exhibit some growth stunting compared to worms administered to uninfected cotton rats but little or no decrease in percent yield (175, 181, 182). While these studies suggest the presence of only minimal concomitant immunity, the number of animals challenged to date with more than one repeat challenge is too small ($n = 3$) (182) to conclude that concomitant immunity does not occur in the setting of numerous infectious challenges.

In contrast to repeat L3 infections, repeated i.v. injections of MF into uninfected cotton rats result in accelerated clearance after each injection (183). In cotton rats first vaccinated subcutaneously with MF, clearance of i.v. injected MF from the peripheral blood is almost instantaneous, showing 98% clearance within 5 min (183). In this study, MF localized to the lungs, were bound by host cells, and became immobilized. This protective effect against microfilaremia by subcutaneous MF administration was also observed when the secondary challenge was by vector-borne transmission of L3s (183).

(vi) Immunity after prior exposure. One experiment suggests that challenge with L3s after cotton rats have cleared a primary infection results in a moderate stunting of adult worm length but does not decrease worm numbers (181).

(vii) Vaccine studies. Few studies have utilized this model for vaccine research. Crude homogenate antigen of adult worms, frozen L3s, and *D. immitis* adult antigen have been shown to be ineffective (181, 182).

(viii) Lessons learned and clinical relevance. The strengths of this model are that it is very permissive to infection and that pathological changes within the lungs may be similar to those of tropical pulmonary eosinophilia (TPE). Interestingly, repeated injections of MF induce a protective immune response that may not develop during a normal infection. Discouragingly, however, this caused dying MF to congregate in the lungs after repeat MF injections. The effect of MF congregation in the lungs on TPE-like pathological changes was not monitored, but it is likely that an MF vaccine could cause more severe symptoms if MF are preferentially trapped within the lungs.

***L. sigmodontis* in jirds. (i) Permissiveness.** Jirds are permissive to infection. Jirds show no gender-specific differences in susceptibility (176). The percentage of larvae that survive to adulthood is highly variable, with a mean of approximately 31% after intradermal injection of 100 L3-stage larvae obtained from dissected mites (176). Despite being an unnatural host for *L. sigmodontis*, jirds display higher percent yields than cotton rats (176).

(ii) Life cycle. Most L3s migrate to the pleural cavity by 4 to 5 days p.i. (184, 185). There are no significant differences in parasite survival within the first 4 months p.i. in the jird compared to the cotton rat (reviewed in reference 176), although occasional nodules around worms can start to be seen as early as 4 to 8 weeks p.i.

in jirds (176). Worm encapsulation and death substantially affect worm yields by 44 weeks p.i. in this model (176). Microfilaremia commences at 8 weeks p.i., peaks by 24 weeks p.i., and declines rapidly thereafter (176). Peak microfilaremia in jirds is incredibly high, with median concentrations of 700,000 MF/ml at 20 weeks p.i. (176). These levels are much higher than those exhibited by cotton rats, which have peak median microfilaria levels of just over 200,000 MF/ml at 20 weeks p.i. (176). From weeks 32 to 52 p.i., the decline in microfilaremia in jirds brings levels back down to those in cotton rats (176). While *L. sigmodontis* preferentially localizes to the pleural cavities of jirds, adult worms can also be found in the peritoneal cavity and pericardial sac.

(iii) Disease. Infected jirds display pathological changes similar to, but more severe than, those displayed by infected cotton rats (176). Pathological sequelae include papillary nodules, mesothelial hyperplasia, alveolar hypertrophy, prominent splenomegaly (178), and tissue adhesions, which can lead to fibrosis of the pleural and pericardial cavities (our unpublished data). High infectious doses can cause mortality at 3 to 4 days p.i. (our unpublished data). Infection with 100 L3 larvae has been reported to cause cachexia and death at 24 to 32 weeks p.i. in approximately one-third of infected jirds (176), although in our laboratory, infection of jirds by subcutaneous inoculation of 80 L3 larvae does not result in appreciable morbidity or mortality (our unpublished data).

(iv) Natural immunity. Despite allowing more L3s to survive to adults, jirds encapsulate worms much sooner than do cotton rats (176). Encapsulation can occur as early as 4 weeks p.i. and becomes pronounced by 12 weeks p.i. (176).

(v) Immunity in the setting of repeated parasite exposures. Repeated infections result in stunting of larval growth and decreased ability of larvae to migrate to the pleural cavity (186, 187). This results in 33 to 48% reduced survival in challenge infections (186). Transfer of both plasma and lymphocytes into naive jirds inhibits larval growth, although neither plasma nor lymphocytes alone are sufficient for this effect (187).

(vi) Vaccine studies. Vaccination of jirds 3 times with irradiated larvae results in protection as high as 98% against L3 challenge (107). Storey and Al-Mukhtar found that this resulted in complete protection from microfilaremia in 11 out of 13 jirds and only transient microfilaremia in the other 2 jirds (107).

(vii) Disease after treatment or vaccination. No disease after treatment or vaccination has been observed. Irradiated larvae do not develop into adult worms in this model (107).

(viii) Lessons learned and clinical relevance. This model, similar to the *L. sigmodontis*-cotton rat model, is not a model of human disease other than possibly TPE. However, perhaps the most relevant information obtained from this model is that it clearly demonstrates very substantial levels of protection after vaccination in an otherwise permissive model. Furthermore, this model is interesting because jirds are in some ways more resistant but in other ways more susceptible to infection than cotton rats. Jirds initially develop higher parasite burdens and higher levels of microfilaremia, yet jirds begin to clear infections much sooner than do cotton rats. Therefore, while jirds develop more pathological sequelae from *L. sigmodontis* infection, this may be due to higher parasite burdens or enhanced immunological responses against the parasites.

***L. sigmodontis* in *Mastomys*. (i) Permissiveness.** *Mastomys* rodents are permissive to infection. All *M. natalensis* rodents infected with 40 L3s develop microfilaremia, and 53 to 71% of inoculated larvae can be recovered at 120 to 319 days p.i. (54).

(ii) Life cycle. Adult worms live within the pleural cavities of *Mastomys* rodents (54). After s.c. inoculation with L3 larvae, microfilaremia can be detected at 56 days p.i., peaks between 100 and 130 days p.i. (54), and typically clears by 360 to 390 days p.i. (188).

(iii) Disease. Infection results in leukopenia and anemia due to intravascular hemolysis and dyshemopoiesis, with reduced stability and increased osmofragility of red blood cells (189). The exact mechanisms underlying these hematological phenomena are unknown.

(iv) Natural immunity. Adult worms transplanted into the pleural or peritoneal cavities of naive *Mastomys* rodents are encapsulated and killed at 10 to 17 days p.i. or 17 to 24 days p.i., respectively (190, 191). Current infection or splenectomy abrogates this protective immune response (190, 191). Mechanisms underlying MF clearance at 360 days after s.c. injection of L3 larvae are not known.

(v) Immunity in the setting of repeated parasite exposures. *Mastomys* rodents that are challenged by s.c. inoculation of L3 larvae and then allowed to reach a postpatent state without anthelmintic therapy are strongly protected against future microfilaremia when challenged with repeated s.c. inoculations of L3 larvae, i.p. implantation of L4 or adult worms, or intravenous administration of MF. Despite this strong protection against microfilaremia, postpatent *Mastomys* rodents are not protected against L3 larvae administered by s.c. inoculation or adults implanted into the peritoneum (188).

(vi) Immunity after prior exposure. *Mastomys* rodents that have been infected and subsequently treated with furazolidone and DEC or amoscanate at 85 to 130 days p.i. showed very minor protection against infective larvae (33%) and microfilaremia (40 to 60%) (188).

(vii) Vaccine studies. s.c. injection of MF obtained from the uterus of adult female worms boosted with i.p. and i.v. injections of MF obtained from peripheral blood of infected animals causes *M. natalensis* to produce an anti-MF antibody that leads to agglutination and death of MF (192). This vaccine protocol is not effective at reducing adult parasite burdens after challenge infection, yet MF levels remain lower in *Mastomys* rodents vaccinated in this manner. Interestingly, agglutinating antibodies produced by vaccinated mice disappear 32 days after infection with L3-stage larvae (192). This disappearance of agglutinating antibodies after infection with L3 larvae may be the reason why Nogami et al. found that repeated subcutaneous injections of MF induced protection against microfilaremia when *Mastomys* rodents were challenged by i.v. inoculation of MF but not when challenged by s.c. inoculation of L3-stage worms (193).

(viii) Lessons learned and clinical relevance. This is not a model for human disease. The benefits of this model include a permissive host and observable effects of immunomodulation on parasite survival. The studies that have been carried out in this model highlight the importance of the immunomodulation that occurs during helminth infections. While naive animals are capable of killing adult worms, this process is hampered once the host has been exposed to infective larvae.

Similarly, infection appears to decrease levels of MF-depleting antibodies induced by prior vaccination. This depletion of antibodies may be the result of cross-reactivity of anti-MF antibodies and antigen present in other stages of the life cycle. Juvenile adult

L. sigmodontis females produce Juv-p120, an antigen that may interact with antibodies directed against the sheath of MF (194), and it is possible that this antigen depletes antibodies that could protect against the MF stage.

***L. sigmodontis* in mice. (i) Permissiveness.** BALB/c, BALB/k, and BALB/b mice are transiently permissive, with BALB/c mice sustaining the longest period of microfilaremia (195). Female BALB/c mice are more susceptible to infection than male BALB/c mice, as measured by both adult worm burden and microfilaremia, but in other strains of mice, males are more susceptible (195, 196). Between 30 and 100% of infected BALB/c mice become microfilaremic, depending on the inoculation protocol (184, 197). In the CBA, C3H, and DBA strains, worms develop to the adult stage, but male spiculae are malformed, preventing microfilaremia (195). All B10 mice are resistant to infection, including ones with H-2^d MHC (195), and 129/SvJ mice are semiresistant (198).

(ii) Life cycle. Three methods of infection are commonly used in the literature: exposure to infected mites, subcutaneous inoculation of L3 larvae obtained from mite dissection, and subcutaneous inoculation of L3 larvae obtained from the pleural cavity of recently infected jirds (184, 198, 199).

In BALB/c mice, L3s enter small-vessel lymphatics shortly after inoculation (200) and then localize preferentially to the pleural cavity by 4 days p.i. A few adult worms can occasionally be found in the peritoneal cavity. Two molts occur within the pleural cavity at 8 to 12 days and 25 to 30 days p.i., and patency commences at 50 days p.i. (184). Adult worm numbers start to decline much earlier than in the natural host. This decline begins at around 70 days p.i. (197), and most worms are cleared by 16 weeks p.i. (201). However, worms can survive as long as 20 weeks p.i. (our unpublished data).

The dynamics of larval survival vary depending on the number of larvae that are inoculated and the source of inoculated larvae (184, 202). In general, 25 to 57% of inoculated larvae survive migration to the pleural/peritoneal cavities (184, 200, 202). When 25 worms were inoculated, the number of surviving adult worms remained steady until about 70 days p.i. (202). However, when 200 worms were inoculated, worm death was accelerated, possibly because of competition for space and resources or due to increased host immune responses (202).

(iii) Natural immunity. While C57BL/6 mice are considered resistant and BALB/c mice are considered susceptible to infection, the differences in parasite burdens do not become striking until after worms reach the adult stage (203). Even at 30 days p.i., there is little difference in the parasite burdens of these two strains of mice, yet worms recovered from C57BL/6 mice at this time point exhibit delayed development and retarded growth (203).

While the mechanisms underlying natural immunity in resistant mice are not completely understood, the magnitude of cellular immune responses likely plays an important role. Compared to BALB/c mice, C57BL/6 mice exhibit increased numbers of T cells, B cells, macrophages, and eosinophils localizing to the pleural cavity (203). C57BL/6 mice develop more of a mixed type 1/type 2 immune response than BALB/c mice, which develop a more polarized type 2 response (203, 204). While this may lead to the conclusion that type 1 responses confer resistance, studies with IL-4-deficient C57BL/6 mice demonstrated that the protective immune response in C57BL/6 mice is dependent on type 2 immunity (204, 205). Of note, it is unclear how necessary antibody responses are for protection in resistant mice. IgG μ chain mutant (μMT) C57BL/6 mice exhibit the same resistance to infection as wild-type C57BL/6 strains (204). Previously thought to be completely deficient in antibody production, μMT mice, which lack the ability to express surface IgM, have recently been shown to have the ability to produce IgE (206). Eosinophils may play a role in protection, as infection of mice deficient in either eosinophil peroxidase or major basic protein significantly increases the number of L3 larvae that survive to the adult stage in the partially resistant 129/SvJ mouse strain (198).

Susceptibility of BALB/c mice appears to be partially due to MHC, as BALB/b mice are more resistant to infection than BALB/c mice (197). However, the role of MHC may be relatively minor, as highly resistant B10.D2 mice display the same MHC as BALB/c (H-2^d) (197). Thus, other factors must also determine susceptibility of mice to *L. sigmodontis*. BALB/c mice show less IgM production, more type 2 skewing, and differential antigen recognition compared to B10.D2 mice (207). More importantly, T regulatory cells appear early in the course of an infection in BALB/c mice, downregulate immune responses, and significantly impair the ability of the immune system to clear worm infections (208, 209). Consistent with the notion that regulatory factors play a role in allowing *L. sigmodontis* infection, overexpression of IL-10 by macrophages has been shown to abrogate protection in the FVB background (205), allowing for patency to develop in an otherwise resistant host (195, 205). This overproduction of IL-10 was associated with a decline in the number of IL-5-producing $CD4^+$ cells and the development of alternatively activated macrophages (205).

Despite the susceptible nature of the BALB/c strain, these mice do, in fact, display protective immune responses and eventually clear the infection. These protective responses depend on IL-5, CD4 T cells, and IFN-γ (201, 210, 211) and involve NK cells (212). While basophils have been shown to augment type 2 immune responses in this model, they are not important in the control of worm infections within the first 8 weeks p.i. (213). IL-4 is not important in defense against the larval and adult stages but is important, along with IL-5, for keeping MF levels under control (201). Experiments with μMT and $JH^{-/-}$ mice suggest that B cells are not important for the immune response and may actually be required for proper worm development (214; our unpublished data). Despite this, BALB.XID mice, which are B1 cell deficient, have been shown to harbor more worms than BALB/c mice 28 days after natural infection (199).

After intravenous injection of MF, C3h/HE and DBA/1 mice clear infection within the first 3 days p.i. SJL, 129/Sv, and C57BL/6 mice, however, clear infection in an intermediate time frame, and BALB/c mice do not clear infection for over 30 days. Early clearance of infection appears to be mediated by innate immune responses, and intermediate/late clearance appears to be associated with MHC. Astoundingly, though, intraperitoneal implantation of even one adult female worm prevents clearance of MF in all strains of mice tested for at least 20 days (215).

(iv) Immunity in the setting of repeated parasite exposures. Current infection with *Litomosoides sigmodontis* provides modest (31%) protection against superinfection with further L3 larvae, suggesting a moderate degree of concomitant immunity in this model (our unpublished data).

(v) Vaccine studies. Vaccine research using this model has been focused primarily on understanding the mechanisms involved in

protection after vaccination with irradiated L3s. This vaccine provides high levels of protection and causes a reduction in worm burden within the first few days after challenge infection (109). Protective immune responses are stage specific, providing immunity to infective larvae that lasts for at least 5 months p.i. (153). Furthermore, protective immunity conferred by vaccination with irradiated larvae does not wane in response to repeated challenges (216).

Protection with the irradiated L3 vaccine is dependent on IL-5 and B cells (214, 217, 218). The current hypothesis on protective mechanisms in this model is that antibodies aid in cytoadherence to incoming larvae, which prevents worm migration into the lymphatics, and enable eosinophil degranulation to kill off infective larvae.

The only other protective vaccine that has been demonstrated in this model is repeated injections of MF adsorbed to alum. This is an intriguing vaccine, as it not only reduces microfilaremia but also accelerates the killing of adults (219). Vaccination with the *Wolbachia* surface protein (WSP) is not protective against future infection, regardless of whether alum or CFA is used as an adjuvant (220).

(vi) Lessons learned and clinical relevance. This model, while not a model of filarial disease, has been useful for characterizing both protective and nonprotective immune responses to filariasis. There are likely several different immunologic pathways responsible for protection or susceptibility. Innate factors, cytokine skewing, and MHC profile can all be associated with a protective immune response albeit at different time points p.i. Type 2 immune responses, especially the cytokines IL-4 and IL-5, appear important for both natural and vaccine-mediated immunity. Additionally, like *L. sigmodontis* infection in *Mastomys*, this model highlights the importance of immune modulation, as a blockade of regulatory pathways leads to increased worm clearance. Indeed, it has been postulated that the susceptibility of BALB/c mice, which develop a strongly polarized type 2 response and thus would be expected to be highly resistant to infection, may be due to their predilection for developing strong immunoregulatory responses (5, 208).

Additionally, this model makes it clear that it is difficult to generalize information about filarial infections from a single model. While other models have shown a predilection for males to become microfilaremic, *L. sigmodontis* appears to be more successful at infecting female BALB/c mice.

Loa loa

For *Loa loa*, the vector is the fly (*Chrysops* species). The natural host is humans. Experimental hosts are *Mandrillus* species.

***L. loa* in *Mandrillus* species: *Mandrillus sphinx* (mandrill) and *Mandrillus leucophaeus* (drill). (i) Permissiveness.** Like humans, *Mandrillus* species infected with *Loa loa* can be amicrofilaremic, transiently microfilaremic, or stably microfilaremic (221, 222). Infection can be achieved either through s.c. injection of L3 larvae or by surgical implantation of adult worms between fascial layers overlying the erector spinae muscles (223, 224).

(ii) Life cycle. Like infection of humans, in *Mandrillus*, adult *L. loa* worms reside in the s.c. tissues, and MF circulate in the blood (Fig. 6) (221). As all vaccine work has used microfilaremia as a measure of protection, we will focus on this portion of the life cycle. In a typical infection, the prepatent period lasts approximately 150 days. By 200 days p.i., microfilaremia peaks and then decreases to a steady-state level (225). The drop in microfilaremia has been referred to as a state of "suppressed infection" (223). During this suppressed infection, MF congregate in the capillaries of the lung and become trapped in the spleen (223). Despite the decrease, circulating MF have been detected at low levels for as long as 1,643 days p.i., which is the longest time that any *Mandrillus* infection has been monitored to date (225).

(iii) Disease. While infected animals are not visibly symptomatic (225), microfilaremic animals develop granulomatous nodules in the spleen, consisting of macrophages, multinucleated giant cells, eosinophils, and degraded MF (226).

(iv) Natural immunity. Natural immunity in this model is exhibited by the host's ability to control microfilaremia. Two sets of data suggest that the "suppressed state" that develops in *Mandrillus* is due to active immunologic clearance of MF. First, implantation of adult worms into drills harboring suppressed infection does not result in a major spike in microfilaremia (223). Second, animals that receive repeated inoculations of L3s at 6-month intervals after the initial infection exhibit very minor increases in microfilaremia (223). Transfer studies and splenectomies suggest that this control of microfilaremia is due primarily to MF clearance in the spleen and not reduced worm fecundity (223). Suppression of MF numbers is associated with the production of antisheath IgM antibodies during the prepatent period (225).

(v) Disease after treatment. In infected animals treated with DEC, MF are rapidly destroyed by the liver rather than the spleen. Overall splenic pathology is not exacerbated by DEC treatment (226).

(vi) Vaccine studies. Vaccine studies in this model have been limited to vaccination with irradiated L3 larvae. Three different trials have been performed, in which mandrills were vaccinated with 50 to 150 L3s irradiated with 40 to 45 kilorads (221, 222, 227). These studies showed a delay in peak microfilaremia in the vaccinated mandrills but no significant decrease in microfilaremia.

(vii) Lessons learned and clinical relevance. While the life cycle and histological findings suggest that this model is very consistent with human loiasis, the high variability of patency status in infected animals and the inability to perform adult worm counts limit the utility of this model for studies of vaccine efficacy. The finding that vaccination with irradiated L3s does not substantially decrease microfilaremia suggests that this approach may not work for human loiasis, as this model is very similar to infection of humans.

Of note, cerebritis has not been reported in this model. Given the clinical importance of posttreatment cerebritis in *L. loa*-infected patients with high-level microfilaremia, it would be helpful to test future vaccines in a *L. loa* model that develops posttreatment cerebritis. While published presently only in abstract form, ivermectin treatment of splenectomized *Loa*-infected baboons was recently reported to cause inflammatory lesions in brain blood vessels (228).

Onchocerca ochengi

For *Onchocerca ochengi*, the vector is the black fly (*Simulium damnosum*) (229). The natural host is cattle. The experimental host is cattle (Table 7).

***O. ochengi* in cattle. (i) Permissiveness.** Cattle are permissive to infection. There is a prevalence of 66 to 71% in areas where the

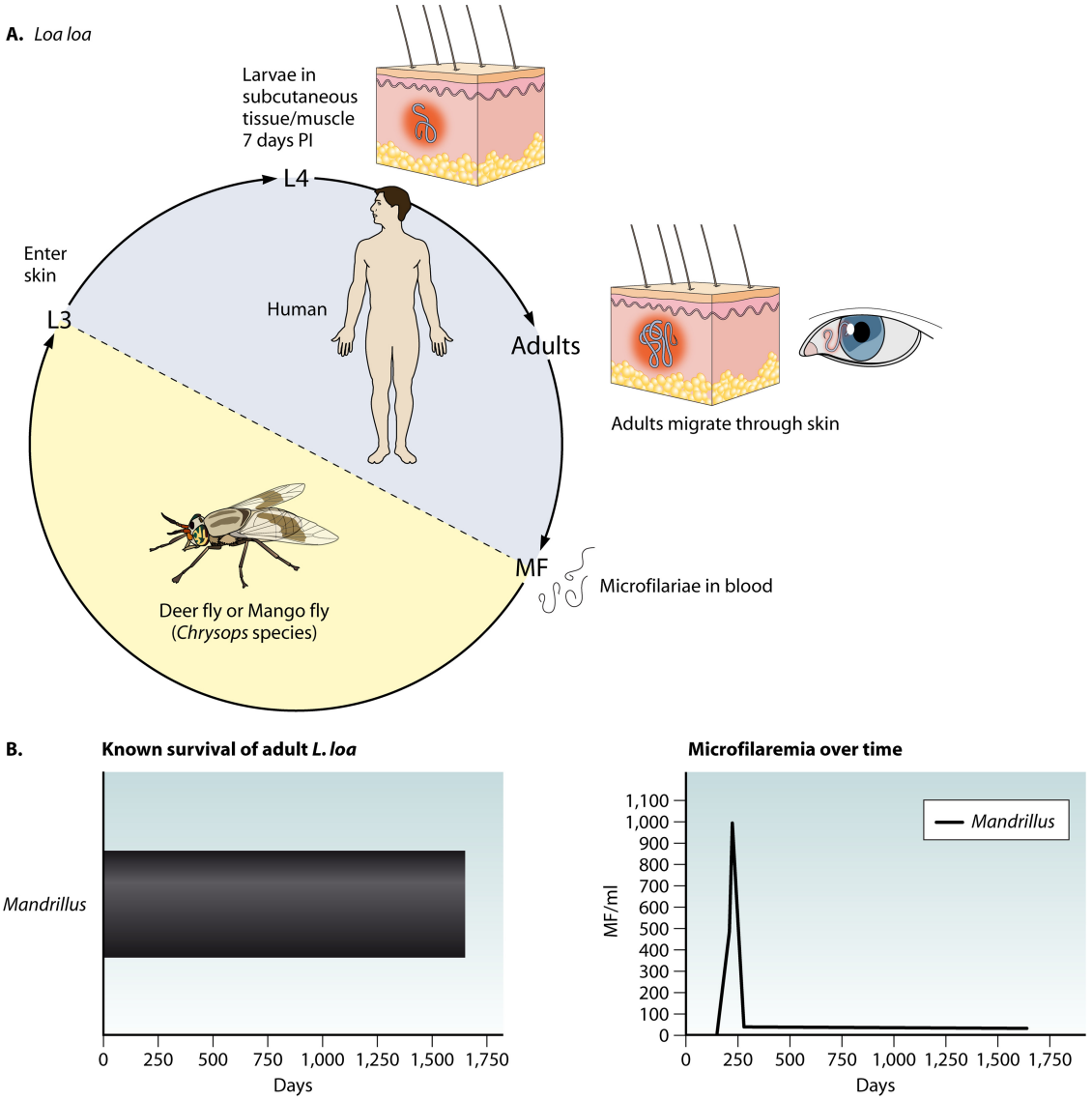

FIG 6 (A) Life cycle of *L. loa* in its natural host, humans. (B, left) Survival of *L. loa* after infection in *Mandrillus* species. (Right) Rough outline of probable course of microfilaremia in *Mandrillus* after infection (225, 258).

disease is endemic (229). Approximately 90% of cattle experimentally infected with at least 350 L3s develop nodules, and 75% of experimentally infected animals develop patent infections within 600 days p.i. (230).

(ii) Life cycle. L3s enter the skin through wounds left by the bite of a black fly (2) and molt to the fourth stage at 2 days p.i. (Fig. 7). After reaching the adult stage, adult female worms each live within their own intradermal nodules, primarily on the ventral aspect and hind legs of the cow (2, 230), and males migrate from nodule to nodule (231). Nodules begin to appear at 180 days p.i., and microfilaridermia onset occurs between 279 and 532 days p.i. (230).

(iii) Disease. Infected cattle do not exhibit dermatitis, ocular lesions, or other pathological sequelae which occur in human onchocerciasis (230). The lack of disease in cattle may be due to immunological differences between humans and cattle, the lower degree of microfilaridermia in cattle (approximately 10-fold less than the maximal levels observed in humans), or the shorter life span of cattle (230). While occasional suppurative inflammatory reactions can develop in *Onchocerca* nodules in cows, these are considered economically insignificant (231).

(iv) Natural immunity. Individual cows express differences in susceptibility to infection (229, 232). In one study, 15% of cows in areas of endemicity were found to be uninfected. These cattle were shown to be more resistant to both infection and pathological consequences of infection (232, 233). However, this protection is not complete, as these putatively immune cattle still became infected when moved to an area where the disease is highly endemic (232).

(v) Immunity in the setting of repeated parasite exposures. Although we do not know whether there is any decreased yield of adult worms during superinfection in this model, currently infected cattle are still susceptible to further infection (233). As older cattle have more nodules than younger cattle, it appears as though substantial protective immunity in cattle does not develop against either infective larvae or adult worms (229). However, there is

some evidence that partial immunity develops against MF, as older cattle have lower microfilarial densities despite harboring more adult worms (229).

(vi) Immunity after prior exposure. Chemically abbreviated infections have not been shown to induce a protective immune response in this model (232–234). In fact, prophylactic ivermectin and curative melarsoprol administrations have both been shown to render cattle more susceptible to future infection (232, 234).

(vii) Vaccine studies. Heterologous infection with *O. volvulus* larvae, which do not survive in cattle, produces a protective effect against infection with *O. ochengi* (231). The level of protection induced from heterologous infection is around 85%, similar to what is seen after inoculation with irradiated larvae (231, 232). Vaccination with irradiated larvae induces high levels of protection against infection when animals live in an area where the disease is highly endemic (232).

Vaccination with 8 different antigens, using both CFA and alum as adjuvants, was shown to have no protective effect against adult worm burdens. However, this vaccination protocol did decrease the number of microfilaridermic animals compared to the control group (235).

(viii) Disease after treatment. Treatment with DEC does not elicit an observable Mazzotti-like reaction (230).

(ix) Lessons learned and clinical relevance. This model has both major benefits and drawbacks. The expense and size of this model make any experiment a very large undertaking. The other major drawback to this model is that it is not a disease model. However, the presence of a normal population in an area where the disease is endemic and the use of natural infection make this model relevant to human infection in terms of infection dynamics. Therefore, this model can be used to determine the effectiveness of vaccine candidates in a real-world application. After vaccination, animals are challenged by allowing them to live in an area where the disease is endemic. The use of this model has demonstrated that vaccination with irradiated larvae induces a protective effect that is long-lived and protective against multiple natural exposures.

The protective effect of *O. volvulus* inoculation is very interesting, as it suggests that heterologous filarial infection with a similar worm that does not thrive within the given host may function similarly to a vaccine. For example, if humans that are first infected with *O. ochengi* become protected against *O. volvulus*, as has been postulated (231), onchocerciasis could be controlled by inoculating people with *O. ochengi* or potentially by keeping many *O. ochengi*-infected cattle in areas where *O. volvulus* is endemic.

Onchocerca volvulus

For *Onchocerca volvulus*, the vector is the black fly (*Simuliidae*). The natural host is human. Experimental hosts are chimpanzees and mice (Tables 8 and 9).

***O. volvulus* in chimpanzees. (i) Permissiveness.** Chimpanzees are permissive to infection. While inoculation of 200 L3s is sufficient to produce consistent infections in this model (236), there are individual differences in susceptibility. Although most chimpanzees develop stable patent infections, some develop only transient microfilaridermia, and others never develop microfilaridermia (237).

(ii) Life cycle. Intradermal and subcutaneous inoculations are each sufficient to induce an infection in chimpanzees (238, 239). In contrast to infection in humans, *O. volvulus* adult worms con-

TABLE 7 Vaccine and repeat infection trials using *O. ochengi* in permissive host cattle[a]

Immunization category	Live worms	Immunization	Adjuvant	Dose	Protection[b]		Reference(s); note(s)
					L3/adult	MF	
Live worms		CAI (melarsoprol)		Living in area of endemicity	↑ susceptibility	Yes	232, 234; similar when ivermectin was used as a prophylactic
		CAI (melarsomine or tetracycline)		Living in area of endemicity	0		233
		Heterologous inoculation of *O. volvulus*		Varied	86%		231
Irradiated larvae	Irradiated L3s			300 3×	84%	Yes	232; challenge by living in area of endemicity = 53% protection
Mixed	Many subunits		CFA/alum	3×	None	42% decrease in prevalence of microfilaridermia	235; OoALT1, OoB8, OoRAL2, OoTMY1, OoCPI, OoB20, OoFAR1, OoFBA

[a] All repeat infection studies are shaded. Repeat infection studies that tested for protective immunity after chemical abrogation of the primary infection are labeled CAI.
[b] Challenge was done by inoculation with L3s unless otherwise stated.

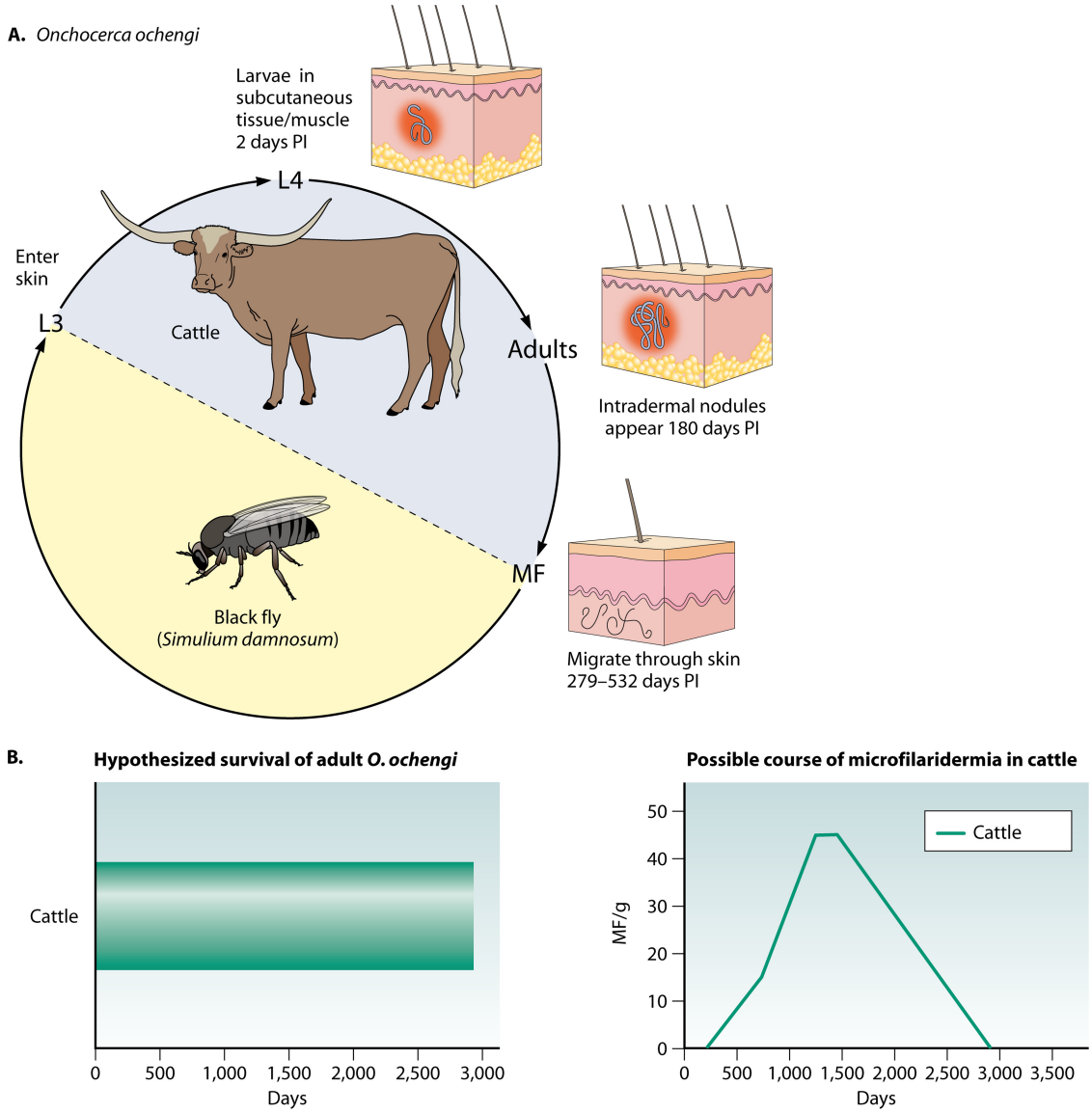

FIG 7 (A) Life cycle of *O. ochengi* in its natural host, cattle. (B, left) Hypothesized survival of adult *O. ochengi* in cattle based on nodule acquisition in naturally infected cattle (229). (Right) Possible course of microfilaridermia in cattle after a single infection. As microfilaridermia has not been monitored after a single infection, this is based on the probable survival of adults and studies of microfilaridermia during ongoing transmission (229).

gregate into worm bundles in sites deeper than subcutaneous tissues (238). In one study, for example, adult worms were most commonly found near the capsule of the hip joint (238). The prepatent period is typically 12 to 23 months and is not affected by inoculum size (Fig. 8) (236, 238). In patent infections, which can last 6 to 9 years, MF migrate throughout the subcutaneous tissues of the host and can be found in higher densities near worm bundles (238).

(iii) Disease. Nodules containing adult male and female worms can be found in the skin and deeper tissues, but no eye lesions in experimentally infected chimpanzees have been reported, possibly due to low infection intensity (238–240).

(iv) Natural immunity. Infected chimpanzees develop immune responses that aid in defense against MF, as it has been shown that plasma from 3- to 4-year-infected chimpanzees contains heat-labile factors, presumably antibodies, that aid in the adherence of neutrophils and eosinophils to MF and in MF killing (237). Even though some chimpanzees never develop patent infections, all infected chimpanzees produce this factor that aids in cytoadherence of neutrophils and eosinophils (237). Neutrophils and eosinophils are equally efficient at killing MF in this model (237).

(v) Immunity in the setting of repeated parasite exposures. Two inoculations with MF result in increased MF killing *in vitro* by neutrophils and eosinophils (237).

(vi) Vaccine studies. Despite showing considerable protection in rodents, vaccination with irradiated larvae was not protective in this model when chimpanzees were administered subcutaneous doses of 1,000 irradiated L3s at 0, 1, and 7 months (241). Interestingly, a typical booster response was not seen after administration of the second and third doses of the vaccine (241). While one of

TABLE 8 Vaccine and repeat infection trials using *O. volvulus*[a]

Host	Immunization category	Immunization	Adjuvant	Dose	Protection (%)[b]		Reference(s); note(s)
					L3/adult	MF	
Chimpanzee (permissive)	Irradiated larvae	Irradiated L3s		1,000 L3s 3×	Insig.	Insig.	241, 242
Mice (nonpermissive)	Live or dead worms	Live L3s		50	54		249
		Live MF		5,000 4×	Insig.		247
		Freeze-killed L3s		25–50 1-2×	0–67		249; protection required 2 doses
		Freeze-killed L4s or L3/4s		1–2×	Insig.		249
	Irradiated L3s	Irradiated *O. lienalis* L3s		50, 25 2×	42		249; insignificant after a single dose
		Irradiated L3s		Varied	0–87		247, 249–252; best protection after 35 kilorads of irradiation; insignificant protection in IL-4$^{-/-}$, granulocyte-depleted, eosinophil-depleted, IgE-depleted, μMT, IL-5-depleted, and IL-4 depleted mice; IFN-γ$^{-/-}$, CBA/J, XID, and EPO knockout mice were still protected; best protection in C57BL/6 mice
	Abundant larval transcript	Ov-ALT-1	Alum	25 μg 3×	39		253; present in esophagus, cuticle, and channels from esophagus to cuticle in L3 *O. volvulus* worms
		Ov-ALT-1	CFA	3×	36		254
	Muscular protein	Ov-tmy-1/psectagB (Gene Gun)		2 μg 3×	Insig.		256; tropomyosin
		Ovtmy-1 cDNA		100 μg 3×	Insig.		256; tropomyosin
	Energy metabolism	Ov-fba-1	CFA	25 μg 2×	50		278; fructose 1,6-bisphosphate aldolase; found where cuticle separates during molting
	Cuticle remodeling	OvChit/pJW (Gene Gun)	Au	3–5×	36–53		255; L3 chitinase
	Protease inhibitor	Ov7	Alum	25 μg 2×	34		34; onchocystatin, a cysteine protease inhibitor, induced a type 2 response regardless of adjuvant used
		Ov7	CFA	25 μg 2×	Insig.		34
	Other	Ov9M	Alum	25 μg 2×	Insig.		34; calponin
		Ov9M	CFA	25 μg 2×	Insig.		34
		OvB20/pJW4303 (Gene Gun)		2 μg 5×	Insig.		256; L3 stage specific, present in cuticle and hypodermis, secreted
		OvB20 cDNA		100 μg 5×	Insig.		256; L3 stage specific, present in cuticle and hypodermis, secreted
		Ov64	Alum	25 μg 2×	40		34; novel L3 antigen; induced very weak antibody responses regardless of adjuvant used
		Ov64	CFA	25 μg 2×	Insig.		34
		OvB8	Alum	25 μg 2×	46		34; novel L3 antigen; induced Th2 response with alum and Th1 response with CFA
		OvB8	CFA	25 μg 2×	Insig.		34
		Ov73k	Alum	25 μg 2×	Insig.		34; novel glycine-rich L4 protein
		Ov73k	CFA	25 μg 2×	Insig.		34

[a] All repeat infection studies are shaded.

[b] Challenge was done by inoculation with L3s unless otherwise stated.

TABLE 9 Vaccine and repeat infection trials using *O. lienalis*[a]

Host	Immunization category	Immunization	Adjuvant(s)	Dose	Protection (%)[b]		Reference(s); note(s)
					L3/adult	MF	
Cattle (permissive)	Homogenate	Sonicated MF		200,000 2×		97	279; challenge was MF by s.c. inoculation
Mice (nonpermissive)	Live or dead worms	Adult male worms		2 1×		55–69	244; challenge was MF by s.c. inoculation
		Live MF		5,000–1,000 MF 1–2×		70–98	244, 280; challenge was MF by s.c. inoculation; IL-5 is necessary for protection in BALB/c mice and important for protection in CBA mice (TRFK-5 treatment hampers protection); removal of neutrophils (NIMP-r14) or macrophages (carbon beads) does not alter protection; protection can be transferred with T cells and plasma
		O. gutturosa uterine forms		20,000		36–70	248; challenge was MF by s.c. inoculation
		Dead MF	Varied	Varied		0–47	244; challenge was MF by s.c. inoculation; best results with 20,000 MF given twice with *Bordetella pertussis*; no protection with CFA or alum as adjuvant
		Adult male *O. gutturosa*		2 1×		0–52	248; challenge was MF by s.c. inoculation; best on day 26
		O. lienalis uterine forms		20,000 2×		66	248; challenge was MF by s.c. inoculation
		O. volvulus uterine forms		20,000 2×		75	248; challenge was MF by s.c. inoculation
		Adult male or female *Dipetalonema viteae*		2 1×		60	248; challenge was MF by s.c. inoculation
		T. spiralis larvae		200 1×		27–81	248; challenge was MF by s.c. inoculation; poor results when challenge was 35 days after vaccination; 81% protection when separated by 90 days
		S. mansoni cercariae		35 1×		Insig.	248; challenge was MF by s.c. inoculation
	Irradiated larvae	Irradiated L3s		Varied	0–64		247; challenge by s.c. implantation of L3 larvae in chambers; protection observed in DBA/2 mice but not in CBA mice
	Other	OvB20	CFA, IFA, PBS	30 μg 3×		0	28; challenge by s.c. inoculation of MF; OvB20 is L3 stage specific, present in cuticle and hypodermis, and also secreted
		rMOv14-MBP	CFA, IFA, PBS	30 μg 3×		48–62	29; challenge by s.c. implantation of L3 larvae in chambers

[a] All repeat infection studies are shaded.
[b] Challenge was done by inoculation with L3s unless otherwise stated.

the vaccinated chimpanzees produced a differential antibody response compared to those of the other chimpanzees and did not develop a patent infection, it is difficult to determine whether this was the result of the vaccine or differences in the host immune response (241, 242). The failure of irradiated larvae to protect against infection may have been the result of downregulation of host immune responses, as strong cellular responses against *Onchocerca volvulus* antigen were induced by the vaccination yet were downregulated after challenge (242).

(vii) Lessons learned and clinical relevance. The lack of protection from vaccination with irradiated larvae in this model is somewhat disheartening, as this model should physiologically most closely mimic human infection. However, there are a few possible explanations for this failed vaccine attempt. The mechanism of protection via vaccination with irradiated larvae is still not completely understood, and the vaccination protocol needs to be adjusted for each model to provide maximum results. The protocol can be altered by dosage of irradiation, number of L3s inoculated, number of vaccinations, and timing of vaccinations.

***O. volvulus* and *O. lienalis* in mice. (i) Permissiveness.** Mice are not permissive to infection; inoculation with infective larvae does not result in systemic infection. Most vaccine studies are carried out using L3s in diffusion chambers implanted into mice or by assessing microfilaridermia after subcutaneous injection of MF (243, 244).

(ii) Life cycle. MF injected subcutaneously into the neck of mice accumulate in the mouse pinnae and are often quantified in the ears before being cleared by the host (244). Ten percent of MF injected into CBA/HT6T6 mice can be recovered at 35 days p.i., after which time MF levels decline (244, 245). While most MF are cleared by 50 days p.i., some can be recovered for as long as 112 days p.i. (244).

For experiments using larvae, diffusion chambers containing L3s are surgically implanted subcutaneously into the host. The larvae can survive for several weeks in the chambers and molt to the L4 stage. Several vaccine studies have used diffusion chambers to assess vaccine efficacy by monitoring L3 survival and development.

(iii) Disease. No clinically apparent disease occurs after either injection of MF or implantation of L3s by diffusion chambers.

(iv) Natural immunity. Mice are able to harbor MF from *Onchocerca*; however, most MF are cleared by 50 days p.i. T cells, eosinophils, and IL-5 are important for MF clearance (244, 246). Depletion of T cells by thymectomy and repeated injections of antithymocyte serum extend the duration of peak microfilaridermia by about a month (244). Passive immunization with sera along with the adoptive transfer of splenocytes from infected mice accelerates clearance of MF (244).

(v) Immunity after prior exposure. Primary exposure of mice to *O. lienalis* MF, L3s, or adult worms results in more rapid clearance of MF after secondary challenge infection (244, 247). Similarly, exposure of mice to MF, freeze-killed eggs, and transplanted adult worms from multiple heterologous parasite species (*Onchocerca cervicalis*, *Onchocerca gutturosa*, *O. volvulus*, *A. viteae*, and *Trichinella spiralis* but not *Schistosoma mansoni*) induces a protective immune response against MF challenge (248). Protection against MF does not correlate with protection against larval stages, as prior MF injections have no effect on larval recovery or development after L3 challenge (247).

(vi) Vaccine studies. *(a) Protection against MF.* Vaccination

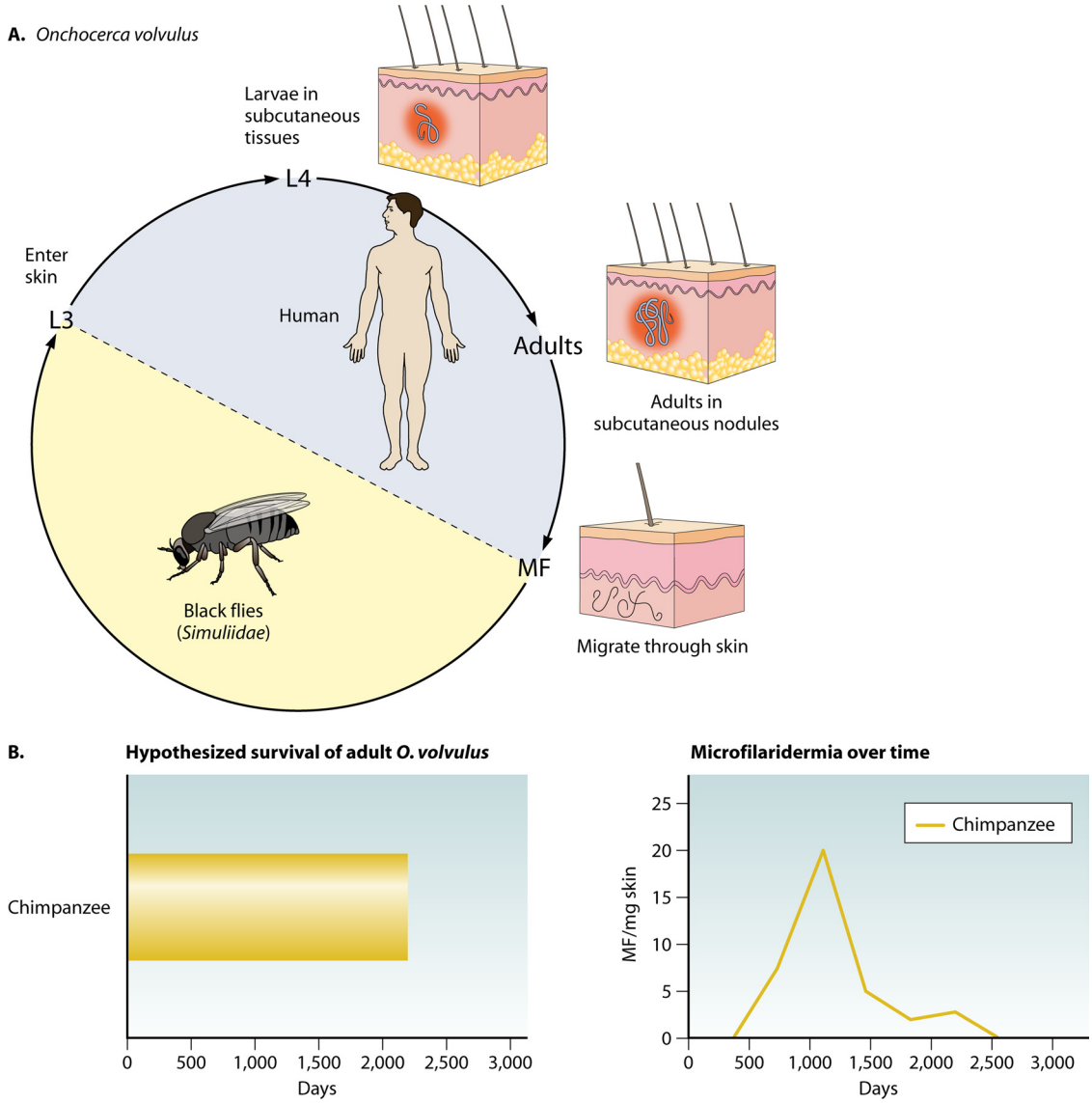

FIG 8 (A) Life cycle of *O. volvulus* in its natural host, humans. (B, left) Hypothesized survival of adult *O. volvulus* in chimpanzees based on microfilaridermia. (Right) Rough outline of the course of microfilaridermia in chimpanzees after infection with 165 L3s based on data reported previously (238).

with dead MF or crude homogenates of MF imparts protection against challenge with MF (244). This effect is enhanced by using *Bordetella pertussis* and endotoxin as adjuvants (244).

(b) Protection against L3s. Mice immunized with even a single dose of irradiated *Onchocerca* L3 larvae show a reduction in L3 survival and molting in implanted diffusion chambers (249). Mice also show cross protection against *Onchocerca* species regardless of which species is used for vaccination (247, 249). The irradiated larval vaccine is efficacious in multiple mouse strains, such as BALB/c, C57BL/6, CBA/J, and 129/SvJ (247, 250, 251), and provides protection ranging from 35 to 85%, depending on the dose of radiation given to the L3s and the number of immunizations (249). This protection is dependent on cytoadherence of immune cells to larvae, IL-5, IL-4, B cells, IgE, and eosinophils but not B1 cells or eosinophil peroxidase (250–252). It is likely that IL-4-driven type 2 immune responses are important for recruiting eosinophils, which are ultimately responsible for worm killing (252).

Immunization with nonirradiated live or freeze-killed larvae can induce a protective immune response similar to that obtained with irradiated larvae. However, immunization with freeze-killed larvae requires multiple doses (249).

(c) Specific antigens. Successful vaccine candidates against the larval stage in this model include *O. volvulus* abundant larval transcript 1 (Ov-alt-1), *O. volvulus* fructose 1,6-bisphosphate aldolase (Ov-fba-1), Ov7, Ov64, and OvB8 (34, 253, 254). Vaccination with *O. volvulus* tropomyosin reduces MF counts after challenge infection (29). Despite showing protection against *A. viteae* in jirds, mice vaccinated with recombinant OvB20, an L3-specific antigen that is excreted as well as present in the cuticle and hypodermis, are not protected against *O. lienalis* MF challenge (28).

(d) DNA vaccines. Of the three DNA vaccines tried in the *Onchocerca*-mouse model, only the use of L3 chitinase has been effective, and this required five immunizations to reach significant protection of 53% (255). Immunizations using plasmids expressing *O. volvulus*

tropomyosin (Ov-TMY-1) or OvB20 elicit high antibody titers but are unsuccessful at protecting mice against challenge with *O. volvulus* L3 larvae, despite the fact that both antigens elicit a protective immune response when the mice are immunized with the respective recombinant protein (256).

(vii) Lessons learned and clinical relevance. This model is a nonpermissive model that utilizes L3s implanted in diffusion chambers or injection of MF to mimic infection. Because of this, and a lack of a disease state, this model is not ideal for vaccine work. Vaccination can induce a more rapid clearance of worms. However, even with effective vaccine candidates, the method of vaccination is essential to eliciting a protective immune response. Some vaccine candidates require a protein vaccine, whereas chitinase can be effective when administered as a DNA vaccine. The main factors important for protective immunity are IL-4, IL-5, B cells, T cells, and eosinophils.

CONCLUSIONS

The intent of this article is to provide, in a single reference, a comprehensive review of vaccine, repeat infection, and natural protection studies conducted using animal models of filariasis. The amount of information extracted from animal studies, the large number of animal models used, and the substantial differences between the various models make it challenging to develop broad conclusions regarding protective immunity and vaccine prospects in filariasis. Nonetheless, keeping these limitations in mind, we believe that there are a number of important lessons that can be drawn from this work.

A Fully Protective Vaccine Strategy Has Not Yet Been Found in a Permissive Model

No vaccine approach has yet demonstrated complete sterilizing immunity in a permissive model of filariasis. Given the complexity of filaria infections and their well-known ability to modulate host immune responses, this is not entirely surprising. Nonetheless, it does suggest that a successful vaccine strategy may require a combination of approaches, such as the use of multiple antigens, antigens from multiple stages, and/or antigens that induce neutralizing responses to specific helminth immunomodulators.

There Is a Paucity of Filaria Vaccine Approaches That Have Been Tested in Animal Models

Despite the enormous burden of disease caused by filaria infections worldwide, the total number of published vaccine studies of animal models of filariasis is quite low. Indeed, from the 1940s to May 2012, only 99 primary research articles were published on filaria vaccines in English-language journals. The approaches taken are even less numerous, as a total of 27 filaria-mammal models have been used, with many simply repeating the same vaccination approaches (especially the irradiated larval vaccine).

We May Not Ever Have Well-Defined Protective Correlates of Immunity That Can Be Used as Predictive Surrogate Markers for Protective Efficacy in the Field of Filaria Vaccine Development

Although we may not ever have well-defined protective correlates of immunity that can be used as predictive surrogate markers for protective efficacy in the field of filaria vaccine development, it does not mean that some correlates of immunity are not known for some animal models. For instance, in both the *Brugia*-mouse and the *L. sigmodontis*-mouse models, T cells, IL-4, IL-5, and IFN-γ play significant roles in worm clearance (119, 120, 124, 201, 210, 211). However, there is a disparity in the overall pathway of clearance for these two models, in that B cells play a major role in clearing *Brugia* during primary infection but no role in clearing *Litomosoides* during primary infection (121, 214). Similarly, during vaccination, some antigens have proven more efficacious when given with a type 1-skewing adjuvant, whereas others have been more efficacious with a type 2-skewing adjuvant (31, 34). These examples suggest that there exist multiple immunological pathways that can lead to worm clearance and that the optimal immunological mechanisms may vary for different vaccine approaches.

Thus, the ideal immune response against any particular filarial antigen likely depends on the host being immunized, the exact filarial infection being prevented, and the worm antigen and life stages being targeted. Consequently, there are likely no specific immunologic parameters that can currently be used to predict vaccine efficacy for all vaccine approaches. Instead, we will likely have to continue to rely on data from experimental challenge studies to determine the best vaccine protocol for every new vaccine approach.

Nonpermissive Models May Overestimate the Protection Obtained by a Particular Vaccine Approach

As shown in Table 10, permissive models are less likely to demonstrate protective immunity than nonpermissive ones. Of the three nonpermissive models that clearly investigated the presence of immunity against a secondary challenge after clearance of the initial infection, two exhibited strong protective immunity and one exhibited modest protective immunity. In contrast, less than half of the tested stably permissive models demonstrated protection upon secondary challenge. Similarly, whereas all tested nonpermissive models exhibited substantial protection after vaccination with irradiated larvae, 3 of the stably permissive models exhibited no protection.

Certain Models May Be Optimal for Conducting Vaccine Research

While investigations with the *Brugia*-mouse model provide important information on why this nonpermissive model is resistant to infection, almost every vaccine approach tried in this model elicits faster clearance. Therefore, even though quite a bit of vaccine research has been done using this model, it may be prudent to validate promising approaches in more permissive models. Particular small-mammal models that appear well suited for vaccine studies include *L. sigmodontis* in BALB/c mice, *L. sigmodontis* in jirds, *Brugia malayi* and *Brugia pahangi* in jirds, and *Brugia malayi* in ferrets.

The *L. sigmodontis*-mouse model is the only permissive murine model of filariasis. This model is well suited for early screening of vaccine candidates, as it is economical and provides easy worm enumeration. Furthermore, the availability of reagents in the mouse allows for immunological studies that would not be feasible in other models. The *L. sigmodontis*-jird model also lends itself to vaccine research, as it allows for easy worm enumeration and is more permissive than the BALB/c model.

Brugia infections of jirds also appear to be very promising models for vaccine research. The life cycle and pathological sequelae have been well studied, infections are long lasting, and a large

proportion of jirds become stably microfilaremic. L3 larvae can be inoculated into the peritoneum to enable easy worm enumeration or can be administered subcutaneously to more closely mimic lymphatic filariasis in humans. Despite the lack of gross lymphedema in this model, jirds infected by the s.c. route develop lymphatic dilations, fibrosis, and other pathological changes that could be used to gauge vaccine safety (90, 94). While there appears to be little difference between the *B. pahangi*-jird and *B. malayi*-jird models, the *B. pahangi* model has been investigated a bit more thoroughly to date, with investigations on both concomitant immunity and protection after repeated infection.

For some of the promising vaccine candidates, it would be prudent to have a small-animal model that can be used to study the effects of vaccination on clinical symptoms of lymphatic disease prior to moving on to expensive monkey or cat studies. For this, the *Brugia malayi*-ferret model would be ideal. This is a permissive model that causes visible leg edema in the host. Unlike *Brugia* infections of mice, which can cause frank lymphedema when inoculated into severely immunocompromised animals such as nude and SCID mice, the *Brugia malayi*-ferret model manifests clinical lymphedema in a fully immunocompetent host.

In terms of larger mammals, inoculation of cats with *Brugia pahangi* also results in a permissive infection with clinically evident lymphedema. These animals not only exhibit symptoms similar to those of humans with lymphatic filariasis but also appear to immunologically mimic humans. This is evident by the large variability in natural protection and the lack of protection after chemically abbreviated infection. Another excellent large-mammal disease model of lymphatic filariasis is *Brugia malayi* in rhesus monkeys. As with humans, there is a range of susceptibility to the infection, and infected monkeys that develop lymphedema exhibit strong immune responses and amicrofilaremia. As both of these models exhibit leg edema, experiments using them would provide an understanding of how a vaccine may affect not only worm numbers but also the development of pathological sequelae. Two other large-mammal filaria models that exhibit lymphedema are *B. malayi* in dogs and *Wuchereria bancrofti* in silvered leaf monkeys (both not covered in this review because no vaccine studies have been conducted with them). Major downsides to large-mammal models include the expense, space, and resources required for the care of these animals.

The Benefits of Studying a *Dirofilaria* Vaccine Have Largely Been Overlooked

D. immitis causes severe disease in cats, dogs, and ferrets. Consequently, this parasite is a common concern of pet owners and often requires routine administration of anthelmintics. In addition to being a concern in veterinary medicine, there have been reports of dirofilariasis caused by *D. immitis* or *D. repens* in humans throughout the Americas, the Mediterranean, Europe, and Japan (4). *Dirofilaria* can be transferred to humans from dogs anywhere where the disease is endemic. Although *Dirofilaria* is increasingly being diagnosed in humans, at the moment, the only method of prevention in humans is prophylactic treatment of animals. Additionally, there are reports of drug resistance in some *D. immitis* strains (257). For these reasons, the development of a vaccine that protects dogs against *Dirofilaria* would thus benefit humans as well as dogs. Additionally, it would provide an opportunity to demonstrate proof of concept for any exceptional filarial vaccine candidates.

TABLE 10 Review of filaria models used for vaccine research[a]

Model	Permissiveness	Concomitant immunity	Secondary immunity	% protection by irradiated L3s	Primary adult worm location	Most similar human infection	Disease model
B. malayi in mice	Nonpermissive		Yes	95–100	Peritoneum/lymphatics	*B. malayi*	Lymphedema in nude mice
B. pahangi in mice	Nonpermissive		Yes	79–100	Peritoneum/lymphatics	*B. malayi*	Lymphedema in nude mice
D. immitis in mice	Nonpermissive		Modest	45			
D. immitis in Lewis rats	Nonpermissive						
O. volvulus in mice	Nonpermissive			0–87			
O. lienalis in mice	Nonpermissive			0–64			
A. viteae in hamsters	Transiently permissive	Modest		60	Subcutaneous tissues	*L. loa*	Glomerulopathy
B. malayi in ferrets	Transiently permissive	Yes			Lymphatics	*B. malayi*	Lymphedema
D. immitis in ferrets	Transiently permissive		Yes		Vena cava/heart	*D. immitis*	Heartworm
L. sigmodontis in albino rats	Transiently permissive			91	Pleural/peritoneal	*Mansonella perstans*	Lung pathology
L. sigmodontis in BALB/c mice	Transiently permissive		No	49–99	Pleural cavity	*M. perstans*	
L. sigmodontis in *Mastomys* species	Transiently permissive		Modest		Pleural cavity	*M. perstans*	
A. viteae in jirds	Stably permissive	Yes		61–100	Subcutaneous tissues	*L. loa*	
B. malayi in cats	Stably permissive			0		*B. malayi*	Occasional lymphedema
B. malayi in jirds	Stably permissive			56–91	Lymphatics/ Peritoneum	*B. malayi*	Histological lymphatic pathology
B. malayi in *M. natalensis*	Stably permissive				Lymphatics	*B. malayi*	
B. malayi in *M. coucha*	Stably permissive		No		Lymphatics	*B. malayi*	
B. malayi in rhesus monkeys	Stably permissive			75	Lymphatics	*B. malayi*	Lymphedema
B. pahangi in cats	Stably permissive	Yes	No	72	Lymphatics	*B. malayi*	Lymphedema
B. pahangi in jirds	Stably permissive	Modest, if any	Yes	39–76	Lymphatics/peritoneum	*B. malayi*	Histological lymphatic pathology
D. immitis in dogs	Stably permissive		Yes	42–88	Heart/pulmonary arteries	*D. immitis*	Dog heartworm
L. loa in mandrills	Stably permissive			0	Subcutaneous tissues	*L. loa*	
L. sigmodontis in cotton rats	Stably permissive	Modest	No		Pleural cavity	*M. perstans*	Reactive lung tissue eosinophilia
L. sigmodontis in jirds	Stably permissive	Modest	Modest	98	Pleural cavity	*M. perstans*	
O. lienalis in cattle	Stably permissive				Connective tissue	*O. volvulus*	
O. ochengi in cattle	Stably permissive		No	84	Intradermal nodules	*O. volvulus*	
O. volvulus in chimpanzees	Stably permissive			0	Subcutaneous tissues	*O. volvulus*	

[a] Row shading used for ease of viewing.

Vaccines That Induce Antibodies against the MF Sheath May Be Beneficial if They Do Not Exacerbate Disease

In many of the models studied, the host develops only transient microfilaremia despite harboring adult worms. This is the nature of latent infections and in most cases is associated with antibodies directed against the sheath of the MF. The only exception to this is *L. sigmodontis* in albino rats, where latency is associated with cellular responses that prevent MF egress from the pleural cavity. Because latency is almost always antibody mediated, it is intriguing to think that a transmission-blocking vaccine could be made against the MF sheath to prevent microfilaremia. This approach has been protective against microfilaremia in at least 10 animal models of filariasis. While many of these models are either nonpermissive or only transiently permissive to infection, two stably permissive models (*L. sigmodontis*-cotton rat and *O. lienalis*-cattle) have demonstrated that a MF vaccine can induce protection that would not otherwise develop. However, there are safety issues that would need to be considered for this vaccine approach, as it is possible that any immune response directed at the MF may induce pathological sequelae. Cotton rats that clear MF of *L. sigmodontis* and ferrets latently infected with *B. malayi* both develop lung lesions which are similar to those observed in human tropical pulmonary eosinophilia (39, 178). Similarly, infected amicrofilaremic rhesus monkeys are more likely to develop lymphedema than microfilaremic monkeys. Probably the strongest data that should urge caution with the MF vaccine are that ferrets vaccinated against *Brugia* MF are more likely to develop lymphedema than naive ferrets after challenge infection (40). Nonetheless, it may be reasonable to consider developing a MF vaccine, since this stage appears particularly susceptible to antibody-mediated clearance.

The Mechanisms Underlying Concomitant Immunity Remain Poorly Understood

Concomitant immunity is a state wherein the host is unable to kill off adult worms but is protected against new challenge infections. This state has been shown in many of the models that have been studied at highly various degrees and time frames. Jirds infected with *A. viteae* develop this immune state very quickly after infection and are highly protected against future infection, yet infected hamsters develop this state only when infected many times with small doses of L3s (10, 18, 23). Cats infected with *Brugia pahangi* develop this state only after 12 infections, and it is not complete until at least 20 infections (73). Jirds infected with *B. pahangi* and cattle infected with *O. ochengi* do not appear to develop concomitant immunity (99, 100). An understanding of the mechanisms underpinning concomitant immunity would undoubtedly aid in understanding immune mechanisms that prevent infection and perhaps give more direction to vaccine research.

A Number of Experimental Vaccines Have Shown Promise

The most thoroughly evaluated vaccination strategy for filariasis is vaccination with irradiated larvae. This method has been shown to be protective in 16 models of filariasis and not effective in 3 models. While this approach is not feasible for human vaccines, it both shows that vaccines against filariasis are possible and provides a framework for future vaccine research. The only major concern with irradiated larval vaccination to date is that 2 out of the 3 nonhuman primate models studied garnered no protection from vaccination. However, this does not mean that there is no irradiated larval vaccine strategy that would work in these models but simply that the specific vaccine strategy tested was not effective. For most of the animal models that have used irradiated larval vaccination, there has been an optimization process applied to obtain protection. Optimization, however, varies from model to model. The amount of radiation that each L3 receives, the number of inoculated L3s, the number of inoculations, and the time from vaccination to challenge are all important for eliciting a protective effect.

Because of the difficulty in optimizing this vaccine protocol, it is not surprising that many of the trials that have been carried out using nonhuman primates have not shown protection. These experiments are expensive and have therefore not used the variety of conditions that the rodent models have used. The only nonhuman primate vaccine study that worked to optimize this approach did show protection in the *B. malayi*-rhesus monkey model.

There are many other vaccines that have been shown to be highly protective in susceptible models of filariasis. OvB20, BmALTII, glutathione *S*-transferase, superoxide dismutase, transglutaminase, thioredoxin peroxidase, collagenase, and CFA_2-6 are all very promising for future research.

Moving Forward

There are many vaccine approaches that have been shown to provide at least 75% protection in permissive animal models of filariasis. While searching for better vaccine candidates may prove fruitful, it is possible that we already have the tools necessary to develop a very effective vaccine approach. Indeed, we may be able to develop a sterilizing vaccine by optimizing the combination of antigens, dosing schedule, vaccine concentration, adjuvant, and route of immunization. Even if a sterilizing vaccine is out of reach, vaccine protocols that decrease worm numbers, decrease pathological symptoms, or block transmission may still be useful against human and animal disease. Although investigations into the development of a filaria vaccine have been ongoing for over half a century, it is clear that relatively little basic research has been done in this direction relative to the terrible disease burden caused by filarial infections in people and animals. Animal studies to date have shown that some degree of protective immunity against most filarial infections can be obtained. Given the many weaknesses likely present within the complex life cycles of these parasites, we believe that continued work in this direction should enable the development of clinically effective vaccines.

APPENDIX

DEFINITIONS

adjuvant Substance added to a vaccine to make the vaccine more immunogenic.

chemically abbreviated infection An infection that was cleared by treatment with anthelmintic therapy.

concomitant immunity Immunity against superinfecting L3 larvae in the presence of an active filarial infection.

latency and latent infection Persistence of adult filarial worms after clearance of microfilariae.

L3 Third-stage larva.

MHC Major histocompatibility complex.

nonpermissive An infection wherein the host does not develop a patent infection (i.e., no microfilaremia or microfilaridermia) following infection with infective larvae.

patency An ongoing infection of adult filarial worms with microfilaremia or microfilaridermia.

permissive An infection wherein the host develops a patent infection

(with development of microfilaremia or microfilaridermia) following infection with infective larvae.

skewing adjuvant An adjuvant that typically results in a specific type of immune response.

stably permissive A permissive host in which microfilaremia or microfilaridermia persists for more than 1 year.

transiently permissive A permissive host in which microfilaremia or microfilaridermia lasts for less than 1 year.

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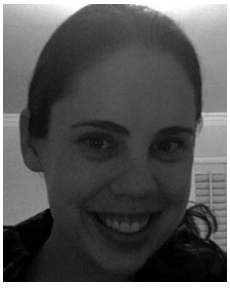
C. Paul Morris is an officer in the U.S. Public Health Service and a fifth-year M.D./Ph.D. student at the Uniformed Services University of the Health Sciences (USUHS). He graduated from California State University, Chico, in 2008 with a B.S. in Biology. While there, he performed research using *Lactobacillus* for the reclamation of industrial waste products under the guidance of Dr. Larry Hanne and Dr. Larry Kirk. After completing the basic sciences portion of medical school at the USUHS in 2010, he joined Dr. Edward Mitre's laboratory. His thesis work focuses on antifilarial vaccine research mainly in the *L. sigmodontis*-BALB/c model of filariasis.



Holly Evans is a Ph.D. candidate at the Uniformed Services University of the Health Sciences in Bethesda, MD. Prior to pursuing a graduate degree in Emerging Infectious Diseases, she received a B.S. in biology and chemistry from the University of Redlands in 2009. Her thesis work is focused on understanding how filarial infections are able to protect the host from experiencing symptoms associated with allergic responses.

Sasha Larsen earned a bachelor's of science (2009) and master's of science (2011) in Biological Sciences from The University of the Pacific in Stockton, CA. She is currently in her second year at the Uniformed Services University of Health Sciences in Bethesda, MD, to earn a Ph.D. in Emerging Infectious Diseases.

Edward Mitre is an Associate Professor in the Department of Microbiology and Immunology at the Uniformed Services University in Bethesda, MD. His laboratory (http://www.usuhs.mil/faculty/edwardmitre-mic.html) studies immune responses toward helminth infections and investigates the mechanisms by which helminths can protect against autoimmune diseases and allergy. Dr. Mitre obtained his medical degree from the Johns Hopkins School of Medicine in 1995 and completed his internal medicine residency at New York University. He then did an infectious diseases fellowship at the National Institutes of Health, followed by postdoctoral research work in helminth immunology as well as clinical training in tropical medicine under the tutelage of Dr. Thomas Nutman at the Laboratory of Parasitic Diseases at the NIH from 2000 to 2005. In addition to his laboratory research and teaching responsibilities at the university, Dr. Mitre regularly attends on internal medicine and infectious diseases consultation services at the Walter Reed National Military Medical Center.