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Studies on Immunization against *Brugia malayi* Infection in the Rhesus Monkey *

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Recent studies on immunity to helminthic infection have shown that some degree of protective immunity may be stimulated by inoculations of attenuated living worms or their metabolites. The present experiments were designed to observe the effects of attempted immunization in the rhesus monkey by the use of attenuated infective larvae of Brugia malayi.

Some effect was observed in animals inoculated with larval incubate, in which microfilaraemia did develop, but at low levels and for short durations. However, the most striking finding was that persistent immunity to challenge infections (expressed as failure to cause microfilaraemia) was obtained in animals vaccinated with large numbers (200) of infective larvae attenuated by X-irradiation at 20 000 R.

Experiments with different numbers of larvae attenuated by different doses of irradiation suggest that there is an optimum combined effect of these two factors in eliciting functional antibody in a quantity sufficient to prevent patent infection in Malayan filariasis.

Excellent reviews on the subject of helminth immunity have been presented by Soulsby (1960), Stoll (1961) and Urquhart et al. (1962). Although vaccination against helminthic infections is far from being a common practice, recently reported studies in this area have contributed much to the knowledge that will probably bring us nearer to making active immunization against worms a reality.

The classical method of producing immunity with vaccines made from dead whole-worm material or from extracts of such material has been generally disappointing (Urquhart et al., 1962; Thorson, 1963). Attempts to provide protective immunity against filarial infection by inoculation with either ground adult worms or infective larvae have not been successful (McFadzean, 1953; Krishnaswami & Pattanayak, 1959).

Some degree of protective immunity has been shown to be stimulated by metabolites of living worms. The metabolites have been introduced by the following procedures: (1) implantation of non-wandering forms into an abnormal site (Stoll, 1958); (2) inoculation with living worms attenuated by artificial means, e.g., X-irradiation (Jarrett et al., 1957; Dow et al,. 1959; Poynter et al., 1960; Hsu et al., 1962; Radke & Sadun, 1963; Miller, 1965); (3) inoculation of a strain of the parasite which would not become patent in the host (Hsu & Hsu, 1961; Hunter et al., 1961; Sadun et al., 1961); and (4) utilization of the metabolites (exoantigens) themselves, obtained from culture media or incubates of the living worms (Thorson, 1953; Campbell, 1955; Soulsby et al., 1959).

The present studies were designed in an attempt to elicit protective immunity in a vertebrate host against filarial infection by inoculations of various vaccines made of or from infective-stage larvae.

MATERIALS AND METHODS

Parasite

The subperiodic strain of *Brugia malayi*, the major human filarial parasite in West Malaysia and

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a natural zoonotic infection in several local animal species, was used in the preparation of vaccines and for the challenge inoculations.

Experimental animals

Rhesus monkeys (*Macaca mulatta*) from India, 1–1½ years old, were used as experimental hosts. This species of monkey has been found to be quite susceptible to this strain of parasite experimentally. All the animals were screened for microfilaraemia at the beginning of the experiment by examination of 2 night blood samples and were found to be negative.

Vector

Infective larvae were obtained mainly from laboratory-bred *Aedes togoi*, supplemented occasionally by wild *Mansonia uniformis* when sufficient numbers of *Ae. togoi* were not available. The mosquitos were fed on 2 cats which had been experimentally infected with *Brugia malayi* (subperiodic) originally isolated from a human infection. Microfilarial counts in the cats at various times of feeding averaged 432 microfilariae per 60 ml of blood. Only third-stage larvae (L_3) were selected. The average recovery rates were 7.6 L_3/mosquito from *Ae. togoi* and 12.7 L_3/mosquito from *M. uniformis*; and the infectivity rates after feeding were approximately 70% and 89% respectively.

Preparation of vaccines

Three types of vaccine were prepared: (1) " SE vaccine ", presumably containing secretory and excretory products (exoantigens) of infective larvae in saline incubate; (2) " IL vaccine ", consisting of infective larvae treated by incubation in immune serum; (3) " XL vaccine ", consisting of infective larvae attenuated by X-irradiation.

As these were initial studies of filarial vaccines, the methods employed in these preparations were somewhat exploratory in nature. SE vaccine was prepared by incubation of 100, 200, or 400 active L_3 in 1.25 ml of sterile Krebs-Ringer solution for 1 hour at 37°C. After incubation, 1.0 ml of each incubate was removed and injected subcutaneously as 100, 200, or 400 SE vaccine respectively. It was assumed that the incubate would contain larval metabolic products in quantities directly proportional to the number of larvae in the incubate.

The above larvae were then placed in tubes containing 1.0 ml of immune serum, also in groups of 100, 200, and 400. After incubation for 1 hour at 37°C, they were removed for subcutaneous injection as 100, 200, or 400 IL vaccine respectively.

The immune serum was obtained from an older rhesus monkey which had never developed patent infection in spite of repeated infective inoculations of infective larvae of subperiodic *Brugia malayi* (Wong, in preparation). It was thought that specific antibodies to the filarial worm might be present in the serum and that infective larvae incubated in these antibodies might be attenuated.

Immediately after dissection from the mosquitos, infective larvae to be X-irradiated were placed in groups of 100, 200, or 400 in flat-bottomed glass dishes (1.5 cm diameter) containing Krebs-Ringer solution. The depth of the solution was then adjusted to 5.0 mm. To reduce exposure time to a minimum, a short X-ray cone giving a target specimen distance of 10 cm and a field area of 50 cm^2 was used. At this distance the dishes were completely covered during X-irradiation.

X-irradiation was delivered by a Dermopan unit with an exposure rate of 670 roentgen/min at the level of the larvae. Radiation factors were 50 KVp, 25 mA with 1.0 mm aluminium filtration. The half-value layer for the beam was 0.75 mm aluminium. The distance from the target to the surface of the suspension was 9.0 cm. Total irradiation doses in the region of 10 000 R, 20 000 R, or 40 000 R were given to produce 9 different XL vaccines—3 each of 100, 200, or 400 infective larvae. These vaccines are designated 100 XL/10 000 R, 200 XL/10 000 R ... 200 XL/40 000 R, 400 XL/40 000 R.

All larvae were as actively motile after irradiation as before. Inoculation into the monkeys was carried out within half-an-hour after irradiation was completed.

Vaccination and challenge

The different types of vaccines were tested in essentially the same manner. Experimental monkeys were inoculated subcutaneously twice, generally at 2- or 4- week intervals, and challenged one month after the last vaccinating inoculum with 100 normal infective larvae. At the same time, a control monkey was inoculated with each vaccinating or challenge inoculum to determine its viability. Assessment of the host's reactions was made by examination of fortnightly samples of night blood for microfilaraemia by the concentration technique of Knott (1939), leucocyte and differential counts, measurement of body temperature, palpation of lymph nodes, and serological tests. In addition, some animals were later sacrificed for recovery of worms, using methods previously described by Buckley & Edeson (1956)

and Ash & Little (1964). This paper reports only the data from parasitological examinations.

RESULTS

Experiment 1

As seen in Table 1, only 1 of the 9 animals vaccinated with SE vaccine failed to develop a patent infection; however, its microfilarial counts were considerably lower than those of the control animal. In 3 animals the counts were never more than 3 microfilariae/0.1 ml of blood and on several occasions no microfilaria was found even by Knott's concentration method. Patency in these animals lasted 9 months or less, in comparison with the 10 or more months of patency seen in the other 5.

The prepatent period, which is counted from the day of the challenge inoculum, is normally found to be about 3 months. On the basis of the range of from 81 days to 131 days found among all the challenge controls (in all experiments), no alteration in prepatency among the test monkeys could be discerned.

Experiment 2

A total of 9 monkeys was inoculated with the 3 different IL vaccines. Three animals were additionally injected with 100 IL vaccine to serve as vaccine controls, i.e., to determine whether or not the so-called attenuated larvae were themselves infective. Table 2 shows that both experimental and control animals became infected. Four animals, representing each of the IL vaccines, developed a low microfilaraemia of short duration (3 months or less). Patent infections of more than 12 months' duration were seen in 5 of the remaining 7 animals. The short prepatent periods observed in 6 of the monkeys in this group are considered to have been derived from the vaccinating inocula.

Experiment 3

As shown in Table 3, microfilaraemia occurred in only 4 of the 9 test animals. None of the animals receiving 400 L_3, regardless of the amount of irradiation received, became patent. When the number of attenuated larvae in the inoculum was 100 or 200, only those receiving approximately 20 000 R did not cause patent infection upon challenge inoculation. Low microfilaraemia of short duration (1½ and 4 months) was seen in the animals vaccinated with 200 XL/10 000 R or 200 XL/40 000 R vaccine respectively. Microfilaraemia in the animals inoculated with 100 XL/10 000 R or 100 XL/40 000 R vaccine persisted for more than 12 months. None of the 6 vaccine control monkeys developed microfilaraemia.

Since these results were observed in single animals, this experiment was repeated using a total of 11 monkeys and testing only 2 of the 9 previous XL vaccines. Four monkeys were each vaccinated with 100 L_3 exposed to 20 000 R and 3 were vaccinated with 200 L_3 also exposed to 20 000 R. Again the interval between the 2 vaccine doses given to each monkey was 2 weeks, with the challenge inoculation with 100 infective larvae carried out a month later. Two monkeys, used as vaccine controls, were given only irradiated larvae, and 2 monkeys were injected only with the challenge inoculum to serve as challenge controls.

Table 4 shows that 5 of the 7 test animals remained negative up to 6 months after challenge (observations are continuing at the time of the writing of this report). The 2 monkeys in which the infection became patent had microfilarial counts of 12 and 45 microfilariae/0.1 ml respectively. Both vaccine controls remained negative while the challenge controls showed good levels of microfilaraemia after a normal prepatent period.

Subsequent experiments

Rechallenge of immune monkeys. Three XL vaccinated animals (A124, A141, and A102 of Experiment 3), which had remained negative for microfilariae for 10 months after the challenge inoculation, were each given a second challenge of 100 L_3. Blood films examined regularly for the following 6 months remained negative for microfilariae in all 3 animals.

Delayed challenge of X-irradiated vaccine controls. Three (A55, A103, and A122) of the 6 vaccine controls, which had not developed microfilaraemia during the 12-month period following their inoculations, were challenged with 100 L_3 each. Two developed no microfilaraemia during the following 6 months while the third became positive after a prepatent period of 143 days. The microfilarial count was very low and reverted to negative after 2 months.

Recovery of adult worms

Two immune monkeys (i.e., those in which microfilaraemia did not occur) and 1 vaccine control (Experiment 3) plus 2 challenge controls were

TABLE 1

RESULTS OF ATTEMPTED IMMUNIZATION OF RHESUS MONKEYS WITH SE VACCINE (EXPERIMENT 1)

Monkey No.	Vaccination dose			Challenge dose	Prepatency (days)	Highest microfilarial count per 0.1 ml	Duration of microfilaraemia (months)
	1st inoc.	Interval (weeks)	2nd inoc.				
A120	100 SE	2	100 SE	100 L_3	74	70	>12
A104	100 SE	2	100 SE	100 L_3	88	2	7
A71	100 SE	4	100 SE	100 L_3	—	—	—
A138	200 SE	2	200 SE	100 L_3	88	225	>12
A107	200 SE	2	200 SE	100 L_3	130	3	9
A146	200 SE	4	200 SE	100 L_3	74	225	>12
A129	400 SE	2	400 SE	100 L_3	130	1	4
A136	400 SE	2	400 SE	100 L_3	74	292	>12
A116	400 SE	4	400 SE	100 L_3	74	95	10
			Challenge control				
A130C	—	—	—	100 L_3	88	1 265	>12

TABLE 2

RESULTS OF ATTEMPTED IMMUNIZATION OF RHESUS MONKEYS WITH IL VACCINE (EXPERIMENT 2)

Monkey No.	Vaccination dose			Challenge dose	Prepatency (days)	Highest microfilarial count per 0.1 ml	Duration of microfilaraemia (months)
	1st inoc.	Interval (weeks)	2nd inoc.				
A137	100 IL	2	100 IL	100 L_3	88	122	>12
A147	100 IL	2	100 IL	100 L_3	47	376	>12
A131	100 IL	4	100 IL	100 L_3	87	30	8
A115	200 IL	2	200 IL	100 L_3	74	6	3
A125	200 IL	2	200 IL	100 L_3	74	29	7
A140	200 IL	4	200 IL	100 L_3	60	3	Died
A105	400 IL	2	400 IL	100 L_3	74	2	1
A119	400 IL	2	400 IL	100 L_3	59	197	>12
A112	400 IL	5	400 IL	100 L_3	33	85	>12
			Vaccine controls				
A113C	100 IL	4	400 IL	—	63	510	>12
A117C	100 IL	—	—	—	174	2	1
A126C	100 IL	—	—	—	63	127	5
			Challenge control				
A133C	—	—	—	100 L_3	104	74	>12

TABLE 3
RESULTS OF ATTEMPTED IMMUNIZATION OF RHESUS MONKEYS WITH XL VACCINE
(EXPERIMENT 3)

Monkey No.	Vaccination dose [a]			Challenge dose	Prepatency (days)	Highest microfilarial count per 0.1 ml	Duration of microfilaraemia (months)
	1st inoc.	Interval (weeks)	2nd inoc.				
A127	100 XL/10 000 R	2	100 XL/10 000 R	100 L_3	102	95	>12
A66	200 XL/10 000 R	3	200 XL/10 000 R	100 L_3	102	5	4
A124	400 XL/10 000 R	2	400 XL/10 000 R	100 L_3	—	0	0
A109	100 XL/20 000 R	2	100 XL/20 000 R	100 L_3	—	0	0
A128	200 XL/20 000 R	4	200 XL/20 000 R	100 L_3	—	0	0
A141	400 XL/20 000 R	2	400 XL/20 000 R	100 L_3	—	0	0
A111	100 XL/40 000 R	2	100 XL/40 000 R	100 L_3	88	188	>12
A132	200 XL/40 000 R	4	200 XL/40 000 R	100 L_3	103	3	1.5
A102	400 XL/40 000 R	2	400 XL/40 000 R	100 L_3	—	0	0
			Vaccine controls				
A55C	100 XL/10 000 R	—	—	—	—	0	0
A61C	100 XL/20 000 R	—	—	—	—	0	0
A103C	100 XL/40 000 R	—	—	—	—	0	0
A121C	—	—	100 XL/10 000 R	—	—	0	0
A122C	—	—	100 XL/20 000 R	—	—	0	0
A46C	—	—	100 XL/40 000 R	—	—	0	0
			Challenge controls				
A145C	—	—	—	100 L_3	130	63	11
A68C	—	—	—	100 L_3	88	400	>12

[a] XL/10 000 R, XL/20 000 R and XL/40 000 R indicate vaccines incorporating larvae irradiated at total doses of 10 000 R, 20 000 R and 40 000 R, respectively.

TABLE 4
RESULTS OF ATTEMPTED IMMUNIZATION OF RHESUS MONKEYS WITH XL/20 000 R VACCINE
(EXPERIMENT 3)

Monkey No.	Vaccination dose			Challenge dose	Prepatency (days)	Highest microfilarial count per 0.1 ml	Duration of microfilaraemia [a] (months)
	1st inoc.	Interval (weeks)	2nd inoc.				
R358	100 XL/20 000 R	2	100 XL/20 000 R	100 L_3	98	12	>3
A51	100 XL/20 000 R	2	100 XL/20 000 R	100 L_3	98	45	>3
R141	100 XL/20 000 R	2	100 XL/20 000 R	100 L_3	—	0	0
A93	100 XL/20 000 R	2	100 XL/20 000 R	100 L_3	—	0	0
A94	200 XL/20 000 R	2	200 XL/20 000 R	100 L_3	—	0	0
A391	200 XL/20 000 R	2	200 XL/20 000 R	100 L_3	—	0	0
A419	200 XL/20 000 R	2	200 XL/20 000 R	100 L_3	—	0	0
			Vaccine controls				
A58C	100 XL/20 000 R	2	100 XL/20 000 R	—	—	0	0
A79C	—	—	200 XL/20 000 R	—	—	0	0
			Challenge controls				
A69C	—	—	—	100 L_3	81	435	>3
A143C	—	—	—	100 L_3	98	84	>3

[a] The data reported represent observations made during the first 6 months after challenge inoculation.

TABLE 5

RECOVERY OF ADULT WORMS FROM 5 EXPERIMENTAL MONKEYS

Monkey No.	Status	Vaccination dose	Challenge dose	Micro-filaraemia	Adult worms recovered
A94	Immune monkey	200 XL/20 000 R (twice)	100 L_3	—	2 (♂ 1 ♀)
A102	Immune monkey	400 XL/40 000 R (twice)	100 L_3	—	
A58	Vaccine control	100 XL/20 000 R (twice)	—	—	
A143	Challenge control	—	100 L_3	+	4 (1♂3 ♀)
A130	Challenge control	—	100 L_3	+	11 (4♂7 ♀)

sacrificed. Attempts were made to recover adult worms to gauge the fate of infective challenge larvae in the immune animal, the viability of X-irradiated larvae after inoculation, and to compare the worm recovery rate of infected animals (challenge controls).

As shown in Table 5, a total of 15 adult worms was recovered from the infected challenge control animals. Two worms, a male and a gravid female with fully developed microfilariae *in utero*, were found in 1 of the 2 immune monkeys. No worm was recovered from the vaccine control.

DISCUSSION

Since absolute resistance to helminthic infection is rarely achieved, one is left to contend with relative or partial immunity. This immunity is usually indicated by significant decreases in rate of migration, degree of development, reproductive capacity and longevity of adults, as well as the final worm burden. In filarial infections, however, the assessment of degrees of immunity is often difficult and problematic. For lack of better parameters, these same indices were considered in the present experiments in comparing the vaccinated and the control monkeys. The rate and degree of development were interpreted from the length of prepatent period; difficulties existed with those which never became patent, no attempt having been made to determine either the migration rate of the larvae or their degree of development. Whether the reproductive capacity and longevity of adult worms could possibly be expressed in the appearance, level and duration of microfilaraemia is questionable—it is not known how many microfilariae are produced by each *B. malayi*, nor whether there is any correlation between the number of microfilariae and the number of adults present. Moreover, the possibility exists that, as with *Dirofilaria immitis*, no microfilaria may be found in the circulating blood although fully mature worms may be found in the heart (Thrasher, 1965). Finally, the assessment of the total worm load depends on the actual recovery of all worms in each host, an infallible technique for which is still lacking.

The use of metabolic products (SE antigens or exoantigens) as vaccines has been shown to elicit some degree of immunity in certain experimental infections: *Trichinella spiralis* (Campbell, 1955; Chute, 1956; Chipmann, 1957), *Nippostrongylus muria* (Thorson, 1953), and *Ascaris lumbricoides* (Soulsby, 1957). Difficulties exist in the collection, concentration, and preservation of such products. It is probable that in the present experiment (Experiment 1) these same difficulties accounted for the result obtained. Moreover, the exsheathing fluid rather than the metabolites may, as pointed out by Soulsby (1960) in the case of *Ascaris*, serve as the main source of functional antigen. Whether this may also be the case in filarial infection has not been determined.

The exact nature of the so-called "functional antigen" is still not clearly defined. Techniques under development in recent years for harvesting, concentrating, characterizing, and standardizing antigens produced during various stages of *in vitro* cultivation (Mills & Kent, 1965; Silverman et al., 1966; Fife et al., 1967; Sadun & Gore, 1967) will undoubtedly aid in achieving this goal.

The deleterious effect of immune serum on worms has been considered in several experiments (Mauss, 1940; Oliver-Gonzalez, 1941; Schwabe, 1957; Wong, 1964). In this experiment (Experiment 2), the immune serum was obtained from a monkey in another experiment (Wong, in preparation) which had been exposed to 20 doses of 20 infective larvae each and had remained negative for microfilaraemia all through the year of the experiment. It was as-

sumed that antibodies which in some way interfered with the larval development had been formed. Unfortunately, no attempt was made to characterize the nature of the immunity until later in the experiment, when a fluorescent antibody technique was designed to test for antibodies against the infective stage as well as the microfilarial stage (Wong & Guest, unpublished data). It was found that antimicrofilarial, but not antilarval, antibodies were demonstrable in the serum. This finding may explain the failure to attenuate the infective larvae used in the vaccinating doses. The question of the existence of stage-specific antibodies becomes of considerable importance in this respect.

Perhaps the most rewarding results were obtained with X-irradiated larvae (Experiment 3). These data lead to speculation on the importance of the sufficiency of functional antigen necessarily present before protective immunity is elicited. Apparently both the degree of attenuation and the number of larvae in the vaccine are contributing factors. The degree of attenuation of the worm should be directly proportional to the dose of radiation to which it was exposed; at any rate, a patent infection was not seen in any of the vaccine controls. Neither did microfilaraemia develop in any of the monkeys which received 400 larvae; however, 2 of the 3 monkeys which received 100 larvae produced patent infections upon challenge. It is possible that the larvae exposed to the highest dose of irradiation (40 000 R) were over-irradiated and probably did not survive long enough to have produced a sufficient amount of functional antigen except when the highest number of larvae (400) was present.

The degree of attenuation which seemed most promising was that accomplished by exposure to a total dose of 20 000 R. However, as shown in Table 4, 2 of the 4 monkeys vaccinated with 100 larvae produced patent infections while none of the 3 monkeys which received 200 larvae exhibited microfilaraemia. These findings again suggest that not only the degree of attenuation, represented by the total irradiation dose, but also the number of larvae used in each vaccine might be contributing to the total effect.

The duration of immunity for more than 10 months, as shown when vaccinated, challenged monkeys were subjected to rechallenge (see under " Subsequent experiments "), is of considerable interest. In addition, the effect of the vaccine seems to persist for a long period. This was demonstrated when vaccine controls were challenged with an infective dose a year later.

Assessment of immunity by recovery of adult worms in Malayan filariasis in the monkey is not an easy task. The techniques developed for their recovery are, to say the least, extremely time-consuming. Moreover, to determine the fate of the attenuated worms used in vaccination would require even more careful and frequent examination.

Although the number of monkeys examined in this experiment barely represents each immune effect seen, the fact that a pair of fully developed adult worms was recovered in a monkey which had consistently shown no microfilaraemia deserves fresh attention and consideration. One wonders if the " immunity " developed was an immune response which either prevented the circulation of microfilariae (Wong, 1964) or inhibited the release of the progeny from the female worm (Taliaferro, 1948). In either case, the possibility of an occult infection cannot be dismissed. Occult filariasis in the form of " eosinophilic lung " (Danaraj, 1958) has been reported in man in Singapore and Malaysia. Microfilariae are undoubtedly produced but are trapped in the lung and other tissues (Webb et al., 1960; Lie, 1962; Danaraj et al., 1966). The presence of filarial antibodies (Danaraj et al., 1959), and specifically microfilarial antibodies (Wong & Guest, 1969), is associated with the syndromes of occult filariasis. It is hoped that the analysis of the various data collected in the present studies, especially the serological studies, may reveal the roles played by the various immune responses in the etiology of filarial diseases.

Although at least one helminthic vaccine has been shown to be of economic value (Urquhart et al., 1962), the results of the present studies do not indicate the practicability of vaccine development and use for Malayan filariasis in the near future. Although some degrees of immunity may be interpreted as having been brought about by vaccination, the problems of assessment of these in Malayan filariasis seem to be compounded by the occurrence of occult infections. Undoubtedly, a better understanding of the basic biology of the filarial parasite would be most helpful towards solving some of these problems.

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RÉSUMÉ

ÉTUDES SUR L'IMMUNISATION CONTRE L'INFECTION À *BRUGIA MALAYI* CHEZ LE SINGE RHÉSUS

Le singe rhésus (*Macaca mulatta*) est réceptif à l'infection expérimentale par *Brugia malayi*, agent de la filariose humaine subpériodique. On a recherché chez cet animal le pouvoir immunisant de trois préparations vaccinales: *a*) un vaccin SE, obtenu par incubation de larves infectantes, et supposé contenir des produits du métabolisme du parasite (exoantigènes); *b*) un vaccin IL, consistant en des doses variables de larves infectantes traitées par contact avec un immunsérum; *c*) un vaccin XL, larves infectantes traitées par des doses plus ou moins élevées de rayons X. Quatre semaines après l'administration de l'un ou l'autre vaccin, les singes ont reçu une injection d'épreuve de 100 larves infectantes de *B. malayi*. La réponse immunitaire a été évaluée sur la base de l'apparition ou de la non-apparition d'une microfilarémie, sur son taux et sa durée; on a également comparé la charge en parasites adultes des singes vaccinés et des animaux témoins.

L'administration des vaccins SE et IL n'a pas empêché l'infection de se développer, mais l'intensité et la durée de la microfilarémie ont été plus faibles chez les animaux vaccinés que chez les singes témoins. Le résultat le plus marquant a été obtenu par l'emploi d'un vaccin XL contenant 200 larves infectantes atténuées par application d'une dose totale de 20 000 R: chez aucun des singes ainsi traités, on n'a pu déceler d'infection après l'inoculation d'épreuve. Il semble que le nombre et le degré d'atténuation des larves contenues dans le vaccin jouent tous deux un rôle dans le déclenchement de la réponse immunitaire. La protection conférée par le vaccin XL s'est maintenue pendant plus de 10 mois comme l'a démontré l'insuccès d'une nouvelle injection d'épreuve. Deux filaires adultes (1 mâle et 1 femelle) parfaitement développées ont été découvertes chez un singe qui n'avait jamais présenté de microfilarémie. Les auteurs envisagent brièvement le problème de l'infection filarienne occulte.

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