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SUSCEPTIBILITY OF THE MARMOSET, *SAGUINUS GEOFFROYI* PUCHERAN, TO INTRAPERITONEAL AND ORAL INFECTIONS WITH *BESNOITIA* (PROTOZOA: TOXOPLASMEA)

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ABSTRACT: Cystic and proliferative stages of two strains of *Besnoitia darlingi* (Brumpt, 1913) and a strain of *B. jellisoni* Frenkel, 1953 were highly pathogenic for marmosets, *Saguinus geoffroyi* Pucheran, when administered by either the intraperitoneal or oral route. The clinical picture of disease was similar for both routes of administration and resembled the acute form of the disease as seen in mice. There was a significant correlation between numbers of parasites in intraperitoneal inocula and the day of death. In general, death after oral administration occurred somewhat later than after intraperitoneal inoculation. But cystic *B. darlingi* killed more quickly than proliferative forms when both were given orally; however, individual variation in monkey hosts may have been as important as strain or stage of the parasite, with regard to time of death. Using relatively large numbers of organisms, all of 22 intraperitoneal infections and 21 of 26 oral infections proved fatal. Parasitemia was detectable at the time of death following both routes of inoculation. When the numbers of organisms were too small to establish a fatal infection they also failed to immunize. Protective immunity, detectable by subsequent intraperitoneal challenge, was established in two monkeys receiving large numbers of parasites by mouth. A monkey which became demonstrably immune to *B. jellisoni* (PN-3) was shown by heterologous challenge to be still susceptible to *B. darlingi* (D-3).

Mouse-adapted *Besnoitia darlingi* (= *B. panamensis*) of lizard origin was reported to be experimentally pathogenic for Geoffroy's marmoset (Schneider, 1965). The disease in this animal was described as an acute, rapidly fatal infection accompanied by considerable ascitic fluid in which numerous proliferative forms of the parasite were found. The disease in the marmoset, in fact, very much resembled the disease in the mouse.

The present paper presents the results of additional studies of marmoset besnoitiosis in which experimental infections were induced with either cystic or proliferative stages of three strains of *Besnoitia* by the intraperitoneal or oral route.

MATERIALS AND METHODS

Geoffroy's marmoset (*Saguinus geoffroyi* Pucheran) abounds in low forest and secondary growth throughout most of Panama. The monkeys could be obtained from street vendors during certain times of the year. If they survived in captivity the first few days, they did well on a diet of fruits supplemented with meat (grasshoppers or baby mice). They would sometimes accept adult mice, eating out the brains, muscles and viscera and throwing away the skin. Almost 100% had microfilariae in the blood and many were infected with a trypanosome.

A total of 53 marmosets were used in the present series of experiments. Administration was by the intraperitoneal or oral route. Intraperitoneal inoculations were made in the area of the right groin with a 21-gauge needle. Orally, parasites were delivered directly into the mouth of the hand-held animal from a small syringe without needle; if given slowly, permitting time to swallow, all monkeys readily accepted the dose once it had been tasted. To avoid trauma, stomach intubation was not used, and anesthetics were avoided as risky and unnecessary.

Strains of *Besnoitia*

Two strains of *B. darlingi* were used: (1) The L-62 strain was isolated from a lizard in 1963 and was maintained by mouse-passage (Schneider, 1965). Other lizards also yielded isolates which are identified in the tables by different "L" numbers. The lizard hosts were all *Ameiva ameiva praesignis* Baird & Girard. (2) The D-3 strain was isolated in 1964 from a common opossum, *Didelphis marsupialis* L. in Panama (Schneider, 1967a). It was maintained in mice. The lizard and opossum isolates are now considered to be conspecific (Schneider, 1967b).

An encysting strain (PN-3) of *B. jellisoni* was made available through the courtesy of Dr. J. K. Frenkel, University of Kansas Medical Center, Kansas City. In the course of subsequent passages through mice the encysting capacity of this strain was lost.

Experimental inocula consisted of mouse peritoneal fluid containing proliferative stages of the parasite. Cystic organisms from natural lizard and opossum infections were also used and, in one case, cystic organisms from the spleen of a mouse which became immune during chemoprophylaxis (see the

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TABLE I. *Survival of marmosets following intraperitoneal inoculation of* Besnoitia darlingi, *strain L-62.*

Monkey	No. of mouse passages of strain	No. of organisms in inoculum	Time of death (days after inoculation)
1	0[1]	2,000,000	6
2	28	1,762,000	14
3	28	880,000	9
4	28	440,000	9
5	28	220,000	11
6	28	110,000	10
7–13	58	25,300	9, 11, 13, 14, 15, 16, 18

[1] Cystic stages from natural lizard infection.

discussion of this phenomenon with regard to murine toxoplasmosis by Frenkel, 1953). Hemocytometer counts of organisms were made after fixing in Hayem's fluid.

Infected monkeys remained alert and active until a few hours to a day before death. Then they became lethargic, but not comatose, and sat huddled with the head bowed between the legs. They could be aroused to convulsive movement if lifted by the tail. Tremors of the hind legs and labored breathing were sometimes noted. A terminal diarrhea was observed in most animals. Rectal temperatures, which varied normally from 99 to 101 F, dropped to 80 to 85 F a few hours before death, as measured with a Thermistemp Tele-Thermometer Ranger (68 to 108 F)[1] equipped with a small animal probe.

Post-mortem findings included the presence of peritoneal fluid and occasionally pleural fluid in varying amounts. A slight splenomegaly was sometimes seen but the other viscera appeared grossly normal. Parasites could be demonstrated in spleen and heart blood by direct microscopic examination. They were also detected by mouse inoculation of blood or homogenates of various organs.

Three control monkeys, not included in the total, were left uninfected to check on the adequacy of cage conditions. Such animals survived while their infected cage-mates died.

[1] Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio.

TABLE III. *Survival of marmosets following intraperitoneal inoculation of proliferative* Besnoitia darlingi *(12th mouse passage of strain D-3).*

Monkey no.	No. of organisms in inoculum	Death (days after inoculation)
22	1,000,000	7
23	100,000	6
24	10,000	8
25	1,000	12
26	100	14

RESULTS

Intraperitoneal infections

Table I presents data pertaining to the survival of marmosets following intraperitoneal inoculation of *B. darlingi*, strain L-62. All of five monkeys given 110,000 to 1,762,000 organisms from the 28th mouse passage died in 9 to 14 days (average, 10.6 days). All of seven monkeys given 25,300 parasites from the 58th mouse passage died in 8 to 18 days (average, 13.7 days). The difference between day of death in these two groups proved to be significant at the 0.05 level of confidence when analyzed by the Mann-Whitney *U* test.[2]

Eight marmosets were inoculated with serial decimal dilutions of *B. jellisoni* obtained from the third mouse passage of an encysting strain (Table II). One monkey given 3 million and one given 300,000 parasites died on the 11th day; two others which received respectively

[2] According to Siegel (1956), this test, which is based on a ranking procedure, constitutes an extremely useful alternative to the *t* test when small groups of nonparametric data are to be compared.

TABLE II. *Survival of marmosets following intraperitoneal inoculation of proliferative* Besnoitia jellisoni *from 3rd mouse passage, strain PN-3.*

Monkey no.	Size of inoculum	Death (days after inoculation)	Size of IP challenge with same strain	Death (days after inoculation)
14	3,000,000	11		
15	300,000	11		
16	30,000	14		
17	3,000	14		
18	300	(22)	250,000	3[1]
19	30	(22)	250,000	11
20	3	(22)	250,000	10
21	0	(22)	250,000	10

( ) = Survived and challenged on this day.
[1] Accidental death.

TABLE IV. *Survival of marmosets following oral administration of* Besnoitia darlingi *of lizard origin. Strains 590 and 591 represent cystic organisms taken directly from lizards.*

Monkey no.	Strain	No. of mouse passages	No. of organisms in inoculum	Death (days after inoculation)	Size of IP challenge with same strain	Death (days after inoculation)	Size of second IP challenge (same strain)	Death (days after inoculation)
27	L590	0	1,387,000	7				
28	L590	0	1,387,000	13				
29	L591	0	10,000,000	7				
30	L591	0	10,000,000	13				
31	L62	47	2,162,500	(32)	515,000	22		
32	L62	105	7,000,000	(28)	345,000	4		
33	L62	105	7,000,000	(28)	345,000	(28)	4,350,000	8
34	L62	105	11,500,000	26				
35	L62	105	11,500,000	16				
36	L62	105	14,000,000	18				
37	L62	105	14,000,000	22				
38	L62	109	6,900,000	32				
39	L62	109	6,900,000	24				
40	L62	109	6,900,000	(28)	455,000	3		
41	L62	109	6,900,000	25				
42	L62	109	6,900,000	10				

( ) = Survived and challenged on this day.

30,000 and 3,000 organisms died on the 14th day. Four monkeys given 300 parasites or less survived for 22 days. The surviving monkeys were then inoculated with a number of parasites of the same strain known to be lethal (250,000); one died in 3 days, probably as a result of inexpert handling, while the others died in 10–11 days. These results indicated that the small numbers of parasites in the primary infections had not established protective immunity.

Table III shows the results of inoculating five monkeys with decimal dilutions of *B. darlingi*, strain D-3. Here, also, larger doses resulted in earlier deaths. It is interesting that an initial inoculum of only 100 parasites of this strain killed a monkey in 14 days.

Oral infections

These are summarized in Tables IV to VI. The oral infections were associated with great variation in the time of death. Whereas a significant correlation was noted between size of intraperitoneal inocula and the day of death, increasing the size of oral doses of parasites did not always produce earlier death. It may be noted, however, that monkeys infected orally tended to live longer than those infected intraperitoneally.

There were five failures (monkeys 31, 32, 33, 40, and 43) among 21 attempts to produce fatal infections by the oral route. In two of these cases (33 and 43) the monkeys survived a subsequent intraperitoneal challenge with a known, lethal number of parasites. When monkey 33 was injected intraperitoneally a third time with a very large inoculum it died 8 days later, but monkey 43 survived a third large injection of parasites. In these cases, protective immunity appeared to have been established by the initial oral exposure. It is thought that monkey 31 may also have been at least partly immunized by its initial oral dose since it survived for the relatively long period of 22 days after a subsequent intraperitoneal challenge with a large number of parasites.

TABLE V. *Survival of marmosets following oral administration of* Besnoitia jellisoni, *strain PN-3.*

Monkey no.	No. of mouse passages	Stage	No. of organisms in inoculum	Death (days after inoculation)	IP challenge (same strain)	Death (days after inoculation)	Second IP challenge (same strain)	Survived
43	12	cystic	1,125,000	(35)	6,725,000	(21)	1,029,000	27[1]
44	13	proliferative	11,900,000	20				

( ) = Survived and challenged on this day.

[1] After 27 days, this monkey was inoculated with approximately 100 proliferative organisms of *Besnoitia darlingi*; it died 16 days later.

TABLE VI. *Survival of marmosets following oral administration of* Besnoitia darlingi, *strain D-3.*

Monkey no.	No. of mouse passages	No. of organisms in inoculum	Death (days after inoculation)
45	0[1]	2,000,000	17
46	5	5,350,000	16
47	28	27,000,000	5
48	28	27,000,000	11
49	28	2,700,000	13
50	28	2,700,000	14
51	42	6,213,000	6
52	42	621,300	10
53	42	62,130	12

[1] Cystic stages from natural infection in opossum.

Monkey 43 was considered to be solidly immune to *B. jellisoni* after the second challenge. Yet when it was inoculated with approximately 100 proliferative organisms of *B. darlingi* it died in 16 days. The immunity in this case did not suffice to protect against a small heterologous challenge.

DISCUSSION

When a relatively large number of parasites was administered, all three strains of *Besnoitia* produced acute, fatal infections in marmosets, regardless of whether the intraperitoneal or oral route was used.

Historically, the few attempts to transfer *Besnoitia* by mouth have produced varied results, perhaps due to the different susceptibilities of the experimental hosts employed. Franco and Borges (1916) failed to infect rats and mice with *Besnoitia besnoiti* by oral or subcutaneous administration of cyst suspensions. These results are best viewed in the light of subsequent failures to infect these rodents with *B. besnoiti* by any route (Pols, 1960). But Pols (1960) was also unable to transmit *B. besnoiti* to two rabbits by oral administration of infected liver and spleen, although the rabbit is highly susceptible to this parasite when inoculated intraperitoneally. On the other hand, Jellison et al. (1956) succeeded in infecting baby mice by introducing cysts and proliferative forms of *B. jellisoni* into the mouth with a capillary pipette. They reported, too, that a number of mother mice developed the infection as a consequence of grooming the newly-infected babies. According to these authors, cystic organisms produced a slightly higher proportion of patent infections than did proliferative forms when given by mouth.

The high susceptibility of Geoffroy's marmoset to laboratory infections with three strains of New World *Besnoitia* has now been repeatedly confirmed. Given relatively large inocula, all of 22 infections by the intraperitoneal route proved fatal. When inocula by this route were too small to kill the host, they also failed to immunize (see Table II, monkeys 18 to 21). In 26 attempts to infect monkeys by mouth there were five failures, i.e., cases of survival of inoculated animals. In two of these, at least some immunity resulted from oral exposure, as demonstrated by failure of subsequent large intraperitoneal inocula to kill. The conditions under which parasites administered by mouth might immunize and not kill were not determined. It should perhaps be pointed out in this connection that some of the marmosets suffered from broken teeth and bleeding gums and that the possibility of infection by contamination of bleeding surfaces cannot be ignored in these cases.

In a similar vein, Rodaniche (1954) orally infected *Saguinus geoffroyi* with a strain of *Toxoplasma gondii* isolated from a *Cebus* monkey. In her experience, gastric intubation of parasites produced irregular results as compared with feeding the inoculum directly into the mouth of the anesthetized animal. The clinical picture and course of the infections thus produced closely resembled acute besnoitiosis with regard to the absence of signs until a few hours or a day before death, then the rapid drop in body temperature and, at autopsy, the presence of peritoneal or pleural fluid containing parasites, sometimes accompanied by a slight splenomegaly.

The picture of acute besnoitiosis in monkeys induced by the intraperitoneal inoculation of parasites could not be distinguished from that which followed feeding the inoculum directly. Parasitemia could be detected just before death in both cases. It is thought that such variations as did occur in time of death were due more to individual variations in the condition of the marmosets than to differences in the strain or stage of the parasites. However, in the case of monkeys 27 to 30, large oral doses of cystic *B. darlingi* from natural infec-

tions in lizards (*Ameiva ameiva*) appeared to kill the hosts in a somewhat shorter time than did similar large doses of proliferative organisms from the mouse-adapted L-62 strain (monkeys 31 to 42); in fact, among the latter there were four failures to kill. These few data suggest that cystic organisms of *B. darlingi* from lizards may be better able to survive stomach passage than proliferative mouse-adapted forms.

In spite of the foregoing it seems unlikely that infections of Geoffroy's marmoset with *Besnoitia darlingi* will be found in nature. *Ameiva* lizards are restricted to the ground and marmosets seldom if ever descend out of the trees for food. Furthermore (although the subject has been little studied), lizards are not known to form part of the natural diet of these monkeys (Moynihan, 1967).

The question of an antigenic relationship between the strains of *Besnoitia* used in these experiments was not purposefully explored here. However, it was noted that one monkey (43) which became demonstrably immune to *B. jellisoni* (strain PN-3) did not survive a small challenge of 100 organisms with *B. darlingi* (D-3), suggesting that there may be a significant antigenic difference between these strains.

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