

Cross-immunity Evidence of the Identity of *Besnoitia panamensis* from Lizards and *B. darlingi* from Opossums

The recent isolation in Panama of *Besnoitia* from two different hosts, a reptile and a mammal, in the same geographic locality, has raised a question regarding the validity of the species names assigned to them. The L-62 strain of *B. panamensis* from lizards (Schneider, 1965, J. Parasit. 51: 340-344) and the D-3 strain of *B. darlingi* from opossums (Schneider, 1967, J. Prot. 14: 78-82) could not be easily distinguished after adaptation to mice. They differed in virulence: the LD₅₀ of *B. darlingi*, strain D-3, in mice was less than 30 organisms at the 96th mouse passage, whereas the LD₅₀ of L-62 strain *B. panamensis* was approximately 2,000 organisms at the 16th mouse passage. Otherwise, they were identical in all obvious respects. In an effort to resolve the question of affinities, cross-infection tests were performed in immunized mice.

The method of inducing immunity to *Besnoitia* in mice was similar to one used by Frenkel (1956, Ann. New York Acad. Sci. 64: 215-251) for immunizing mice against *Toxoplasma*. Groups of 10 NIH all-purpose mice weighing 20 to 30 g were inoculated intraperitoneally with 66,000 to 68,000 living parasites and at the same time were started on a regimen of sulfadiazine (60 mg of the sodium salt in 100 ml of drinking water). Treatment was maintained for 3 weeks, after which immunity was usually established in survivors. At this time drug was removed from the drinking water and, after 24 hr, the survivors were challenged with the homologous strain. Those

mice which lived another 2 weeks after challenge were considered to be demonstrably immune and were rechallenged at this time with the heterologous strain. The homologous and heterologous inocula used for rechallenge contained 100,000 to 200,000 organisms. The combined results of two experiments are summarized in Table I.

Not all mice responded to the above procedure by developing immunity. Some died during the initial 3-week period on drug. An occasional breakthrough, with accompanying death of the mouse, occurred after removal of drug from the drinking water. In all, less than half of the mice so treated reached the stage where they could be challenged with heterologous organisms. This may have been due to insufficient drug intake or to lack of susceptibility of the parasites to sulfadiazine.

In mice which survived and became demonstrably immune to one strain, cross-immunity between the two strains appeared to be essentially complete. These data, coupled with the fact that the strains were isolated in the same geographic site in Panama, strongly support the hypothesis that they are conspecific. On this basis, *Besnoitia panamensis* Schneider, 1965, becomes a synonym of *B. darlingi* (Brumpt, 1913).

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TABLE I. Cross-immunity in murine besnoitiosis, between *B. panamensis* (L-62) and *B. darlingi* (D-3). Challenge inocula consisted of 100,000 to 200,000 parasites inoculated intraperitoneally.

Primary inoculation with:	Challenged with:	Number of mice	Number of deaths (days) after second homologous or heterologous challenge	Survived at least 60 days after second homologous or heterologous challenge
<i>panamensis</i>	<i>panamensis</i> ¹	9	1 (24)	8
<i>darlingi</i>	<i>darlingi</i> ²	8	—	8
<i>panamensis</i>	<i>darlingi</i> ²	10	—	10
<i>darlingi</i>	<i>darlingi</i> ²	8	1 (34)	7

¹ Thirty nonimmune control mice died in an average 7.5 days.

² Ten nonimmune control mice died in an average 7.5 days.