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VACCINATION OF *CALLITHRIX JACCHUS* (LINNÉ, 1758) MARMOSETS WITH THE PF STRAIN OF *TRYPANOSOMA CRUZI*

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Callithrix jacchus *marmosets, vaccinated more than once with high doses of the PF strain of* Trypanosoma cruzi, *showed a certain degree of parasitemia related to the number of parasites injected. Thirty days after vaccination, all animals were alive and showed no apparent morbid symptoms. The relationship between the dose of injected trypanosomes and the observed parasitemias is discussed and analysed as well as the immunologic incompetence of the experimental animals used.*

As already demonstrated by us in a previous study Menezes (1980), the PF strain of *Trypanosoma cruzi* shows no infecting power for *Callithrix jacchus geoffroyi* marmosets (Humboldt, 1812) when injected in adequate doses and administered by a convenient route. The data presented here concern the vaccination of *C. jacchus* (Linné, 1758) marmosets with the same strain but with a different immunization program.

MATERIAL AND METHODS

Marmosets – Twenty-two animals out of a group of 64 *Callithrix jacchus* species, divided into two groups, were utilized. The marmosets were captured in the forests of the littoral of Bahia, Brazil.

Group B included 10 animals, and group C, 12 animals.

18,6% of all the animals gave positive xenodiagnosis and/or blood cultures and obviously were taken out of the experiment as the animals that presented sorological positive tests.

At the end of the selection remained only the twenty-two animals above mentioned.

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In spite of the ECG alterations presented by few of these marmosets, they have negative xenodiagnosis, blood culture and sorological tests.

Group B – consisted of 10 animals (6 males and 4 females) weighing an average of 241 g before vaccination. Two xenodiagnosis, 2 hemocultures, and 2 electrocardiograms were taken for all marmosets before administering the first vaccination (Table I). The first vaccination consisted of 30,000 parasites/g body weight injected subcutaneously into each animal (Vaccine 115-V_1). A second vaccination was administered 15 days later by the same route but with the number of flagellates/g body weight reduced to 20,000 (Vaccine 115-V_2). Xenodiagnosis, blood culture and ECG were repeated after 30 days.

TABLE I
Group B Marmosets

Nº	*Marmoset*	*Sex*	*Before Vaccination*				*30 Days After Last Vaccination*				*Notes*
			Weight	*Xeno-Diag.*	*Blood Culture*	*ECG*	*Weight*	*Xeno-Diag.*	*Blood Culture*	*ECG*	
1	27 A	M	235g 218g	– –	– –	N N	209g	–	–	N	– = negative N = normal
2	42 A	F	214g 232g	– –	– –	N N	225g	–	–	N	
3	49	M	260g 228g	– –	– –	N N	222g	–	–	N	
4	56	M	245g 289g	– –	– –	N	212g	–	–	N	
5	69	M	260g 257g	– –	– –	N N	222g	+	–	N	+ = positive
6	71	F	260g 241g	– –	– –	N N	230g	–	–	N	
7	73	F	211g 203g	– –	– –	N N	198g	–	–	N	
8	78	M	249g 230g	– –	– –	RABB-LAB 1/2 B RABB-LAB 1/2 B	244g	–	–	RABB LAB 1/2 B	RABB = Right anterior Branch Block LAB 1/2 B = Left anterior Hemi-Block
9	86	F	250g 240g	– –	– –		252g	–	–	N	
10	88	M	227g 216g	– –	– –	N N	225g	–	–	N	
			$\bar{X}$ = 241g				$\bar{X}$ = 224g				

Group C – consisted of 12 animals (7 males and 5 females) weighing an average of 241 g. The animals were tested the same way as group B (Table II). The first vaccination was identical to that used for Group B (Vaccine 115-V_1), prepared as described below, and administered by the same route. The second vaccination (Vaccine 115-V_2), also prepared as described below, was the same as used for Group B. Doses and route were the same as used for Group B. Group C received a third subcutaneous vaccination containing 10,000 p/g (Vaccine 115-V_3) 15 days after the second. Xenodiagnosis, blood culture and ECG were taken for all animals 30 days after the third vaccination.

Vaccines

115-V_1. Parasites of the PF strain of *T. cruzi* Menezes & Albuquerque (1970) were cultured in flasks containing saline, in which a dialysis bag containing Warren medium was immersed (Warren, 1960). The saline-Warren medium ratio was 2 : 1 (Warren-Nakamura-saline). Culturing was carried out for 25 days at 28°C. The saline solution was centrifuged once at 1500 rpm and the sedimented parasites were resuspended in sterile saline and observed. Ninety percent were mobile forms. Final concentration was 3×10^7 parasites/ml and the percentage of trypomastigotes was 0.5% (1000 parasites were counted on a slide stained with Giemsa).

TABLE II
Group C Marmosets

Nº.	Marmoset	Sex	Before Vaccination				30 Days After Last Vaccination				Notes
			Weight	Xeno-Diag.	Blood Culture	ECG	Weight	Xeno-Diag.	Blood Culture	ECG	
1	3 A	F	213g 215g	– –	–	N N	207g	+	–	N	– = negative + = positive N = normal
2	16 A	M	221g 211g	– –	– –	N N	188g	–	–	N	
3	51	M	239g 244g	– –	– –	N N	247g	–	–	N	
4	63	F	313g 285g	– –	– –	RBB RBB	191g	–	+	RBB	RBB = Right Branch Block
5	68	F	192g 187g	– –	– –	N N	189g	+	–	N	
6	84	M	275g 278g	– –	– –	N N	258g	–	–		
7	85	F	308g 301g	– –	– –	VES N	247g	+	+	N	VES = Ventricular Extra Systoles
8	89	M	252g 238g	– –	– –	N N	244g	–	–	N	
9	91	M	195g 209g	– –	– –	AAR AAR	199g	–	–	N	AAR = Atrial Arrhythmia
10	96	M	259g 258g	– –	– –	N N	256g	–	–	N	
11	97	F	230g 217g	–	– –	N N	193g	–	–	N	
12	100 A	M	220g 230g	– –	– –	N N	229g	–	–	N	
			$\bar{X}$ = 241g				$\bar{X}$ = 229g				

115-V₂. The same PF strain was cultured in two flasks containing the same Warren-Nakamura-saline medium as described above. The culture was incubated for 25 days at 28°C and treated as the 115-V_1 vaccine. Ninety percent of the observed forms were mobile. Final concentration was 4×10^7 p/ml and the percentage of trypomastigotes, determined on a Giemsa-stained slide, was 0.2%.

115-V₃. The PF strain of *Trypanosoma cruzi* was cultured for 19 days in a flask containing Warren medium in a cellophane bag. This medium, however, was slightly modified according to Ribeiro dos Santos (1980), as follows: 10g% tryptose (Oxoid), 8.0g% yeast extract (Oxoid), and 200mg% folic acid were added to the Warren medium. After boiling and stirring, the medium was filtered on paper and distributed among various dialysis bags. The bags were immersed into PBS solution, pH 7.2, with 5 g% glucose added. The culture medium-PBS saline solution ratio was 1:2. The medium was sterilized in an autoclave at 115°C for 15 minutes. After culturing of the PF strain, the PBS solution was centrifuged and the sediment washed 3 times in sterile physiological solution. The final parasite concentration was 3×10^7 p/ml with 1% trypomastigotes.

Xenodiagnosis. Boxes containing 6 *Rhodnius neglectus* nymphae, 5th instar were used. Each box was applied to the skin of each animal's abdomen and left there for at least 30 minutes. The animals, under sedation with 0.1 ml Inoval* injected subcutaneously, were tied to a restraining table. The insects were maintained at room temperature (an average of 28°C with ± 50% humidity) and their intestinal contents were examined 30 and 40 days after the blood meal.

Hemoculture. Two tubes of Warren medium were used for each animal. Blood from each animal (0.2 ml) was added to each 5 ml of medium. The tubes were placed in an incubator, kept at 28°C for 30 and 45 days, and examined.

*Inoval, Johnson & Johnson Lab. (phentanyl, 0.50mg/10 ml; dropenol, 25 mg/10ml).

Electrocardiogram. All ECG tracings were made with a FUNBEC ECG-3 apparatus after the animals had been sedated with 0.1 ml Inoval injected subcutaneously. The 12 classical derivations were taken and always compared to those taken at the beginning of the the experiment for each animal.

Serology. Blood was collected from the animals on filter paper and stored frozen (−20°C). Indirect immunofluorescence tests, direct and indirect agglutination tests for *T. cruzi* were carried out in different laboratories. The results obtained were so uneven that we decided not to include them in this paper.

RESULTS

Xenodiagnosis. Of the 10 animals in Group B, i.e. the marmosets which received 50,000 p/g in 2 doses at a 15 days interval, only one showed a positive xenodiagnosis (Fr 115-69) 45 days after the second vaccination (1 triatomine out of the 6 utilized). See Table I. Of the animals in Group C, i.e. the 12 marmosets which received 80,000 p/g in 3 doses at 15 days intervals, 3 showed positive xenodiagnosis, 1 also showing positive hemoculture (Table II). Only 6 out of the 18 triatomines used gave positive results.

Hemocultures. All hemocultures carried out for the animals in Group B after the second vaccination were negative. The 12 hemocultures (24 tubes) carried out for Group C after the last vaccination, two were positive (for two animals). One of these animals also showed positive xenodiagnosis, as reported above.

Electrocardiogram. According to the reports of the cardiologist who assisted us in this study, no significant differences were found between the original tracings and the tracings obtained after the various vaccinations (Tables I and II).

DISCUSSION AND CONCLUSIONS

In 1968 we had already demonstrated that when animals are injected with excessively high doses of parasites, some will show parasitemia detectable by xenodiagnosis and hemoculture (Menezes, 1970). The use of immunodepressants may also lead to the appearance of parasitemia (detected by xenodiagnosis or hemoculture) in certain cases (Menezes, 1972, 1974). These facts led us to suggest that the PF strain is a mixture of virulent and avirulent mutants selected according to the pressures of the medium in which the parasites develop. This concept seems to have received indirect support by the observations of Hungerer, Enders & Swisler (1976), who verified a strict correlation between doses of injected parasites and the appearance of parasitemia. This does not seem to be an exceptional case to us since it has been known for a long time that there are optimum vaccine doses for each animal species (especially when the vaccine contains live antigens) which must not be exceeded. However, what compelled us to insist on this point is the real worry about the possibility that experimental animals, and eventually human beings, may be infected by this type of vaccination.

Tagliaferro & Pizzi (1955) had already demonstrated that macrophages from animals sensitized to *T. cruzi* have a more efficient phagocytic action on these parasites than macrophages from normal animals.

Hungerer, Enders & Swisler (1976) verified that the parasites from so-called avirulent strains, do not actively penetrate cells in macrophage cultures, but are phagocyted and lysed in the cytoplasm, as observed with the light and electron microscope. However, when the number of parasites presented to the cells is very high, the macrophage cytoplasm becomes saturated with parasites, so that not all of them are destroyed, since some will escape the lytic action of cellular enzymes. Although Hungerer does not consider this

phenomenon to be immunotolerance, we continue to believe it to be, based on the fact that the intimate mechanism, or mechanisms, of immunologic tolerance are not yet fully known, so that this may very well be one of them.

The hypothesis whereby the composition of the PF strain, and possibly of other strains considered to be avirulent, is seen as a mixture of mutants seems to be once more indirectly confirmed by the fact that virulent forms have developed in triatomines from samples of the PF strain.

In 1932, Dias demonstrated the possibility of culturing *T. cruzi* in the celomic cavity of triatomines. In 1977, Ribeiro, Belda Neto & Pereira Barreto, showed that several *T. cruzi* strains, including the PF strain, when submitted to passage through the celomic cavity of "barbeiro" bugs, reacquired their lost or attenuated virulence. This research was carried out using only one sample of each strain. I repeated the experience with Miyasaka (Meneses & Miyasaka, 1980), using the PF strain, and only one of the six samples examined gave positive results (17%) by infecting all tested mice. Thus it can be seen that both the celomic cavity of triatomines and the cytoplasm of macrophages saturated with a mixture of mutants seem to act as selecting media by favoring the growth of virulent forms of this parasite, and consequently contributing to infecting animals inoculated with it.

Previous treatment of so-called avirulent strains with ethidium bromide (Hungerer, Enders & Swisler, 1976) or with sodium azide (Menezes & Yamashita, 1979; Menezes 1975, 1976a, 1976b and 1980) do not induce parasitemias demonstrable by the parasitologic means currently used.

This working hypothesis of the existence of a mixture of mutants in a low-virulence strain is being tested in our laboratory, and everything leads us to believe that it may be possible to isolate clones of flagellates devoid of virulence to laboratory animals, at least from the PF strain.

Despite the existence of research demonstrating the immunogenic role of subcellular (Segura et al, 1974), and molecular (Molteni, London & Ekdesman, 1973; Lemos & Menezes, 1978; Fruit et al, 1978) fractions, the best immunogenic responses continue to be obtained with low or no virulence live parasites. After this work had been started I get in acquaintance with the literature pertaining to the immunologic status of marmosets (Gengozian, Kateley & Nickerson, 1978; Harvey Jr. et al, 1974) in which I learned that the marmosets are abnormal in both bursal and thymic immune systems.

One very important and pertinent objection to the animals used in my experiments was that they were not bred in captivity.

To my own knowledge, until the begining of this experiment (June, 1977) we had not, in Brazil, a center of primatology, except that of Manguinhos Institute that in an island of the Baia de Guanabara to bred Rhesus monkeys.

However, all the animals used in my works were held for more than 30 days in the facilities of the Department while I did the tests for the selection of the animals.

In accordance with Whitney, Johnson & Cole (1973), less than 1% of the over 60,000 primates used annually in research in the United States are bred in that country.

Thus we conclude that the PF strain, as used up to now, is devoid of virulence even to callithricid primates, as long as administered in adequate doses and by adequate routes (Menezes, 1980), but can induce parasitemias in these animals when given in high

doses. A correlation seems to exist between the number of injected parasites and the number of cases showing positive parasitemia (Tables I and II). We believe that this obstacle will be removed by previously treating the strains with ethidium bromide or sodium azide, or by the use of clones of totally avirulent parasites. The immunological abnormalities demonstrated by the marmosets are other important point to be considered and I hope to bring more data on this in a further paper.

RESUMO

Sagüis da espécie *Callithrix jacchus* vacinados mais de uma vez, com altas doses de tripanosomas da cepa PF do *T. cruzi,* apresentam certo grau de parasitemia relacionado com o número de parasitas injetados. Trinta dias após a vacinação todos os animais estavam vivos e sem manifestações aparentes da doença. A relação entre a dose de tripanosomas injetados e as parasitemias observadas é discutida e analisada bem como a incompetência imunológica dos animais usados.

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