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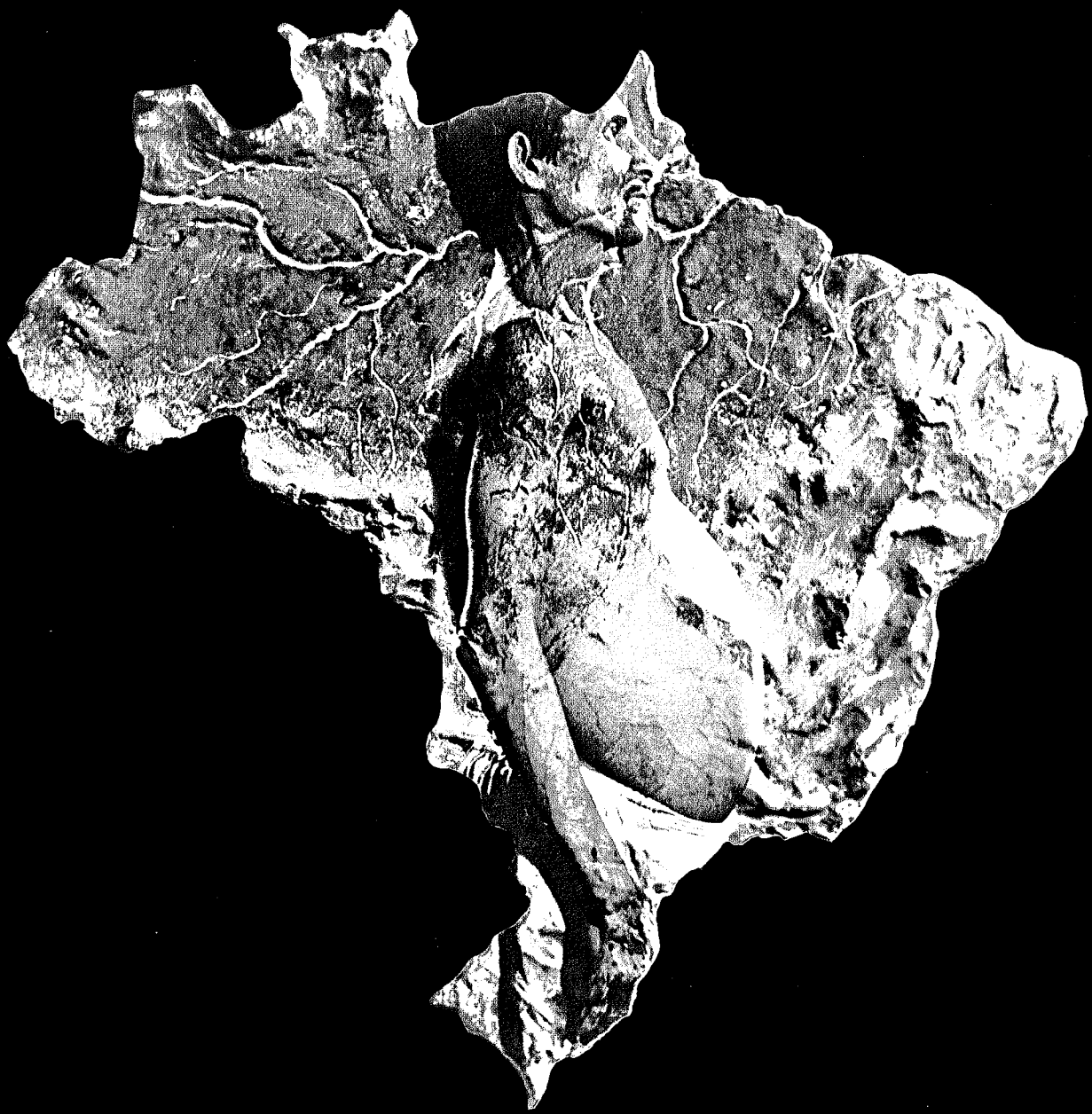

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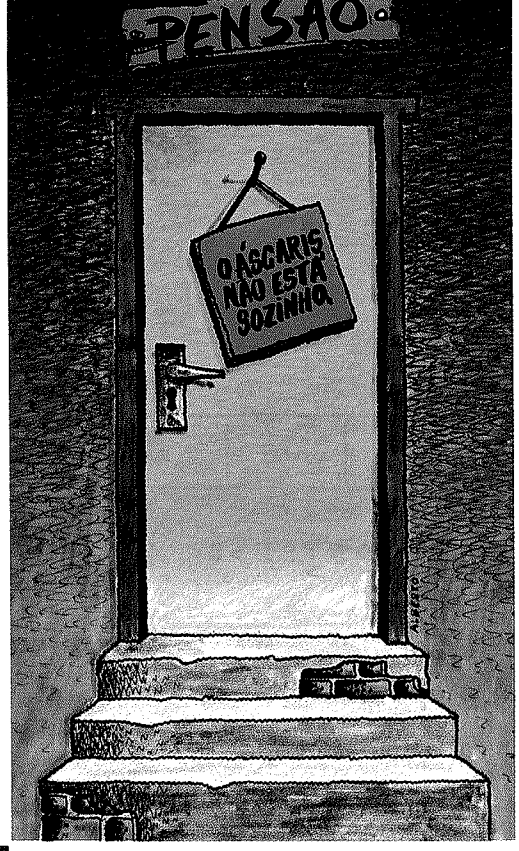

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(1) Texto baseado em opinião proferida pelo Prof. Dr. Rubens Campos, Prof. Titular da Cadeira de Parasitologia da Universidade de São Paulo, durante o VI Congresso da Federação Latino-Americana de Parasitologia, São Paulo, 1983.

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CELL MEDIATED IMMUNE RESPONSE IN CANINE ANTIRABIES VACCINATION

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SUMMARY

The Authors studied the cell mediated immunity (CMI) and humoral antibody levels raised in response to canine antirabies vaccination and revaccination. The vaccine used was the FUENZALIDA & PALACIOS which is routinelly utilized in human and canine vaccination in Brazil. The evaluation of CMI was done by inhibition of peripheral leukocyte migration and antibody levels were measured by serum neutralization and complement fixation tests. Five dogs were used and each animal received one daily dose of 1 ml of the vaccine for five days and one booster 210 days after the initial schedule. All animals showed moderate antibody levels after the initial immunization and a clear secondary immune response following the booster dose. Cell mediated immune response however occurred only following the initial immunization and could not be detected after the booster dose. The Authors concluded that CMI occurred in canine antirabies primo-vaccination under the experimental conditions employed and that although a secondary cell mediated immune response was not detected in this study its existence cannot be rulled out but remains to be studied by means of other immunization experiments.

INTRODUCTION

The protective role played by the different immunological mechanisms raised in responses to antirabies vaccine is still poorly understood.

The most studied and still controvertial aspect is undoubtedly the antiviruses antibody level. In some situations, circulating antibodies are clearly involved in protection [5,7,8], however, natural infection may coexist with the presence of high levels of humoral antibodies [19] or on the other hand solid immunity may be seen in its absence [19].

In experimental models, the administration of interferon or its production, induced by antirabies and other antiviral vaccines or non biological inducers, proved to be protective if interferon was present before or soon after the challenge. Despite of the fact that, commercial vaccines are not efficient inducers of interferon synthesis and also in practice, vaccination is usually done a few days after exposure, a solid protection is undoubtedly obtained.

In spite of several previous suggestions on the role of cell mediated immunity mechanisms in antirabies protection, it was only in 1974 that WIKTOR et al. [16] showed the ability of rabies virus antigens to stimulate immunized rabbits lymphocytes **in vitro.** After that, RAMANNA [10] demonstrated that the citotoxicity mediated by immunized rabbits lymphocytes against BHK-21 cells infected by rabies viruses, preceded the detection of circulating antibodies.

In addition WIKTOR et al. [18] showed that previous administration of antirabies anti-

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bodies to mice depresses both cell mediated immunity (CMI) and humoral immunity (HI).

More recently SANTOS et al.[11] demonstrated the development of CMI in response to human antirabies vaccination.

The purpose of this paper is the study of CMI and humoral antibody levels in dogs in response to antirabies vaccination and revaccination.

The choice of dogs as experimental animals is due to the fact that they are part of the epidemiological chain that leads to human rabies and are themselves subjected to the disease.

The FUENZALIDA & PALACIOS vaccine[6] was used because it is the usual vaccine employed in human and canine antirabies vaccination in Brazil; and the schedule was more intense than usual in order to obtain more evident and easily interpreted responses.

MATERIALS AND METHODS

1. **ANIMALS** — Five dogs from 6 to 48 months old, without defined race and kept in individual cages during the study were used. Four of them (No. 1-4) were received at the age of 2 months, born from non vaccinated mothers and kept in isolation before immunization. The last dog (No. 5) was sent to Instituto Pasteur de São Paulo for observation. No clinical signs of rabies were detected and according to its owner it had not been submitted to previous vaccination.

2. **VACCINATION SCHEDULE** — For five days each animal received one daily dose of FUENZALIDA & PALACIOS vaccine and one booster 210 days after, by intra muscular route. Leukocytes from animals number 1 and 2 were used before immunization to check the antigen for mitogen or toxic activities in the leukocyte migration inhibition test (LIF).

3. **ANTIGENS** — FUENZALIDA & PALACIOS vaccine was used in the immunization and complement fixation reactions. Briefly, it contains 2% of nervous tissue of newborn mice infected with fixed rabies virus, PVI strain (sent to Instituto Butantãn by Instituto Pasteur de Paris in 1956), 0.5% of phenol and 1/10 000 of merthiolate as preservatives and it is inactivated by ultra violet irradiation. The antigen used as control in complement fixation reactions consisted of a suspension of normal newborn mice nervous tissue prepared according to the technique used in the production of antirabies vaccine. The antigen used in LIF was similar to the FUENZALIDA & PALACIOS vaccine except that it contains 10% of nervous tissue and no preservatives. The control antigen was prepared with normal newborn mice nervous tissue.

4. **BLOOD SAMPLING** — Heparinized blood samples for LIF were collected asseptically by jugular puncture. Sera for complement fixation reactions (CF) and Serum neutralization test (SN) were obtained from blood samples collected without heparine and kept at −25°C.

5. **SN** — the test was performed as described by ATANASIU[2], using the fixed virus CVS strain.

6. **CF** — the reactions were performed according to the technique described by ALMEIDA[1], adapted to the rabies system by PEREIRA & PEREIRA[10], with the complement expressed in 50% hemolytic units.

7. **LIF** — the inhibition of peripheral blood leukocyte migration in the presence of proper antigen dilutions was performed according to the Soborg's technique with modifications[11]. Heparinized blood was collected under sterilized conditions. The buffy coat was separated and ajusted to $3x10^7$ cells/ml in Eagle's minimal essential medium containing 10% of normal human serum (NHS). Polyethylene capillary tubes 7 cm long filled with the cell suspension were sealed at one end and centrifuged at 150 g for 10 minutes. They were then cut at the cell-fluid interphase and placed in Sykes-Moore migration chambers.

The chambers were filled with medium containing 10% NHS and the proper antigen (vaccine or tissue control antigen), in several concentrations. Each test was performed in duplicate. After an 18 hours incubation period at 37°C, the migration areas were measured using a screen microscope, and the migration index calculated as:

$$\frac{\text{mean area of migration with antigen}}{\text{mean area of migration without antigen}}$$

Values of 0.75 or less were considered positive.

RESULTS

Results obtained after primo-vaccination in LIF, SN and CN reactions are presented in Table I. From this data we can observe that all

dogs showed a cell mediated immune response detected by LIF between the 8th and 15th day approximately. Antibodies were detectable in all immunized animals; one of them (No. 5) however, showed earlier synthesis and higher levels than the others.

T A B L E I

Evolution of peripheral leukocytes migration inhibition index (LMI) and antibody levels (in dogs) measured by serum neutralization (SN) and complement fixation tests (CF) (according to the time after the beginning of immunization)

Dog	Test	Days							
		0	5	8	12	15	20	28	35
1	LMI (*)	.89	1.02	.65	.58	.70	1.05	.96	ND
	SN	< 5	< 5	8	56	42	42	ND	51
	FC	—	—	—	2.3	56	56	ND	74.8
2	LMI	.95	.80	.51	.67	.72	1.08	.90	ND
	SN	< 5	< 5	11	19	21	ND	ND	52
	FC	AC	AC	AC	AC	AC	AC	ND	AC
3	LMI	.83	.83	ND	.71	.62	.76	.98	ND
	SN	< 5	< 5	ND	31	25	31	39	ND
	FC	—	—	ND	15.2	18	70	AC	ND
4	LMI	.92	1.08	.70	.53	.66	ND	.88	.95
	SN	< 5	< 5	5	39	39	43	35	28
	FC	—	—	—	28	28	38.8	38.8	32
5	LMI	.87	1.10	.71	.47	.51	1.05	ND	1.10
	SN	< 5	10	478	338	229	281	ND	265
	FC	—	—	87.5	56	89	67.3	ND	65.8

—, negative; ND, Not done; AC, anticomplementar.
48 LD50 were used in all SN tests.
(*) values of 0.75 or less were considered positive.

Data concerning to revaccination are shown in Table II. No CMI was observed and antibody levels were higher than those obtained in the primo-vaccination. Dog No. 5 was the only one that still had high levels of antibodies at the time of booster. LIF and CF reactions with control antigens were always negative.

DISCUSSION

Our results show that antirabies vaccination of dogs induce specific cellular immunity in the experimental conditions employed.

The results of LIF were positive for the 5 dogs in a farily regular way, from the 8th to 15th days after the beginning of vaccination. Similar results were obtained with human beings [11] and guinea-pigs. Regarding to the presence of circulating antibodies, however, one of the dogs (No. 5) presented earlier detectable and higher levels than the others, that were still present at the time of booster.

Cellular immune response was not observed after the booster on the 210th day of primo-

T A B L E II

Evolution of peripheral leukocytes migration inhibition index (LMI) and antibody levels (in dogs) measured by serum neutralization (SN) and complement fixation tests (CF) (according to the time after the booster dose)

Dog	Test	Days				
		0	3	6	12	20
1	LMI (*)	.89	.99	.84	1.10	.98
	SN	< 5	< 5	< 5	13	145
	FC	—	—	—	74.8	88.9
2	LMI	1.10	1.08	.90	ND	.93
	SN	< 5	9	93	ND	232
	FC	AC	AC	AC	ND	85.6
3	LMI	.87	.83	1.10	.78	.85
	SN	< 5	< 5	338	ND	ND
	FC	—	—	28	ND	ND
4	LMI	.94	.80	.77	.86	1.01
	SN	6	ND	11	363	93
	FC	—	ND	—	122	135
5	LMI	.86	.83	1.07	.85	.98
	SN	40	19	625	ND	420
	FC	—	—	74.7	ND	112

—, negative; ND, Not done; AC, anticomplementar.
48 LD50 were used in all SN tests.
(*) values of 0.75 or less were considered positive.