

## CELL MEDIATED IMMUNE RESPONSE IN HUMAN ANTIRABIES REVACCINATION

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### SUMMARY

The occurrence of secondary cell mediated immune response (CMI) in human antirabies immunization was studied.

The Fuenzalida & Palácios vaccine was used because it is routinely used in Brazil.

CMI was evaluated by lymphoblastic transformation indices obtained in whole blood culture in the presence of rabies and control (nervous tissue) antigens.

Eleven volunteers submitted to revaccination constituted the group under study, while three other volunteers submitted primo vaccination were utilized as control group.

A clear secondary CMI to rabies antigen was detected in all the revaccinated volunteers who showed earlier and more intense response than the control group.

Response to the control antigen, however, present in all the components of the first group was not detectable in two out of the three primovaccinated and very low in the third one.

**KEY WORDS:** Human antirabies revaccination; Cellular immunity in rabies; Antirabies immune response

### INTRODUCTION

The involvement of the different immunological mechanisms in protection against rabies is still poorly understood. Neutralizing antibodies are produced in response to active immunization but their actual role in protection is not yet well established.

In some situations circulating antibodies seem to be clearly involved in protection<sup>3,7,12,13</sup>. However, high antibody titers have been found in humans and animals with rabies<sup>21</sup> and also, immunity was demonstrated in the absence of detectable antibodies<sup>19,32</sup>.

In addition inadequately immunized animals, presenting high antibody titers, may die earlier than non-immunized ones when challenged with rabies virus<sup>4,8,9,21,27</sup>. There are evidences suggesting that this phenomenon, known as "early death", is related to the presence of specific antibodies<sup>22</sup>.

In experimental models protection can be obtained by administration of interferon or an induced of its synthesis, either before or immediately after the challenge<sup>5,6,32,33</sup>. In practi-

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ce, however, protection is undoubtedly achieved with a vaccination schedule, frequently initiated some days after natural infection and there is no evidence that our commercial vaccines are good inducers of interferon synthesis.

The protective role played by cell mediated immunity (CMI) in several viral infections is already well recognized<sup>11,16</sup> but its importance in the critical events involved in the host-rabies virus interaction is still obscure.

Recent observations have demonstrated the occurrence of CMI in response to antirabies vaccination in humans, dogs, rabbits, hamsters, guinea-pigs and mice<sup>18,20,23,25,26,28,31</sup>.

A direct correlation, under experimental conditions in mice, between CMI and survival against street rabies virus challenge by intra cerebral route has been established<sup>30</sup>.

Secondary CMI was demonstrated in mice after inoculation of either a vaccine booster dose or street rabies virus and this response was not inhibited by previous administration of neutralizing antibodies<sup>29</sup>. In contrast, passive immunization of nonvaccinated animals inhibits CMI to rabies antigens<sup>30</sup>.

The purpose of this paper was to study the development of CMI in previously vaccinated persons after revaccination with the Fuenzalida & Palácios vaccine, routinely used in most Latin-American countries.

## MATERIALS AND METHODS

### Revaccinated individuals

Eleven volunteers, previously vaccinated against rabies and working in rabies research or diagnosis laboratories with ages from 22 to 56 years constituted the group under study. Ten of them received one dose of antirabies vaccine and the other one, (JL) who had been naturally exposed to rabies virus, received one daily dose for three consecutive days.

### Primo-vaccinated individuals

Three volunteers who had never been exposed to rabies antigen were immunized with one daily dose of vaccine for five consecutive days. They constituted the control group sin-

ce CMI has been previously reported to occur with this immunizing schedule<sup>26</sup>.

### Antirabies vaccine

The Fuenzalida & Palácios vaccine<sup>10</sup> is routinely used in our country for human vaccination. The vaccine was prepared at Instituto Butantan, São Paulo, Brazil. Briefly, this vaccine is prepared from newborn mouse brain (myelin free) previously inoculated by intracerebral route with fixed rabies virus, strain PV<sub>1</sub>. It is inactivated by U.V. radiation and contains 2% nervous tissue, 0.5% phenol and 1:10.000 thimerosal.

### Antigens for lymphoblastic transformation assay

Rabies antigen (R-Ag) was prepared essentially as the vaccine except that it contained 10% nervous tissue and no preservatives.

Control rabies antigen (C Ag) was the same as R-Ag but prepared from non-inoculated newborn mice.

### Lymphoblastic transformation assay

The assay was performed as described by HALL & GORDON<sup>14</sup> with few modifications. Briefly, heparinized blood was collected and diluted 1:10 in RPMI — 1640 (Gibco-Grand Island Company, N.Y.). All control and test lymphocyte cultures were set up in triplicate.

Test cultures consisted of 1.0 ml of 1:10 whole blood plus 100  $\mu$ l of a 1:4 dilution of either rabies antigen or control rabies antigen. Test cultures were incubated for 144 hours at 37°C under 5% CO<sub>2</sub> humidified atmosphere. In previous experiments with the primovaccinated group maximum lymphocyte stimulation was obtained at that time of culture and antigen dilution, which also showed no toxic or mitogenic activity on lymphocytes from non-immunized individuals (unpublished results).

Control cultures consisted of 1.0 ml of 1:10 whole blood plus 100  $\mu$ l of either phytohemagglutinin — P (PHA — Difco, Detroit, Mchi) or RPMI — 1640. Control cultures were incubated for 72 hours under the same conditions.

Six hours before the end of incubation period 10  $\mu$ l of <sup>3</sup>H thymidine (specific activity

$2.49 \times 10^{11}$  Bg/mol, New England Nuclear, Boston, Mass) were added to each culture.

After incubation, cultures were centrifuged and the supernatants discarded. The sediment received 2.0 ml of 3% cold acetic acid and was centrifuged and the supernatants discarded. The sediment received 2.0 ml of 3% cold acetic acid and was centrifuged at  $400 \times g$  for 10 minutes. Pellets were resuspended in 1.0 ml of 7% cold trichloroacetic acid and centrifuged.

The new pellets were resuspended in 1.5 ml of methanol and centrifuged. Supernatants were carefully removed and pellets were solubilized with Protozol (New England Nuclear, Boston, Mass) by incubation at  $50^{\circ}\text{C}$  for 30 minutes. Immediately after 5.0 ml of scintillation fluid was added to each tube. After an overnight incubation each sample was counted for 1 minute in a Beta scintillation counter (Beckman LS350).

Lymphoblastic transformation was expressed as an index (LTI) obtained as follows:

$$\text{LTI} = \frac{\text{mean cpm of cultures with R Ag or C Ag}}{\text{mean cpm of cultures without antigens}}$$

Indices higher than 2.0 were considered as positive. PHA was used as a control, to assure that every lymphocyte culture under assay could respond normally to mitogenic stimulation.

## RESULTS

Table I shows lymphoblastic transformation indices obtained with blood samples from the primo-vaccinated volunteers in the presence of R Ag and C Ag at different intervals since the beginning of vaccination.

Positive results (index  $> 2$ ) with R Ag were obtained since the 7<sup>th</sup> day, reached a maximum at about the 14<sup>th</sup> day and were still positive at the 35<sup>th</sup> day, when the last assay was performed. Response to C Ag in this group was very low, with results usually negative, except for the indices shown by volunteer DRV at days 10, 14 and 21.

In Table II are presented the same data referent to the revaccinated group.

T A B L E I

Lymphoblastic transformation indices (LTI) obtained in whole blood cultures of primo-vaccinated volunteers. Indices are expressed for both, rabies antigen (R-Ag) and control antigen (C-Ag)

Volunteers	Day								
		0	3	7	10	14	21	28	35
DRV	R Ag	0.75	1.19	2.0	9.78	12.07	11.45	6.25	3.56
	C Ag	0.65	1.02	1.50	3.32	3.79	2.32	1.40	1.28
CRZ	R Ag	1.95	1.70	3.52	7.66	5.98	2.89	2.83	2.51
	C Ag	1.23	1.88	1.82	1.94	1.38	1.81	1.55	1.42
MHTA	C Ag	1.25	1.52	0.80	0.93	1.09	1.95	1.63	1.42
	R Ag	1.26	1.46	5.40	8.35	10.47	8.05	4.51	3.0

This group showed positive results to R Ag earlier than the primo-vaccinated group and with higher indices. Three out of the nine volunteers tested at day zero were already positive and three days after the booster dose all indices were higher than 2.5. Within this group response had a maximum at 21<sup>th</sup> day, persisted at high levels, up to the 35<sup>th</sup> day and all, except one, were still positive 110 days after the booster, when the last assay was carried out. The revaccinated group showed also positive responses to C Ag, although with indices much lower than those obtained with R Ag.

Figure 1 represents the evolution of the mean indices of each group obtained in the presence of R Ag and C Ag.

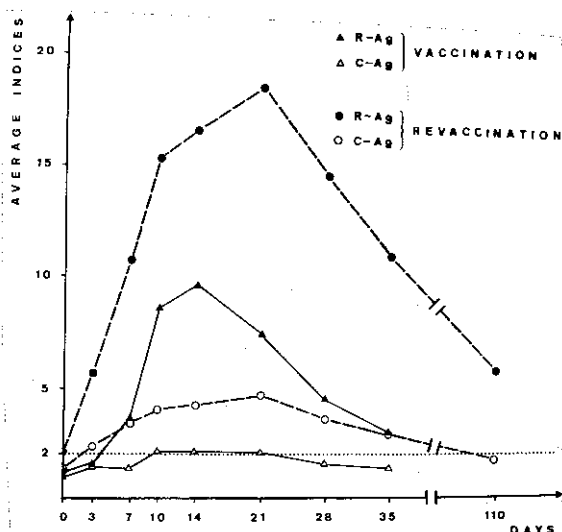


Fig. 1 — Evolution of average lymphoblastic transformation indices in antirabies vaccination and revaccination (R-Ag: rabies antigen; C-Ag: control rabies antigen).

T A B L E II

Lymphoblastic transformation indices (LTI) obtained in whole blood cultures of revaccinated persons. Indices are expressed for both rabies antigens (R-Ag) and control antigens (C-Ag)

Day		0	3	7	10	14	21	28	35	110
Volunteers										
JBFS	R Ag	1.47	2.35	3.18	5.36	6.28	5.82	3.20	2.43	1.82
	C Ag	1.65	1.28	1.27	1.98	2.40	2.23	2.08	1.58	1.28
DLFS	R Ag	1.9	11.97	16.75	NR	7.90	5.61	5.38	6.12	4.56
	C Ag	1.48	2.49	4.30		3.41	2.49	2.45	2.12	1.29
PCSS	R Ag	1.45	2.88	5.18	7.12	12.80	19.19	14.78	8.45	3.28
	C Ag	1.08	2.31	2.31	2.28	3.72	4.12	3.80	3.18	1.17
MSC	R Ag	1.92	2.64	5.38	7.53	7.72	5.91	5.04	6.15	2.73
	C Ag	1.60	1.82	2.64	2.93	2.52	3.01	2.34	1.98	1.13
PJARO	R Ag	2.45	4.70	8.83	15.18	12.35	9.82	7.80	6.31	3.45
	C Ag	1.81	2.13	3.93	4.34	3.65	3.13	2.24	1.58	1.33
SCM	R Ag	4.27	5.83	6.34	10.99	12.15	12.08	8.35	6.42	2.86
	C Ag	1.84	2.02	3.28	5.14	4.42	5.70	3.30	2.85	1.12
EADP	R Ag	2.80	6.91	9.29	12.13	13.95	7.02	10.18	6.35	3.76
	C Ag	1.43	2.33	3.80	2.93	4.50	3.82	3.25	2.24	1.21
CMA	R Ag	ND	12.20	14.15	16.18	22.31	21.18	16.08	12.08	4.82
	C Ag		4.32	4.85	6.30	6.82	7.31	5.20	4.81	2.08
ELBC	R Ag	1.37	8.82	20.36	38.16	30.07	28.45	18.12	8.14	4.20
	C Ag	1.40	2.21	4.91	5.85	6.00	3.80	2.78	2.48	1.98
MELS	R Ag	ND	8.63	24.18	34.41	37.82	56.91	40.38	29.40	18.43
	C Ag		2.64	4.38	6.07	6.21	11.43	8.45	7.83	4.03
JL	R Ag	1.43	3.79	4.94	8.32	18.20	32.15	32.28	28.40	15.75
	C Ag	1.68	1.86	2.20	2.32	2.95	4.83	3.72	3.43	3.53

ND = not done

## DISCUSSION

It was demonstrated a clear secondary cellular immune response in humans receiving a booster dose of the Fuenzalida & Palacios vaccine.

The response, evaluated by lymphoblastic transformation of peripheral lymphocytes in the presence of rabies antigen was positive in all individuals since the third day after the booster dose and remained so up to the 110th day in 10 out of the 11 volunteers under study.

The response of the revaccinated group came earlier, was more intense and lasted longer than the response of the primo-vaccinated group. However, the revaccinated group showed CMI to nervous system components (C-Ag) with relatively high indices, which was not observed in the primo-vaccinated group.

The development of CMI to C-Ag might be related to post-vaccine accidents which are known to be more frequent in revaccinated persons and seem to be due to immune response to these antigens 1,2,15,17.

CMI against R-Ag was detectable before the booster dose in three out of nine revaccinated volunteers one of them (SCM) showing a relatively high index (LTI of 4,25). This group consisted of technicians who work at a rabies diagnosis laboratory and had already been submitted to several revaccinations before this study.

Although there are some references on the occurrence of CMI in human antirabies<sup>26</sup> we were not able to find any study about CMI in human revaccination, in the literature.

We observed a rather large fluctuation in the intensity of CMI presented by the different

volunteers of the revaccinated group. Although the data we collected do not provide us an explanation for that, there are some aspects we consider to be of importance and to deserve a closer look in the future: differences in the individual antibody levels at the time of booster administration; number and schedules of revaccination each person was submitted; differences in the time lapsed between last revaccination and the beginning of the study; variations in the individual response to rabies antigen.

Additional studies are needed not only to improve our knowledge on the occurrence of CMI in antirabies vaccination and revaccination, but also on the role played by CMI in the prevention of disease development.

### RESUMO

#### Resposta imune mediada por células na revaccinação anti-rábica humana.

Foi estudada a ocorrência de resposta imune celular na re-imunização anti-rábica humana.

Usou-se a vacina Fuenzalida & Palácios a qual é rotineiramente usada no Brasil.

A resposta imune celular foi avaliada pelo índice de transformação blástica obtido em cultura de sangue total, na presença de antígeno rábico e antígeno controle (sistema nervoso).

O grupo em estudo constituiu-se de 11 voluntários submetido a revaccinação, enquanto três outros, submetidos a primo-vaccinação foram utilizados como grupo controle.

Detectou-se uma clara resposta imune celular secundária contra antígeno rábico em todos os voluntários revacinados, cujas respostas foram mais rápidas e mais intensas do que as do grupo controle.

Observou-se também, resposta ao antígeno controle em todos os indivíduos do grupo de revacinados. No grupo de primovacinados, resposta ao antígeno controle foi fracamente observada em somente um indivíduo.

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