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IMMUNOMODULATORY EFFECT OF GLUCAN ON THE RESPONSE TO EXPERIMENTAL ANTIRABIES VACCINATION

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SUMMARY

The objective of the present study was to determine the stimulatory response to antirabies vaccination promoted by glucan in mice. Glucan increased both resistance to infection and antibody titres and this effect was more evident when glucan was used at dose of 0.5 mg, administered intraperitoneally before, during and after immunization and when the challenge virus was applied to the foot-pad.

KEY WORDS: Glucan; Antirabies vaccination; Rabies.

INTRODUCTION

Glucan is a polyssacharide consisting of chain glicopyranose molecules, joined by B1-3 glycoside links, extracted from the cell wall of *Sacharomyces cerevisiae*. The administration of glucan to rats and mice stimulates the mononuclear phagocytic system, with a simultaneous increase in weight and size of the spleen, liver and lungs, and an increase in number of activated macrophage [22]. Glucan also potentiates antibody synthesis, exerts a potent effect on hemathopoiesis, and increases the activity of B and T lymphocytes [4, 21].

It has been shown in animal system that glucan increases resistance to several infectious agents such as *Candida albicans* [19], *Mycobacterium leprae* [5], *Staphylococcus aureus* [11], *Murine Hepatitis Virus* [20], *Leishmania donovani* [10] and others. Its activity in intensifying the immune response has also been observed in several neoplasias [1, 6]. The conditions in which these results were obtained varied, with the need for a different experimental model in each case.

In the present study we determined the stimulatory effect of glucan on the response to antirabies vaccination, and established a model capable of demonstrating it clearly.

MATERIAL AND METHODS

Animals: Swiss albino mice weighing 11 to 16g from Instituto Pasteur, São Paulo (Brazil).

Virus: CVS (Challenge Virus Standard) samples in a 20% suspension (Weight/Volume) of infected mouse brain in distilled water, with 2% horse serum (Titre in mice: $10^{7.2}$ LD_{50}/0.03 ml). The suspension was aliquoted and stored at - 70°C until use.

Antirabies Vaccine: We used FUENZALIDA & PALÁCIOS vaccine [8] routinely employed in human vaccination in Brazil, prepared at Instituto Butantan (São Paulo-Brazil), lot 8608162. Briefly, the vaccine consists of a suspension of brain tissue from newborn mice previously inoculated with

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fixed rabies virus, strain PV_1, by the intracerebral route. The vaccine is inactivated by U.V. radiation and presents a final concentration of 2% nervous tissue, 1:1.000 phenol and 1:10000 thimerosal.

Glucan: Glucan was prepared by the procedure of HASSID et al. [9]. Five glucan suspensions were prepared in a 0.85% NaCl solution containing 0.05, 0.125, 0.25, 0.5 and 1mg/0.2ml.

Immune Adherence Hemagglutination Test: The test was perfomed essentially as previously described [23]. Briefly, equal volumes (25 µl) of serial dilutions of serum and rabies antigen (Rabies Vaccine - Pfizer Química Ltda - São Paulo - Brazil) (1:15) were incubated in 96 well plastic U-bottom plates (Petecil) at 37°C for 30 minutes. After the addition of 25µl of a 1:120 dilution of fresh guinea-pig serum as source of complement, the mixture was further incubated at 37°C for 40 minutes. A selected "0" type erythrocyte suspension (1%) was then added (25µl/Well) and the plates were allowed to stand at room temperature for 2 hours. The hemagglutination patterns were read and the titers recorded as the reciprocal of the highest serum dilution showing positive agglutination.

Vaccination and Challenge: The vaccination was made according to the schedule for potency test recommended by the National Institutes of Health (NIH), Bethesda, MD, USA. Briefly, each mouse in a group of 16 animals received 2 doses i.p. of 0.5 ml of one of the vaccine dilutions (containing 0.08, 0.4, 2.0 or 10.0 mg of nervous tissue/ml) with a one week interval. The challenge was done with 5 to 50 LD_{50} of the CVS virus suspension, on the 14th day. Day zero was considered to be the first day of vaccination. The results were evaluated by determining the effective dose 50%, (ED_{50}) [15], which corresponds to the amount of nervous tissue present in the vaccine (expressed in mg) that induces a protective immune response in 50% of the mice.

The effect of glucan administration on the antirabies immune response was evaluated in terms of increasing resistance to infection in experiments with different challenge routes, different quantities of glucan per dose, different routes and different periods of glucan administration (in relation to the immunization period). In addition, the kinetics of antibody production with or without glucan administration was also evaluated.

Route of Challenge: Three experiments were perfomed, all of them following the same vaccination protocol established by the NIH for the vaccine potency test, including additional groups of mice that received 5 doses of glucan (0.5 mg/dose by i.p. route on days -4, -1, 2, 6 and 9) in addition to the same immunization schedule. A different inoculation route of the challenge virus was used in each experiment, i.e., intracerebral (i.c.), intraperitoneal (i.p.) and foot-pad. (see Table 1).

Table 1

Effect of glucan administration to vaccinated mice challenged by different routes on the ED_{50} values.

Challenge Inoculation Route	LD_{50}	Treatment	ED_{50}
Intracerebral	48	5 Doses of Glucan + Vaccine	0.86
		Vaccine	1.14
Intraperitoneal	38	5 Doses of Glucan + Vaccine	0.12
		Vaccine	0.16
Foot-pad	31	5 Doses of Glucan + Vaccine	0.44
		Vaccine	0.81

Effective Glucan Dose: Five groups of mice were submitted to vaccination according to the NIH protocol. Each group of mice received five 0.2 ml injections i.p. route of a glucan suspension containing 1.0, 0.5, 0.25, 0.125, and 0.05 mg/dose, respectively, on day -4. -1, 2, 6 and 9. An additional group received the same vaccination schedule with the administration of saline replacing the glucan doses. The challenge was made via foot-pad (see Table 2).

Table 2

Effect of different doses of glucan administered to vaccinated mice on the ED_{50} values.

GLUCAN (mg/dose)	ED_{50} (mg)
1.0	1.19
0.5	0.48
0.25	0.53
0.125	0.67
0.05	0.95
0.0	0.95

Glucan Administration Route: Rabies virus was titered in series of six fold dilutions (10^{-1}; $10^{-1.78}$; $10^{-2.55}$ and $10^{-3.33}$) by the foot-pad route in 4 groups of mice on the 14th day. Three of them were

vaccinated i.p. with 2 doses of 0.5 ml (containing 2.5 mg of nervous tissue/ml) on days zero and 7. Each animal of the two first groups received 5 doses of 0.5 mg/dose of glucan (days -4, -1, 2, 6, 9) i.p. or s.c.; saline, instead of glucan, was administered to the 3rd group and the 4th group was used as control (see Table 3).

Table 3

Effect of different routes of glucan administration to vaccinated mice on the LD_{50} values of rabies virus.

TREATMENT	LD_{50}
INTRAPERITONEAL GLUCAN + VACCINE	$10^{-1.66}$
SUBCUTANEOUS GLUCAN + VACCINE	$10^{-1.86}$
VACCINE	$10^{-1.88}$
NO TREATMENT	$10^{-2.55}$

Glucan Administration Period: Two groups of animals were submitted to vaccination acording to the NIH protocol and received 5 doses of glucan (0.5 mg/dose, i.p.). In one group, glucan was administered before and during the vaccination (days -4, -1, 2, 6 and 9) and in the other, only after the immunization period (days 9, 12, 14, 16 and 19). An additional group receiving vaccine only was used as control. In this case the challenge was perfomed on day 21 by foot-pad inoculation and the results are expressed as ED_{50} (see Table 4).

Table 4

Effect of glucan administration at different periods of immunization on the ED_{50} values.

TREATMENT	ED_{50}	% (*)
VACCINE	0.18	100
GLUCAN BEFORE, DURING AND AFTER VACCINATION	0.11	61
GLUCAN AFTER VACCINATION	0.19	105

$$(*)\ \frac{ED_{50}\ \text{OF THE GROUP WITHOUT GLUCAN}}{ED_{50}\ \text{OF THE GROUP WITH GLUCAN}} \times 100$$

Antibody Production: The kinetics of the antibody response was studied in mice inoculated with 5 doses of glucan (0.5 mg/dose on days -4, -1, 2, 6 and 9) and vaccinated on days 0 and 10 (dilution of vaccine containing 2.5 mg of nervous tissue/ml). This kinetics was compared to that of control group. Every 5 days, 15 animals from each group were bled and the sera pooled for antibody titration by immunoadherence hemagglutination.

RESULTS

Challenge Route: The results of this test are presented in Table 1. In the experiment using the i.c. route, the ED_{50} value of the group receiving glucan was equal to 75% of the ED_{50} value of the group that did that did not receive the drug, indicating that protection may be obtained with a smaller quantity of vaccine. Similar results were obtained with the i.p. route, although with lower ED_{50} values. When the animals were inoculated via foot-pad, the ED_{50} values were intermediate between those of the two former groups and the animals receiving glucan had an ED_{50} corresponding to 54% of that of the animals treated with vaccine only.

Effective Dose of Glucan: As it can be seen in Table 2, the lowest ED_{50} value was obtained in the group tested with 0.5 mg doses and the results obtained with doses of 0.05 mg did not differ from those of the control group.

Glucan Administration Route: Table 3 shows the results expressed as DL_{50} obtained for each group. The administration of glucan was effective in increasing vaccine activity only by the i.p. route.

Glucan Administration Period: The results obtained in this experiment are summarized in Table 4. The vaccine ED_{50} was similar in the group receiving glucan only after vaccination and in the control group (0.19 mg and 0.18 mg, respectively). The group receiving glucan prior to, during and after vaccination had an ED_{50} equivalent to 60% of the ED_{50} of the control group.

Antirabies antibody production: The potentiation of antibody synthesis by glucan was demonstrated by an increased titer of immuneadherence hemagglutination in the group treated with glucan in comparison to the group without treatment (Table 5). The Mann-Whitney test showed that this

Table 5

Antirabies antibody levels evaluated by the immune adherence hemagglutination test in vaccinated mice with and without glucan treatment (0.5 mg/dose).

	TREATMENT	
DAYS	GLUCAN + VACCINE	VACCINE
05	0	0
10	20	10
15	80	40
20	320	80
25	320	80

increase was only significant after the 15th day, with U=0 and for the adopted rejection level.

DISCUSSION

A clear stimulatory effect of glucan was observed on the response to an experimental antirabies vaccination in mice. The results allowed us to establish the basis for a model capable of presenting this function in a clear fashion. This activity can be detected either by increased resistance to infection or by the potentiated antibody synthesis.

Initially, the most convenient route for inoculation of the challenge virus was selected. In these experiments, the period of use, the number of doses and the glucan concentration per dose were based on literature data obtained with other biological models [11, 14, 18, 19, 20]. The intraperitoneal route for glucan administration was chosen because, besides presenting satisfactory results in preliminary experiments, it was innocuous to the animals and easy to execute.

The best results were obtained with the foot-pad route, which is simple to handle. An antigen increase of 84% was needed in the immunization of control to reach the ED_{50} obtained in those treated with glucan, contrasting with a 33% difference observed in animals challenged by other routes (Table 1). Thus, in subsequent experiments the challenge was always carried out by the foot-pad route.

The lowest ED_{50} was obtained by the use of 0.5 mg of glucan per dose (Table 2). Depending on the experimental model, a wide variety of optimun doses have been described by different authors [11, 14, 18, 19, 20].

In the study of glucan activity related to the period of drug administration and immunization, we delayed the moment of challenge. This modification reduced considerably the ED_{50} values as can be seen in Table 4. There was no significant difference between the untreated group and the group treated with glucan after vaccination. However, the earlier injection of glucan before vaccination resulted in an even lower ED_{50} than in the other groups. Many authors have obtained similar results, verifying that the administration of glucan before the challenge with a variety of infectious agents shows a reduction of mortality which was not observed when, the administration was carried out after the challenge [11, 14, 19, 20]. In our experimental model, glucan administration in the absence of vaccination was ineffective, probably because of the short incubation period of the infection (5-6 days).

When the subcutaneous and intraperitoneal routes of glucan administration were compared, even though the former route of vaccination showed favorable results in Venezuelan equine encephalitis [14], our experiment showed that the intraperitoneal route is more effective, with 67.4% more virus being necessary to obtain 1 DL_{50} than in the group treated with vaccine alone.

The antibody level found after vaccination of the glucan-treated animals was significantly higher than of the control group (Table 5), with fourfold differences obtained with samples from days 20 and 25.

Therefore, the administration of glucan increases the effect of experimental antirabies vaccination in mice. A greater resistance to infection associated with higher antibody levels can be clearly observed with early (before and during vaccination) administration of the 0.5 mg/dose of glucan by the intraperitoneal route and with use of foot-pad challenge.

This protective activity of glucan when associated with vaccination enabled us to conclude that glucan is an important activator of the specific immune response. Further studies are needed to clarify the immunologic mechanisms involved in the protective activity of glucan and its potential clinical application.

RESUMO

Efeito imunomodulatório da glucana na resposta à vacinação anti-rábica experimental.

No presente trabalho verificou-se a atividade estimuladora da resposta à vacinação anti-rábica experimental determinada pela glucana em camundongos. Esta atividade pôde ser detectada por aumento da resistência à infecção e pela resposta imune mais intensa em termos de títulos de anticorpos. Estes resultados foram mais evidentes quando a glucana foi utilizada em doses de 0.5mg, administrada por via i.p., antes, durante e após a imunização e o desafio feito no coxim plantar da pata posterior.

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