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CHARACTERISTICS OF CELLULAR AND HUMORAL IMMUNE RESPONSES AFTER IMMUNIZATION WITH DIFFERENT RABIES VACCINES

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Summary. — Three rabies vaccines were compared: 1. the Fermi type vaccine, a phenol-treated suspension of brain tissue from infected sheep; 2. a virus grown in sheep brain, purified from the contaminating material to 85—90% and inactivated with β-propiolactone; 3. and the MNIIP-74 strain cultured in Japanese quail embryo cells and inactivated with β-propiolactone. A single immunization of mice with any of the preparations resulted in about 50% inhibition of splenocyte migration after 2 days; by day 15 the inhibition was 95—98%. By day 45 the migration index returned to the initial level. Increased ability to blast transformation in splenocytes was found by day 15, reached the maximum (160 to 212% of the control level taken for 100%) by day 30, and then began to decrease. The most marked change in blast transformation was brought about by the purified cerebral vaccine, while the less marked one by the tissue culture vaccine. The titre of virus-neutralizing antibodies reached a maximum after 15—30 days, 60 days after immunization it dropped twice. The resistance of mice to intracerebral infection with the rabies virus shortly after immunization might be due to cellular protective factors, while at later intervals it correlated with the level of virus-neutralizing antibodies.

Key words: rabies vaccines; splenocyte migration inhibition; blast transformation of splenocytes; virus-neutralizing antibodies; post-vaccinal immunity

Introduction

The aim of this work was to compare three rabies vaccines with different content of impurities as to their ability to influence upon the cellular immunity factors, and then to compare the dynamics of cellular and humoral immunity indexes with the resistance to rabies. The Fermi type vaccine which contains 12—14 mg/ml of brain tissue proteins, is highly reactogenic, and sometimes causes severe neurological complications. The purified, propiolactone-inactivated vaccine from sheep brain contains 1.5—1.8 mg/ml protein, shows no neuroallergic activity in experimental animals (Karakuyumchan *et al.*, 1981)

and is far less reactogenic than the Fermi type when administered to humans (Unanov *et al.*, 1984). The tissue culture vaccine prepared of the virus grown in culture contains 0.4% human albumin as a stabilizer, and possesses a weak reactogenity (Bektemirova *et al.*, 1983).

The mechanism of immunity in rabies or antirabies vaccination is not sufficiently understood. The degree of resistance to infection is usually in good correlation with the level of serum neutralizing antibodies. For this reason many workers, e.g. Crick and Brown (1976) or Nicholson *et al.* (1979) believe that humoral immunity is the principal, if not the single protection factor against rabies, but in some cases in man rabies may develop despite of the high antibody level (Ramanna and Pal, 1980*a*) as the presence of antibodies in the serum or cerebrospinal fluid does not prevent the fatal outcome (Schuller *et al.*, 1979). Antiserum injection to infected animals in the majority of cases only prolonges the incubation period (Baer and Yager, 1977). As shown in animal studies (Wiktor *et al.*, 1976; Wiktor and Koprowski, 1980), an intensive production of rabies antibodies does not always result in resistance to the disease, and hence the antibodies do not play a decisive role in the immunity. As to cellular factors of immunity, there is evidence that splenocytes from immunized mice have a protective effect (Prabhakar *et al.*, 1981) whereas cellular responses may cause immunopathological states (Guillon and Tsiang, 1980).

According to some investigators (Baer and Yager, 1977; Atanasiu, 1982), the main factor of protection against rabies is the induction of interferon production.

In this study we demonstrate a somewhat greater stimulation of cellular immunity by cerebral vaccines as compared to the cultural one. This did not depend on the amount of contaminating proteins and was probably determined by some properties of the virus itself that resulted from its reproduction in different cells.

Materials and Methods

The rabies vaccines. The following vaccines were used: 1. the Fermi type which is a phneol-treated 5% sheep brain tissue suspension; 2. a virus grown in sheep brain, purified to 85—90% from contaminating materials and inactivated with β-propiolactone (Karakuyumchan *et al.*, 1981; Pille *et al.*, 1981); 3. a vaccine from fixed rabies virus, strain MNIIVP-74 that was cultured in Japanese quail embryo cells and inactivated with β-propiolactone (Bektemirova *et al.*, 1983).

Splenocyte migration inhibition test. Agarose (300 mg) was dissolved in 32 ml of hot distilled water, and supplemented with 4 ml foetal calf serum, 4 ml of 10× concentrated medium 199, and 0.5 ml of a 7.5% Na_2CO_3 solution; pH of the medium was brought to 7.2—7.4. The mixture was poured into Petri dishes, and wells 4 mm in diameter were made after it solidified. The spleens of immunized mice were mechanically grinded, and the cells suspended in medium 199 containing 10% foetal serum at the concentration of $2 \cdot 10^6$ cells per 0.01 ml. This quantity of cells was added to each well, followed by 2—5 LD_{50} of the MNIIVP-74 virus (the LD_{50} value is given for intracerebral administration to mice). The plates were incubated for 24 hr in a wet chamber at 37 °C. Thereafter, the area occupied by the cells around each well was measured at 80—120-fold magnification. In order to do this, 8 diameters of each circle were measured by means of the microscope scale, and the average was used to calculate the circle area. Splenocytes of non-immunized mice were used as controls and the splenocyte migration in the experimental group was expressed as per cent of the control value.

Blast transformation of splenocytes. The spleens of immunized mice were used to prepare of a suspension containing 10^2 cells per ml of the RPMI-1640 medium containing 20% of foetal

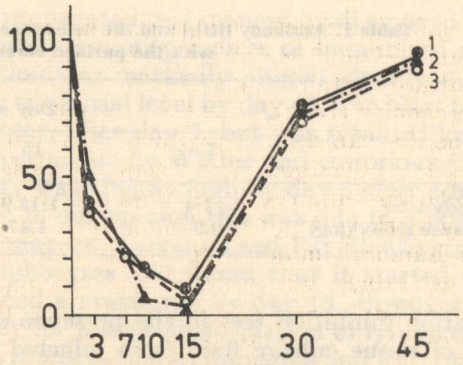

Fig. 1.
Migration of lymphocytes from immunized mice in the presence of rabies virus
1 — Fermi vaccine; 2 — purified brain vaccine; 3 — tissue culture vaccine
Abscissa: days post-vaccination; ordinate: intensity of lymphocyte migration (% of control).

cattle serum and 0.006% of glutamin. The suspension was distributed by 2 ml portions into scintillation vials which were then incubated for 24 hr at 37 °C. ^{3}H-thymidine was then added to the vials to final concentration of 800—1000 kBq/ml together with 2—5 LD_{50} of MNIIVP-74 rabies virus per vial. After another incubation for 24 hr at 37 °C the degree of blast transformation was estimated by incorporation of the label into the acid-insoluble fraction of the cells. The results were expressed as percentage of the control with splenocytes of intact mice.

Antibodies to rabies virus were assayed in the neutralization test. About 100 LD_{50} units of the rabies virus strain CVS were added to the series of two-fold serum dilutions, and after one hr incubation at 22 °C the mixture was injected intracerebrally to mice. The results were scored as the number of dead and live animals by 14 days later.

The resistance of immunized mice was assessed by comparing with the resistance of intact animals of the same age after intracerebral administration of the CVS virus in serial 10-fold dilutions; the observation period was 21 days.

Results

All the vaccines used, the Fermi type, the purified and inactivated vaccine from the brain-propagated virus and the cell culture-propagated and inactivated virus, had immunogenicity indices ranging from 0.9 to 1.1 as determined by the National Institute of Health method. The vaccines were injected intraperitoneally to mice weighing 10—12 g at 0.3 ml to each. On days 3, 7, 10, 30 and 45, 4—5 animals were sacrificed, and their splenocytes assayed in the

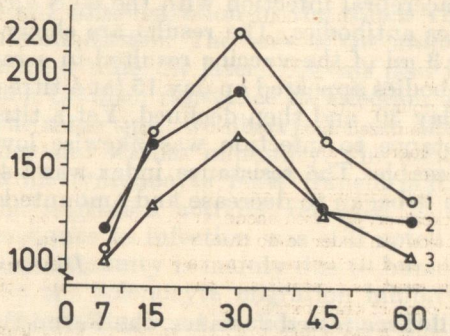

Fig. 2.
Blast transformation of lymphocytes from immunized mice in the presence of rabies virus
Designations as in Fig. 1.
Abscissa: days post-vaccination; ordinate: intensity of lymphocyte blast transformation (% of control)

Table 1. Antibody titres and the resistance to infection in mice immunized with the purified cerebral vaccine

	Day after immunization			
	7	15	30	60
Antibody titre	<1:4	1:13.9	1:13.9	1:5.7
Resistance index (log)	0.9	1.47•	1.15	0.5

migration inhibition test in the presence of the strain MNIIVP-74 added in form of tissue culture fluid from infected Japanese quail embryo cells. The results are given in Fig. 1.

Even on day 3 after immunization the splenocytes of the animals showed a different response to the virus as compared to control splenocytes: their migration ability was reduced two-fold. The most pronounced migration inhibition was observed on day 15, the migration amounted to 2.25—4.86% of the control level. By day 30 the value started to increase and by day 45 it reached the initial level. All the vaccines tested had the same effect on the intensity of splenocyte migration and its dynamics.

Fig. 2 demonstrates the ability of the vaccines to affect blast transformation of splenocytes of immunized mice. On day 7 there was almost no difference between the experimental and control groups, whereas by day 15 the blast transformation index increased markedly in all the three immunized groups. The capacity of blast transformation reached a maximum 30 days after immunization (159.7—212.2% of the control level). It may be noted that the greatest effect was produced by the purified cerebral vaccine, the response to the Fermi vaccine was somewhat less pronounced and the response to the cultural vaccine was the weakest. By day 60 mice of the latter group had the same blas t transformation index as control animals while mice immunized with the Fermi and purified vaccines had still retained enhanced splenocyte blast transformation ability.

In mice immunized with the purified cerebral vaccine the resistance to intracerebral infection with the CVS virus was tested along with the titre of rabies antibodies. The results are shown in Table 1. A single administration of 0.3 ml of the vaccine resulted in a rather weak antibody production. The antibodies appeared on day 15 (at a titre of 1:13.9), remained at the same level on day 30, and then declined. Yet a titre of 1:6.5 was found by day 60. The resistance to infection was likewise low, although a certain dynamics was noticeable. The resistance index was 1.47 log 15 days after the vaccination, then it began to decrease and amounted to 0.51 log by day 60.

Discussion

All three tested vaccines, the Fermi type, the purified and devoid of neuroallergic properties vaccine of the brain-propagated rabies virus, and the tissue

culture vaccine prepared of a virus propagated in Japanese quail embryo cells, induced significant changes in cellular immunity indices of immunized mice. The ability of splenocyte migration was markedly altered already 3 days after immunization, and returned to the initial level by day 45. The blast transformation ability rised somewhat later, after day 7, but was retained longer: the peak occurred on day 30. In experiments by Wiktor and coworkers (1974) the splenocyte transformation ability was already high by day 8 after administration of a live virus or a vaccine; in our opinion this was due to a possible intravenous inoculation of the immunogens. Ramanna and Pal (1980*b*) studied blast transformation of rabbit lymphocytes and found that it started from day 6 after immunization and reached a maximum by day 13. However, the same workers did not find any ability for blast transformation of lymphocytes neither in vaccinated subjects nor in rabies patients (Ramanna and Pal, 1980*a*); this might be a result of too much antigen inoculation (5 ml of sheep brain vaccines a day for 14 days) or of a late examination.

We found some difference in the response to our three vaccines. The blast transformation response was most profound in response to the purified vaccine and the less, to the tissue culture vaccine, the Fermi type vaccine being intermediate. It appears that cerebral antirabies vaccines are more active with regard to cellular immunity than the tissue culture vaccine. Since the Fermi vaccine contains some live virus, there was an opinion that this could be responsible for its stronger effect on the immune system as compared to tissue culture vaccine. However, this is not consistent with the fact that the purified vaccine, which is free from live virus, shows high activity. A comparative study (Wiktor *et al.*, 1974) of intact and propiolactone-inactivated rabies viruses did not reveal any difference in their ability to stimulate splenocytes. The observed difference between the cerebral and the tissue culture vaccines cannot be due to contamination with brain tissue either. In our blast transformation experiments we used only the virus propagated on Japanese quail embryo cells, which is free from brain tissue, regardless of the vaccine used for immunization. In some experiments (data not shown) we tested the influenza virus instead of the rabies virus as one of the controls; the transformation was absent in this case. All this indicates that the reaction was rabies-specific, and the difference was due to a certain property of the virus dependent on the host cell.

The resistance of mice to intracerebral administration of the rabies virus was enhanced already 7 days after immunization. The sera of the animals lacked virus-neutralizing antibodies at that time. At later intervals post immunization there was a clear correlation between resistance to infection and the level of antirabies antibodies. At the same time, we observed some difference between the dynamics of resistance and cellular immunity. The highest resistance was recorded by day 15 while lymphocyte blast transformation reached the maximum by day 30. The migration activity returned to the control level by day 45 while some resistance to infection was still noted on day 60. It appears that post-vaccination immunity is mainly due to humoral factors. Cellular factors, as revealed by the splenocyte migration inhibition test, for instance, may contribute to the resistance at earlier intervals after immunization, prior to production of neutralizing antibodies.

References

Atanasiu, P. (1982): Róle de l'inteferon dans l'immunité antirabique. *Comp. Immun. Microbiol. Infect. Dis.* **5**, 123—127.

Baer, G. M., and Yager, P. A. (1977): A mouse model for post-exposure rabies prophylaxis: the comparative efficacy of two vaccines and of antiserum administration. *J. gen. Virol.* **36**, 51 — 58.

Bektemirova, M. S., Pille, E. R., Matevosyan, K. Sh., and Nagieva, F. G. (1983): Rabies vaccine prepared from the virus grown in Japanese quail embryo cell cultures. *Acta virol.* **27**, 59—64.

Crick, J., and Brown, P. (1976): Rabies virus and the problems of rabies vaccination in man. *Trans. R. Soc. trop. Med. Hyg.* **70**, 196—201.

Guillon, J.-C., and Tsiang, H. (1980): Róle de l'interferon et du thymus dans la pathogenese de l'infection rabique chez la souris. *Ann. Virol. Inst. Pasteur* **131**E, 229—245.

Karakuyumchan, M. K., Nadaichik, L. V., Pille, E. R., and Rozina, E. E. (1981): Freeing rabies virus of the neuroallergenic factor from brain tissue. *Acta virol.* **25**, 155—158.

Nicholson, K. G., Cole, P. J., Turner, G. S., and Harrison, P. (1979): Immune responses of humans to a human diploid cell strain of rabies virus vaccine: lymphocyte transformation, production of virus-neutralizing antibody, and induction of interferon. *J. infect. Dis.* **140**, 176—182.

Pille, E. R. Karakuyumchan, M. K., and Nadaichik, L. V. (1981): Properties of rabies vaccine freed of neuroallergenic factor from brain tissue. *Acta virol.* **25**, 304—308.

Prabhakar, B. S., Fischman, H. R., and Nathanson, N. (1981): Recovery from experimental rabies by adoptive transfer of immune cells. *J. gen. Virol.* **56**, 25—31.

Ramana, B. Ch., and Pal, S. R. (1981*a*): Cellular and humoral immune response in human beings vaccinated with rabies vaccine *J. Commun. Dis.* **12**, 139—143.

Ramana, B. Ch., and Pal, S. R. (1980*b*): In vitro transformation of rabbit lymphocytes in rabies virus infection. *Natn. Inst. anim. Hlth* **20**, 75—76.

Schuller, E., Helary, M., Allinquant, B., Gibert, C., Vachon, F., and Atanasiu, P. (1979): IgM and IgG antibody responses in rabies encephalitis. *Ann. Microbiol. Inst. Pasteur* **130**A, 365 — 372.

Unanov, S. S., Pille, E. R., Levchenko, E. N., Karakuyumchan, M. K., Yuminova, N. V., and Shkolnik, R. Ya. (1984): The results of immunization of human beings with a new rabies vaccine containing no neuroallergen factor of brain tissue (in Russian). *Vop. Virus.* **29**, 437 — 441.

Wiktor, T. J., Kamo, I., and Koprowski, H. (1974): In vitro stimulation of rabbit lymphocytes after immunization with live or inactivated vaccine. *J. Immunol.* **112**, 2013—2019.

Wiktor, T. J., Koprowski, H., Mitchell, J. R., and Merigan, T. C. (1976): Role of interferon in prophylaxis of rabies after exposure. *J. infect. Dis.* **133** (suppl.), A260—A265.

Wiktor, T. J., and Koprowski, H. (1980): Antigenic variants of rabies virus. *J. exp. Med.* **152**, 99—112.