Acta virol. **31** : 269—270, 1987

IN VIVO OPSONIZATION OF JAPANESE ENCEPHALITIS VIRUS BY PERITONEAL MACROPHAGES IN INFANT MICE

S. D. KELKAR

Division of Virology, National Institute of Virology, P.O.B. No. 11, Pune-411001, India

Received May 12, 1986

Summary. — Anti-JE antibody in nonneutralizable concentration and Concanavalin A are synergistic in protecting 10-day-old mice from lethal JE virus challenge by i.p. route.

Key words: Concanavalin A; macrophages; Japanese encephalitis virus

Inoculation of 3 doses (125 μg) of Concanavalin A (Con A) by i.p. route into 9—10-day-old mice effectively protected them against lethal Japanese encephalitis (JE) virus infection (Kelkar, 1982). Further studies revealed that Con A-induced macrophages alone or together with some other local factors prevented the virus spread to target organs protecting mice from lethal JE virus infection (Kelkar and Gogate, unpublished data). A single dose or 3 doses of Con A induced more or less equal number of peritoneal exudate (PE) cells, namely about 2×10^6 cells/infant mouse. However, a single dose of Con A did not protect against JE virus infection.

In order to potentiate the capacity of macrophages (induced by a single dose of Con A) to take up JE virus more effectively, in vivo opsonization was attempted. Infant mice (8—10-day-old) were inoculated with a single dose of Con A (125 μg/mouse/0.05 ml). Controls were inoculated with phosphate buffered saline (PBS). At 48 hr p.i. when peritoneal population reached peak level, 0.03 ml of different dilutions of anti-JE mouse serum was inoculated by i.p. route. Controls were given similar dilutions of normal mouse serum. After 3—4 hr 2 dex of JE virus was inoculated i.p., and percentage of survivors was calculated.

The results are presented in Table 1. Among control mice which were given anti-JE immune serum diluted 1 : 400, the survival rate was 50 percent while there were no survivors among mice which were given normal mouse serum. In contrast among mice which were administered Con A and anti-JE immune serum (1 : 400) the survival rate was 87.5 per cent. The survival among Con A-treated mice which received higher dilutions (1 : 800, 1 : 1200) of immune serum was 68.7 per cent, while in mice treated immune serum only (without Con A) the survival rates were 12.5 and 0 per cent, respectively ($p < 0.001$). Similarly, in mice treated with Con A and normal mouse serum the survival rates were 25 and 12.5 percent, respectively ($p < 0.001$). It appears that in the presence of JE-specific antibody, Con A-induced macrophages take up the virus more effectively. Macrophages are non-permissive for JE virus, thus its multiplication does not take place in them. The data

Table 1. Protection of infant mice primed with Con-A and anti-JE serum against challenge with JE virus

No.	Con A 125 μg i.p. (−48 hr)	Different dilutions of JE immune/normal mouse serum i.p. (−4 hr)	Mortality ratio	% Survival
1.	Con A 125 μg	Anti-JE mouse serum-1 : 400*	2/16	87.5
2.	Con A 125 μg	Anti-JE mouse serum-1 : 800	5/16	68.7
3.	Con A 125 μg	Anti-JE mouse serum-1 : 1200	5/16	68.7
4.	Con A 125 μg	Anti-JE mouse serum-1 : 1600	5/14	64.3
5.	Con A 125 μg	No serum —	14/16	12.5
6.	Con A 125 μg	Normal mouse serum -1 : 400	7/8	12.5
7.	Con A 125 μg	Normal mouse serum -1 : 800	6/8	25
8.	Con A 125 μg	Normal mouse serum -1 : 1200	7/8	12.5
9.	Con A 125 μg	Normal mouse serum -1 : 1600	7/8	12.5
10.	PBS	Anti-JE mouse serum-1 : 400*	4/8	50
11.	PBS	Anti-JE mouse serum-1 : 800	7/8	12.5
12.	PBS	Anti-JE mouse serum-1 : 1200	8/8	0
13.	PBS	Anti-JE mouse serum-1 : 1600	7/8	12.5
14.	PBS	Normal mouse serum -1 : 400	8/8	0
15.	PBS	Normal mouse serum -1 : 800	8/8	0
16.	PBS	Normal mouse serum -1 : 1200	8/8	0
17.	PBS	Normal mouse serum -1 : 1600	7/8	12.5

* 1 : 400 concentration of anti-JE serum was in part protective
** Except these, all mice were challenged with 2 dex of JE virus by i.p. route.

support the view that efficient phagocytosis of JE virus protects mice from lethal infection.

Infant mice are susceptible up to 11 days to JE virus inoculation by i.p. route. Since 12—13 days, the mice started to develop resistance to the virus given by i.p. route. However, when PE cells from different age groups were examined, there was very slow increase in the number of PE cells. On day 14 PE cell count was only 0.3×10^6 per infant mouse, but the mice were already partially resistant to JE virus challenge by i.p. route. By day 21 the PE cell count was 1.2×10^6 per infant mouse and mice were fully resistant to i.p. challenge. It seems that it is not the number of macrophages alone that is important for the uptake of virus but also the functional characteristics of the macrophages.

The role of macrophages in resistance to JE has been substantiated by the fact that silica treatment of mice leads to susceptibility of mice to JE (A. B. Kulkarni, personal communication). Thus, it appears that the low level of neutralizing antibody and Con A are synergistic in protecting mice from JE virus challenge by i.p. route. The protective effect is probably due to increase clearance of virus by macrophages.

Reference

Kelkar, S. D. (1982): Protection against Japanese encephalitis virus in infant mice by Concanavalin A. *Indian J. Med. Res.* **76**, 47—52.