PROTECTIVE ROLE OF THERMOLABILE VIRUS-NEUTRALIZING INHIBITORS DURING EXPERIMENTAL VIRUS INFECTION

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The sera of normal rabbits contain thermolabile virus-neutralizing inhibitors of vaccinia virus. The higher the content of inhibitors in the serum, the more resistant the animal to intradermal infection with vaccinia virus.

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There is no unanimity regarding the protective role of virus-neutralizing inhibitors [3, 4, 8] of native sera of normal animals. Individual authors [2, 5-7] have reported the positive protection effect of virus-neutralizing inhibitors of importance in the nonspecific resistance of the body to influenza infection.

In the present investigation the protective role of thermolabile virus-neutralizing inhibitors was studied in experimental infection with vaccinia virus.

EXPERIMENTAL METHOD

Virus. Vaccinia virus was used as the culture fluid of infected chick embryonic fibroblasts, obtained 5 days after infection.

Sera. Native sera of normal rabbits were obtained by cardiac puncture. The sera were separated from the clot and stored at −15°.

The virus-neutralizing activity of the native sera was determined in a culture of chick fibroblasts, 0.4 ml of the test native serum being mixed with 1.6 ml of virus taken in serial dilutions with a coefficient of 0.5 log. The final dilution of each serum was 1 : 5. After contact between virus and serum for 2 h at 37°, 1 ml of the mixture was added to the tubes containing cell culture and left for 15 min at 37° to allow adsorption of uninactivated virus. The controls were the same dilutions of virus mixed with normal serum, preliminarily heated for 30 min at 56°. After adsorption of virus for 15 min, the mixture of serum with virus was removed, the cells were washed twice or three times with buffer solution, and the maintenance medium was poured off and incubated. The results of the neutralization reaction were read by counting the number of primary plaques by hemadsorption of chick erythrocytes [1], from which the number of tissue plaque-forming units (PFU) could be estimated.

Titration of Vaccinia Virus in Rabbits' Skin. Rabbits weighing 2.5-3 kg were used. Dilutions (1 : 10) of infective culture fluid containing from 6 to 0.1 log PFU virus /0.2 ml were injected intradermally into the shaved lateral surface of the animals' back. The results were read on the 5th day, taking note of the presence of inflammation at the places of injection, its character (size of the papules, severity of the necrosis), and were expressed as the titer of virus just causing the development of skin lesions.

EXPERIMENTAL RESULTS

During intradermal titration of the infective activity of the virus in individual normal rabbits, considerable differences were found in their individual sensitivity to the virus.

The minimal dose of virus causing inflammation at the place of intradermal injection ranged from 0.5 to 3.5 log PFU/0.2 ml for individual animals. The titer of virus, determined in skin doses (SD) from its final dilution, capable of causing inflammation by a single intradermal injection, varied from one rabbit to another even though they obtained identical doses of virus (in PFU). In animals with increased sensitivity

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TABLE 1. Relationship between Intradermal Activity of Vaccinia Virus and Inhibitory Power of Normal Rabbit Sera

Rabbit No.	Dose of vaccinia virus (in log PFU) neutralized by serum (1 : 5) in cell culture	Titer of virus by intradermal infection (in reciprocals of log SD)
10	0.5	6.0
19	0.5	6.0
9	1.0	6.0
59	1.0	6.0
62	1.0	6.0
69	1.0	6.0
5	1.5	5.0
24	1.5	5.0
28	1.5	5.0
30	1.5	5.0
32	1.5	5.0
37	1.5	5.0
44	1.5	5.0
25	2.0	4.0
41	2.0	4.0
67	2.0	4.0
31	2.5	4.0
21	2.5	3.0

to virus, which developed inflammation after intradermal injection of as little as 0.5-1.0 log PFU of virus, its titer was maximal (−6.0 log SD; rabbits Nos. 3, 7, 46). In animals with moderate sensitivity, which developed inflammation after injection of 1.5 log PFU/0.2 ml, the titer of the virus only reached −5.0 log SD (rabbits No. 40,42, 43, 55). Finally, in the more resistant animals (which developed inflammation after injection of not less than 2.5-3.5 log PFU virus/0.2 ml), the titer of virus did not exceed −4.0 log SD (rabbits Nos. 4, 38, 39, 21).

In the study of the virus-neutralizing activity of normal native sera of these animals in cell culture, considerable differences were found in their ability to neutralize the cytopathogenic action of vaccinia virus.

The dose of virus neutralized by native sera of individual rabbits varied from 0.5 to 2.5 log PFU. An inverse relationship was found between the inhibitory activity of the rabbit sera and the titer of virus just causing intradermal changes in the rabbits (see Table 1).

Table 1 shows that in animals with a low content of virus-neutralizing inhibitors (rabbits Nos. 9, 10, 19, 62, 59), the activity of the virus was maximal by intradermal titration, reaching −6.0 log SD. If the inhibitory activity of the serum was moderately high, neutralizing 1.5 log PFU of virus, the intradermal activity of the virus was lowered and the titer fell to −5.0 log SD (rabbits Nos. 5, 24, 28, 30, 32, 37, 44). With high virus-neutralizing power of the sera, neutralizing 2.0-2.5 log PFU of virus, the intradermal activity of the virus was minimal (−3.0 to −4.0 log SD; rabbits Nos. 21, 25, 31, 41, 67). It should be emphasized that in animals with a high level of virus-neutralizing inhibitors in the blood, along with low intradermal activity of the virus, a lower intensity of inflammatory changes was also observed in all parts of the lesion (smaller papules, absence of necrosis or ill-defined necrosis), which was observed parallel with low reproduction of virus in the intradermal focus.

The results described above demonstrate the ability of thermolabile virus-neutralizing inhibitors to exhibit protective functions and to restrict reproduction of the virus directly in vivo.

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