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"VACCINIAL DISEASE" OF WHITE RATS AS A MODEL FOR THE DETERMINATION OF THE IMMUNITY TO VACCINIA VIRUS

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Summary. — The so-called vaccinial disease of white rats may serve as a model for the determination of the intensity of the immunity to vaccinia virus in animals immunized either actively or passively. Immunity of high degree arises after active immunization by live or gamma-irradiated smallpox vaccine. The time of resistance depends on the method of inoculation and on the dose of immunizing virus. This finding makes it possible to employ the method for the titration of immunogenicity od smallpox vaccine. To secure complete defence against vaccinial disease, the titre of virus neutralizing antibodies in passively immunized irradiated rats should be not lower than 1 : 320.

Introduction

Two indicators are usually employed to characterize the intensity of the immunity of animals to vaccinia virus: 1) antibody titre and 2) intensity of skin lesions after treatment with different virus dilutions (Groth, 1923; Gastinel *et al.*, 1931; Barg and Boroday, 1937; McClean, 1945; Collier *et al.*, 1955; Mastyukova, 1956; Morozov and Konstantinova, 1957; Mastyukova and Yaroslavskaya, 1958; Amies, 1962; and others).

It is well known that both the titre of smallpox antibodies and skin reaction to virus inoculation give but approximate characteristics of the intensity of smallpox immunity (Force, 1914; Peterson, 1922; Boek, 1946; Agerty, 1947; Downie and McCarthy, 1958; Mineyeva, 1959; Kempe, 1960).

More adequate is the test suggested by Solovyov and Mastyukova (1956) according to which immune rabbits are inoculated intracerebrally with a definite quantity of LD_{50} of neurovaccinia virus. The strong point of this method is its exactness. However, the strength of the challenge may decrease the virtual resistance of animals.

In our previous paper (Svet-Moldavskaya, 1968) we described a method of establishing in irradiated white rats the "vaccinial disease", a peculiar toxic-infectious lethal process. We also showed that "vaccinial disease" cannot be induced in animals immunized before irradiation. That is why we used the so-called "vaccinial disease" as a model to test the resistance of animals immunized either with "live" or inactivated smallpox vaccine. The possibility of passive prophylaxis against "vaccinial disease" by immune serum was also investigated.

Materials and Methods

Animals. White Wistar rats weighing 150—200 g were used.

Immunization. Two types of antigens were used:

1) Dried smallpox vaccine prepared of strains Tashkent or EM-63 was employed. For subcutaneous immunization, the rats were inoculated once in each hind leg with 0.2—0.5 ml of the vaccine reconstituted with saline. The doses of virus varied from 1.2×10^7 to 3.0×10^7 pock-forming units (PFU). In intravenous immunization, the rats received 1 ml of the vaccine into the femoral vein (4.6×10^6—3×10^7 PFU).

2) Liquid smallpox vaccine strain EM-63 inactivated by gamma-irradiation Co^{60}, integral dose $(1.5—1.75) \times 10^6$ rad, dose rate 400 rad/sec. The titre of the vaccine before inactivation was 10^8 PFU/ml. One ml of the inactivated vaccine was once inoculated into the femoral vein of the rats.

Challenge. In different periods after the immunization (from 3 weeks to 18 months) the rats were totally irradiated with Co^{60} (dose rate 45 r/min; the integral dose for each rat — 400 r). Twenty-four hours later the animals were inoculated intravenously with 1 ml of vaccinia virus strain Tashkent reconstituted in saline. Control non-vaccinated rats irradiated with the same dose of gamma rays were inoculated in parallel. The results were read 6—8 days later by comparing the death rate to the 100% deaths in control groups.

In experiments on *passive immunization*, rats were irradiated and the next day injected intravenously with 3 ml of serum from the rats immunized with "live" vaccine strain Tashkent. After 24 hours, blood was taken from the hearts of the animals to check the titre of passively transferred antibodies and the animals were inoculated with the lethal dose of the vaccine strain Tashkent. Control irradiated rats were injected in parallel.

Antibody titres were determined by the neutralization reaction on chick embryo chorioallantoic membranes according to Boulter (1957).

Results

Table 1 shows the results of three experiments on the extent of immunity in rats actively immunized by smallpox vaccine. The data presented illustrate the resistance to lethal dose of virus in actively immunized rats. The rats proved to be completely resistant for 2 months after subcutaneous injection

Table 1. Resistance of irradiated rats previously immunized with "live" smallpox vaccine

Method of immunization	Strain of virus	Dose of virus (PFU)	Time of challenge	Results*	
				Experiment	Control
Subcutaneous	Tashkent	1.2×10^7	21 days	0/10	5/5
			2 months	0/10	5/5
			9 months	9/10	9/10
		3×10^7	6 months	0/10	5/5
			9 months	0/10	5/5
Intravenous		3×10^7	1 month	0/10	3/3
			2 months	0/10	5/5
			18 months	0/10	5/5
			1 month	0/10	9/10
Intravenous	EM-63	4.6×10^6	2 months	0/8	10/10
			4 months	0/6	5/5
			6 months	0/7	5/5
			9 months	1/10	10/10

* Numerator: number of dead animals; denominator: number of experimental animals.

with vaccine containing 1.2×10^7 PFU (strain Tashkent), regaining their susceptibility 9 months after immunization. An increase in the dose up to 3×10^7 PFU prolonged the period of insusceptibility of the animals to 9 months at least (period of observation).

When injected intravenously, the rats remained completely insusceptible for 18 months (period of observation).

Table 2. Resistance of irradiated rats previously immunized with inactivated smallpox vaccine

Time of challenge	Results*	
	Experiment	Control
1 month	0/10	10/10
2 months	0/10	5/5
4 months	0/8	Not done
6 months	0/7	5/5
9 months	1/10	10/10

* See Table 1.
The rats were intravenously immunized with virus strain EM-63; dose of virus before inactivation = 3.6×10^8. PFU.

Similar results were obtained by intravenous immunization of rats with EM-63 strain vaccine diluted 1 : 10 (titre 4.6×10^6 PFU). The death of one out of 10 experimental rats can be attributed to a lower dose of immunizing virus as compared to strain Tashkent.

Table 3. Increase of antibodies in immunized rats after irradiation and intravenous inoculation of virus

Method of immunization	Animal No.	Antigen-smallpox vaccine	Antibody titre before challenge	Time of challenge (months)	Antibody titre after challenge	Rate of antibody titre increase
Subcutaneous	1	Live	320	6	2560	8
	2		640		2560	4
	3		640		2560	4
Intravenous	4		1280		5120	4
	5		1280		5120	4
	6		320		2560	8
	7		1280		5120	4
	1	Inactivated	320	4	2560	8
	2		160		1280	8
	3		40		160	4
	4		80		640	8
	5		320		2560	8
	6		80		640	8

The results of the study on the resistance of irradiated rats previously immunized with inactivated vaccine (Table 2) showed a high immunizing capacity of smallpox vaccine inactivated by gamma rays.

In a number of experiments we checked the circulating antibody titre before irradiation and challenge of immunized rats. We hoped to determine in this way the minimal antibody titre sufficient to defend the rats against "vaccinial disease".

Table 3 shows the results of two experiments: one carried out 6 months after immunization with live vaccine, the other 4 months after immunization with inactivated vaccine. In spite of individual differences, antibody titres

Table 4. Passive prophylaxis against "vaccinial disease" by serum from rats immunized with "live" smallpox vaccine

Antibody titres in sera from rats		Results*	
immunized with live vaccine	passively immunized	Resistance test	Control
5120	1280	0/1	5/5
	640	0/1	
	320	0/4	
	160	1/2	
2560	640	0/4	5/5
	320	0/1	
1280	40	2/7	5/5
	40	2/7	
	80	0/2	

* See Table 1.

in rats treated with live vaccine were higher than those in rats immunized with inactivated vaccine. Nevertheless, all the animals withstood the irradiation and the inoculation with a lethal dose of the virus.

After challenge, the antibody titres as a rule increased 4—8 times. Resistance of the animals seemed to depend more on their readiness to the secondary response than on the antibody titre before challenge as such.

In our next experiments we attempted to determine the virus neutralizing antibody titre necessary for passive prophylaxis against "vaccinial disease". Table 4 presents the results of three such experiments. In the first experiment, the virus neutralizing antibody titre in the donors' serum was 1 : > 5120. Irradiated recipients displayed a titre varying from 1 : 160 up to 1 : 1280. All the recipients, except one with a titre of 1 : 160, survived. In the second experiment, the donors' serum contained antibodies in a titre of 1 : 2560. The recipients displayed various titres from 1 : 320 up to 1 : 640; all the animals survived. In the third experiment, the donors' serum titre was 1 : 1280, the recipients showed titres from 1 : 40—1 : 80, and only 4 out of 16 animals survived.

Discussion

Our results show that "vaccinial disease" of white rats may be employed as a model to determine the resistance of animals immunized with vaccinia virus.

Active immunization resulted in a stable and strong immunity of rats against "vaccinial disease".

Intravenous inoculation proved to be more effective than subcutaneous inoculation, probably because of wide distribution of virus in the lymphoid system, this fact being responsible for intensive immunogenesis. The intensity of the immunity proved to be directly proportional to the immunizing dose of the virus, which enabled us to use this test for titrating immunogenicity of smallpox vaccine.

The experiments on passive immunization of irradiated animals against "vaccinial disease" revealed a high defensive capacity of immune serum. Preliminary data suggest that, to provide complete defence of recipients against "vaccinial disease", the titre of virus neutralizing antibodies in their serum should be not lower than 1 : 320.

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