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The Use of Hyperimmune Antivaccinia Gamma-globulin for the Prevention and Treatment of Smallpox

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Smallpox prophylaxis studies having shown that the classical Jennerian type of vaccination may in some instances prove inadequate to prevent the development of the disease or may be contra-indicated for certain persons, attempts were made to evolve a method which could supplement or replace such vaccination. Investigations reported in this paper have demonstrated that the administration of gamma-globulin obtained from the sera of various animals hyperimmunized with vaccinia virus gives highly effective results in the prevention of different forms of experimental vaccinial infection. Encouraging results were also obtained with this gamma-globulin given prophylactically to 13 persons who had been in close contact with smallpox patients during an outbreak of this disease in Moscow in 1960. The author suggests that hyperimmune antivaccinia gamma-globulin of animal origin may also prove effective in the treatment of postvaccinal complications.

More than 150 years have passed since Jenner's discovery of the prophylactic value of vaccination against smallpox, and experience since then has amply demonstrated that this is a highly effective means of combating the disease.

While allowing smallpox vaccination its due appreciation, we should not forget that there are nevertheless problems in smallpox prevention. Thus, for instance, vaccination may prove to be ineffective if administered after exposure. There are two views on this problem, which has been under thorough study for a long time. One, originating in Pirquet's time (Pirquet, 1907), is based on the fact that about seven days are required for adequate immunity to be formed; immunization during the second half of the incubation period will therefore be ineffective. This is the opinion held at the present time by Downie and associates (Pierce et al., 1958). The other view is based mainly on clinical observations which suggest that vaccination may not be very effective even if performed during the first days of the incubation period. This view has been put forward by, for instance, Kempe (1954). Both theories are in agreement that, in a number of cases, vaccination after contact with a smallpox patient may prove ineffective; and instances are known in which vaccination has proved ineffective after prolonged and close contact with a smallpox patient, presumably because the degree of immunization conferred was inadequate in relation to the size of the infecting dose of virus.

It should also be noted that vaccination is not always harmless, as has been demonstrated by experience with mass vaccination and revaccination carried out in connexion with smallpox outbreaks in New York, France and Moscow (Greenberg, 1948; Crosnier, 1956; Marennikova & Petrosov, 1961; Smelov & Kalamkaryan, 1961).

Thus, the inadequacy of delayed vaccination or of vaccination after prolonged contact with a smallpox patient and the existence of a certain number of persons for whom vaccination is contra-indicated render it necessary to develop new methods for smallpox prevention which are complementary to the classical Jennerian vaccination.

Seroprophylaxis is one such method, the efficiency of which has been shown by Kempe and associates (1956, 1961) in investigations carried out in 1953 and 1960 in Madras. These scientists divided smallpox contacts into two groups, one of which received the usual vaccination, and the other vaccinia hyperimmune gamma-globulin in addition to vaccination. This gamma-globulin was produced from the serum

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of persons who had received smallpox vaccination 3-4 weeks previously. Subsequent observations demonstrated that the attack and mortality rates were much lower in the group of persons given seroprophylaxis.

The beneficial effects of preparations containing smallpox antibodies are not limited to prophylaxis. Observations of earlier authors (Béclère et al., 1896; Teissier & Marie, 1912) have demonstrated that such preparations, (e.g., convalescent serum, serum of vaccinated persons, and antivaccinial sera of immunized animals) are capable of a therapeutic effect in smallpox. This is especially important since so far no chemotherapeutic or antibiotic agent at our disposal has been found which will effectively treat smallpox.

From all the foregoing it was concluded that research should be undertaken into the development of a serum preparation that might be used both for smallpox prevention and treatment and for treatment of postvaccinal complications such as generalized and gangrenous vaccinia, vaccinial eczema, etc. As a result of research work begun in 1955, at the Metchnikov Institute of Vaccines and Sera, Moscow, we obtained antivaccinia gamma-globulin, an active preparation from the sera of animals hyperimmunized with vaccinia virus, containing virus-neutralizing antibody in high titre (Marennikova et al., 1958).

PREPARATION OF HYPERIMMUNE ANTIVACCINIA GAMMA-GLOBULIN

To obtain the immune antivaccinia serum the animals (rabbits, swine, sheep and calves) were immunized with vaccinia virus grown in the developing chick embryo. The animals were repeatedly inoculated intramuscularly or intravenously with increasing doses.

The gamma-globulin fraction was obtained from the immune sera by alcohol precipitation in the cold. The content of antihaemagglutinins and virus-neutralizing antibody was determined in the samples obtained.

ANTIHAEMAGGLUTINATING AND VIRUS-NEUTRALIZING ACTIVITY OF ANTIVACCINIA GAMMA-GLOBULIN

The antihaemagglutinin titres in the gamma-globulin are compared with those in the initial immune sera in Table 1. A rise in antihaemagglutinin concentration (of 10.6 to 13.6 times) was noted in the gamma-globulin obtained from immune rabbit, sheep and calf sera whether the initial serum titres were high or low. With the swine material, however, it is noteworthy that we failed to detect the presence of antihaemagglutinins either in the initial sera or in the gamma-globulin.

TABLE 1

ANTIHAEMAGGLUTINATING ACTIVITY OF HYPERIMMUNE ANTIVACCINIA GAMMA-GLOBULIN AND INITIAL IMMUNE ANIMAL SERA

Type of gamma-globulin	No. of samples	Average antihaemagglutinating titre		Concentration factor
		Initial serum	Gamma-globulin	
Rabbit	7	225.7	2 400	10.6
Swine	5	0	0	—
Sheep	5	230	4 350	13.6
Calf	3	80	853	10.7

Table 2 shows virus-neutralizing antibody titres of different gamma-globulins in comparison with those of the initial immune sera. It will be seen that the antibody titre of rabbit gamma-globulin which completely neutralized 100 ID_{50} of virus (in the intradermal test in rabbits) was on the average 6.4 times higher than that of the initial serum. In gamma-globulin obtained from immune swine serum the virus-neutralizing antibody titre was 32 times higher than that of the initial serum when tested against 10 ID_{50} of virus, and in gamma-globulin obtained from immune sheep serum it was 5 times higher when tested against 1000 ID_{50} of virus.

Experiments were also carried out with a view to investigating the virus-neutralizing activity of

TABLE 2

VIRUS-NEUTRALIZING ACTIVITY OF INITIAL IMMUNE SERA AND HYPERIMMUNE ANTIVACCINIA GAMMA-GLOBULIN

Animal	Infective doses (ID) of virus neutralized	Average neutralizing antibody titre	
		Initial serum	Gamma-globulin
Rabbit	100	24	155
Swine	10	160	5 120
Sheep	1 000	128	640

TABLE 3

ACTIVITY OF VARIOUS HYPERIMMUNE ANTIVACCINIA GAMMA-GLOBULIN PREPARATIONS FROM HUMAN AND ANIMAL SERA

Titre of :	Human gamma-globulin				Hyperimmune gamma-globulin from animal sera shown			
	Normal (7 batches)	Hyperimmune						
		Sweden	England	USSR	Rabbit	Swine	Sheep	Calf
Antihaemagglutinin	17	160	320	160	2 560	0	2 560	512
Antibody completely neutralizing 10 ID_{50} for rabbit	5.4	32	32	32	5 120	5 120	620 [a]	512

[a] Neutralizing 1 000 ID_{50} for rabbit.

gamma-globulin in monolayer cultures on monkey-kidney tissue. These showed that up to 500 000 cytopathogenic units of virus were neutralized by hyperimmune swine gamma-globulin in a dilution of 1 : 5 and up to 10 000 units in a dilution of 1 : 50.

Investigations of the activity of hyperimmune rabbit serum and gamma-globulin against variola virus demonstrated that this was also neutralized by these preparations, the antibody titres being the same as in the experiments with vaccinia virus.

Table 3 gives comparative data on the experiments carried out in our laboratory on the activity of antivaccinia gamma-globulin obtained from the sera of hyperimmunized animals and that of gamma-globulins obtained in various countries from the sera of recently vaccinated humans.

PROPHYLACTIC AND THERAPEUTIC EFFECT IN ANIMALS

In the light of the data presented above, it was considered possible to begin a study of the prophylactic and therapeutic effect of antivaccinia gamma-globulin in experiments on animals.

Chick embryos were used in the initial experiment. As shown in Table 4, gamma-globulin administered prophylactically into the allantoic cavity two hours or 30 minutes before the inoculation of a lethal dose of virus completely prevented the development of infection and the death of embryos. The virus could not be detected in suspensions of chorio-allantoic membranes of surviving embryos, when studied by Grot's method in rabbits.

The right-hand portion of Table 4 shows that when the virus dose was increased tenfold, in the

TABLE 4

PROPHYLACTIC EFFECT OF HYPERIMMUNE ANTIVACCINIA GAMMA-GLOBULIN ADMINISTERED BEFORE OR AFTER INJECTION OF VACCINIA VIRUS INTO ALLANTOIC SAC OF CHICK EMBRYO

Time when gamma-globulin administered	1 LD_{50} of virus			10 LD_{50} of virus		
	Proportion of dead embryos [a]	Presence of infection in : [b]		Proportion of dead embryos [a]	Presence of infection in : [b]	
		Surviving embryos	Dead embryos		Surviving embryos	Dead embryos
2 hours before infection	0/5	0		ND	ND	ND
30 minutes before infection	0/5	0		1/10	0	±
30 minutes after infection	1/5	0	±	7/9	0	+
2 hours after infection	2/5	0	+	9/9		+
Controls [c]	5/5		+	4/4		+

[a] Expressed as the number of chick embryos killed by specific infection over the number infected.

[b] + = positive skin reaction in rabbit; ± = few vesicles on rabbit skin; ND = not done.

[c] Gamma-globulin not administered.

experiment involving the administration of gamma-globulin 30 minutes before infection, 9 of the 10 embryos survived without signs of specific infection.

The results obtained in experiments involving the administration of gamma-globulin after infection were less clear-cut. Thus, the administration of gamma-globulin 30 minutes and two hours after infection with 1 LD_{50} of virus resulted in the survival of 4 of 5 and 3 of 5 embryos respectively.

The prophylactic effect of gamma-globulin on the development of vaccinal keratitis in rabbits was also studied. Virus-containing suspensions were applied to the scarified rabbit cornea, and gamma-globulin was given 30 minutes before or 30 minutes after infection ; this completely prevented the development of vaccinal keratitis.

Antivaccinia gamma-globulin was also observed to have a distinct therapeutic effect in all eight rabbits infected intravenously with a lethal dose of virus. This dose provoked generalized vaccinial infection, accompanied by generalized eruption on the skin and mucosa, terminating in the death of the animals on the third to fifth day after infection.

The daily intravenous administration of 1 ml of antivaccinia gamma-globulin to such animals for a period of 5-7 days prevented their death and the development of generalized infection, the administration of the gamma-globulin being begun on the first day, two hours after infection. Analogous results were obtained with intramuscular administration of gamma-globulin. In this case, however, the gamma-globulin was administered in much greater doses (5 ml daily for 5-10 days).

Finally, mention should be made of a study that has recently been started on the prophylactic and therapeutic effect of antivaccinia gamma-globulin in experimental vaccinal encephalitis of rabbits provoked by the administration of 1000 LD_{50} of neurovaccine virus. In the course of these experiments it has been demonstrated that the prophylactic suboccipital administration of the preparation prolonged the incubation period and the infectious process by two or three times that in control rabbits, although it did not prevent the death of the animals.

PROPHYLACTIC AND THERAPEUTIC EFFECT IN MAN

In view of the above results obtained in animal experiments and in safety testing with hyperimmune antivaccinia gamma-globulin, its use was considered justified for the seroprophylaxis and specific therapy of smallpox during an outbreak of this infection in Moscow in 1960.

A group of 42 persons who had been in close contact with smallpox patients (the clinical diagnosis being confirmed by direct isolation of the virus) were under observation. Antivaccinia gamma-globulin was prophylactically administered to 13 of these individuals, it being injected intramuscularly in a single dose of 9 ml for adults and 6 ml for children.

The likelihood of these persons developing smallpox was particularly great as all had been in contact with the patients in the infectious stage; some had been in contact with more than one patient; four had been vaccinated in childhood or infancy only; all but three were not revaccinated until the fifth day after contact or later (see Table 5); and none had a measurable antihaemagglutinin titre.

The fact that none of these 13 contacts developed the disease may be attributed, at least in part, to the action of the antivaccinia gamma-globulin they received. This view is strengthened by comparison with the remainder of the contact group, who were given no prophylactic inoculation. These persons, numbering 29 in all, were members of the patients' families, medical staff, etc., who had had about the same degree of contact as the treated group; 13 of these 29 persons developed smallpox.

Notwithstanding the comparatively small number of persons observed, we nevertheless consider that these data point to the prophylactic activity of the hyperimmune antivaccinia gamma-globulin.

To treat smallpox, the preparation was used in two patients during the prodromal period and in two at the height of the disease.

In the first two, antivaccinia gamma-globulin was administered twice—on the third and on the fourth day of the disease. The first patient (40 years of age) received 24 ml, and the second (an 8-year-old boy) 12 ml.

The course of the disease was mild in these patients, being clinically characterized as smallpox without eruption (variola sine exanthemata) in one and as varioloid in the other.

In the patients to whom gamma-globulin was administered at the height of the disease, we noted a distinct improvement of their general condition and a favourable effect on the cutaneous lesions.

CONCLUSIONS

In conclusion it may be stated that experimental evaluation of the antivaccinia gamma-globulin obtained from sera of hyperimmunized animals has

TABLE 5

RESULTS OF USE OF HYPERIMMUNE ANTIVACCINIA GAMMA-GLOBULIN IN 13 PERSONS IN CLOSE CONTACT WITH SMALLPOX PATIENTS

Source of infection	Recovery of variola virus	Contacts			Day of disease when contact occurred	Day after contact on which		Outcome
		Contacts	Age (years)	Previous vaccinations		Revaccinated	Anti-vaccinia gamma-globulin administered	
Patient A	From nasopharynx and skin lesions on 9th day of disease	1	30	No smallpox vaccination marks	8th	2nd	5th	Remained well
		2	30	Vaccinated and revaccinated	5th-6th	6th	8th	Remained well
		3	67	Vaccinated in childhood	3rd-5th	10th	10th	Remained well
		4	20	Vaccinated and revaccinated	6th	5th	7th	Remained well
Patient B	From nasopharynx and skin lesions on 9th day of disease	5	60	Vaccinated in childhood	2nd	9th	11th	Remained well
Patient C	From nasopharynx and skin lesions on 9th day of disease	6	25	Vaccinated and revaccinated	8th	3rd	5th	Remained well
		7	20	Vaccinated and revaccinated	8th	3rd	5th	Remained well
Patient D	From skin lesions on 14th day of disease	8	36	Vaccinated in childhood	11th	5th	11th	Remained well
		9	49	Vaccinated and revaccinated	12th-13th	6th	10th	Remained well
Patient E	From skin lesions on 16th day of disease	10 [a]	10	Vaccinated in infancy	1st-9th	15th [b]	22nd [b]	Remained well
Patient F	From skin lesions on 17th day of disease	11 [c]	30	Vaccinated and revaccinated	12th-13th	5th	10th	Remained well
		12 [c]	28	Vaccinated and revaccinated	12th-13th	5th	10th	Remained well
		13	25	Vaccinated and revaccinated	12th-13th	5th	10th	Remained well

[a] Was also in contact with patient G (not shown) from the 1st to the 6th day of disease.
[b] Counting from the first day of contact.
[c] Was also in contact with patient H (not shown) from the 3rd to the 6th day of disease.

demonstrated that it possesses high specific activity, considerably exceeding that of analogous preparations obtained from sera of recently vaccinated humans. Experimental evaluation of the prophylactic effect of this preparation in vaccinial and smallpox infection has also established its high efficacy in this respect; and the preliminary results obtained in humans are in agreement with the experimental data.

Finally, it may be mentioned that an early report on the production and experimental study of antivaccinia gamma-globulin prepared from the sera of hyperimmunized animals was submitted in 1958 to the WHO Study Group on Recommended Requirements for Smallpox Vaccine.[1] Since then the preparation has been studied in other laboratories and its high activity confirmed.[2] In 1961 an analogous preparation from the sera of hyperimmunized sheep was produced in England.[3] Further studies in this field should be directed to the development of the international standard for antivaccinia gamma-globulin and the testing of its efficacy in large-scale field trials.

[1] Marennikova, S. S., Ponomareva, N. A., Ogorodnikova, Z. I. & Durasova, M. N. (1958) *Production and experimental study of antismallpox gamma-globulin* (unpublished working document WHO/BS/IR/63 Rev. 1).

[2] Gispen, R.—personal communication, 1959.

[3] Evans, D. G.—personal communication, 1961.

RÉSUMÉ

Appliquée sous la forme jennérienne classique, la vaccination contre la variole ne prévient pas à coup sûr l'apparition de la maladie; elle n'est pas non plus exempte de tous risques, et peut même être contre-indiquée chez certains individus. Ces notions découlent de diverses enquêtes qui ont incité à perfectionner la vaccination par inoculation, voire à envisager de la remplacer par d'autres méthodes. Kempe *et al.* (Madras, 1953) ont montré que la séroprophylaxie utilisant la gammaglobuline de sujets vaccinés contre la variole depuis 3-4 semaines augmentait nettement le pouvoir protecteur du vaccin. Par la suite, d'autres auteurs devaient apporter la preuve que la gammaglobuline se révélait efficace dans le traitement de la variole et des complications postvaccinales.

En vue de développer les premières acquisitions faites en ce domaine, l'Institut Metchnikov, de Moscou, s'est attaché depuis 1955 à mettre au point des préparations de gammaglobuline anti-vaccinale faites à partir du sérum de divers animaux hyperimmuns au virus de la vaccine et possédant des titres d'anticorps antivarioliques élevés. La fraction gammaglobuline de ces sérums renferme en effet la majeure partie des anticorps, comme il ressort d'une étude approfondie des processus d'immunisation.

Utilisées dans un but préventif ou curatif, les préparations de gammaglobuline ont fait la preuve de leur efficacité dans différentes formes d'infection vaccinale provoquée expérimentalement. Administrée à titre prophylactique à un lot de 13 sujets qui avaient été au contact de victimes de l'épidémie de variole observée à Moscou en 1960, la gammaglobuline a donné des résultats prometteurs: aucun cas de variole n'a été à déplorer après ce traitement, tandis que parmi 29 personnes qui avaient été exposées à la variole dans des conditions identiques, 13 étaient atteintes par la maladie. Par ailleurs, la gammaglobuline appliquée à la phase prodomique de l'affection a produit une nette atténuation de l'évolution clinique.

Ces résultats donnent à penser que les préparations de gammaglobuline antivariolique extraite du sérum d'animaux hyperimmums pourraient également être efficaces dans le traitement des complications postvaccinales.

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