THE ANTIBODY RESPONSE IN MAN FOLLOWING INFECTION WITH VIRUSES OF THE POX GROUP

III. ANTIBODY RESPONSE IN SMALLPOX

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(With 3 Figures in the Text)

The development of antibody in the course of smallpox is of interest in relation to the pathology and pathogenesis of the disease. We have been especially concerned with the time of appearance of antibody, more particularly neutralizing antibody, in relation to the development of skin lesions and the subsequent severity of illness. The influence of previous vaccination on the antibody response to infection has been studied and for this purpose the information in the preceding paper (McCarthy, Downie & Bradley, 1958) has been useful for comparative purposes. The sera have been obtained from some of the cases in outbreaks of smallpox in this country during the years 1944–53. Mention of some of our findings has been previously made (Downie, 1947 & 1951; McCarthy & Downie, 1948; Downie & McCarthy, 1954). A study of the antibody present in sera from cases of alastrim has already been published (McCarthy & Downie, 1953) but these findings have been included in the present paper as the antibody response of variola minor patients seems to be identical with that found in variola major.

Previously vaccinated contacts of smallpox patients occasionally suffer from a febrile illness similar to the pre-eruptive stage of smallpox but fail to develop a rash—*variola sine eruptione*. We have examined sera from a few persons who suffered from such mild infections and the results of these tests have served to confirm the variolous nature of these illnesses. Quite apart from these minimal infections, the clinical diagnosis of modified smallpox in the vaccinated is sometimes difficult, and, in the absence of virus isolation, help is sometimes obtained by the examination of serum for antibody. The data presented in this paper pertaining to smallpox cases of varying severity, both vaccinated and unvaccinated, when studied along with the results in the previous paper, establish the value of antibody tests in diagnosis and, we believe, indicate the most appropriate kind of test in particular instances.

MATERIALS AND METHODS

Sera

Fifty-nine sera were examined from fifty-one cases of smallpox who had never been vaccinated up to the time of their infection; twenty-one sera from nineteen alastrim cases are included. Of the thirty-two cases of variola major seven died, although the overall mortality among unvaccinated cases of variola major in the outbreaks studied was over 50 %. There were eighty-one sera from sixty-six

smallpox patients who gave a history of prior vaccination and of these three were fatal. Normal sera from healthy unvaccinated persons were included in all tests.

Tests for antibody

Variola virus was used in tests for neutralizing antibody; this technique and the details of the haemagglutination inhibition and complement-fixation tests have been described in the preceding paper.

RESULTS

It has been our custom to regard complement-fixation titres of 1/5 or higher, antihaemagglutinin titres of 1/20 or higher and neutralization of 50 % or more of variola virus on the chorio-allantois as evidence of the presence of the corre-

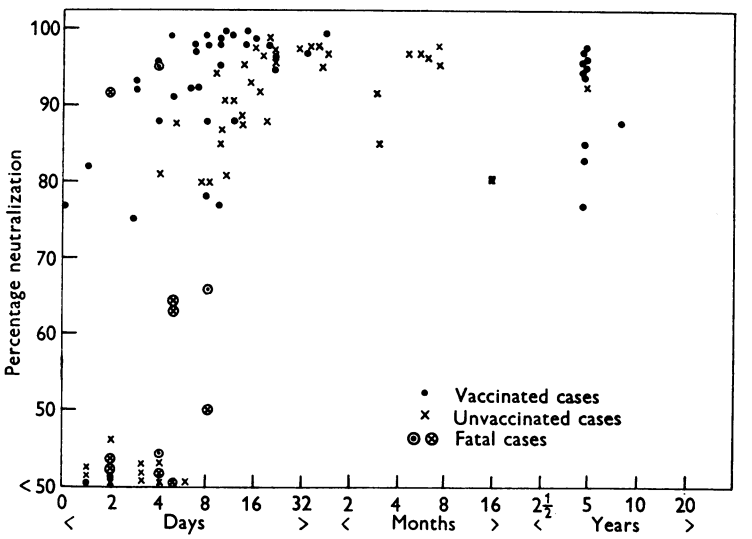

Fig. 1. Variola-neutralizing antibody in sera from cases of smallpox.

sponding antibody. By these criteria all sera taken after the ninth day and before 5 weeks after the onset of illness had all three antibodies (Figs. 1, 2 and 3); but in the vaccinated cases the time of appearance, the titre and the persistence of these antibodies differed from that found in unvaccinated cases.

Neutralizing antibody

Unvaccinated cases

Excluding sera from those who subsequently died four of seven sera examined between the third and ninth day of illness had fairly high levels of neutralizing activity, i.e. from 80 to 90 % (Fig. 1). All sera had neutralizing antibody after the sixth day. The level reached was higher than that seen following primary vaccination, but of the same order as that in generalized vaccinia and in some persons following revaccination (see preceding paper, McCarthy, Downie & Bradley, 1958, Figs. 2 and 6). Up to 8 months from the onset of illness, there was no appreciable fall and, in one case examined, a high level of antibody was present after 4½ years.

It should be noted, however, that where, as here, neutralization levels with undiluted sera are high, differences in antibody content can only be determined by testing the sera in dilutions, and this was not done in all instances.

Vaccinated cases

Neutralizing antibody was present earlier in these cases than in the unvaccinated (Fig. 1). It is possible that some antibody had persisted from the prior vaccination; with the exception of one fourth-day serum from a patient who subsequently died, all sera showed neutralizing antibody after the second day of illness. The patient who showed antibody on the first day of illness was a vaccinated nurse who suffered from *variola sine eruptione* and the patient whose serum showed antibody on the second day was a mild case with a modified rash. It is to be noted that not

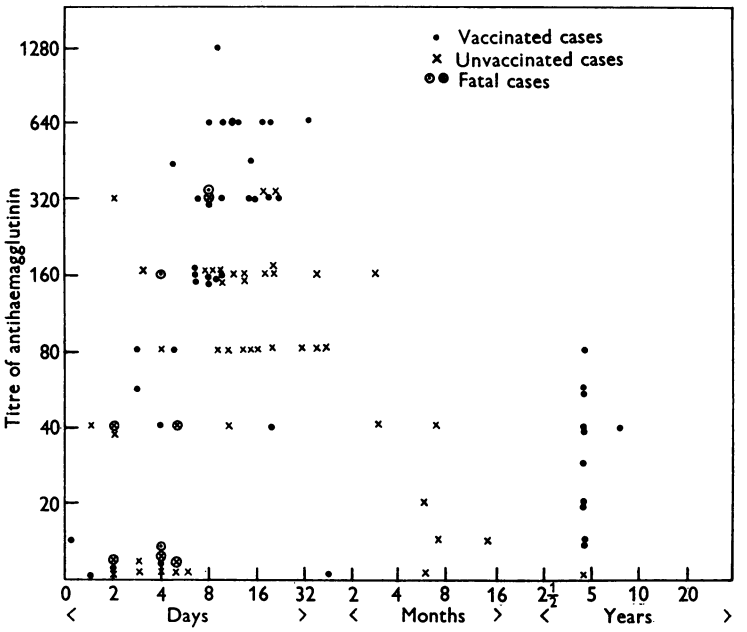

Fig. 2. Antihaemagglutinin in the sera from cases of smallpox.

only did antibody appear earlier in the vaccinated, but it also reached high levels sooner than in the unvaccinated (Fig. 1). After 5 years the level of antibody was still relatively high.

Antihaemagglutinin

Unvaccinated cases

In a few patients this antibody appeared as early as the second or third day of illness but in some had not appeared until a few days later (Fig. 2). All showed antihaemagglutinin after the seventh day. This is in agreement with the findings of Collier, Smit and v. Heerde (1950) although the titres we observed were not so high nor so greatly in excess of post-vaccination titres as they report. It would appear that antihaemagglutinins in the unvaccinated cases do not persist at a high level for more than a few months.

Vaccinated cases

Antihaemagglutinin is present from early in the illness as in unvaccinated cases. The titres reached are, however, higher and seem to persist longer (Fig. 2); nine of eleven sera examined after 5–8 years were still positive at a dilution of 1/20 or higher.

Unvaccinated cases *Complement-fixing antibody*

These appeared about the eighth to tenth day of illness and continued to rise to a peak titre of 1/40 or 1/80 about the fourteenth to sixteenth day, and, although declining, remained at a detectable level for several months (Fig. 3). It should be

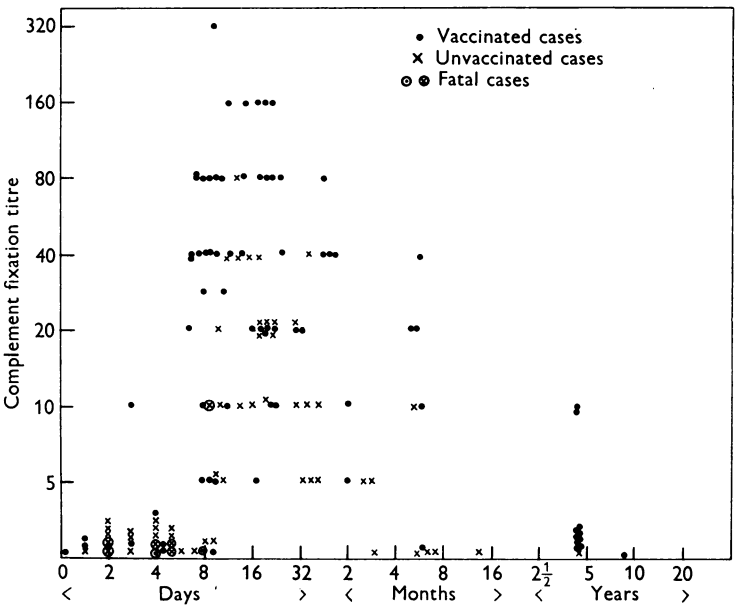

Fig. 3. Complement-fixing antibody in the sera from cases of smallpox.

noted that in contrast with our findings in vaccinated or revaccinated persons (McCarthy, Downie & Bradley, 1958) all the smallpox patients showed complement-fixing antibody in response to their infection.

Vaccinated cases

Complement-fixing antibody can be seen to have appeared earlier and to have reached higher levels than in unvaccinated cases (Fig. 3). The titres in many instances were higher than those found after vaccination or revaccination. Antibody was still present in four of five cases examined at 6 months but was present in only two of ten sera examined after $4\frac{1}{2}$ years.

Unvaccinated cases *Antibody response in fatal cases*

From the seven fatal unvaccinated cases (variola major) no specimens of serum were obtained later than 8 days from the onset of illness. Only one specimen,

taken on the eighth day, showed complement-fixing antibody (Fig. 3) and the other specimens were obtained before a rise in such antibody was to be expected in unvaccinated patients. Three showed antihaemagglutinins (Fig. 2) and although the number of specimens from fatal cases is small the results do not appear to indicate a failure of production of antihaemagglutinin. On the other hand, the neutralizing antibody levels were, with one exception, relatively low or absent (Fig. 1). The exceptional case was a middle-aged woman who died of confluent smallpox and whose serum after 2 days' illness failed to fix complement, was not tested for antihaemagglutinin, but showed a high level of neutralizing antibody when tested on two separate occasions. This was a most unexpected finding which suggests that the vaccination history may have been erroneous.

Vaccinated cases

Of the sixty-six cases of variola major from whom serum was available three died. All were men over fifty years old who had been vaccinated in infancy but not since. One of these cases died on the tenth day of confluent smallpox. On the eighth day of illness his serum failed to fix complement, had an antihaemagglutinin titre of 1/320 but its neutralizing activity was comparatively poor. The second patient died of haemorrhagic smallpox after 4 days; blood, taken after death, contained 500–1000 infective virus particles in 0·1 ml.; the serum was not tested for complement-fixing antibody but showed no antihaemagglutinin or neutralizing antibody. The third patient also died of fulminating smallpox on the fourth day of illness. Serum obtained several hours before death showed no complement-fixing antibody, but had an antihaemagglutination titre of 1/160 and had a high level of neutralizing activity. Virus was present in lysed blood clot which also yielded a pneumococcus on culture.

From these limited observations it would be unjustifiable to draw firm conclusions; but in at least two of the patients death occurred in spite of the presence of good levels of neutralizing antibody in the serum after several days' illness. In all probability it is the antibody level at the commencement of illness, or just before, which might effectively limit the viraemia. It would appear likely that if viraemia were heavy at the onset of illness then a subsequent antibody response, even if fairly prompt, might come too late to prevent widespread dissemination of the virus to cells of the skin and other organs where it would grow unimpeded by antibody. This view is supported by the observations of Downie *et al.* (1953) on the unfavourable prognostic significance of a severe viraemia, or the presence of virus antigen in detectable amounts, in the blood during the early stage of illness.

Variola sine eruptione

In the Table there are listed six close contacts of smallpox cases who, 10 or more days after contact, developed febrile illnesses without eruption. The first two cases occurred in the Middlesex outbreak in 1944 (Bradley, Davies & Durante, 1946). The first, a nurse, was revaccinated on the day of contact with a resulting early accelerated type of skin 'take'. Ten days later she had a rise of temperature which

reached 102·4° F. after 3 days and had returned to normal after two more days. The levels of neutralizing and complement-fixing antibody were higher than would be expected after revaccination and indicate that the febrile attack was due to an abortive smallpox infection. The second case, also a nurse, had had her primary vaccination 12 months previously and was not revaccinated. She had an illness which seemed clinically to be chickenpox and because there was smallpox in the hospital, crusts and a specimen of blood were sent to us for examination; these examinations excluded the posssibilty of smallpox infection at this time. However, 4 days later and 12 days after close contact with smallpox patients she

Table. *Cases of* variola sine eruptione

Patients' age and sex	History*			Antibodies in sera		
	Last vaccination	Contact with smallpox	Serum collection	Neutral.†	C.F.	A.H.A.
28 years (F.)	D − 10 days (revaccinated)	D − 10 days	D + 17 days	N.T.	160	N.T.
			D + 36 days	1000 R.I.D.	80	N.T.
			D + 177 days	100 R.I.D.	20	N.T.
20 years (F.)	D − 1 year (primary)	D − 12 days	D − 4 days	77 % C.A.M.	—	10
			D + 8 days	98 % C.A.M.	80	160
			D + 44 days	99 % C.A.M.	40	—
			D + 175 days	N.T.	20	N.T.
26 years (F.)	D − 4 years (primary)	D − 25 to D	D + 7 days	N.T.	80	N.T.
Adult (M.)	D − 7 years (revaccinated)	D − 12 days	D + 10 days	98 % C.A.M.	320	1280
33 years (F.)	D − 33 years (primary)	D − 12 days	D + 10 days	99 % C.A.M.	5	320
			D + 19 days	N.T.	20	320
33 years (M.)	D − 4 years (revaccinated)	D − 10 days	D + 11 days	100 % C.A.M.	30	160

* D = day of onset of illness; N.T. = not tested.

† Neutralizing antibodies expressed as either number of rabbit skin infecting doses of vaccinia virus neutralized (R.I.D.), or percentage reduction in variola pock count on the chorio-allantois (C.A.M.) effected by serum.

suffered a febrile illness (temperature 103° F.) lasting 3 days but no rash followed. Subsequent specimens of serum showed the development of high titre antibody by all three kinds of test.

The third patient is 'nurse L' mentioned by Boul & Corfield (1946). As she had not been revaccinated since her primary vaccination 4 years previously the finding of complement-fixing antibody in so high a titre as 1/80 in serum taken 7 days after the onset of her illness is evidence that this was due to smallpox infection. The last three subjects had been vaccinated some years before contact with cases in the 1953 outbreak of variola major (Lyons & Dixon, 1953). Two were the son and daughter of a missed fatal case of smallpox and the third a pathologist who had carried out an autopsy on another missed case. The high titres of antibody, particularly complement-fixing, in the absence of recent vaccination in these three persons provide the evidence that the febrile illnesses which they experienced were abortive attacks of smallpox.

In cases of this kind isolation of virus from blood on the first day of fever might

provide definite proof of smallpox infection. Such specimens were not available to us, but the findings in the Table provide evidence, not hitherto available, establishing the variolous nature of such 'contact fevers'.

DISCUSSION

Most reported studies on serum antibodies in smallpox cases have been made with vaccinia virus and its antigens. For the *in vitro* tests—complement fixation and haemagglutination inhibition—this choice seems to be justified. It has not been possible to show differences when vaccinial antigens were substituted for variola antigens in these tests and there are practical advantages in the use of the former. In neutralization tests on the other hand, where one is estimating protective antibody, the use of variola virus would seem desirable on general principles and is preferable for reasons which have been previously mentioned (McCarthy, Downie & Armitage, 1958).

In the unvaccinated smallpox cases, with one exception where there was some doubt as to the vaccination history, neutralizing antibody was usually present from the fourth or fifth day. At this time, although the rash is developing, the temperature has come down and, in most cases, the patient feels better. Antihaemagglutinin sometimes appeared earlier and complement-fixing antibody somewhat later. If one reckons the incubation period of smallpox at 12 days, it would seem that the antibody response in unvaccinated smallpox cases occurs a day or two later, after initial infection, than it appears after primary vaccination. Presumably this is due to the larger infecting dose and more rapid evolution of the vaccinial infection. On the other hand, the degree of response in smallpox is greater in that antibody levels are higher, particularly neutralizing antibody; and whereas only some persons show complement-fixing antibody after primary vaccination, this antibody, in our experience, is always to be found in smallpox convalescents. These differences are not unexpected when one considers the severity of infection and consequent greater antigenic stimulus in smallpox. De Jong (1956) failed to demonstrate complement-fixing antibody in five of forty cases of alastrim, but, of those sera from alastrim cases that we examined after the ninth day of disease, all gave positive results (McCarthy & Downie, 1953).

In the smallpox cases who had had previous vaccination, antibody appeared earlier and levels generally were higher for all three antibodies. This was not surprising as the smallpox infection must be considered as providing a secondary antigenic stimulus in persons who had previously been immunized. While the antihaemagglutinin titres in unvaccinated cases were not very much higher than after primary vaccination, the levels in the vaccinated cases were considerably in excess of those seen after primary or after revaccination. For reasons mentioned previously (McCarthy, Downie & Bradley, 1958), the titres found by us both after vaccination and in smallpox cases were considerably lower than those recorded by Collier *et al.* (1950), but in vaccinated smallpox cases the relative differences in titre approached those reported by Collier *et al.* (1950). These authors, however, do not give clear information about the vaccination histories of their smallpox patients.

In many instances there was no close correlation between the titre of the three antibodies in individual smallpox sera. A similar lack of correlation was evident in the sera from vaccinated and revaccinated persons. The three antibodies seem to be independent of each other as shown by Chu (1948) in the sera of vaccinated rabbits. It would seem that one is not justified in regarding the antihaemagglutinin titre as a measure of immunity to infection, as can be done in relation to influenza virus infection.

In the fatal cases from which serum was examined neutralizing antibodies were, for the most part, absent or present only in low titre; a fairly high level was present, however, in two patients on the second and fourth day of illness. The antibody level in these two patients was, of course, unknown at the onset of illness, when severe viraemia must have occurred, but it seems unlikely that antibody was high at this time. The presence of antibody within a few days of onset failed to prevent the progress of the disease to a fatal termination.

When laboratory tests for the confirmation of a clinical diagnosis are required such confirmation is readily achieved in the average case by the demonstration of virus or virus antigen in the blood or in the skin lesions of the patient. It is in the retrospective diagnosis of missed atypical cases, or in the confirmation of abortive infections without skin lesions, that tests of the patient's serum for antibody may give useful information. Such infections usually occur in previously vaccinated individuals and blood samples are rarely taken in the early stages of the disease; so that determination of a rise in titre of antibody by the examination of an early and late specimen of serum is not usually possible. As neutralizing antibody may be present for years after vaccination, the examination for this antibody will not usually be very informative in previously vaccinated persons, unless the titre is high as determined by tests with serum dilutions. Antihaemagglutinins may, in some cases, still be present for a few years after vaccination, so that in a single specimen of serum, only a high titre could be interpreted as indicating an atypical variola infection. Complement-fixing antibodies on the other hand are not always present after vaccination or revaccination and are not to be found six or eight months later. The presence of demonstrable complement-fixing antibody, therefore, in a patient who has not been vaccinated within the previous year, is good evidence of a recent variola infection. Later samples of serum may show that the titre diminishes from three weeks after the onset of infection, as in the recent case reported by Andres, Lieske, Lippelt, Mannweiler, Nielsen, Peters & Seelemann (1958). The demonstration of complement-fixing antibody in the serum of patients has been used by itself as a laboratory diagnostic test in smallpox (Sindo & Nisimura, 1940 and Sindo, Nisimura & Nagai, 1941), but it is not usually positive at a sufficiently early stage of the disease to be useful as a routine diagnostic method.

SUMMARY

From 117 cases of smallpox 140 sera have been examind for antihaemagglutinin, for complement-fixing and for variola-neutralizng antibody.

In smallpox patients who had not been vaccinated prior to infection, variola-neutralizing antibody and vaccinial antihaemagglutinin were present in all sera

examined after the sixth day of illness. Complement-fixing antibody was not found until the eighth day of illness but was present in all examined after the ninth day.

In previously revaccinated smallpox patients variola-neutralizing antibody was present in all sera examined after the third day of illness and antihaemagglutinins in all after the fourth day. Complement-fixing antibody was present by the seventh day and was found in all sera examined after the ninth day. Not only did antibody in general appear earlier in previously vaccinated smallpox patients but the titres were generally considerably higher. In ten fatal cases antibody titres were low except in two in whom the level of variola-neutralizing antibody was quite high—in one case on the second day and in the other on the fourth day of illness.

In six variola contacts, who suffered febrile attacks without rash, antibody studies indicated the variolous nature of their illnesses. In these and other atypical smallpox infections, when laboratory confirmation of clinical diagnosis is only requested at a late stage of the disease, a high titre of antibody, particularly complement-fixing antibody, may enable a firm diagnosis to be made.

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