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A note on the immunogenicity of ultra-violet irradiated vaccinia virus in man

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INTRODUCTION

Collier, McClean & Vallet (1955) reported that vaccinia virus inactivated by exposure to ultra-violet irradiation under strictly controlled conditions stimulated immunity in rabbits and, to a lesser extent, in monkeys. This was measured by relative resistance to challenge with living virus and by the titration of circulating antibody after two injections of the irradiated vaccine. The immunity induced in rabbits was so satisfactory and uniform as to justify an attempt to immunize man with irradiated virus as a means of diminishing the risk of complications and reducing the severity of the reaction to subsequent Jennerian vaccination with living virus. This paper records tests in man of ultra-violet irradiated vaccine.

METHODS

The Lister Institute strain of vaccinia virus was used. A partially purified suspension was prepared from crude vaccinial pulp by differential centrifugation. Its infectivity before irradiation, estimated from pock counts in the chorioallantoic membranes of chick embryos (Westwood, Phipps & Boulter, 1957), was 4×10^7 infectious units/ml.

The elimination of bacterial contaminants, irradiation of the suspensions and subsequent tests for inactivation of the virus were done by the methods described previously (Collier *et al.* 1955). The test for inactivation of virus was more rigorous, however; 200 ml. instead of 8 ml. of irradiated suspension were tested after 125-fold concentration on the centrifuge.

In some of the rabbit and monkey experiments previously described, suspensions dried with 0·5 % peptone were used. We thought it undesirable to inject peptone subcutaneously into human volunteers. Gelatine (0·5 % (w/v)) proved to be as good a stabilizer as peptone both during freeze-drying and subsequent storage. Tests of immunogenicity in rabbits were made both before and after drying. To avoid any risk of contamination with living virus in the laboratory environment, the freeze-drying was done at the Microbiological Research Establishment, Porton, by Dr B. Record to whom we are much indebted.

One batch of freeze-dried irradiated vaccine was used for all the tests on human volunteers; during the experiments it was repeatedly tested for its immunogenicity in groups of five rabbits both by challenge with living virus and by titration of circulating antibody (Collier *et al.* 1955). The results were uniformly satisfactory.

Human volunteers were given five times the dose of vaccine used for rabbits. Concentration was effected by reconstituting the dried vaccine to 1/5 of its original volume. A sample of blood was taken from each man before vaccination; two doses of vaccine were given subcutaneously at intervals of 14 days; and a fortnight after the second dose the men were bled again and challenged by vaccination with living virus. With two exceptions which are noted (Table 2) the men included in this trial had no record of previous vaccination and no detectable vaccination scars; the two exceptions were men vaccinated in early childhood.

RESULTS

Two pilot tests (Table 1) were made, each on six volunteers. In the first, three did not react to challenge with living virus, one had an accelerated reaction and two had typical primary reactions. The sera of all six men contained antibody but, in contrast with the results when rabbits were immunized, there was no correlation of the antibody titres with the reaction to challenge. In the second pilot test,

Table 1. *The response of* 12 *male volunteers to challenge with potent smallpox vaccine after immunization with irradiated vaccinia virus*

	Volunteer no.	Skin reaction	Antibody titre*
Test 1	4	Primary	8
	5	Primary	8
	3	Accelerated	4
	1	Negative	8
	6	Negative	8
	2	Negative	16
Test 2	8	Primary	< 4
	9	Primary	< 4
	10	Primary	< 4
	11	Primary	< 4
	7	Primary	8
	12	Negative	16

* Reciprocal of the highest dilution of serum neutralizing virus infection in the scarified skins of normal rabbits.

five of the six men had normal primary reactions to vaccination. Only two had circulating antibody. Since about half the men in these two tests developed skin immunity, a larger test was made on 25 volunteers (Table 2). Clearly, a vaccine consisting of virus inactivated by ultra-violet irradiation is unlikely to be useful in the immunization of man. The reaction to challenge was unmodified in 15 of the 25 men; 10 of these 15 had no detectable circulating antibody. There was poor correlation between reaction to challenge and the presence of circulating antibody. Ten of the 25 had skin immunity and, of these, seven (including the two previously vaccinated), had measurable circulating antibody; thus, skin immunity generally, but not always, indicates circulating antibody but not vice versa.

When these tests were complete, the irradiated preparation was tested in rabbits; it induced resistance to challenge and titres of circulating antibody similar to

those obtained in previous studies. Thus the failure in man could not be attributed to deterioration of the vaccine.

DISCUSSION

The difference between rabbits and men in their immune response to irradiated virus was much greater than expected; the rabbits consistently produced circulating antibody and resistance to challenge, whereas in the men, the immune response was not uniform. Previous experience had warned us that it might prove easier to immunize rabbits than men. In their investigation of the immunity following intracutaneous and subcutaneous vaccination with elementary body suspensions of vaccinia, Henderson & McClean (1939) found that suspensions of living virus, given either intracutaneously or subcutaneously, produced a firm immunity in rabbits; but human volunteers were not protected unless a vesicle was accidentally produced in the epidermis at the point of needle entry. In a

Table 2. *The response of* 25 *male volunteers to challenge with potent smallpox vaccine after immunization with irradiated vaccinia virus*

Volunteer no.	Skin reaction	Antibody titre*
14	Primary	< 4
17	Primary	< 4
19	Primary	< 4
22	Primary	< 4
25	Primary	< 4
28	Primary	< 4
29	Primary	< 4
30	Primary	< 4
31	Primary	< 4
32	Primary	< 4
33	Primary	> 8
18	Primary	16
21	Primary	16
20	Primary	> 32
23	Primary	> 32
36	Modified	< 4
37	Modified	< 4
13	Modified	4
35	Modified	4
27	Modified	> 8
15	Modified	> 32
26†	Modified	> 8
34†	Modified	> 8
24	Negative	< 4
16	Negative	> 32

* See Table 1.
† Previously vaccinated many years ago.

recent study of vaccinia virus inactivated by formaldehyde, Amies (1961) reported that the immunogenic properties of the virus decreased in proportion to the fall in infectivity; samples in which no living virus could be detected were not immunogenic. Rabbits inoculated with formalinized suspensions containing only a

trace of living virus produced a firm immunity to challenge and circulating antibody. On the other hand, rabbits immunized with completely inactivated virus were fully susceptible to vaccination although they had high titres of circulating antibody demonstrable by neutralization tests in tissue cultures. Similar tests in the skin of a living rabbit, however, failed to show any antibody. Amies suggests that this result indicates impairment of the antigen in completely inactivated virus so that it induces a less avid antibody. Full susceptibility to vaccinia virus can exist in the presence of detectable antibody, as we found in some of our volunteers.

Mahnel (1961), on the other hand, reported that vaccinia virus inactivated by 0·03 % formalin stimulated immunity in rabbits after one intramuscular injection. However, as the degree of immunity depended on the length of the period between injection and challenge and not on the mass of antigen injected, a suspicion must remain that the inactivation of virus infectivity was incomplete. Appleyard (1961) recently obtained a soluble immunizing, or 'serum blocking', antigen from cells and tissues infected with either vaccinia or rabbit pox virus. It will be interesting to see whether this antigen also proves to be much more effective in the rabbit than in man.

In addition to the doubts cast by our results on the efficacy of non-infectious smallpox vaccine they also draw attention to an apparently anomalous situation. Skin immunity usually, but not always, indicates circulating antibody, but the presence of antibody does not necessarily confer immunity to skin infection with vaccinia virus. This problem is being studied.

SUMMARY

A preparation of vaccinia virus inactivated by ultra-violet irradiation under strictly controlled conditions, and shown to produce neutralizing antibody and resistance to challenge in rabbits, was active in only about half of 37 human volunteers. It was not, therefore, likely to be usefully immunogenic in man. In the largest single test, 15 of 25 men had unmodified primary skin reactions when challenged with living virus, although five of these had circulating antibody. On the other hand, only three of 10 men who responded to challenge with modified or negative reactions, had no circulating antibody.

In man the presence of circulating antibody does not necessarily indicate resistance to infection in the skin, nor does skin immunity always indicate the presence of circulating antibody.

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