Immunofluorescence Test for Persistent Poxvirus Antibodies

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Summary

A collection of 408 human sera and 165 monkey sera were examined for vaccinia and monkeypox antibodies respectively by immunofluorescence (IF). Neutralization (N) was performed for comparison in 266 of the human and all monkey sera.

Sera of persons examined within 10 years after vaccination showed similar results with IF and N. Only one of 93 sera tested in this period showed discordant results, being IF--N+. 91 sera were IF+N+ and one IF−N−.

Out of 73 samples, which were taken more than 10 years after vaccination, 74 per cent were IF− and 77 per cent N+. Discordant results were obtained almost exclusively in these late samples, 8 of which were IF−N+ and 7 IF+N−. The median titre of IF decreased from 512 in the most recently vaccinated group to 32 in the group which was vaccinated ≥51 years previously. The median titre of N decreased from 361 to 28 in the same period.

No positive reactions were found in 138 human pre-immunization sera by IF, neither in 100 of these pre-sera when tested by neutralization. Sera of 154 wild-caught monkeys were negative with one exception being IF+N− in dilutions up to 8.

Immunofluorescence appeared a specific and sensitive test for persistent vaccinia antibodies.

1. Introduction

It is known that neutralizing antibodies after smallpox vaccination may persist for many years. These antibodies have a protective effect against smallpox by checking viremia. Virus neutralizing antibodies can be titrated by reduction of pock- or plaque-count. The assay of these antibodies, however, is laborious and plaque-count reduction seems to give difficulties when applied in some systems.

More recently fluorescent antibody techniques were applied to variola infected cell-counting (5) and to detection of ectromelia virus (3). Immunofluorescence was referred to as a more sensitive and reliable method for ectromelia antibody assay than hemagglutination inhibition.

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This report describes a comparative study of immunofluorescence (IF) and neutralization (N) as methods of testing for persistent vaccinia antibodies. Monkeypox antibodies were involved to some extent in this study.

2. Materials and Methods

2.1. Virus Strains

The Elstree vaccinia strain was used for preparing suspensions and antigens in serological techniques.

2.2. Reference Serum

A reference antiserum (AK) was prepared from the blood of a donor who had been vaccinated against smallpox. This serum was used for parallel tests throughout this study.

2.3. Test Sera

Sera of 138 non-vaccinated persons were taken at the time of primary vaccination. Sera from 100 of them were collected again during convalescence of primary vaccination, 94 in the 3rd or 4th week and 6 in the 5th—10th week after inoculation. Sera of 170 vaccinated persons were collected at the time of revaccination.

A collection of 154 sera of wild-caught cynomolgus monkey sera (Macacus irus) from Kuala Lumpur were received through the intermediary of Dr. I. Arita, Smallpox Eradication Unit WHO and were described elsewhere (1).

Seven cynomolgus immune sera were prepared against the Copenhagen isolate of monkeypox virus. Two monkeypox immune baboon sera were sent by Dr. S. S. Kalter. Sera of two orang utans which had suffered from naturally acquired monkeypox nearly 6 years previously (6), were received from Dr. L. L. E. Beuvery, Rotterdam Zoo.

2.4. Neutralization

A 50 per cent pock count reduction technique in chick chorioallantoic membranes as described by Boultet (2) was used. Sera were inactivated at 56° C for 30 minutes. Frozen stock suspension of vaccinia Elstree virus (—70° C) in one-test-ampoules was thawed and diluted with 10 per cent skimmed milk in distilled water. Sera of vaccinated individuals were titrated with 4-fold serum dilutions in 10 per cent skimmed milk. Sera of non-vaccinated were screened in 1-4 dilution. Pock count reduction ≥50 per cent was accepted as a positive reaction. The Copenhagen isolate of monkeypox virus was used for neutralization tests in monkey sera.

2.5. Immunofluorescence

An indirect method of immunofluorescence was used. The antigen consisted of vaccinia infected BHK-21 cells produced in suspension culture. The cells were concentrated by low speed centrifugation to 2.10⁶ cells per ml of Eagle’s medium with 10 per cent calf serum and 5 per cent dimethylsulfoxide. Ampoules were filled with 1 ml of the concentrated cells. After sealing the ampoules were kept at 4° C for 1 hour and then cooled at a speed of 1° C per minute for storing in liquid nitrogen.

Smears were made from an ampoule of frozen cell suspension by thawing in a water bath at 37° C and two washings with Eagle’s medium with 10 per cent calf serum to remove DMSO. After the last washing the cells were suspended into 0.3 ml Eagle’s medium with 10 per cent calf serum. Two smears were prepared on each of a series of fluorescence-free microscopic glass slides. The smears were dried for ½ hour. Fixation took place during submersion in precooled methanol at —70° C for ½ hour. The antigen preparations were stored in containers at —70° C.

Coded test sera were absorbed with hamster kidney powder and with 3 volumes 20 per cent hamster brain-egg yolk suspension. Two fold dilutions of the absorbed test sera in veronal buffered saline pH 7.2 containing 10 per cent fresh guinea pig serum were put on the smears for the virus-antibody reaction as described previously (4). The slides were washed for 10 minutes by submersion in phosphate buffered salt solu-