An Antigenically Atypical Strain of Variola Virus

(Accepted 21 July 1976)

SUMMARY

The Kuwait-5-67 strain of variola virus, although indistinguishable from variola virus in its other properties, behaved atypically in immuno-diffusion tests with antivaccinia serum. The precipitation line pattern produced was similar to that of cowpox virus but lacked one of the main precipitation bands given by all other strains of variola, alastrim and vaccinia viruses tested. Trypsin treatment of cowpox virus preparations revealed the missing precipitation line but did not do so with the Kuwait-5-67 strain. The antivaccinia serum absorbed by Kuwait-5-67 strain lost its ability to interact with this strain, but still gave precipitation lines with variola, vaccinia and cowpox viruses.

Although in recent years the variability of variola virus has been examined in relation to certain markers (Bedson, Dumbell & Thomas, 1963; Sarkar & Mitra, 1966, 1967, 1968; Shafikova & Marennikova, 1970; Dumbell & Huq, 1975), differences in the antigenic properties of separate strains of this virus have not previously been reported. In this paper we present results obtained with the Kuwait-5-67 strain of variola virus which show a difference.

The Kuwait-5-67 strain was isolated from an outbreak of smallpox in Kuwait in 1967 which affected 41 people with a mortality rate of 38%. The infection was imported from Pakistan (Arita, Shafa & Kader, 1970) and the isolate was made from crusts taken from a 25-year-old man (Mefreh Ali) with clinical features typical of smallpox at the scabbing stage. Retrospective analysis revealed that the patient was in the third generation of the outbreak. The Kuwait-5-67 strain was isolated by two methods simultaneously: on 12-day-old chick embryos (CE) and in a continuous line of human amnion cells. Focal c.p.e., typical of variola virus, was observed as early as 48 h post inoculation. Pock lesions—white, prominent, with distinct margins, 0.5 to 1 mm in diam.—developed on the chorioallantoic membranes (CAM) of all inoculated CE. Further tests of this strain showed that it gave no reaction on rabbit’s scarified skin (dose $10^6$ pox forming units [pk.f.u.]), and only a soft, pink infiltrate, 7.5 mm in diam., following intracutaneous inoculation. The strain did not cause pock development on the CAM at an incubation temperature of 38.7 °C (dose of 100 pk.f.u.). At 38.5 °C, 8% of pocks (compared with 100% at 35 °C) developed. Inoculated with this dose, 25% of infected CE died with an average survival time of 5.9 ± 0.17 days. Following inoculation on the CAM with $10^5$ pk.f.u. the virus accumulated in CE liver to $10^7$ pk.f.u/g.

The patient’s specimen itself (crust suspensions) gave negative results in gel precipitation tests (GPT) with antivaccinia serum. Similar tests on the isolate showed that it belonged to the poxvirus group, but that the precipitating lines were different from those formed by the reference strain of variola virus (Harvey). Two modifications of GPT were devised for the further study of the antigenic properties of Kuwait-5-67 strain, using 0.9% agarose (1 mm thick). In the first experiment the immune serum and virus antigens were placed in the wells (4 mm in diam., 4 mm apart). In the second experiment GPT was performed according to
Fig. 1. Immunoprecipitin test with the Kuwait-5-67 strain of variola virus. Wells 1, 3 and 5 contained the Kuwait 5-67 strain; well 2, Harvey strain; well 4, vaccinia strain, well 6, cowpox strain. Central wells: (a) antivaccinia serum; (b) antivaccinia serum absorbed with the Kuwait strain.

Gispen & Brand-Saathof (1974) with templates. The antiserum to vaccinia virus was prepared by hyperimmunization of rabbits (Gispen, 1955). For special tests the serum (undiluted and in dilution 1:2 and 1:4; Gispen & Brand-Saathof, 1974) was absorbed by Kuwait-5-67 strain. Treatment with trypsin was made according to Rondle & Dumbell (1962). CAM as well as CAM suspensions were treated with trypsin (SPOFA-CSSR). The latter were first subject to centrifugation for 30 min at 2000 and 6500 g. Sixty-seven variola virus strains from 13 different countries, 3 alastrim virus strains, 4 vaccinia virus strains (EM-63, B-51, Tashkent, Lister Institute strain) as well as two cowpox virus strains (Brighton and that obtained from Holland in 1973) were all tested in the form of 20 % CAM suspensions with 0.8 to 2 × 10^8 pfu/ml activity.

All strains of variola, alastrim and vaccinia viruses tested with the sole exception of Kuwait-5-67 strain gave the same line patterns and complete serological identity in tests with the several batches of antivaccinia serum used. Precipitation bands formed by Kuwait-5-67 strain differed greatly and were similar but not identical to those of cowpox virus; the latter formed a fine extra line which appeared later (Fig. 1a). We found the same precipitation pattern with all preparations of Kuwait-5-67 strain, i.e. 12 CAM suspensions from the first to tenth passages, and also with antigens prepared after passage twice in both monkey kidney cell cultures and in a continuous line of human amnion cells.

For a more thorough study of the serological relationship between Kuwait-5-67 and cowpox viruses, special tests were performed. Because the missing precipitation line might depend on antigen present in a non-diffusable form (as was demonstrated by Rondle & Dumbell, 1962, for cowpox virus) we have examined this possibility with Kuwait-5-67 strain. Extracts of Kuwait-5-67 strain were therefore treated with trypsin, but unlike cowpox virus, no additional zones of precipitation were obtained. Control experiments confirmed that the trypsin treatment released an additional antigen with cowpox virus, the treated preparation forming a distinct spur with the untreated one. Furthermore, antivaccinia serum absorbed with Kuwait-5-67 and hence no longer able to form a precipitation zone in GPT still retained the ability to precipitate with variola, vaccinia and cowpox viruses. However, with the latter the precipitation zone was less distinct (Fig. 1b). Virtually the same results were obtained in a newly developed two-step GPT (Chumakov et al. 1974; Maltseva & Marennikova, 1976), when Kuwait-5-67 was the initial (absorbing) virus and variola, vaccinia and cowpox were used as test viruses. In this case, in addition to the precipitation zones formed by the interaction of the serum with the absorbing virus (Fig. 2a), new zones appeared with the test viruses in all wells except the control with Kuwait-5-67 (Fig. 2b).

The experiments performed clearly demonstrated the peculiar behaviour in GPT of the
Two-step gel precipitation test. Well 1, Kuwait strain; well 2, Harvey strain; well 3, vaccinia virus; well 4, cowpox virus. Central well, vaccinia antiserum. (a) Result before adding test virus; (b) result after adding test virus.

variola virus strain Kuwait-5-67. By its antigenic pattern (in this test) it appeared closer to cowpox virus. However, we have failed to show similarity of these viruses either by trypsin treatment or by the serum absorption test. More complete information on the degree of these differences may be obtained by further studies. However, even at this early stage it may be considered that for the first time the existence of an atypical variola virus strain has been shown, and this should be taken into consideration in diagnostic work.

Research Institute of Virus Preparations
Moscow, 109388, U.S.S.R.

S. S. MARENNIKOVA
E. M. SHELUKHINA
G. R. MATSEVICH
N. A. HABAHPASHEVA

REFERENCES


(Received 13 October 1975)