

## Studies on a Strain of Vaccinia Virus Defective in Surface Antigen Production (36461)

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Appearance of surface antigen(s) with virus specificity has been demonstrated on cells infected with poxviruses by immunofluorescence and mixed agglutination (1-3). All poxviruses tested, including strains of vaccinia, variola, cowpox, and fibroma virus, have been found to produce surface antigen. The antigen was considered to be among the early viral-coded antigens, since synthesis occurred in the presence of DNA inhibitors (1, 2).

A strain of vaccinia virus, V1-010 was obtained as part of a virology proficiency test survey. When this strain was examined for production of surface antigen it was found to be defective. Experiments performed on this strain and their relevance to production of surface antigens by poxviruses are the subject of the present report.

*Materials and Methods. Vaccinia strains.* V1-010 was received from Dr. Charles T. Hall, National Communicable Disease Center (NCDC), Atlanta. The strain was identified as originating from the Lister strain and a summary of the passage history at NCDC was as follows: rabbit skin 959; cell cultures—primary rabbit kidney 2, primary human amnion 1, AV3 1; chicken embryo chorioallantoic membrane (CAM) 1; cell cultures—FL 2, HEP-2 1. A strain designated here NY was originally derived from commercial calf lymph (4) and has been passaged in our laboratory in primary rhesus monkey kidney cell cultures. V1-010 and NY strains were passaged in a continuous line of rabbit kidney cells (BIRK) established in this laboratory (5). The infectivity titer for both strains was  $3 \times 10^7$  PFU/ml deter-

mined in the BGM line of African green monkey kidney cells (6).

*Antisera.* Antiserum to V1-010 (No. 286) was obtained from a rabbit immunized with virus grown in BIRK cells. The animal was injected intradermally and after a rest period of 4 weeks, a booster injection was given intravenously. Rabbit antiserum against NY (No. 254) was produced following a series of intravenous injections of virus propagated on CAM. A rabbit serum against surface antigen, anti-S (No. 27) was supplied by Dr. Y. Ueda, Dept. of Enteroviruses, National Institute of Health, Tokyo. Anti-S serum was prepared by immunization with crude surface antigen fraction (early antigen complex), obtained from rabbit kidney cell cultures infected with DI strain of vaccinia virus grown in the presence of cytosine arabinoside (Y. Ueda, unpublished data).

Neutralizing antibody titers as determined by 80% plaque reduction in BGM cell cultures for Nos. 286 and 254 were 640 and 2560, respectively; antibody titers were 64 and 128, respectively, as determined by the hemagglutination-inhibition (HI) test. The anti-S serum (No. 27) did not contain any detectable antivaccinia antibodies by neutralization or HI tests. This serum had an antibody titer of 300,000 as determined by the mixed agglutination test for surface antigen.

*Cell cultures.* HEP-2 cells were used throughout this study for detection of surface antigen. The cells were grown in Eagle's basal medium (BME) in a base of Hanks' balanced salt solution, penicillin (200 units/ml) and streptomycin (200  $\mu$ g/ml), supplemented with 10% unheated fetal calf serum (Gray Laboratories, Fort Lauderdale). After virus inoculation, the cultures were main-

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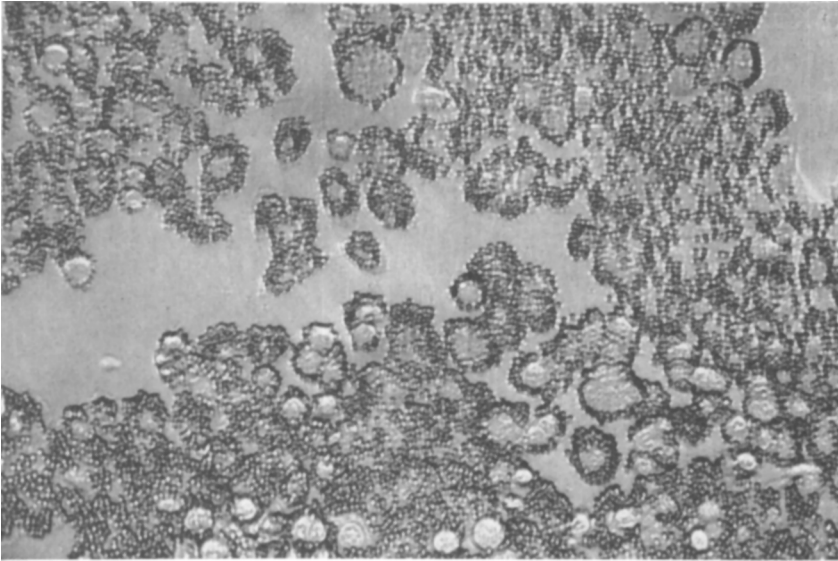


FIG. 1. Surface antigen on HEp-2 cells infected with NY strain of vaccinia, detected by mixed agglutination with anti-S serum (No. 27) at a dilution of 1:3000. Indicator cells adhere to cell surface diffusely (*D-type*) (100 $\times$ ).

tained in BME in a base of Earle's saline (sodium bicarbonate, 180 mg/100 ml), containing 3% fetal calf serum and antibiotics.

*Detection of surface antigen by mixed agglutination.* Culture tubes were inoculated with either V1-010 or NY at a low input multiplicity, approximately 0.03 PFU/cell, in order to avoid any effect of surface antigen present in the inoculum (M. Ito, unpublished data). The cultures were incubated at 36° for 20 hr. To prevent synthesis of late antigens which appear on the cell surface in some experiments, cultures inoculated with the vaccinia strains were maintained in the presence of cytosine arabinoside (Ara-C, 20  $\mu$ g/ml, Nutritional Biochemicals Corp., Cleveland). The presence of surface antigen was detected by the mixed agglutination procedure performed essentially as previously described (5, 7). Antisera were employed at dilutions of 1:1000–3000, which were previously determined as optimal for detection of surface antigen.

*Results.* The first indication that V1-010 had unusual surface antigen properties occurred when mixed agglutination experiments were performed using anti-S serum. The mixed agglutination results were completely negative with cell cultures infected with

V1-010 strain. A strong reaction, in which the indicator erythrocytes diffusely adhered to the infected cell surface (*D-type*) was obtained as expected with the NY strain (Fig. 1).

An experiment was conducted in which cell cultures were inoculated with V1-010 or NY strains in the presence and absence of Ara-C. Mixed agglutination tests were performed using anti-S, anti-V1-010, and anti-NY sera (Table I). In the absence of Ara-C, cultures infected with V1-010 strain yielded strong *D-type* mixed agglutination reactions with either homologous or anti-NY serum. Different reactions were observed when the sera were tested with cultures containing Ara-C. Although positive mixed agglutination results were obtained, the pattern was distinctly different and was found to appear on single cells (*S-type*) (Fig. 2). Anti-V1-010 serum also produced the *S-type* mixed agglutination reaction when tested against cultures infected with NY strain in the presence of Ara-C.

Hemagglutination and hemadsorption of sensitive chicken erythrocytes were also tested for V1-101 and NY strains (Table I). Positive results were obtained with both strains in the absence of Ara-C.

TABLE I. Comparison of Surface Antigen Reactions of Cells Infected with Two Vaccinia Virus Strains.

Vaccinia strain	Ara-C (20 µg/ml)	Surface antigen <sup>a</sup>			Hemagglutination <sup>b</sup> and hemadsorption <sup>c</sup> with chicken RBC
		S	antiserum V1-010	NY	
V1-010	Absent	Neg.	D <sup>d</sup>	D	Pos.
	Present	Neg.	S <sup>e</sup>	S	Neg. <sup>e</sup>
NY	Absent	D	D	D	Pos.
	Present	D	S	D	Neg. <sup>e</sup>

<sup>a</sup> Detected by mixed agglutination with each of the antisera at a dilution of 1:3000.

<sup>b</sup> Microtiter test using 0.5% suspension of susceptible chicken RBC at room temperature (25°).

<sup>c</sup> Infected cells were treated with 0.5% suspension of susceptible chicken RBC for 30 min at 36°.

<sup>d</sup> *D-Type* mixed agglutination reaction as shown in Fig. 1.

<sup>e</sup> *S-Type* mixed agglutination reaction as shown in Fig. 2.

Comparative infectivity titrations of V1-010 and NY were performed in cell cultures by plaque procedure and pock count on CAM. Equivalent titers were obtained for both viruses in the two assay systems.

*Discussion.* Surface antigens of poxviruses have been considered to be early antigens (1, 2) as distinguished from those which appear on the cell surface, such as hemagglutinin, late in the growth cycle (M. Ito, unpublished data). The positive results obtained with anti-V1-010 and anti-NY sera by mixed agglutination did not support the notion that V1-010 was completely defective in production of surface antigen as might have been assumed from the results with anti-S serum. Experiments performed with Ara-C showed that V1-010 strain synthesized surface antigen which could be distinguished from the NY strain serologically as well as by the pattern of mixed agglutination, *S-type* versus *D-type*.

Our experiments have led to the speculation that there are at least two types of surface antigens produced by poxviruses, which may be designated as major and minor. V1-010 strain would be deficient in synthesis of major antigen components, whereas NY strain produced both antigens. We have recently obtained further data (M. Ito, unpublished data) by indirect immunofluorescence which supports the concept of the existence of major and minor surface antigens. Ueda and Tagaya (personal communication)

have recently examined the V1-010 strain by direct immunofluorescence and complement fixation, and have confirmed that this strain is defective in production of surface antigen. These investigators also tested the Lister strain of vaccinia in their laboratory, which is the parent of V1-010, and found it to be defective.

V1-010 strain is not apparently defective in producing the late surface antigens such as hemagglutinin. Also *D-type* mixed agglutination reaction was obtained when cell cultures infected with either V1-010 or NY strains in the absence of Ara-C were tested by mixed agglutination with anti-V1-010 serum.

At present, the biological role of surface antigens in the growth of poxviruses is not completely understood. The fact that V1-010 strain appeared to grow equally well in cell cultures and on the CAM as the NY strain, indicates that the major surface antigen may not be an essential factor in viral growth. In addition, since hemagglutinin is synthesized by V1-010 in the absence of major surface antigen it could be concluded that this surface antigen is not a precursor of hemagglutinin.

To the best of our knowledge this is the first report of a mutant strain which is defective in the synthesis of surface antigen among those DNA containing viruses (poxviruses, herpesviruses, adenoviruses, and papovaviruses) which are known to produce surface

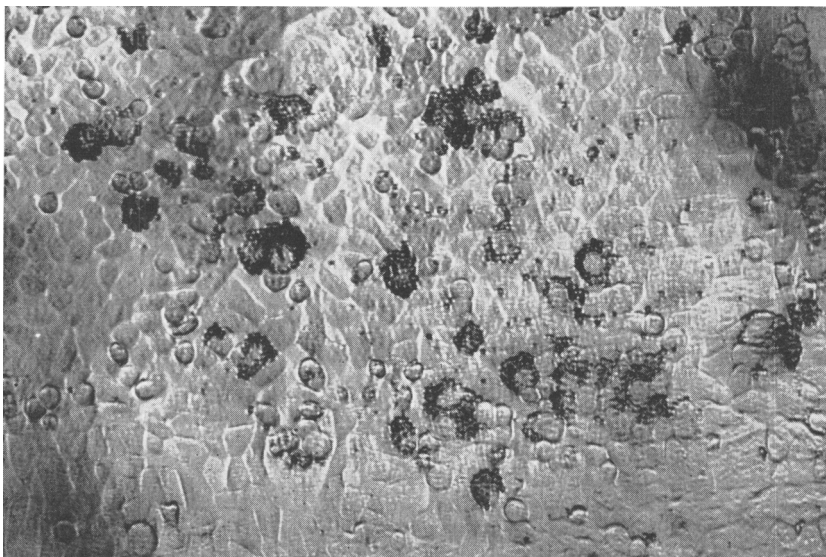


FIG. 2. Surface antigen on HEp-2 cells infected with V1-010 strain of vaccinia in the presence of cytosine arabinoside, detected by mixed agglutination with anti-V1-010 serum at a dilution of 1:3000. Indicator cells adhere to single cells (*S-type*) (100 $\times$ ).

antigens during the course of replication or transformation.

*Summary.* A strain of vaccinia, V1-010, was discovered which is defective in surface antigen production as detected by mixed agglutination. Evidence for existence of major and minor surface antigens on vaccinia-infected cells was obtained. Minor surface antigen could be distinguished from the major antigen serologically as well as by the pattern of mixed agglutination reactions. V1-010 was capable of producing minor surface antigen and hemagglutinin.

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