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Cell Surface Alterations Induced by Vaccinia and Newcastle Disease Viruses[1] (38486)

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(Introduced by H. C. Lichstein)

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Virus-infected mammalian cells often exhibit characteristic changes in surface properties such as the appearance of new antigens (1, 2) hemadsorption (3) increased membrane permeability (4) agglutinability by plant lectins (5, 6) and altered electrophoretic mobility (7, 8).

Our previous studies on Newcastle disease virus (NDV) and vaccinia-infected cells identified some of the plasma membrane alterations which followed infection by these viruses (9, 10). The present investigation was undertaken to compare the effect of NDV and vaccinia virus infection on the surface properties of the intact cell. In this regard three parameters were investigated: hemadsorption, the occurrence of concanavalin A (con A) binding sites and electrophoretic mobility.

Methods. Cell cultures. HEp-2 cells were grown in suspension culture as previously described (4), and monolayers of these cells were prepared by seeding cell suspensions into 6 cm Falcon dishes at a concentration which yielded confluent monolayers after 48 hr incubation at 37°.

Viruses. The IHD strain of vaccinia virus and the California strain of NDV were propagated as described earlier (4). Vaccinia virus and NDV were assayed on HEp-2 cell monolayers by plaque titration (11) or hemadsorption focus assay (12), respectively.

Cell electrophoresis. Electrophoretic migration of virus-infected or unintected cells was measured at 37° ± 0.01° in a cylindrical tube apparatus (13). Cells were removed from suspension by centrifugation, washed 3 times with Hank's balanced salt solution, (HBSS) pH 7.35 and resuspended in HBSS for the electrophoretic mobility studies. The migration times of at least 48 cells were obtained for each mobility determination with reversal of polarity after each measurement. Measurements were made only on single cells. Before each experiment the reproducibility of the electrophoresis system was examined by determining the mobility of human erythrocytes.

Assay for con A binding sites. The details of the erythrocyte adsorption assay for con A binding sites will be published elsewhere. Briefly, the procedure used was as follows: five virus-infected or sham-infected monolayers were washed twice with phosphate buffered saline (PBS) pH 7.2 and exposed to 1000 μg/ml of con A (Sigma) in PBS for 20 min at 4°. After removing the con A and two additional washings, 2.0 ml of a 0.5% suspension of fresh mouse erythrocytes in PBS was added to each dish. The plates were agitated at low speed in a reciprocating shaker for 20 min after which the unattached red blood cells were removed and the monolayers washed twice with PBS. Next were added 4.0 ml of a 0.005% saponin solution in PBS to lyse the adsorbed erythrocytes. The supernatant fluids were centrifuged at 2500 *g* for 5 min and optical density measurements of released hemoglobin were made at 410 nm against a blank derived from fluids of infected cells treated in the manner described with exception of the con A step.

Assays. The protein content of cells was determined by the method of Oyama and Eagle (14) using crystalline bovine serum albumin (Pentax) as the standard. The sialic acid (*N*-acetyl-neuraminic acid) content of cells was measured as follows. Approximately 1×10^7 cells were suspended in 2.0 ml of 0.1 *N* H_2SO_4 and hydrolyzed for 60 min at 80° after which the cellular debris was removed by centrifugation. The clear supernatants were recovered and assayed for free

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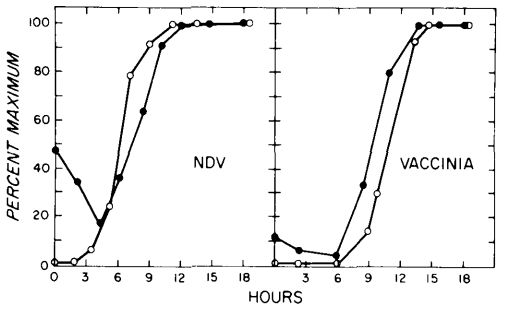

FIG. 1. Relationship between virus synthesis (●) and hemadsorption (○) in NDV or vaccinia-infected cells. HEp-2 cells (1×10^6/ml) were exposed to virus (input multiplicity 20) for 30 min at 37°, after which the cells were washed twice with HBSS and resuspended in growth medium. Incubation in suspension culture at 37° was continued. At the indicated times samples were removed, a portion of the sample (cells and fluids) was disrupted by brief sonic treatment and the lysate titrated for virus content. The remaining cells were washed twice in HBSS, mixed with fresh chicken erythrocytes and scored for hemadsorption (10). Each point on the curves represents an average of three determinations.

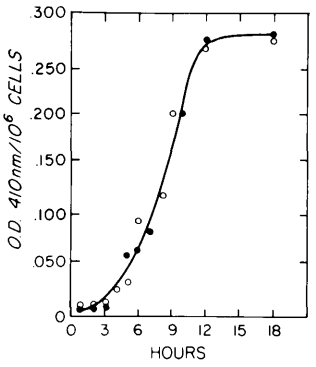

FIG. 2. Kinetics of appearance of con A binding sites on NDV (●) or vaccinia-infected (○) HEp-2 cells. Monolayers of cells were infected with 0.5 ml of virus (input multiplicity 20). After 30 min at 37°, the monolayers were washed twice with HBSS, 5.0 ml of growth medium added and incubation was continued at 37°. At the indicated times the plates were processed for con A binding activity as described in Methods. Each point on the curve represents an average of five determinations per time sample.

sialic acid by the method of Warren (15) and employing the precautions suggested by Warren when working with hydrolyzates of whole cells. The chromophore was first extracted with isoamyl alcohol followed by cyclohexanol. Optical densities of the cyclohexanol supernatant were measured at both 532 and 549 nm (15).

Results. Kinetics of appearance of hemadsorption and con A binding sites. Figure 1 compares the temporal relationship between NDV and vaccinia virus synthesis and the respective hemadsorption and reactions from representative experiments. It is clear that the majority of NDV infected-cells were hemadsorption positive at a time coincident with, or preceding virus maturation. In contrast, vaccinia progeny preceded hemadsorption by approximately 1.5 hr. These results emphasized a temporal difference in the relationship between virus synthesis and the appearance of virus-specific materials at the cell surface.

The next experiment was designed to study the time of appearance of con A binding sites on the surfaces of NDV and vaccinia-infected cells. As determined by the mouse erythrocyte binding assay (Methods), the kinetics of appearance of con A binding sites on both the NDV and vaccinia-infected cells was strikingly similar (Fig. 2). These experiments suggest that at least for the vaccinia-infected cell, the con A and hemadsorption sites are not the same since con A binding was assayable much earlier than hemadsorption.

Effect of virus infection on the electrophoretic migration of cells. The preceding experiments demonstrated alterations in the surface properties of both NDV and vaccinia-infected cells. It was therefore not unreasonable to assume that the ionogenic properties of the NDV or vaccinia-infected cells might be appreciably altered. To test this possibility HEp-2 cells derived from suspension cultures were infected with NDV or vaccinia virus and at selected intervals thereafter mobility measurements were made. Mobility determinations on sham-infected cells were made at each time that infected cells were examined.

Table I shows that a significant reduction ($P = <0.001$) in the anodal mobility of NDV-infected cells occurred during the first 30 min of infection. This reduced mobility was maintained throughout the entire infectious cycle. On the other hand, the mo-

TABLE I. ELECTROPHORETIC MOBILITY OF NDV OR VACCINIA-INFECTED HEp-2 CELLS.

Hours post infection[a]	Sham-infected cells (μm)[b]	NDV-infected cells (μm)	Vaccinia-infected cells (μm)
0.5	-1.119 ± 0.030	-0.761 ± 0.047[c]	-1.075 ± 0.030
3	-1.091 ± 0.053	-0.756 ± 0.041	—
6	-1.065 ± 0.034	-0.743 ± 0.032	—
10	-1.134 ± 0.042	—	-1.131 ± 0.037
15	-1.100 ± 0.045	—	-1.135 ± 0.033
18	-1.106 ± 0.031	-0.746 ± 0.028	-1.086 ± 0.038

[a] Approximately 3×10^8 cells from suspension culture were washed twice with HBSS and divided into three aliquots. Two aliquots of cells were infected with either NDV or vaccinia virus (input multiplicity 20) for 30 min at 37°. The remaining sham-infected cells were treated in like manner, except that exposure to growth medium replaced virus. The cells were then resuspended in growth medium (1×10^6 cells/ml) and incubation in suspension culture at 37° was continued. At the indicated times samples (1×10^6 cells) were removed, washed three times with HBSS and resuspended in the same medium for electrophoretic mobility measurements as described in Methods.

[b] Mobility (μm) = μm sec^{-1} v^{-1}. cm. Values are expressed as means ± standard error.

[c] $P = <0.001$ as compared with mobility values for sham-infected cells by Student's paired t test.

TABLE II. EFFECT OF NEURAMINIDASE TREATMENT ON THE ELECTROPHORETIC MOBILITY OF NDV OR VACCINIA-INFECTED HEp-2 CELLS.[a]

Hours post infection	Sham-infected cells (−NANase)	Sham-infected cells (+NANase)	NDV-infected cells (+NANase)	Vaccinia-infected cells (+NANase)
0.5	-1.119 ± 0.030	-0.686 ± 0.029	-0.664 ± 0.047	-0.591 ± 0.063
3	-1.091 ± 0.053	-0.717 ± 0.032	-0.732 ± 0.031	—
6	-1.065 ± 0.034	-0.688 ± 0.027	-0.637 ± 0.022	—
10	-1.134 ± 0.042	-0.664 ± 0.033	—	-0.664 ± 0.033
15	-1.009 ± 0.029	-0.649 ± 0.028	—	-0.649 ± 0.028
18	-1.092 ± 0.040	-0.789 ± 0.037	-0.746 ± 0.028	-0.721 ± 0.024

[a] Virus or sham-infected cells (see Table I) were washed twice with HBSS and exposed to *Vibrio cholera* neuraminidase (Sigma) as follows: 1×10^6 cells were suspended in 1.0 ml of HBSS (pH 7.35) containing 2 units of neuraminidase. The suspension was then incubated for 30 min at 37° with frequent agitation. Following enzyme treatment, the cells were washed three times with HBSS, resuspended in the ame medium and examined for electrophoretic mobility as described in Methods.

sbility of vaccinia-infected cells remained unaltered over an 18 hr period as compared with sham-infected HEp-2 cells ($P = >0.1$). As a working hypothesis it was assumed that the decrease in mobility of HEp-2 cells following NDV infection was most likely due to the removal of surface sialic acid by viral neuraminidase, a major component of the NDV envelope (16). It was of interest, therefore, to examine the effect of neuraminidase on the mobility of both vaccinia and NDV infected cells as compared with sham-infected cells. From data in Table II it is clear that neuraminidase treatment of sham-infected cells had a significant effect ($P = <0.001$) on mobility, which was comparable to NDV-infection per se. Furthermore, neuraminidase treatment of HEp-2 cells subsequent to NDV infection failed to affect a further reduction in anodal mobility. These data show that NDV or neuraminidase treatment effectively removed a major ionogenic component of the host cell. Since terminal sialic acid residues contribute materially to the net negative charge on cell surfaces, it is likely that the removal of this moiety by neuraminidase caused the decrease in anodal mobility (21, 22). Vaccinia-infected cells behaved as sham-infected or NDV-infected cells, following treatment with neuramini-

TABLE III. SIALIC ACID CONTENT OF VACCINIA OR NDV-INFECTED CELLS.

	Treatment[a]	Sialic acid (μg/mg protein)
Uninfected	−	2.6[d] ± 0.28
Uninfected	+	1.2 ± 0.17
NDV-infected[b]	−	1.3 ± 0.09
NDV-infected	+	1.2 ± 0.12
Vaccinia-infected[c]	−	4.2 ± 0.03
Vaccinia-infected	+	2.9 ± 0.07

[a] Neuraminidase treatment as described in Table II.

[b] Eighteen-hour infected cells (Table I).

[c] Fifteen-hour infected cells (Table I).

[d] Values expressed as mean ± standard error.

dase. These results suggest that ionogenic properties of the vaccinia-infected cell were not appreciably altered from the normal state even though con A or hemadsorption binding sites were readily detectable.

These experiments led to a consideration of the amount of sialic acid which might be removed from HEp-2 cells either by NDV infection or treatment with neuraminidase. In Table III are presented data from experiments in which the total sialic acid content of NDV or vaccinia-infected cells was determined before and after neuraminidase treatment. Brief neuraminidase treatment (30 min) of sham-infected cells or NDV infection resulted in a 50% reduction in sialic acid content. Neuraminidase treatment of sham or vaccinia-infected HEp-2 cells removed comparable amounts of sialic acid (1.3 μg), even though vaccinia-infected cells contained appreciably more sialic acid.

Discussion. In these experiments HEp-2 cells infected with NDV or vaccinia virus were examined for alterations in their surface properties by various methods. Hemadsorption of erythrocytes to the NDV-infected cells is conditioned by the association of viral hemagglutinin with the plasma membrane (17) in preparation for the maturation of the virus (18). While the hemadsorption of chicken erythrocytes to vaccinia-infected cells has not been linked to virus maturation, this is clearly a virus-directed function (19). We employed hemadsorption as a means of examining the temporal appearance of virus-specific products at the cell surface. In the case of NDV infection, hemadsorption preceeded virus maturation while in the vaccinia-infected cell this reaction followed virus synthesis. In light of these data, it is noteworthy that the kinetics of con A binding was practically identical for both NDV and vaccinia-infected cells. Under our experimental conditions in which more than 95% of the cells were infected (multiplicities of 20), these results suggest that the appearance of con A binding sites may be an early and nonspecific response to virus infection, unrelated to events having to do with the integration of viral products into the plasma membrane. In experiments not reported here we examined the binding of fluorescein- and I^{125}-labeled con A to vaccinia-infected HEp-2 cells. Thus, preliminary studies with high vaccinia multiplicities revealed an early (30 min) binding of con A to cells. Moreover, the fluorescein-labeled con A binding which we observed (a patchy fluorescence) closely resembled that recently described for NDV-infected cells by Poste and Reeve (20). Taken as a whole, these studies suggest that con A binding sites, which apparently appear very early in vaccinia and NDV infections, may represent a generalized response of the plasma membrane to virus adsorption, entry, or other early viral functions. Experiments are in progress which test this hypothesis.

The electrophoretic mobility of NDV-infected cells was significantly reduced when compared with sham-infected cells ($P = < 0.001$). This was not an unexpected finding in light of studies with myxoviruses (7, 8). It is noteworthy, however, that vaccinia-infected cells failed to show any significant ($P = > 0.1$) alteration in mobility over the entire virus infectious cycle. Thus, it is clear that the plasma membrane of the vaccinia-infected cell can undergo considerable modification (the appearance of con A and hemadsorption binding sites) without an appreciable change in ionogenic properties. Pertinent to this point is the recent finding that con A binding sites on the NDV-infected cell do not increase upon infection. Rather, the increased agglutinability of NDV-infected cells is related to the redistribution (clustering) of existing binding sites (20). Therefore, even if con A binding sites do contribute to the ionogenic properties of the cell surface,

there would probably be little change in the net surface charge on the cell when these sites are redistributed as a result of virus infection.

When HEp-2 cells, either uninfected or vaccinia-infected, were exposed to neuraminidase, cellular mobility was greatly reduced. This finding indicated that these cells responded to the enzyme in like manner. These data appear anomalous in light of results (Table III) which showed vaccinia-infected cells to contain considerably more sialic acid. Taken at face value these findings suggest that a major portion of the sialic acid synthesized by the vaccinia-infected cell was inaccessible to neuraminidase. It is tempting to speculate that the bulk of sialic acid in vaccinia-infected cells is intracellular and thus not susceptible to enzymatic action (21, 22). Such a view, however, must be tempered with caution since it has been convincingly demonstrated by Nordling and Mayhew (23) that neuraminidase can enter different types of mammalian cells with the resultant release of intracellularly-bound sialic acid. Even if this situation applied to vaccinia-infected cells, our results show that a considerable portion of the sialic acid synthesized by these cells remained associated with the cell after enzyme treatment. The intriguing question remains as to what function, if any, sialic acid has in vaccinia-infection. In this regard it is noteworthy that some virus-transformed cells contain increased amounts of a sialic acid-rich component as compared with untransformed cells (24). Furthermore, such transformed cells had higher sialyl-transferase levels. While a virulent vaccinia infection cannot be equated with a virus transformation system, it is conceivable that the vaccinia-infected cell, which is known to synthesize new species of glycoproteins (25), might also synthesize more sialic acid. Future experiments will be concerned with this problem.

Summary. A comparison was made of HEp-2 cell surface changes induced by NDV or vaccinia virus infection. Three parameters were examined as a function of time after infection: the kinetics of hemadsorption and the appearance of concanavaline (con A) binding sites, and alterations in electrophoretic mobility of single cells. The kinetics of appearance of con A binding sites was strikingly similar for both virus infections, whereas hemadsorption preceded NDV synthesis and followed vaccinia synthesis. These data suggest that in the vaccinia-infected cell the hemadsorption and con A binding sites are different. NDV infection or exposure of sham-infected cells to bacterial neuraminidase significantly reduced their anodal mobilities. This also occurred after enzyme treatment of vaccinia-infected cells. Measurements of the sialic acid content of NDV or sham-infected cells before and after neuraminidase treatment indicated the exposure to the enzyme or NDV materially reduced the sialic acid content of cells. Vaccinia-infected cells contained considerably more sialic acid than did normal cells. For the vaccinia-infected cell a change in surface properties as detected by hemadsorption or increased con A binding was not reflected in a change in electrophoretic mobility.

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