INFECTION AND IMMUNITY, Dec. 1970, p. 713-715
Vol. 2, No. 6
Printed in U.S.A.
Copyright © 1970 American Society for Microbiology

Cell Surface Antigen Induced by Venezuelan Equine Encephalomyelitis Virus

NICHOLAS HAHON

Aerobiology and Evaluation Laboratories, Fort Detrick, Frederick, Maryland 21701

Received for publication 6 August 1970

By immunofluorescence staining, a specific surface antigen induced by Venezuelan equine encephalomyelitis virus was detected on L-929 cells. Formation of the antigen was independent of viral ribonucleic acid synthesis.

Deoxyribonucleic acid (DNA) virus-specific surface or membrane antigens induced on established cell cultures or on tumor cells by oncogenic viruses such as simian virus 40, polyoma, Epstein-Barr, Shope papilloma, and herpesvirus of Marek's disease (2, 4, 8, 9, 11, 12, 17), and by nononcogenic agents, i.e., some poxviruses of the vaccinia group (15, 18), have been demonstrated by the immunofluorescence technique. Other than the detection of a new cell surface antigen in Moloney virus-induced lymphomas and in cell cultures (10, 16), reports of similar findings with other ribonucleic acid (RNA) oncogenic or nonocogenic viruses are limited (19). This report describes the induction of a specific cell surface antigen by the RNA nononcogenic Venezuelan equine encephalomyelitis (VEE) virus.

MATERIALS AND METHODS

A stock pool of the Trinidad donkey brain strain of VEE virus was prepared from infected McCoy cell monolayers (6); its titer was 8×10^8 cell-infecting units (CIU) per ml when assayed by the immunofluorescent cell counting technique (6). Monolayers of L-929 (mouse fibroblast) cells were grown and maintained in medium 199 with 5% fetal calf serum, 50 μg of streptomycin per ml, and 75 μg of kanamycin per ml. The procedure for infecting confluent cell monolayers grown on 15-mm (diameter) cover slips has been described in detail elsewhere (6). To demonstrate cell surface antigen, infected cell monolayers were incubated at 35 C for 7 to 8 hr, rinsed with phosphate-buffered saline (PBS), *p*H 7.1, and, without fixation, stained by the direct immunofluorescence technique. Surface antigen was unstainable by acetone fixation of cells for 5 min at room temperature (2, 8, 10, 12, 15, 18, 19). The preparation of monkey antiviral serum, conjugation of the globulin fraction with fluorescein isothiocyanate, and procedures for staining and counting infected cells have been described previously (6).

RESULTS AND DISCUSSION

Surface antigen appeared on VEE virus-infected cells as bright rings of fluorescence (Fig. 1); uninfected cells did not fluoresce. The specificity of the staining was confirmed by the absence of surface fluorescence on cells infected with VEE virus and stained with variola, chikungunya, or yellow fever fluorescein-conjugated antiviral serum. Similar results were obtained when cell monolayers were infected with any of these three viruses and stained with conjugated VEE antiserum. Further incidence of staining specificity was obtained by demonstrating that cell surface antigen fluorescence was blocked when cells were first treated with unlabeled monkey antiviral serum prior to staining with conjugated antiviral serum. Induction of cell-surface antigen was not limited as to the source of virus, the strain of virus, or the L-929 cell line; the phenomenon was elicited by both infective mouse brain and chick embryo preparations, by two different attenuated VEE virus strains (1, 7), and on guinea pig lung, McCoy, and baby hamster kidney (BHK21/C13) cell lines. Induction of cell surface antigen by VEE virus is dependent on the presence of infectious virus particles. Virus inactivated by either ultraviolet irradiation or heat (56 C, 16 hr) failed to induce the formation of cell surface antigen. Furthermore, the number of cells showing surface antigen was reduced approximately 93% when virus was mixed with antiviral serum before it was added to cell monolayers. Additional evidence that the manifestation of cell surface antigen is associated with the presence of infectious virus particles was noted by the demonstration of a linear relationship between relative virus concentration and the number of cells showing surface antigen. This finding suggests that each cell exhibiting fluorescent surface antigen results from infection by a single infective virion or aggregate not divisible by dilution. The dependency of surface antigen induction on viable virus is consistent with observations on the direct association between infection or production (or both) of Epstein-Barr virus and cell membrane antigen (4).

To compare the rate of appearance of fluores-

cent surface antigen on cells with that of cytoplasmic viral antigens, cell monolayers were infected with approximately 10^8 CIU of virus and, 1 hr after virus attachment, a 1:10 dilution of antiviral serum was added to each cell monolayer to prevent a secondary cycle of infection by extracellular virus. During incubation at 35 C for 20 hr, representative cell monolayers, at 4-hr intervals, were either washed with PBS or fixed with acetone and then stained. Fluorescent cell surface antigen became visible 4 hr after infection and preceded the appearance of cytoplasmic viral antigen by 4 hr (Fig. 2). The number of cells showing surface antigen was maximal between 8 and 12 hr; the number was equivalent to those with cytoplasmic fluorescence at 12 hr. In view of the quantitative relationship established earlier, it is feasible to assay for VEE virus infectivity (by counting cells with fluorescent surface antigen) and to assess the neutralizing content of serum. The latter prospect is based on the observation cited earlier wherein a reduction in the number of cells with surface antigen resulted when virus inoculum was first mixed with antiviral serum. The assay of Moloney leukemia virus based on cell membrane fluorescence has been found applicable for estimating the antiviral activity of serum (16).

The possibility that surface antigen may be derived from the outer protein layers of penetrated virions deposited on cell surfaces was examined by incorporating inhibitors of protein synthesis, puromycin (50 μg/ml) or cycloheximide (100 μg/ml), into maintenance medium, which was then added to cell monolayers immediately after virus attachment. Cell monolayers to which only maintenance medium was added showed surface antigen at 8 hr when stained without fixation and

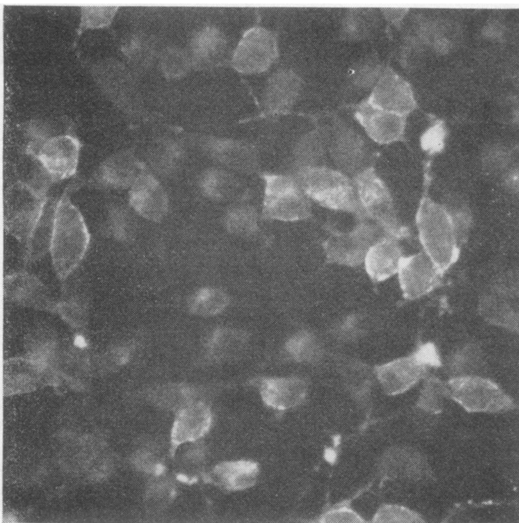

FIG. 1. *Immunofluorescence on the surface of L-929 cells infected with VEE virus. Eight hours after infection, cells were stained, without fixation, with labeled anti-VEE monkey globulin. × 126.*

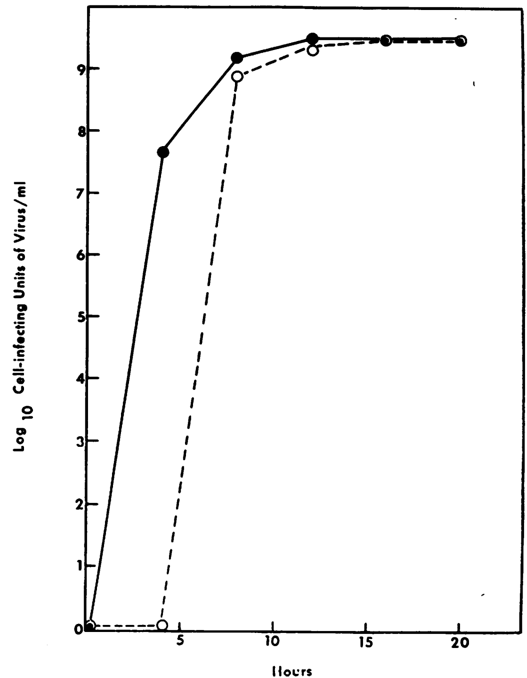

FIG. 2. *Rate of appearance of immunofluorescent surface (○) and cytoplasmic (●) antigens in L-929 cells infected with VEE virus. Cell-infecting units of virus calculated by averaging the number of fluorescent cells per microscopic field, multiplied by the number of fields per cover slip, the reciprocal of the dilution of virus inoculum, and a volume factor for conversion to milliliters (6).*

TABLE 1. *Effect of interferon on formation of VEE virus antigens in L-929 cells*

Treatment	No. of immunofluorescent cells/50 mic roscopic fields	
	Surface antigen	Cytoplasmic antigen
Interferon[a]	218	13
Control	215	198

[a] Interferon was prepared in L-cells with VEE virus as the inducer and was assayed by the immunofluorescent cell reduction technique (13). Cell monolayers were treated for 24 hr at 35 C with 660 units of interferon or maintenance medium (control) before the addition of virus inoculum. They were then incubated at 35 C for 8 hr and stained without prior fixation to detect surface antigen or they were incubated at 35 C for 20 hr and fixed with acetone before staining to detect cytoplasmic viral antigen.

showed cytoplasmic viral antigens at 20 hr when appropriately fixed. However, neither surface nor cytoplasmic antigens could be demonstrated in cell cultures that had been treated with inhibitors of protein synthesis. The introduction of infectious viral RNA onto cell monolayers resulted in the formation of cytoplasmic viral antigens as well as cell surface antigen. This constitutes additional evidence that cell-surface antigens are not deposits of viral protein coats.

That the addition of cytosine arabinoside to HeLa cell cultures infected with vaccinia virus suppresses the formation of cytoplasmic viral antigens but does not impair the synthesis of surface antigen is indicative of the latter's independency of viral DNA replication (18). To determine whether the production of surface antigen induced by VEE virus is dependent on the replication of viral RNA, cell monolayers were treated for 24 hr with L-cell interferon, an inhibitor of viral RNA synthesis (3, 14), before they were infected with virus. The results (Table 1) show that the formation of cell surface antigen was unaffected by interferon, but the number of cells with cytoplasmic antigen, an indicator of viral replication (6), was reduced by approximately 93%. These data suggest that the specific surface antigen is a newly synthesized protein coded by an early function of the viral genome, a fraction thereof, or a component of the cell membrane modified by virus infection. That cell membranes possess protein-synthesizing mechanisms has been established by the recent demonstration that isolated membranes of animal cells actively participate in protein synthesis (5).

LITERATURE CITED

1. Berge, T. O., I. S. Banks, and W. D. Tigertt. 1961. Attenuation of Venezuelan equine encephalomyelitis virus by *in vitro* cultivation in guinea pig heart cells. Amer. J. Hyg. 73:209–218.
2. Chen, J. H., and H. G. Purchase. 1970. Surface antigen on chick kidney cells infected with the herpesvirus of Marek's disease. Virology 40:410–412.
3. DeSomer, P., A. Prinzie, P. Denys, Jr., and E. Schonne. 1962. Mechanism action of interferon. I. Relationship with viral ribonucleic acid. Virology 16:63–70.
4. Dunkel, V. C., and R. F. Zeigel. 1970. Studies on the relation of membrane immunofluorescence to Epstein-Barr virus infection. J. Nat. Cancer Inst. 44:133–144.
5. Glick, M. C., and L. Warren. 1969. Membranes of animal cells. III. Amino acid incorporation by isolated surface membranes. Proc. Nat. Acad. Sci. U.S.A. 63:563–570.
6. Hahon, N., and K. O. Cooke. 1967. Primary virus-cell interactions in the immunofluorescence assay of Venezuelan equine encephalomyelitis virus. J. Virol. 1:317–326.
7. Hearn, H. J., Jr. 1960. A variant of Venezuelan equine encephalomyelitis virus attenuated for mice and monkeys. J. Immunol. 84:626–629.
8. Irlin, I. S. 1967. Immunofluorescent demonstration of a specific surface antigen in cells infected or transformed by polyoma virus. Virology 32:725–728.
9. Ishimoto, A., and Y. Ito. 1969. Specific surface antigen in Shope papilloma cells. Virology 39:595–597.
10. Klein, E., and G. Klein. 1964. Antigenic properties of lymphomas induced by the Moloney agent. J. Nat. Cancer Inst. 32:547–568.
11. Klein, G., G. Pearson, J. S. Nadkarni, J. J. Nadkarni, E. Klein, G. Henle, W. Henle, and P. Clifford. 1968. Relation between Epstein-Barr viral and cell membrane immunofluorescence of Burkitt tumor cells. J. Exp. Med. 128:1011–1020.
12. Kluchareva, T., K. L. Shachanina, S. Belova, V. Chibisova, and G. I. Deichman. 1967. Use of immunofluorescence for detection of specific membrane antigen in simian virus 40-infected nontransformed cells. J. Nat. Cancer Inst. 39:825–832.
13. Kozikowski, E. H., and N. Hahon. 1969. Quantitative assay of interferon by the immunofluorescent cell-counting technique. J. Gen. Virol. 4:441–443.
14. Lockart, R. Z., Jr., T. Sreevalson, and B. Horn. 1962. Inhibition of viral RNA synthesis by interferon. Virology 18: 493–494.
15. Miyamoto, H., and S. Kato. 1968. Immune hemadsorption by cells infected with poxviruses. Biken J. 11:343–353.
16. Nordenskjold, B. A., E. Klein, T. Tachibana, and E. M. Fenyö. 1970. Tissue culture assay for Moloney leukemia virus. J. Nat. Cancer Inst. 44:403–412.
17. Tevethia, S. S., M. Katz, and F. Rapp. 1965. New surface antigen in cells transformed by simian papovavirus SV40. Proc. Soc. Exp. Biol. Med. 119:896–901.
18. Ueda, Y., M. Ito, and I. Tagaya. 1969. A specific surface antigen induced by poxvirus. Virology 38:180–182.
19. Yamanouchi, K., F. Kobune, A. Fukuda, M. Hayami, and A. Shishido. 1970. Comparative immunofluorescent studies on measles, canine distemper, and rinderpest viruses. Arch. Gesamte Virusforsch. 29:90–100.