CHANGES IN CELL MEMBRANE MICROVISCOSITY ASSOCIATED WITH ADSORPTION OF VIRUSES

Differences between fusing and non-fusing viruses

Avigdor LEVANON and Alexander KOHN
Department of Virology, Israel Institute for Biological Research, Ness Ziona, Israel

Received 6 November 1977

1. Introduction

We have demonstrated earlier [1] that adsorption of various DNA and RNA viruses to susceptible cells was accompanied by a marked increase in the fluidity of the lipids in host cell membranes. This increase in fluidity was detectable within a few minutes of adsorption as a decrease in the fluorescence polarization of a fluorescent probe, DPH, embedded in the cell membranes.

In this communication we demonstrated that paramyxoviruses, in contrast to other viruses that we studied, induce an increase in fluorescence polarization (i.e., microviscosity) of the membranes of the adsorbing cells. We present here a model explaining these opposing results.

2. Materials and methods

2.1. *Cells*

The following cells and cell lines were used: BHK-21, 3T3, HeLa and chick embryo fibroblasts (CEF). CEF were grown in M-199 medium; all other cells were grown in Eagle's minimal essential medium (MEM F12, Gibco) supplemented with 10% calf serum.

2.2. *Viruses*

Encephalomyocarditis (EMC) and West Nile (WN) viruses were grown in BHK-21 cells. Polyomavirus was grown either in BHK-21 or in 3T3 cells. Two vaccine strains of poliovirus (P712 = type 2, and LSC = type 1) were grown in secondary cultures of monkey kidney cells. These viruses were purified as described [2]. Influenza viruses A, strains Mel and WSN, as well as Newcastle disease (NDV) and Sendai viruses were propagated in the chorioallantoic membranes of 10-day old embryonated eggs and harvested as allantoic fluid. Influenza and ND viruses were purified by low and high speed centrifugation followed by sucrose density gradient purification [3]. Sendai virus was purified by zonal sedimentation in sucrose gradient [4].

2.3. *Fluorescent labeling of cells*

The polarization of fluorescence is determined by the rotation of 1,6-diphenyl-1,3,5-hexatriene (DPH) molecule during the lifetime of its excited state (11.4 nsc). The rotation of this probe is in turn dependent on the 'microviscosity' of the environment in which the probe is embedded [5]. Rapid rotation leads to depolarization. Fluorescence polarization and fluorescence intensity are obtained by measurements of fluorescence parallel and perpendicular to the direction of polarization of the excitation beam.

2.4. *Preparation of labeled cells*

DPH in tetrahydrofuran (2×10^{-3} M) 0.1 ml as dispersed into 100 ml phosphate buffered saline (pH 7.2) by vigorous stirring at room temperature. Cells in monolayer were incubated with 5 ml DPH suspension for 30 min at 37°C. The cells were then trypsinized, washed three times in PBS and suspended

to a concentration of about 10^6 cells/ml. Alternatively, the cells were first trypsinized from a monolayer, washed, labeled in a suspension of DPH (as above) and then washed twice with 5 ml PBS.

2.5. *Measurement of fluorescence polarization*

Virus suspension, 0.3 ml, of a desired concentration were added to 2.7 ml DPH-labeled cells at 37°C. Fluorescence polarization of the cells treated with virus was determined at 1 min intervals, from 0–20 min, in Microviscosimeter MV-1 (Elscint, Haifa, Israel). Excitation beam was at 365 nm, the readings were taken at two independent cross-polarized channels at 430 nm. Polarization values (*P*) were read directly off the instrument, and the corresponding microviscosity was calculated from Perrin's formula [5].

3. Results

The changes in fluorescence polarization value, *P*, of various DPH-labeled cells 5 min after contact at 37°C with a standard concentration of a number of viruses is shown in fig.1. The time of 5 min has been chosen, since after the first 3–5 min there was no additional change in the values of fluorescence polarization, *P*.

Comparison of the effects of various viruses on different cells, as shown in fig.1 permit to group the viruses into two classes. In one, containing Newcastle disease and Sendai viruses (fusing viruses), the adsorption of viruses to cells caused an increase in the lipid microviscosity of the cell membranes, as calculated from the increase in the values of *P*. In the other group, RNA and DNA viruses, both enveloped and naked, such as poliomyelitis, EMC, polyoma, West Nile and influenza viruses induce a decrease in the lipid microviscosity of the DPH-labeled cells, within less than a minute of addition of the virus to the cells.

The kinetics of changes in fluorescence polarization of chick fibroblasts, HeLa and BHK cells labeled with DPH, due to adsorption of enveloped viruses of two classes, is shown in fig.2. Influenza and West Nile viruses induced a decrease in microviscosity, while Sendai and Newcastle disease viruses had an opposite effect. The changes were discernible already after 1 min adsorption.

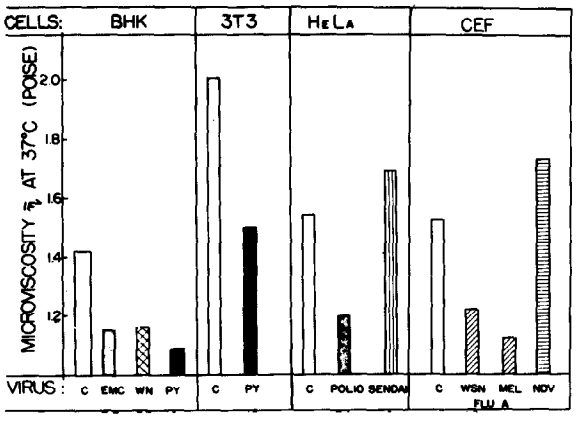

Fig.1. Comparison of changes in fluorescence polarization induced by animals viruses in different cells. Encephalomyocarditis (EMC), West Nile (WN), polyoma (Py) and polioviruses were added to about 2–3 × 10^6 cells at a m.o.i. of 15 p.f.u./cell. Influenza A viruses (Mel and WSN) were added at a multiplicity of about 100 hemagglutination units (HAU)/10^6 cells. The effects of viruses on fluorescence polarization of DPH-labeled cells 5 min after adsorption at 37°C are expressed in terms of microviscosity [5].

4. Discussion

Attachment of viruses to animal cells labeled with fluorescent probe, DPH, induces in them very rapidly a change in the fluorescence polarization of the probe.

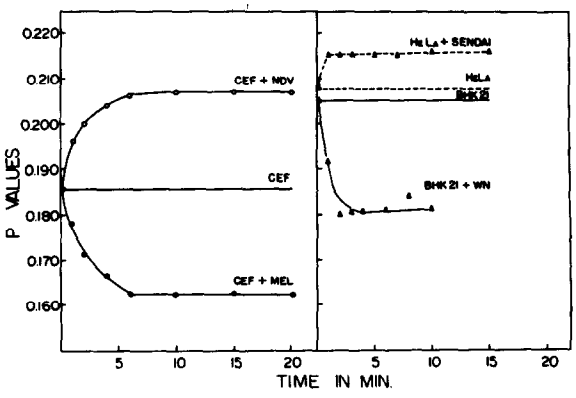

Fig.2. Comparison of kinetics of change in fluorescence polarization induced by enveloped RNA viruses: West Nile WN (▲); Influenza A-Mel (●); Newcastle disease virus NDV (○); and Sendai viruses (△), in HeLa, CEF and BHK-21 cells.

It is difficult to find a simple explanation for this effect which would be based on molecular events occurring in the cell membranes adsorbing the virus, since some viruses induce an increase of the microviscosity of the host cell membranes, while others act in an opposite direction. Thus fusing paramyxoviruses (NDV, Sendai) cause an increase in the microviscosity of cell lipids (fig.1,2,[6]). This effect may be explained by an assumption that during the process of fusion of the virus envelope with the cell membrane their respective lipids intermix [7,8]. Paramyxoviruses penetrate their host cells by a process in which the first step, following adsorption, is the fusion of the viral envelope with the cell membrane [9]. Since the microviscosity of the envelopes of NDV and Sendai viruses is considerably higher ($P = 0.31–0.32$) that that of the membranes of the cells in which they grew or to which they adsorb ($P = 0.18–0.21$) the microviscosity of the mixed phospholipids is indeed expected to have some intermediate value which is higher than that of the cell membranes. Other enveloped viruses, however, such as influenza and West Nile, as well as a number of naked viruses (EMC, polio, polyoma), induce a decrease in the microviscosity of the lipids of the adsorbing cells. All these viruses are believed to gain entry into the host-cell cytoplasm by a process akin to phagocytosis [10]. For influenza virus the term viropexis (sinking in) was coined [11]. For these viruses, therefore, the intermixing hypothesis is not applicable: influenza and WN viruses have envelopes with higher microviscosity than the cells in which they matured [12–14]; moreover, naked viruses have no lipids in their structure, and therefore cannot contribute lipids for mixing with the cell lipids.

The effects on membrane fluidity of viruses that enter cells by phagocytosis fit the findings [15] that phagocytosis leads to an increased fluidity of the membrane of the phagocytic cell (as measured by fluorescence polarization of DPH).

One should perhaps mention the results [16] that within 30 s interaction between Sendai virus with chick erythrocytes a clustering of intramembranal particles (proteins) occurred. Similar clustering has been observed [17]. Such a clustering would obviously change the existing protein lipid interaction in the plane of the membrane. Additional evidence supporting the hypothesis that viruses induce movement of integral proteins in the membrane is the observation [1] as well as that of [7] that at temperatures below 10°C the viruses, though adsorbing normally, do not affect the fluidity of the phospholipids in the cell membrane.

It is important to note that trypsinization of cells, which should affect the external proteins did not affect the cell–virus interaction, as measured by fluorescence polarization [1], or by ESR in the study [18] on fluidity changes of chick erythrocytes agglutinated by influenza virus. These findings are compatible with an assumption that only changes in the integral proteins of the membrane would contribute to the fluidity changes of membranal lipids.

The paradoxical finding that the rapid movement and clustering of intramembranal particles induced by paramyxoviruses [7,17] occurs at the same time as the increase in the microviscosity of the host cell phospholipids can be interpreted by assuming that these two chains of events are not causally linked, but produced by two independent mechanisms.

The results of our studies on non-fusing viruses, shown in fig.1, seen in this perpective may be interpreted as follows: A specific virus–receptor interaction is followed by a non-specific phagocytosis and this would account for the increase in membrane fluidity of the 'infected' cells [15]. When the attachment of the virus to the cells is prevented by some chemicals such as e.g., protamine in the case of EMC virus [1], or by treatment with neuraminidase which destroys the myxoviruses receptors [18], or by prior interaction between the virus and its specific antibody (unpublished observations), the change in the membrane microviscosity is diminished or abolished.

It is probable that rearrangement of the virus and cell proteins in the cell membrane might be held responsible for the observed changes described here, in our previous studies [1], as well as in other investigations [6,7,18].

Acknowledgements

A.K. is an established investigator of the Chief Scientists Bureau, Ministry of Health, Israel. We are grateful for the technical assistance of Mrs J. Gitelman.

References

[1] Levanon, A., Kohn, A. and Inbar, M. (1977) J. Virol. 22, 353–360.
[2] Phillips, B. A., Summers, D. F. and Maizels, J. V. jr. (1968) Virology 35, 216–226.
[3] Bean, W. J. and Simpson, R. W. (1975) J. Virol. 16, 516–524.
[4] Kohn, A. (1975) J. Gen. Virol. 29, 179–187.
[5] Inbar, M., Shinitzky, M. and Sachs, L. (1973) J. Mol. Biol. 81, 245–253.
[6] Nicolau, C. (1977) personal communication.
[7] Maeda, T., Asano, A., Ohki, K., Okada, Y. and Ohnishi, S. (1975) Biochemistry 14, 3736–3741.
[8] Okada, Y., Kim, J., Maeda, Y. and Koseki, I. (1974) Proc. Natl. Acad. Sci. USA 71, 2043–2047.
[9] Morgan, C. and Howe, H. M. (1968) J. Virol. 2, 1122–1132.
[10] Dales, S. (1973) Bacteriol. Reviews 37, 103–135.
[11] Fazekas de St. Groth, S. (1948) Nature 162, 294–295.
[12] Moore, N. F., Barenholz, Y. and Wagner, R. R. (1976) J. Virol. 19, 125–135.
[13] Sefton, R. M. and Gaffney, R. J. (1974) J. Mol. Biol. 90, 343–358.
[14] Barenholz, Y., Moore, N. F. and Wagner, R. (1976) Biochemistry 15, 3563–3570.
[15] Berlin, R. D. and Fera, J. P. (1977) Proc. Natl. Acad. Sci. USA 74, 1072–1076.
[16] Bächi, T., Aguet, M. and Howe, C. (1973) J. Virol. 11, 1004–1012.
[17] Lalazar, A., Reichler, Y. and Loyter, A. (1976) Proc. 5th Eur. Cong. Electron Microscopy (Ben-Shaul, Y. ed) Vol. 2, pp. 330–332, Tal International Publ.
[18] Lyles, D. S. and Landsberger, F. R. (1976) Proc. Natl. Acad. Sci. USA 73, 3497–3501.