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INTERACTION BETWEEN SENDAI VIRUS AND KB CELL LINES WITH ALTERED CELL FUSION ABILITY: A STUDY EMPLOYING ELECTRON SPIN RESONANCE

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Summary

The nature of the interaction between Sendai virus and Sil mutant cells was examined by measuring a change in ESR spectrum of spin-labeled phosphatidylcholine molecules on the viral envelope. When spin-labeled virus was incubated with the Sil cells that had a reduced ability to respond to virus-induced cell fusion, interchange of the phospholipid molecules between viral envelope and cell surface membrane occurred to a smaller extent than that observed with parental cells. Moreover, the degree of the interchanging correlated with the degree of the fusion capacity of the mutant lines. The results show that the mutant cells carry such a lesion(s) on their surface membranes that the viral envelopes can hardly fuse into them.

Introduction

We have isolated and characterized lines of mutant KB cells (the Sil cell lines), which have a reduced ability to respond to Sendai virus-induced cell fusion [1]. Sil cells adsorbed essentially the same amount of Sendai virus as did wild-type cells (KB cells), although Sil cells had reduced amounts of sialic acid compared to KB cells. Penetration of the virus into Sil cells was markedly affected at high multiplicities of infection [1]. Based on these findings, we proposed that Sil cells might have a defect(s) in a step of fusion between cell membrane and virus envelope (i.e. envelope fusion) [1]. To confirm this further, we examined the interchange of phospholipid molecules between viral

Abbreviation: PC*, spin-labeled phosphatidylcholine where β-fatty acid chain is replaced with the 4′,4′-dimethyloxazolidinyl-*N*-oxy derivative of 12-keto-stearic acid.

envelope and cell surface membrane by using an electron spin resonance (ESR) method.

Previously, Maeda et al. [2] studied virus-cell interaction with Sendai virus which carried spin-labeled phosphatidylcholine molecules (PC*) in the envelope. These authors showed that the characteristic exchange broadening in the ESR spectrum of the heavily labeled virus disappeared rapidly when the PC*-virus was incubated with red blood cells at 37°C. These results directly indicated interchange of phospholipid molecules between viral envelopes and the red blood cell membrane, and demonstrated the feasibility of this method to examine early steps of the virus-host cell interactions.

By applying this method, we demonstrate here that Sil cells have a reduced ability to respond to Sendai virus-induced phospholipid interchange as compared with parental cells. This supports our hypothesis that Sil cells have a defect(s) in the step of envelope fusion.

Materials and Methods

Cells and viruses. All cell lines used in the experiments are derivatives of KB cells. Most of the materials, methods and procedures employed in the paper, with the exception of those given below, were described previously [1].

Sil mutant lines were selected by repeated incubations of non-mutagenized KB cells with increasing doses (from 640 hemagglutinating units/ml to 5120 hemagglutinating units/ml) of Sendai virus which is a potent cytocidal agent in the absence of Ca^{2+} [1]. Sil-3 cells were isolated from cloned Sil-1 cells by additional challenges of Sendai virus (5120 hemagglutinating units/ml).

Sendai virus, strain Z, was used throughout these studies, except that Sendai virus, strain Fushimi, was employed for the elution experiments. Influenza virus, strain A_0PR8, was donated by Dr. T. Iwasaki. The viruses were propagated in the chorioallantoic cavity of 10-day-old chick embryos for 2 days (influenza virus) or 3 days (Sendai virus) at 36°C. The allantoic fluid was clarified by centrifugation at 1000 $\times g$ for 10 min, and subjected to sedimentation at 40 000 $\times g$ for 60 min. Precipitated viruses were suspended in phosphate-buffered saline (25 600 hemagglutinating units/ml) and used without further purification. This concentration of Sendai virus corresponds to about 2 mg proteins/ml or 0.6 mg lipids/ml although the ratio of hemagglutinating units to virus proteins or lipid varies slightly from a preparation to a preparation. They were stored at −75°C and thawed just before use. Treatment of Sendai virus with trypsin (bovine, grade 3, Miles-Seravac) was carried out at 37°C for 20 min at a concentration of 20 μg/ml in 10 mM phosphate buffer (pH 7.2). Soybean trypsin inhibitor (400 μg/ml) was added at the end of incubation and kept on ice. Under these conditions, the trypsin treatment caused a 70% decrease in hemolyzing activity of the virus.

ESR measurement. Procedures to prepare PC*-virus carrying spin-labeled phosphatidylcholine molecules (PC*) on the envelope and those for ESR measurement were described previously [2,3]. Briefly, cells at a late exponentially growing stages ($1.5 \cdot 10^5$—$2.0 \cdot 10^5$ cells/cm^2) were harvested with 0.25% trypsin, washed and suspended in a cold fusion buffer (107 mM NaCl/6.8 mM KCl/0.8 mM $MgSO_4$/4 mM $CaCl_2$/10 mM HEPES-NaOH, pH 7.8).

After adjusting the cell number to $2 \cdot 10^7$ cell/ml by use of a Coulter Counter, the cell suspension was kept in an ice-water bath until use. The reaction mixture essentially consisted of 500 μl of the cell suspension, 160 μl 20 mM adenine, 130 μl 50 mM inosine, 25 μl 50 mM $CaCl_2$, 650 μl Tricine-buffered saline (pH 7.8) and 50 μl PC*-virus preparation. This mixture was kept in an ice-water bath for 10 min, and centrifuged to remove unadsorbed virus. Sedimented cell · virus complexes were immediately used for the measurement.

ESR spectra were measured with a commercial X-band spectrometer (JEOLCO Model ME-2X) at approx 23°C. The extent of phospholipid interchange in the various systems was compared with the initial rate estimated from the increase of the peak height after 5 min.

Results

Interchange of PC between viral envelope and cell membrane*

The ESR spectrum of PC* molecules incorporated into membranes depends on their relative concentration. At higher concentrations, a high degree of magnetic spin-spin exchange interaction occurred and thus exchanged broadening of the spectrum is evident. When intermixing of phospholipid molecules takes place between densely PC*-labeled and unlabeled membranes, PC* molecules transfered to the unlabeled membrane is magnetically diluted and its ESR spectrum changes from an exchange-broadened one to a sharp one without any exchange interaction. Thus, the interchange of PC* molecules could be con-

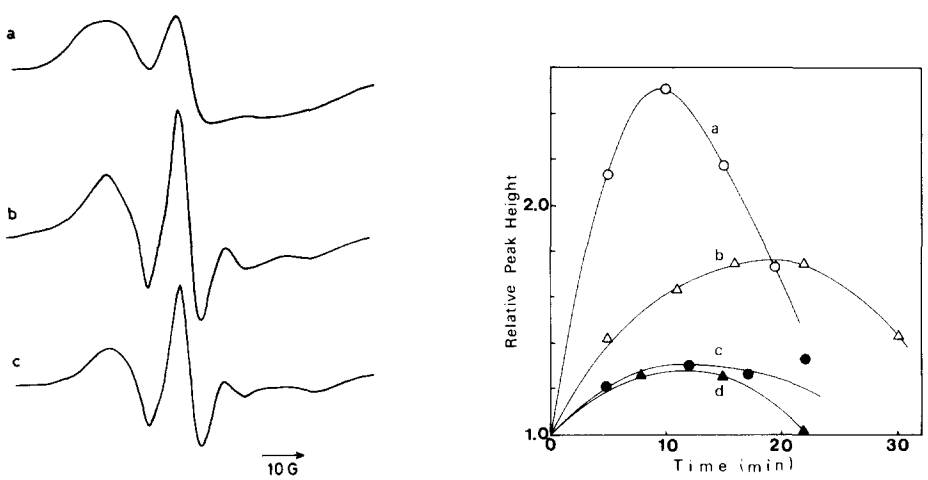

Fig. 1. Change in ESR spectrum of PC*-Sendai virus when incubated with KB cells. PC*-Sendai virus and KB cells were mixed at 0°C (final concentrations, 800 hemagglutinating units/ml and $4 \cdot 10^6$ cells/ml, respectively) and the resulting pellet of cell aggregates was taken into quartz capillary tube and incubated at 37°C for 0 min (a), 10 min (b), and 22 min (c). The ESR spectrum was recorded at 23°C.

Fig. 2. Time course of intermixing of PC* between Sendai virus and cells. PC*-Sendai virus, or PC*-influenza virus was incubated with either parental KB cells or mutant Sil-1 cells. The reaction mixture contained $4.4 \cdot 10^6$ cells/ml and 560 hemagglutinating units/ml of Sendai or 4500 hemagglutinating units/ml of influenza virus. Sendai virus plus parental cells (a) or Sil-1 mutant cells (b); influenza virus plus parental cells (c) or Sil-1 mutant cells (d). The relative peak height represents the ratio of the central peak height at given time to that at the initial time in ESR spectra.

veniently followed by observing the increase in central peak height in the spectrum [2,3].

As shown in Fig. 1, when PC*-Sendai virus was incubated with wild-type KB cells at 37°C, the ESR spectrum changed rapidly from an extremely broad one to a sharp one and the central peak height in the spectrum grew correspondingly. The spectral change is consistent with an interchange of PC* between viral envelope and KB cell membrane as described previously [2,3]. The time course of the change in spectral peak height is shown in Fig. 2a. The peak height increased rapidly, reaching 2 to 3 times the height of the initial peak after 10 min of incubation, and subsequently decreased. This decrease in peak height will be discussed later. In contrast, when Sil-1 cells were used instead of the parental cells (Fig. 2b), the rate of the increase in the peak height was much less (34%). These results suggest that Sil mutant cells have a reduced ability to respond to Sendai virus-induced phospholipid intermixing.

To estimate the degree of PC*-intermixing reactions occurring independently of the fusion event, influenza virus of 8 times higher hemagglutinating titer was used instead of Sendai virus. Influenza virus has both hemagglutinating and neuraminidase activities, but lacks fusing and hemolysing activities. When densely PC*-labeled influenza virus is incubated with either mutant or parental cells, the increase in the peak height should reflect the non-specific intermixing of PC*. As shown in Figs. 2c and d, the rate of increase in peak height dropped in both types of cell, reaching only 18% of the level observed between Sendai virus and wild type cells. These results indicate that the phospholipid intermixing induced by Sendai virus is a result of specific interaction between the envelope of Sendai virus and the cell membrane. Furthermore, lack of a difference in the influenza virus-induced spectral changes between these two cell types reveals that the non-specific intermixing of phospholipid is not affected in Sil cells. Thus, the reduced ability of Sil cells to respond to Sendai virus-induced phospholipid intermixing is closely correlated with Sil mutation.

Effect of trypsin-treatment of virus particle

To confirm further that the changes in ESR spectrum are actually related to the fusion event between viral envelope and cell membrane, trypsin-treated Sendai virus was used. Trypsin treatment of the virion is known to inactivate fusing and hemolysing activities of the virus without affecting either neuraminidase or hemagglutinating activities [4,5]. Inactivation of these two activities is associated with selective cleavage of F glycoprotein [5]. As shown in Fig. 3, when the trypsin-treated PC*-virus was incubated with either mutant or parental cells, the peak height increase in both cases was only 27% that of the control with untreated PC*-virus. This value was comparable to that observed when the parental cells were incubated with PC*-influenza virus. These results indicate that the observed changes in the ESR spectra are dependent on the function of the F glycoprotein.

Isolation and characterization of Sil-3 cells

To confirm further the observations described above, we isolated a cell line from Sil-1 cells as the latter mutant is still leaky with regard to its resistance to the virus-induced cytolysis and cell fusion. As shown in Fig. 4, the new mutant

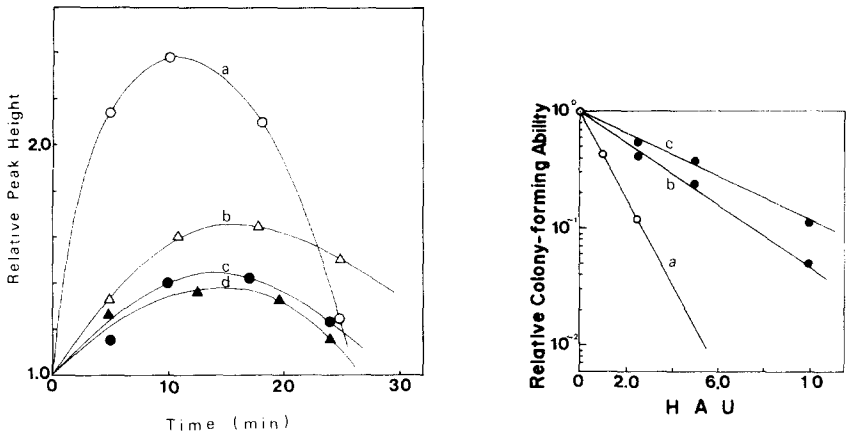

Fig. 3. PC* interchange with trypsin-treated Sendai virus. Either parental KB or Sil-1 cells were mixed with trypsin-treated or untreated PC*-Sendai virus. Parental cells plus untreated (a) or trypsin-treated virus (c); Sil-1 mutant cells plus untreated (b) or trypsin-treated virus (d). The trypsin treatment was carried out as described in Materials and Methods.

Fig. 4. Sensitivity of Sil mutant cells to ultraviolet-inactivated Sendai virus. The effects of various concentrations of ultraviolet-inactivated Sendai virus on the plating efficiency of KB (a), Sil-1 (b) and Sil-3 (c) cells were compared in the absence of Ca^{2+}.

line, designated as Sil-3, showed higher resistance to virus-induced cytolysis than does the Sil-1 mutant line. When the former cells of confluent monolayer in a 60 mm plastic dish were treated with 500 hemagglutinating units of Sendai virus, no polykaryocyte could be detected after 30 min of incubation at 37°C while a few small polykaryocytes were observed in the case of Sil-1 cells. Under the same conditions, parental KB cells almost formed a single huge polykaryocyte in the dish. Thus, the ability of Sil-3 cells to respond to the virus-induced cell fusion was reduced simultaneously. In addition, both Sil-1 and Sil-3 cells could adsorb nearly the same amount of virus particles over a wide range of virus concentrations: when their abilities to support viral adsorption were determined at virus concentration of 320 hemagglutinating units/ml, the fraction of virus particles adsorbed was 17% for Sil-1 cells and 15% for Sil-3 cells, respectively.

Furthermore, the possibility that the increased resistance of Sil-3 cells to cytolysis was a secondary result of the increased elution of absorbed virus particles from the cells was excluded by the results shown in Fig. 5 which demonstrate that the kinetics of elution of adsorbed virus particles from Sil-3 cells was essentially the same as that from Sil-1 cells.

Phospholipid intermixing with Sil-3 cells

When Sil-3 cells were incubated with PC*-Sendai virus, the ESR spectrum changed only slightly and the increase in the peak height was considerably lower than that of Sil-1 cells (Fig. 6). The correlation between decrease in the spectral change and reduced fusion ability of a series of mutant lines is consistent with the notion that the Sendai virus-induced phospholipid intermixing is closely associated with the fusion event between virus envelope and cell membrane.

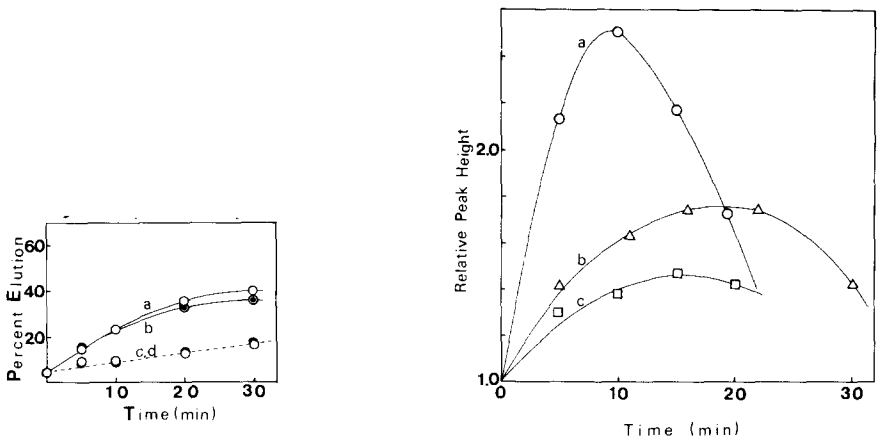

Fig. 5. Elution of Sendai virus from cells. Trypsinized cells were washed once with fusion buffer and resuspended in the same buffer at a cell density of $2.0 \cdot 10^6$/ml. To this suspension, ^{3}H-labeled Sendai virus, strain Fushimi, was added at a final concentration of 47 plaque-forming units/cell (a, b) or 24 plaque-forming units/cell (c, d), and incubated at 0°C for 30 min. Unadsorbed virus was removed by washing twice with fusion buffer. The cells were then resuspended in the same buffer to give an initial cell density, and incubated at 37°C. After times indicated, 1.0 ml of samples were withdrawn and immediately centrifuged. The radioactivity of eluted virus in the supernatant liquid was counted in the Nuclear-Chicago Mark II liquid scintillation counter after addition of 15 ml PCS-solubilizer (Amersham/Searle Corporation). a and c, Sil-1 cells; b and d, Sil-3 cells.

Fig. 6. PC* interchange in mutant lines. PC*-Sendai virus was incubated with parental KB cells (a) or Sil-1 cells (b) or Sil-3 cells (c). All procedures are the same as described in Fig. 1.

Discussion

Virus-cell interaction in the Sil mutant cells was studied by examining exchange of phosphatidylcholine molecules between viral envelope and cell surface membrane. The results are essentially in agreement with those for the Sendai virus-red blood cell system [2,3], except that the reduction of PC* molecules occurred in the present system. The loss of spin-labels by the reduction of the nitroxide radicals in PC* molecules generally causes magnetic dilution and affects the line shape in ESR spectrum. This raises the possibility that the observed increase in the peak height (1) is due to the loss of the labels in the virion and it depends on the PC*-reducing activity of the cell which acts outside the cell, or (2) is due to the loss of the labels after intermixed with cell membrane and it depends conversely on the cellular activity which acts only in the cell. However, these two possibilities can be eliminated due to the following reasons. (1) The loss of the labels was estimated by the double integration of a whole spectrum. During an incubation for 10 min at 37°C, the loss was about 30% with KB-Sendai virus and about 10% with either KB-influenza virus or Sil-1-Sendai virus. (2) When the PC*-molecules in Sendai virus were reduced with cystein and Fe^{3+}, the maximum incrase of the central peak height, caused by a gradual and homogeneous loss of the labels, was only about 30%. (3) The signal increase in the experiments with trypsin-treated Sendai virus or influenza virus was much less than those with the active Sendai virus (Figs. 2 and 3). (4) The PC*-reducing activities were determined by incubating either cell type with

lightly PC*-labeled liposomes (PC*/PC = 0.01) which do not show any exchange broadening. After incubation at 37°C for 10 min, KB, Sil-1 and Sil-3 cells were found to reduce 12%, 5% and 8% of spin-labels, respectively. Thus, it was concluded that the observed spectral changes are induced largely by the exchange of phospholipid molecules between viral envelope and cell membrane.

When either type of mutant cell was incubated with the PC*-virus, the phospholipid exchange occurred at a markedly decreased level and the degree of the intermixing correlated with the degree of the fusion capacity of the mutant lines. These results favor our postulation that Sil mutant cells have a defect(s) in the surface membrane which affects certain process of the envelope fusion. Since the mutant cells can adsorb Sendai virus almost as well as do their parental cells, the affected membrane component(s), rather than receptors to the HN spike protein of the virus, may be the component with which the F spike protein of the virus interacts to cause envelope fusion.

Moreover, the extensive loss of the spin-labels in the KB-Sendai virus system suggests that the spin-labels exchanged with cell membrane are more susceptible to reduction than those in the virion. Gaffney observed [6] that such a reaction can also take place vigorously in living cells. Loss of the labels in this way could well explain why the peak height increased less in KB cells (about 3 times at the maximum) than in red blood cells (5—6 times). Presumably, this also make the difference in interchange in KB and Sil cells less defined.

Acknowledgement

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