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Altered interaction between Sendai virus and a Chinese hamster cell mutant with defective cholesterol synthesis

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An amphotericin B-resistant mutant (AMB^r-1) isolated from the Chinese hamster V79 cell line is defective in a pathway for sterol synthesis and contains a much reduced free cholesterol level as compared with the parental V79. The character of the plasma membrane of AMB^r-1 was compared with that of V79 by measuring the fusion with the envelope of the Sendai virus and also by measuring membrane fluidity: AMB^r-1 was found to be more sensitive to Sendai virus-induced cytolysis than V79. Both assays for membrane-permeability change and electron spin resonance (ESR) study showed an enhanced response to the fusion between viral envelope and plasma membrane in AMB^r-1 cells. Measurement of the fluorescence polarization for 1,6-diphenyl-1,3,5-hexatriene suggested that the membrane of AMB^r-1 was more fluid than that of V79. This aberrant nature of the cell membrane of AMB^r-1 might be caused by the altered membranous sterol content.

Cholesterol is one of the major components in biological membrane and clearly important in influencing its fluidity [1,2]. However, the effect of membrane cholesterol on cell fusion is complex [3]. Many studies have been carried out to clarify the role of cholesterol in membrane fusion between viral envelope and artificial liposomes [4–9]. However, little is known regarding its effect on fusion between the viral envelope and the membrane of living cells. Thus it is interesting to ask whether fusion between viral envelope and cell membrane is altered in growing animal cell lines with low sterol content.

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We have previously selected a Chinese hamster cell mutant which is resistant to a polyene antibiotic, amphotericin B [10]. The amphotericin B-resistant clone (AMB^r-1) was shown to be defective in a pathway of lanosterol formation [11] and the growth is dependent upon exogenous sterols under conditions in which lipoprotein is limited [12,13]. The resistant phenotype was found to show recessive character in hybrids between wild-type strain and the resistant clone [14]. The free cholesterol content of AMB^r-1 cells was 50–60% that of parental V79 cells [10,11]. In this study, we characterize the membrane of AMB^r-1 cells by measuring fusion with Sendai virus-envelope and also by measuring membrane fluidity.

We first examined the cellular sensitivity of AMB^r-1 and V79 to the cytolytic effect of Sendai

virus. In the absence of Ca^{2+}, most cultured cells were extensively lysed but not fused during incubation with Sendai virus [15]. Sendai virus-induced cytolysis is thought to be initiated by the fusion between viral envelope and plasma membrane [16,17]. Assay for Sendai virus-induced cytolysis was carried out according to the procedure of Toyama et al. [17]. The effect of ultraviolet-inactivated Sendai virus on the plating efficiencies of V79 and AMB^r-1 was compared (Fig. 1). AMB^r-1 cells was shown to be more sensitive to the cytolytic effect of the virus than V79 cells. The cytolytic effect of Sendai virus appeared to be about 2-fold higher in AMB^r-1 than in the parental cells, as examined from the slope of the inactivation curve (Fig. 1).

Fusion of the viral envelope with cell membrane is known to induce an increase of the permeability of the cell membrane to small molecules as well as cytolysis [16,18]. Sendai virus-induced changes in membrane permeability were assayed by the influx of extracellular fluorescent molecules into the cytosol (Fig. 2A) or efflux of intracellular metabolites into the medium (Fig. 2B) according to the method of Impraim et al. [16] and Wyke et al. [18]. Measurement of the influx of extracellular fluorescent materials into the cytosol was carried out in the presence or absence of Ca^{2+} using calcein (3,3′bis[*N*,*N*-di(carboxymethyl)-aminomethyl]fluorescein, Dojindo Loboratories, Kumamoto, Japan) which is used as a non-permeable fluorescent marker across the cell membrane [19]. We also examined the efflux of intracellular metabolites by measuring the leakage of [^{3}H]choline from the prelabeled cells. Fig. 2 shows the virus-induced change in membrane permeability of V79 and AMB^r-1 in the presence or absence of Ca^{2+}. In the absence of Ca^{2+}, the increase of membrane permeability determined by influx of calcein into the cytosol or efflux of [^{3}H]choline into the medium was observed in V79 and AMB^r-1. However, both influx (Fig. 2A) and efflux (Fig. 2B) were 1.5- to 2-fold higher in AMB^r-1 in comparison with V79, indicating that AMB^r-1 was more sensitive to the virus-induced change in membrane permeability than the parental V79. On the other hand, only a slight if any influx of calcein was observed in the presence of Ca^{2+} (Fig. 2A) in both cell lines. Leakage of [^{3}H]choline was

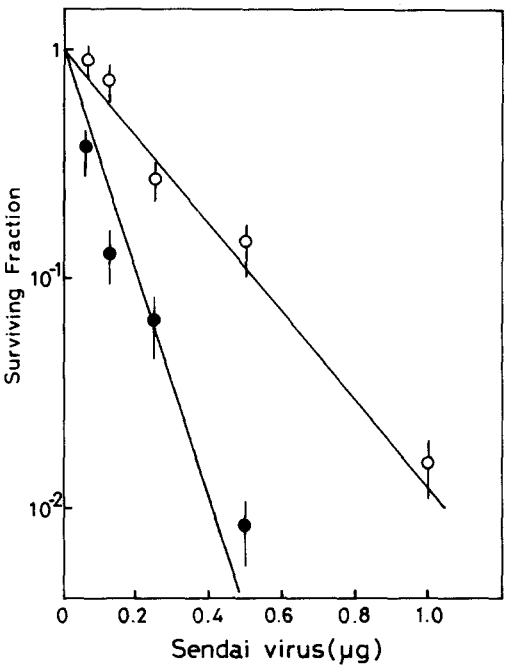

Fig. 1. Dose effect of Sendai virus on the surviving fraction of V79 cells (○) and AMB^r-1 cells (●) in the absence of Ca^{2+}. Cells were grown in monolayer in minimal essential medium (MEM) containing 10% newborn calf serum (NCS) [12]. Sendai virus (strain Z) was prepared from the allantoic sac of chicken embryos [20]. Ultraviolet inactivation was carried out by irradiation with a germicidal lamp for 5 min according to Toyama et al. [17]. Cells at a late exponentially growing stage were harvested by brief treatment with 0.05% trypsin (Difco) in phosphate-buffered saline containing 1 mM EDTA. After washing with phosphate-buffered saline, cells in suspensions ($2 \cdot 10^6$ cells/ml) were mixed with various concentrations of virus in 0.5 ml phosphate-buffered saline, kept in ice for 15 min and then incubated for a further 30 min at 37°C. After cells were diluted in MEM containing 10% NCS and incubated overnight, the number of cells adsorbed to the dishes was scored. Each point with error bar represents the average from duplicate determinations.

also low in the presence of Ca^{2+} in comparison with that in the absence of Ca^{2+}. Pasternak and his colleagues [16,18] reported the repair of the virus-induced changes in membrane permeability by Ca^{2+}. Our present data also suggest that the repair of membranous damages caused by Sendai virus occurs similarly in AMB^r-1 and V79.

The enhanced response of AMB^r-1 to Sendai virus-induced cytolysis and changes in membrane permeability may be caused by increased absorption of virus to mutant cells. The ability of cells to adsorb ^{125}I-labeled Sendai virus was examined.

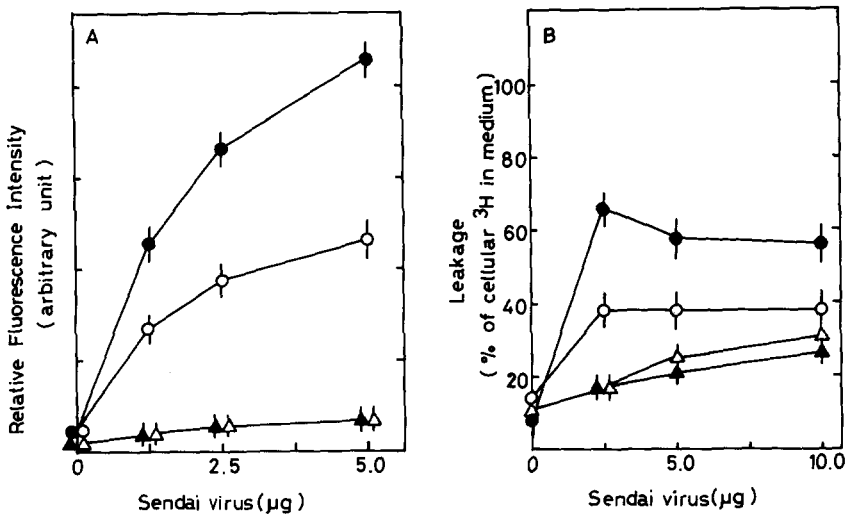

Fig. 2. Sendai virus-induced changes in membrane permeability. V79 (○, △) and AMB^r-1 (●, ▲) cells in monolayer were pre-labeled with [^{3}H]choline (0.1 μCi/ml) in MEM for 30 min at 37 °C and transferred into suspension as described in the legend of Fig. 1. Cells ($4 \cdot 10^6$ cells) in suspension were mixed with virus in 0.2 ml phosphate-buffered saline and kept in ice for 30 min for absorption. After washing with ice-cold phosphate-buffered saline, cells and virus were further incubated in phosphate-buffered saline (○, ●) or phosphate-buffered saline containing 1 mM $CaCl_2$ (△, ▲) in the absence (A) or presence (B) of 0.1 mM calcein for 20 min at 37 °C. After centrifugation, the radioactivity in the supernatant was measured in B. The cell pellets were washed with phosphate-buffered saline containing 1 mM $CaCl_2$ and dissolved in 0.5% Triton X-100, and then the fluorescence intensities at 530 nm (excitation at 495 nm) were measured in A. In B, total radioactivities of [^{3}H]choline inside $4 \cdot 10^6$ cells were $2.6 \cdot 10^3$ cpm in V79, and $2.2 \cdot 10^3$ cpm in AMB^r-1. Each point with error bar represents the average from duplicate trials.

Cells in suspension were incubated with various concentrations of ^{125}I-labeled virus (5–20 μg of viral protein) for 15 min in ice, washed and then cell-associated radioactivities were measured. Similar amounts of virus were found to be adsorbed to both V79 and AMB^r-1 cells (Table I).

TABLE I

BINDING OF ^{125}I-SENDAI VIRUS TO PARENTAL AND MUTANT CELLS

^{125}I-labeled virus was prepared by the method of Fraker and Speck [30]. The specific radioactivity of the ^{125}I-virus was 2530 cpm/μg viral protein. Cells ($2 \cdot 10^6$ cells) were incubated with the indicated amount of radiolabeled virus in 0.1 ml phosphate-buffered saline (0.15 M NaCl/10 mM phosphate, pH 7.2) on ice for 15 min. After washing twice by centrifugation with cold phosphate-buffered saline, the radioacitivity associated with the cells was measured.

Input virus (μg)	Adsorbed virus (% of input)	
	V79	AMB^r-1
5	55.3	52.1
10	50.6	51.4
20	52.3	50.7

To examine the cellular capacity for fusion with the viral envelope, we carried out phospholipid intermixing experiments between viral envelope and cell membrane, using spin-labeled phosphatidylcholine (PC*). Procedures to prepare PC*-labeled virus and those for electron spin resonance (ESR) measurement were described previously [20–24]. When fusion of the viral envelope with cell membrane occurred. PC* preincorporated into the viral envelope rapidly diffused away from the fused site and intermixed with lipids of the target cell membrane. Since the viral envelope contained a high concentration of PC*, the intermixing is expected to cause dilution of PC*, weakening the spin-spin exchange interaction, and resulting in increase in the ESR peak height.

When PC*-labeled Sendai virus was incubated with wild-type V79 cells at 37 °C, the central peak height of the ESR spectrum changed gradually, reaching about 3.5-times the height of the initial peak after 10 min of incubation (Fig. 3). When PC*-labeled virus was incubated with AMB^r-1 cells, the rate of the increase in the peak height was much faster than that of V79: the peak height

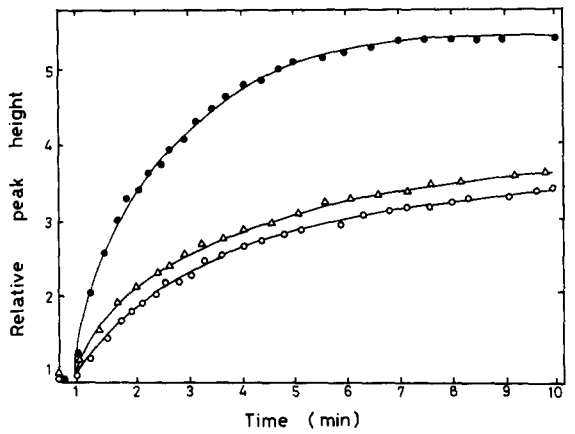

Fig. 3. Time course of ESR peak height increase for PC*-labeled Sendai virus. Cells ($1 \cdot 10^7$ cells) in suspension were mixed with 10μg of PC*-virus in 0.2 ml of Hanks' balanced-saline solution buffered with 20 mM Hepes (4-(2-hydroxyethyl-1-piperazine-ethanesulfonic acid) at pH 7.4 (Hanks/Hepes) containing 0.2% bovine serum albumin (BSA), kept in ice for 15 min for absorption, and washed by centrifugation after addition of 1.5 ml of Hanks/Hepes. The pellet was resuspended with 10 μl of the same buffer and ESR spectrum was measured at 37° C. The time course of the central peak height divided by the initial peak height at time 0 is plotted. Typical data from several experiments of each cell line, V79 (○), AMB^r-1 (●), VH1204 (△), are shown.

increased rapidly, reaching more than 5-times the height of the initial peak after 8–10 min of incubation. The time course of the change in the relative peak height observed in AMB^r-1 was similar to that observed in erythrocyte membrane [25]. Fig. 3 apparently shows that the initial rate of fusion with AMB^r-1 cells was about 2-fold higher than that with V79. VH1204, a hybrid clone between V79 and AMB^r-1, showed similar sensitivity to amphotericin B and similar cholesterol content as the parental V79 cells [14]. VH1204 also showed the similar time course of the peak height-increase as V79 cells, indicating that the enhanced response to fusion between viral envelope and cell membrane in the mutant was recessive.

The free cholesterol content of AMB^r-1 was 50–60% that of parental V79 [10,11]. In our present study, AMB^r-1 cells showed a more enhanced response to fusion with viral envelope than V79. In general, depletion of cholesterol increases the fluidity of biological membrane [1,2] and a number of studies have suggested that an increase in membrane fluidity favors cell fusion [3]. We examined the membrane fluidity of AMB^r-1 and V79 by measuring fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH). Cells at $3 \cdot 10^6$ cells/ml in suspension in phosphate-buffered saline (0.15 M NaCl/10 mM phosphate, pH 7.2) were mixed with 1/1000 volume of 1.5 mM DPH (Wako Chemicals, Osaka, Japan) dissolved in ethanol. The suspensions were incubated at 37° C for 30 min with shaking. The cells were then washed three times with phosphate-buffered saline and resuspended in phosphate-buffered saline. The fluorescence intensities of DPH through filters both L39 and B390 (Hoya, Tokyo, Japan) with excitation at 359 nm was measured at various temperatures in a Union Giken FS-501-A fluorescence polarization analyzer (Fig. 4). Fluorescence polarization values in both cell lines decreased with increase of temperature. However, within the range of 20° C to 37° C, the polarization values of AMB^r-1 cells were always lower than those of V79 cells, which indicated that the membrane of AMB^r-1 cells was more fluid than that of V79 cells.

In our present system, AMB^r-1 with a reduced sterol content apparently shows increased fluidity of the cell membrane as compared with the parental V79 (Fig. 4). The mutant also shows an enhanced response to fusion with the Sendai virus-envelope (Figs. 1–3). A hybrid clone between V79 and AMB^r-1 with similar cholesterol content as V79 showed a similar response to fusion with the viral envelope as V79 (Fig. 3), indicating a close relationship between the cholesterol level and the sensitivity to fusion with Sendai virus. Although the correlation between increased membrane fluidity and fusion with the viral envelope has not been clarified completely, these changes of cell membrane might be caused by altered sterol metabolism in the mutant cells.

Two independent studies indicated that cholesterol is required for fusion of Sendai virus with liposomes. Kundrot et al. [5] showed an absolute requirement of cholesterol for Sendai-virus induced lysis of liposomes containing glycophorin as a viral receptor. Hsu et al. [7] reported that the presence of cholesterol enhances the fusion between Sendai virus and liposomes containing phosphatidylcholine and mixed gangliosides. However, Tsao and Huang [4] reported that

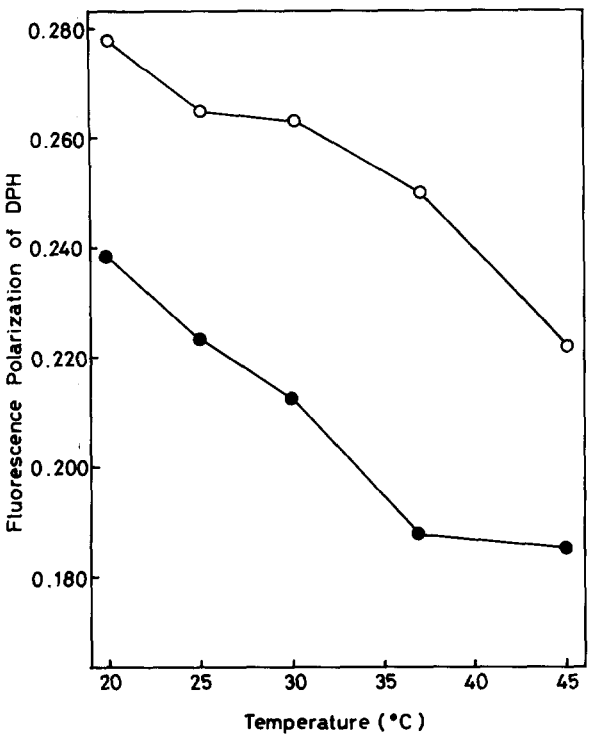

Fig. 4. Fluorescence polarization of diphenylhexatriene (DPH) in V79 (○) and AMB^r-1 (●) cells. Suspension cells in phosphate-buffered saline ($3 \cdot 10^6$ cells/ml) were mixed with 1.5 μM DPH and incubated for 30 min. Cells were washed three times with phosphate-buffered saline and resuspended in phosphate-buffered saline. Average values of fluorescence polarization from ten determinations were plotted as a function of temperature. The standard error of each point was less than 5% of the value.

cholesterol was not required for the lytic response of ganglioside-containing liposome induced by Sendai virus, and also that the presence of cholesterol suppressed the leakage response. They suggested the presence of cholesterol in the target membrane may positively or negatively modulate the membrane leakage induced by Sendai virus, depending on the lipid composition or the viral receptor (glycoprotein or ganglioside) in the target membrane. In our present study, we have employed two isogenic cell lines with low and high sterol contents to question the requirement of cholesterol for the fusion process in cultured living cells. We showed that a mutant with reduced cholesterol content is more sensitive to fusion with Sendai virus than the parental cell with higher cholesterol content. Low cholesterol content in the cell membrane might cause modification of the lipid composition or membrane proteins, and these changes in the mutant cell membrane might induce enhanced fusion with viral envelope. Alternatively, cholesterol is required for fusion with viral envelope, but above a certain level it may inhibit fusion.

Relevant somatic cell variants with altered response to fusion either with cells or/and with viruses have been isolated. Roos and his colleagues [26–28] have isolated somatic cell variants resistant to poly(ethylene glycol) (PEG), a potent fusion agent. The content membrane fatty acids in the resistant clone is different from that in the parental clone [27], and the variant shows an aberrant response to fusion with enveloped viruses (cited in Ref. 28). Toyama et al. also isolated mutants resistant to Sendai virus-induced cytolysis [17]. Their mutant showed a reduced response to fusion with cell membrane as well as fusion with viral envelope [17,23]: the mutant was deficient in a sialyltransferase activity [29]. These studies with several variants suggest involvement of various cellular factors in the fusion processes. Further isolation of mutants with altered fusion steps could be a effective way to understand other cellular factor(s) necessary for fusion.

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References

1 Tanaka, K. and Ohnishi, S. (1976) Biochim. Biophys. Acta 426, 218–231.
2 Radda, G.K. and Vanderkooi, J. (1972) Biochim. Biophys. Acta 265, 509–549
3 Lucy, J.A. (1978) in Membrane fusion (Poste, G. and Nicolson, G.L., eds.), pp. 267–304, Elsevier/North-Holland Biomedical Press, Amsterdam.
4 Tsao, Y. and Huang, L. (1985) Biochemistry 24, 1092–1098
5 Kundrot, C.E., Spangler, E.A., Kendall, D.A., MacDonald, R.C. and MacDonald, R.I. (1983) Proc. Natl. Acad. Sci. USA 80, 1608–1612.
6 Breisblatt, W. and Ohki, S. (1976) J. Membrane Biol. 29, 127–146.
7 Hsu, M.-C., Scheid, A. and Choppin, P.W. (1983) Virology 126, 361–369
8 Kielian, M. and Helenius, A. (1984) J. Virol. 52, 281–283.

9 Mooney, J.J., Dalrymple, J.M., Alving, C.R. and Russel, R.K. (1975) J. Virol. 15, 225–231.
10 Hidaka, K., Endo, H., Akiyama, S. and Kuwano, M. (1978) Cell 14, 415–421
11 Kuwano, M., Masuda, A., Hidaka, K. and Akiyama, S. (1983) Somat. Cell. Genet. 9, 659–672.
12 Hidaka, K., Akiyama, S. and Kuwano, M. (1980) Exp. Cell Res. 128, 215–221.
13 Kuwano, M., Akiyama, S., Takaki, R., Okano, H. and Nishimoto, T. (1981) Biochim. Biophys. Acta 652, 266–273.
14 Hidaka, K., Akiyama, S. and Kuwano, M. (1981) J. Cell. Physiol. 106, 41–47.
15 Okada, Y. and Murayama, F. (1966) Exp. Cell Res. 26, 108–118
16 Impraim, C.C., Foster, K.A., Micklem, K.J. and Pasternak, C.A. (1980) Biochem. J. 186, 847–860
17 Toyama, S., Toyama, S. and Uetake, H. (1977) Virology 76, 503–515
18 Wyke, A.W., Impraim, C.C., Knutton, S. and Pasternak, A. (1980) Biochem. J. 190, 625–638
19 Straubinger, R.M., Hong, K., Friend, D.S. and Papahadjopoulos, D. (1983) Cell 32, 1069–1079
20 Maeda, T., Asano, A., Ohki, K., Okada, Y. and Ohnishi, S. (1975) Biochemistry 14, 3736–3741.
21 Maeda, T., Asano, A., Okada, Y. and Ohnishi, S. (1977) J. Virol. 21, 232–241
22 Maeda, T., Kuroda, K., Toyama, S. and Ohnishi, S. (1981) Biochemistry 20, 5340–5345.
23 Koyama, A., Maeda, T., Toyama, S., Ohnishi, S., Uetake, H. and Toyama, S. (1978) Biochim. Biophys. Acta 508, 130–136
24 Yoshimura, A., Kuroda, K. Kawasaki, K., Yamashina, S. and Ohnishi, S. (1982) J. Virol. 43, 284–293.
25 Kuroda, K., Kawasaki, K. and Ohnishi, S. (1985) Biochemistry, 24, 4624–4629.
26 Roos, D.S. and Davidson, R.L. (1980) Somat. Cell Genet. 6, 381–390
27 Roos, D.S., Robinson, J.M. and Davidson, R.L. (1983) J. Cell Biol. 97, 909–917
28 Roos, D.S. and Choppin, P.W. (1984) Proc. Natl. Acad. Sci. USA 81, 7655–7626
29 Toyama, S., Koyama, H. and Toyama, S. (1983) J. Biol. Chem. 258, 9147–9152
30 Fraker, P.J. and Speck, J.C. (1978) Biochem. Biophys. Res. Commun. 80, 849–857