INTERACTION BETWEEN HAEMAGGLUTINATING VIRUS OF JAPAN AND HUMAN En (a−) ERYTHROCYTES LACKING MAJOR SIALOGLYCOPROTEIN

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1. Introduction

Haemagglutinating virus of Japan (HVJ, synonym of Sendai virus) has been an useful tool to investigate the molecular mechanism of membrane fusion. The virus envelope is composed of two membrane proteins, HANA and F proteins [1]. The HANA protein binds sialic acid or oligosaccharides with sialic acid on erythrocyte surface, causes aggregation of cells at 0°C and also expresses neuraminidase activity at 37°C [1]. The F protein is responsible for lipid intermixing between the envelope and cell membrane and also between the fusing cell membranes [2] as well as haemolysis and fusion [3]. It is important to identify the membrane components on erythrocyte surface with which the two envelope proteins interact and to know how the F protein causes chemical or structural modifications of cell membrane to effect these phenomena. In this regard the use of a genetic variant of erythrocyte with defined alterations in membrane components provides a particularly interesting system. In this paper we studied the interaction between HVJ and human erythrocytes of blood group En(a−) compared with normal erythrocytes En(a+). The rare En(a−) completely lacks the major sialoglycoprotein (glycophorin) and have an altered sugar composition of band 3 [4]. The major sialoglycoprotein represents ~75% of total sialoproteins of normal erythrocytes and carries 30–50% of total cell surface carbohydrates [4–6]. The results have shown that the virus binding, virus-induced lipid intermixing, haemolysis and fusion occurred with both En(a−) and normal En(a+) cells to similar extent.

2. Materials and methods

HVJ, z strain was used throughout. The virus and spin-labelled or radioiodinated derivatives were prepared as in [2]. The spin label used was phosphatidylcholine with 12-nitroxide stearic acid attached at the 2-position. Virus concentration was expressed as haemagglutination unit per milliliter (HAU/ml). Whole blood from an En(a−) donor G. W. was kindly supplied by Dr D. J. Leikola, Finnish Red Cross Blood Transfusion Service, Helsinki [7] and had been stored in liquid nitrogen before use.

Adsorption of the virus to cells or reconstituted vesicles composed of egg lecithin and the major sialoglycoprotein was determined as follows. Radioiodinated virus (25 μl, 2500 HAU/ml) was mixed with 175 μl of various concentrations of erythrocytes or reconstituted vesicles in Tris-buffered saline (140 mM NaCl, 5.4 mM KCl, 20 mM Tris–HCl, pH 7.6) at 0°C and incubated for 15 min. After centrifugation at 700 × *g* for 15 min, the radioactivity in the supernatant was counted by a scintillation counter. The reconstituted vesicles were prepared by the method in [8]. For the measurement of haemolysis and fusion, erythrocytes (2×10^8 cells/ml) were incubated with various concentrations of HVJ in 1 ml Tris-buffered saline for 15 min at 0°C and then for 30 min at 37°C. Finally 3 ml of the chilled buffer was added and centrifuged at 700 × *g* for 15 min. Haemoglobin content in the supernatant was measured spectrophotometrically. Fusion of erythrocytes was observed under a phase contrast microscope. Lipid intermixing was measured as in [2]. Briefly, spin-labelled HVJ was added at 400 HAU/ml to erythrocyte

suspension (2.5×10^8 cells/ml) in Tris-buffered saline at 0°C. The cell aggregates were taken into a quartz capillary and the ESR spectrum was periodically measured at 37°C with a JEOLCO Model ME-X spectrometer.

3. Results and discussion

3.1. *Adsorption of HVJ to En(a−) cells and reconstituted vesicles containing the major sialoglycoprotein*

Sialic acids on the cell surface are known to be receptors for paramyxoviruses [9]. It is interesting to examine whether the absence of the major sialoglycoprotein in En(a−) cells modified the adsorption of HVJ to these cells. The sialic acid content of En(a−) cells is markedly reduced to only 45% of normal [6]. However, the adsorption curve (fig.1) shows that En(a−) cells bind the same amount of HVJ as En(a+) cells. Lack of the major sialoglycoprotein does not affect binding of the virus to the erythrocyte surface. Thus either the sialoglycoprotein is not a receptor for the virus or other receptors are present on surface.

The ability of the major sialoglycoprotein to bind HVJ was therefore investigated using the reconstituted vesicles. Binding of HVJ to the vesicles and to normal erythrocytes was measured and compared (fig.2). The result indicates that the vesicles carrying the sialoglycoprotein bind slightly less HVJ than normal erythrocytes containing an equivalent amount of the sialoglycoprotein. In the binding measurements the amount of unadsorbed virus was determined from the radioactivity in the supernatant. This method gives a low estimate for the reconstituted vesicles since all the vesicles did not sediment by the centrifugation. The amount of the major sialoglycoprotein on erythrocytes was calculated assuming 5×10^5 copies/cell [10]. In the reconstituted vesicles, a half of the sialoglycoprotein was assumed to expose outside the vesicles [8]. This assumption gives an upper limit to the number of available sialoglycoproteins. Therefore, it is very likely that the major sialoglycoprotein has a similar binding constant for HVJ to the receptors on normal erythrocytes. Although the major sialoglycoprotein may be a receptor for HVJ in normal erythrocyte, other components on the cell surface substitute equally well in En(a−) erythrocytes.

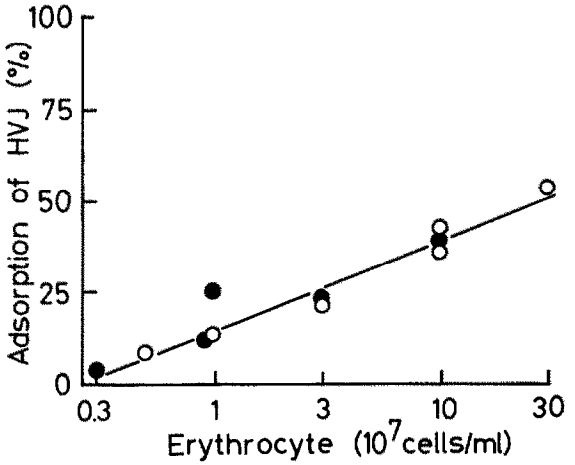

Fig.1. Adsorption of HVJ to En(a+) cells (○) and En(a−) cells (●). After radioiodinated HVJ (300 HAU/ml) was incubated with various concentrations of erythrocytes for 15 min at 0°C, the amount of bound virus was determined.

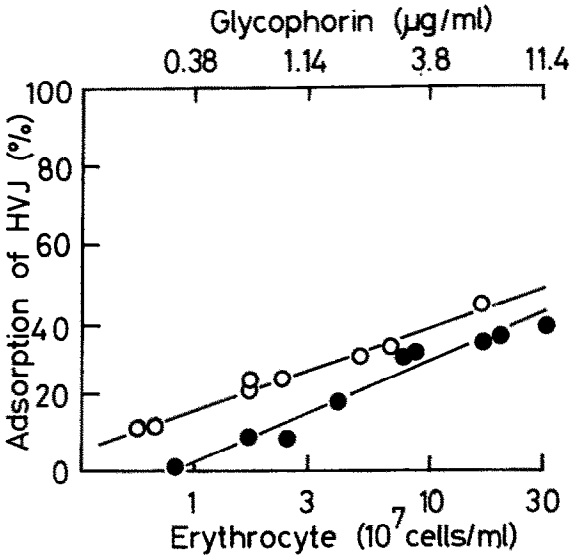

Fig.2. Adsorption of HVJ to normal erythrocyte (○) and reconstituted egg lecithin vesicles carrying the major sialoglycoprotein (glycophorin) (●). After radioiodinated HVJ (300 HAU/ml) was incubated with various concentrations of erythrocytes or vesicles for 15 min, the radioactivity of supernatant was determined. The amount of the bound virus was plotted against the concentration of glycophorin.

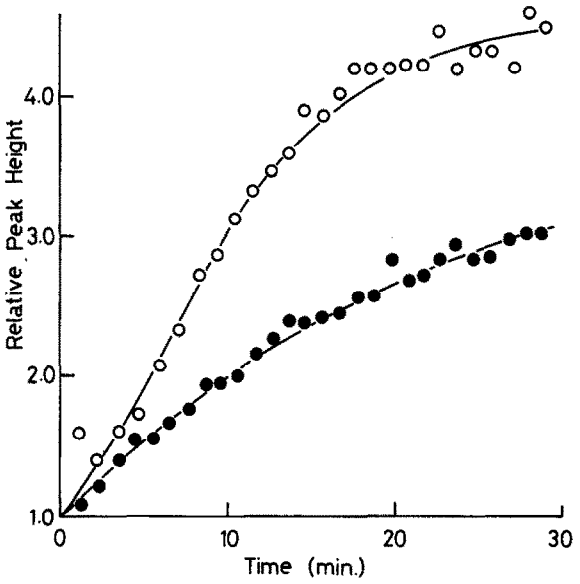

Fig.3. Transfer of spin-labelled phosphatidylcholine from HVJ envelope to En(a+) (○) or En(a−) (●) cell membranes. Spin-labelled HVJ (400 HAU/ml) and erythrocytes (2.5×10^8 cells/ml) were incubated at 37°C. The central peak height of the ESR spectrum is plotted against incubation time.

3.2. *Lipid intermixing, haemolysis and fusion*

In order to determine whether absence of the major sialoglycoprotein affects expression of the F protein-induced phenomena, lipid intermixing, haemolysis and fusion of En(a−) cells were studied. The lipid intermixing between the virus envelope and erythrocyte is an essential step before or during the envelope fusion [2]. When the spin-labelled HVJ was incubated with En(a−) cells at 37°C, the ESR spectrum changed rapidly in quite similar way to normal erythrocytes. The spectral peak height increased rapidly (fig.3). This change shows transfer of spin-labelled phosphatidylcholine from the viral envelope to erythrocyte membrane. After 10 min incubation about 12% of the spin-labelled lipid in the envelope moved into En(a−) cell membrane, while the value with En(a+) cells was 26%. The difference between the two curves in fig.3 is partly due to partial haemolysis during transportation to Kyoto. A recent result with En(a−, Ss−) cells showed much closer similarity to the control curve (to be published). The virus-induced haemolysis of En(a−) cells was also

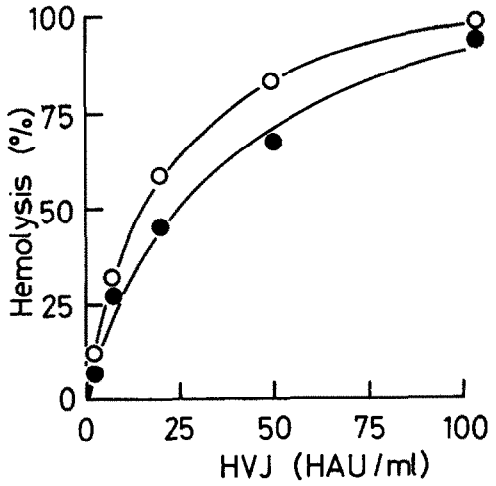

Fig.4. Dose response curve of HVJ-induced haemolysis of En(a+) cells (○) or En(a−) cells (●). Erythrocytes (2.5×10^8 cells/ml) were mixed with various concentrations of HVJ at 0°C and incubated for 30 min at 37°C.

quite similar to that for En(a+) (fig.4). Giant fused En(a−) cells produced by HVJ were observed under the phase contrast microscope (fig.5). These results indicate that HVJ causes essentially the same kind of reactions with both En(a−) and En(a+) cells and that the function of F protein does not require presence of the major sialoglycoprotein. The possibility remains that the modified band 3 in En(a−) cells subsitutes for the major sialoglycoprotein in mediating the reaction of F protein.

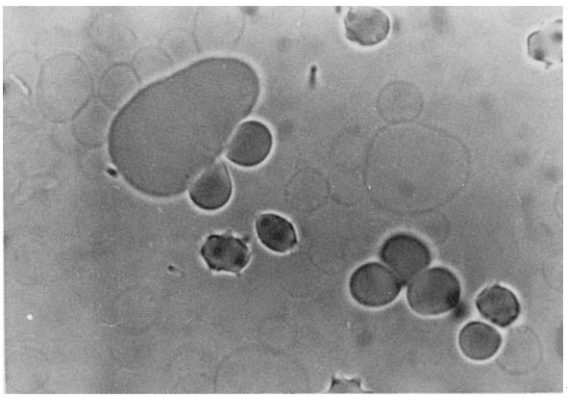

Fig.5. A light micrograph of fused En(a−) cells. The cells (2.5×10^8 cells/ml) were incubated with 600 HAU/ml of HVJ at 37°C.

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