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Analysis of virus-cell binding characteristics on the determination of Japanese encephalitis virus susceptibility

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Summary. The susceptibility of fourteen established cell lines to infection with Japanese encephalitis virus (JEV) was assayed using an indirect fluorescent antibody technique. In kinetic studies, the degree of binding and internalization of JEV allowed the identification of high susceptibility and low-susceptibility cells. Scatchard analysis showed that JEV specifically bound to high-susceptibility Vero cells with greater affinity than to low-susceptibility NRK cells. Microinjection of viral genomic RNA into NRK cells induced highly efficient production of viral antigen and infectious virions. A hemagglutinin-inhibiting monoclonal antibody against JEV (MAb 301) inhibited the binding of JEV to the Vero and NRK cells. JEV was found to bind to a 74K molecule present in the membrane fraction of Vero cells and this binding was inhibited by MAb 301. Importantly, the 74K molecule was not detected in the membrane faction of NRK cells. These results suggest that early events in the JEV-cell interaction influence the susceptibility of cells to infection, and in particular suggests that the 74K molecule may be a possible candidate or component of the cellular receptor for JEV.

Introduction

The early events of viral infection, especially the interaction between viral attachment proteins and cellular receptors, are one of the factors determining viral tissue tropism. Recently, the cellular receptors for a number of viruses have been identified. For example, the CD4 molecule is the receptor for human immunodeficiency virus [7, 20], and CD21, the receptor for Epstein-Barr virus [10, 11, 25]. Major human rhinovirus [13], polio virus [21, 23], and mouse hepatitis virus [9, 33]

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enter cells by binding to the receptors that belong to the immunoglobulin superfamily. Integrin VLA-2 has been recently identified as a receptor for echovirus-1 [4]. The identification of specific cellular receptors has contributed not only to advance in the study of the virus-cell interactions, but also to the development of antiviral strategies [5].

Japanese encephalitis virus (JEV), a member of the family *Flaviviridae*, is an important human pathogen that causes encephalitis in Southeastern and far eastern Asia. The mechanisms of the early events in flaviviral infection remain poorly understood. Several studies on the early events of flavivirus infection have been performed with West Nile virus [12], yellow fever virus [15], and Kunjin virus [26], and these have shown that the entry of flavivirus into cells occurs by an endocytotic mechanism. In the case of dengue virus type 4, it has been reported that cell susceptibility appears to be determined largely at the stage of viral binding [1]. However, the cellular receptors for the flaviviruses have not been identified, and the relationship between the entry mode of JEV and cell susceptibility remains unclear.

In the present study, we have investigated, firstly, whether early events of JEV-cell interaction, specifically virus-cell binding, influence the susceptibility of cells to infection and, secondly, if specific JEV-binding molecules in the cell membranes of susceptible cells could be identified.

Materials and methods

Cells

A number of cell lines were used for the present studies (Table 1). N18TG and N115 cells were generous gifts of Dr. Takehiko Amano (Mitsubishikasei Institute of Life Sciences, Tokyo, Japan). PC12h cells were generously donated by Dr. Hiroshi Hatanaka (Osaka University, Osaka, Japan). B50, B82, and B104 cells were generously provided by Dr. Yoichiro Kuroda (Tokyo Metropolitian Institute for Neuroscience, Tokyo, Japan). Vero cells were cultured in Eagle's minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS). B104 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 4.5 g/l D-glucose, 10% horse serum (HS), and 5% FBS. PC12h cells were grown in DMEM supplemented with 5% HS and 10% FBS. *Aedes albopictus* C6/36 cells were grown in MEM supplemented with 10% FBS and 200 μM MEM non-essential amino-acid solution (GIBCO). The other cell lines were cultured with DMEM supplemented with 10% FBS.

Viruses

JEV (JaGAr-01 strain) was propagated in C6/36 cells. [^{35}S]methionine-labeled JEV was prepared by a modification of the method of Takegami et al. [30]. Briefly, 24 h after infection, the culture medium was changed to methionine- and cystine-free medium. After 1 h of incubation, 740 kBq/ml of Expre^{35}S^{35}S[^{35}S] Protein Labeling Mix (NEN Research Products, Du Pont) was added and the cells were cultured for an additional 45 h.

[^{3}H]uridine-labeled JEV was prepared by a modification of the method of Takeda et al. [29]. Briefly, 18 h after infection, the culture medium was changed to medium containing 0.1 μg/ml Actinomycin D (SERVA) and 20 μM thymidine. After 1 h of incubation in this medium, 1.11 M Bq/ml of [^{3}H]uridine (American Radiolabeled Chemicals, Inc.) was added and the cells were cultured for an additional 49h.

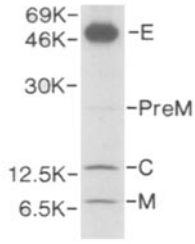

Fig. 1. SDS-PAGE of purified, radiolabeled JEV. ^{35}S-labeled JEV was prepared and purified as described in materials and methods. Polyacrylamide gels, 16% were used for separation, and the dried gel was subjected to autoradiography. *E* E (envelope) glycoprotein; *PreM* PreM protein; *C*, C (core) protein; *M* M (membrane) protein

Purification of JEV from the harvested medium was carried out by the method of Takegami et al. [30], with minor modifications. The purity of the virus preparations was checked by analysis of the virus structural proteins using sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) (Fig. 1). The infectivity titers were measured by plaque assay in Vero cells by standard procedures.

Assay of JEV infectivity

Approximately 1.6×10^5 cells grown in poly-L-lysine-pretreated 8-well-chamber slides (Nunc) were infected with JEV at a multiplicity of 100 PFU/cell. After two days of culture, the cells were fixed with cold acetone for 5 min and stained with anti-JEV rabbit serum followed by fluorescein isothiocyanate-conjugated goat anti-rabbit IgG [F(ab)'$_2$] (Cappel). The anti-JEV serum was prepared from rabbits immunized by two intravenous inoculations of purified JEV (JaGAr-01 strain) (2×10^9 PFU/rabbit). Cells showing fluorescence were counted in three areas of each well.

Binding and internalization assays

For binding assays, cell monolayers plated on microtiter wells were pretreated with MEM supplemented with 0.2% bovine serum albumin, 25 mM HEPES, and 0.1% sodium azide (binding medium) for 2 h at 4 °C. After incubation with diluted [^{35}S]-labeled JEV at 4 °C for indicated times, the cells were washed 3 times with binding medium, lysed with 1% SDS, and transferred to glass microfiber filters (Whatman).

For internalization assays, MEM supplemented with 0.2% bovine serum albumin and 25 m M HEPES was used as assay medium. The cells were pretreated with the medium for 2 h and incubated with diluted [^{3}H]-labeled JEV for 90 min at 4 °C. After washing 3 times with the assay medium, the temperature of the cultures was changed from 4 °C to 37 °C, and the cultures were incubated for 5 to 180 min. Viral particles bound to the cell surface were removed by 15 min incubation with 0.125% trypsin (SIGMA), followed by centrifugation of the cells in PBS(-) containing 1 mM phenylmethanesulfonyl fluoride, 1 m M benzamide, and 1 mM benzamidine hydrochloride (SIGMA). The resulting cell pellets were lysed with 1% SDS and transferred to glass microfiber filters.

Radioactivity in the microfiber filters was determined in Econofluor (NEN Research Products, Du Point) with a Beckman LS 1801 scintillation counter.

Scatchard analysis of the binding of JEV to cell lines

Radiolabeled JEV in increasing amounts (from 11 to 1, 440 PFU/cell) was added to cell monolayers (2×10^5) cells grown in 48-well plates and incubated at 4 °C for 180 min. Then the cell monolayers were washed, and radioactivity was determined. The amount of specific binding was determined by

subtracting the amount of nonspecific binding from the amount of total binding. Nonspecific binding was estimated from the counts bound in the presence of a constant amount (8.0×10^3 PFU/cell) of excess unlabled JEV. Then the specific binding data was presented in the form of Scatchard plots.

Microinjection of JEV RNA

Viral genomic RNA was prepared from purified JEV by phenol extraction. The purity of the RNA preparation was checked using denaturing agarose gel electrophoresis. Preliminary experiments confirmed the absence of infection if the RNA was only added into the medium. Cells were plated in individual 35 mm dishes to give ~60% confluent monolayers after overnight incubation. Cells were injected with 0.5 pl of an aqueous solution of 10 μg/ml genomic RNA using automated injection systems (Zeiss). The injection introduce ~1000 copies of the viral genome per cells (assuming all of the RNA in the preparation to be full length genomic RNA). The cells were then incubated in growth medium at 37 °C for indicated times. The culture supernatants in 35 mm dishes were collected and served for plaque assay. The cells were fixed with methanol at room temperature for 1 h and processed for indirect immunofluorescence as described above.

Fractionation of cultured cells

Cultured cells were disrupted using a Dounce homogenizer containing 4 volumes of SEAT buffer (0.25 M sucrose, 1 m M EDTA, 10 m M acetic acid, and 10 m M triethanolamine, pH 7.4) [28]. The homogenate was centrifuged at 240 **g** for 15 min, the pellet suspended in SEAT buffer, and the previous step repeated. The two supernatant fractions were combined and centrifuged at 42 000 **g** for 1 h . The 42 000 **g** supernatant was then collected and re-centrifuged at 105 000 **g** for 2 h. The final pellets were used as crude membrane extracts. These were treated with N-(D-gluco-2,3,4,5,6-pentahydroxyhexyl)-N-methylnonanamide (MEGA-9, Dojindo Laboratories) at final concentration of 1.0%, and aliquots were loaded onto mono Q HR5/5 anion exchange columns (Pharmacia).

Binding of JEV to electroblotted cellular proteins

Two ml fractions collected from the anion exchange column were concentrated with Centricon-30 (Amicon) by centrifugation at 4,500 rpm for 30 min in an angle rotor. Concentrated aliquots (10 μl) were diluted with an equal volume of 2 × sample buffer (125 m M Tris-HCl, pH 6.8, 2% SDS, 20% glycerol, 10% 2-mercaptoethanol, and 0.01% bromophenol blue) and boiled for 4 min. Electrophoresis in 8% polyacrylamide gels was performed on 1 mm thick slab gels in a Laemmli buffer system [22].. Electrophoresed proteins were transferred to Immobilon PVDF transfer membranes (Millipore) by the method of Towbin et al. [31]. Membranes were blocked with PBS containing 2% non-fat dried milk at 37 ° C for 2 h, followed by washing three times with MEM supplemented with 0.2% bovine serum albumin (MEM-BSA). [^{35}S]-labeled JEV diluted in MEM-BSA was incubated with the membrane at 4 °C overnight. After washing three times with MEM-BSA, the membrane was subjected to autoradiography.

Preparation of Fab fragment of monoclonal antibodies (MAbs)

Fab fragments of MAbs were prepared with an ImmunoPure Fab Preparation Kit (Pierce). MAb 301 and MAb 503 have been described previously [17, 18]. Briefly, MAb 301 cross-reacts with all the Flaviviruses and has high hemagglutinin-inhibiting (HI) activity. In contrast, MAb 503 reacts specifically with JEV, and possesses very high neutralization activity. MAb 301 does not cross-react with the epitope recognized by MAb 503.

Results

JEV infectivity of different cell lines

The susceptibility of each cell line to JEV infection was determined by indirect immunofluorescence methods. Two hundred cells were examined in each area and the number of cells showing specific fluorescence were counted. JEV infectivity was expressed as a percentage of the average number of positive cells. The infectivities following infection with 100 PFU/cell were found to differ significantly between the various cell lines (Table 1). Cells were classified as having either a high-susceptibility or low-susceptibility to infection.

Binding and internalization of JEV to cell lines

To investigate the differences in viral infectivity among the cell lines, we examined binding and internalization of JEV to the high-susceptibility and low susceptibility cell lines (Fig. 2). Vero cells were selected as high-susceptibility, and NRK cells were selected as low-susceptibility cell lines respectively. The binding kinetics of radiolabeled JEV to these two cell lines was determined (Fig. 2a). The counts of cell-bound virus were as a function of time from 2 to 180 min. The level of binding of the high-susceptibility Vero cells was found to be much higher than that of the low-susceptibility NRK cells. To determine what proportion of bound JEV could be internalized in the two cell lines, we plotted the count of cell associated virus after removal of surface radioactivity after 0 to 180 min of incubation at 37 °C (Fig. 2b). It could be demonstrated that the percentage of virus internalized into the high-susceptibility Vero cells was significantly higher than that into the low susceptibility NRK cells. We then performed Scatchard analysis to show the relative affinity

Table 1. Comparison of JEV infectivity in various cell lines

Cell line	Species	Tissue origin	JEV infectivity (%)[a]	Reference
Vero	African green monkey	Kidney	100	36
B104	Rat	Brain tumor (neuronal)	80	27
PC12h	Rat	Pheochromcytoma	56	14
G8-1	Mouse	Skeletal muscle myoblasts	47	6
MMT	Mouse	Mammary tumor	38	
FR	Rat	Skin	30	
N18TG	Mouse	Neuroblastoma	26	2
B50	Rat	Brain tumor (glial)	19	27
N115	Mouse	Neuroblastoma	16	2
L6	Rat	Skeletal muscle myoblasts	15	34
B82	Rat	Brain tumor (glial)	13	27
3Y1	Rat	Embryonic fibroblasts	5	16
C6	Rat	Glioma	3	3
NRK	Rat	Kidney	0.2	8

[a] Infectivity is expressed as percentages of the average number of cells showing fluorescence

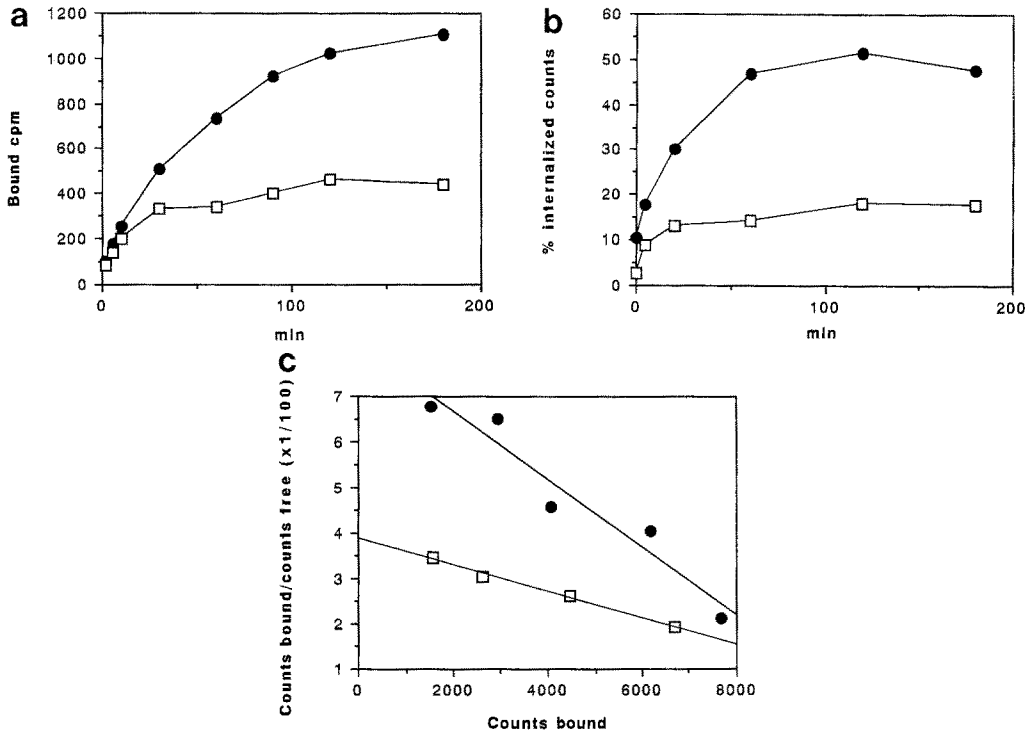

Fig. 2. a Binding of JEV to Vero and NRK cells. [^{35}S]methionine-labeled JEV (1.6×10^3 PFU/cpm) was added to cell monolayers (2×10^5 cells) grown in 48-well plates at 30 PFU/cell. After incubation at 4 ° C for the indicated times, the cell monolayers were washed and then lysed, and radioactivity was determined. **b** Internalization of JEV by Vero and NRK cells. [^{3}H]uridine-labeled JEV (4.9×10^2 PFU/dpm) was added to cell monolayers (8×10^5 cells) grown in 12-well plates at 30 PFU/cell. After preincubation at 4 °C for 90 min, the cells were washed and then warmed to 37°C for the indicated times. Surface radioactivity was removed by the method described in Materials and methods. Vertical axis shows internalized counts as percentage of pre-bound counts. **c** Scatchard plot of radiolabeled JEV bound to Vero cells vs. NRK cells. The activity of the radiolabeled JEV preparation used was 7.0×10^2 PFU/cpm. Data derived from the specific binding to Vero and NRK cells as described in Materials and methods. The results of **a** and **b** represent the mean values of two independent assays. The result of **c** represent the mean values of three independent assays. Vero cells (●) and NRK cells (□) were compared

constant (Fig. 2c). The Scatchard plots for both Vero and NRK cells were linear, but NRK cells had a more flattened curve compared to the Vero cells.

Microinjection of JEV RNA

To investigate the difference between Vero and NRK cells in their capacity to support viral replication, we examined microinjection of viral genomic RNA into Vero and NRK cells (Table 2). Purified genomic RNA was injected into the almost same number of cells within the marked square (62–89, described in Table 2). Infection was monitored by counting infected cells and also by counting infectious virus released in the media using a plaque assay at 12, 24 and 48 h post injection. Both Vero and NRK cells produced viral antigens and released the infectious virus.

Table 2. Microinjection of JEV RNA

Cell	Hours after injection	Number of injected cells[a]	Number of cells showing fluorescence[b]	Titer[c]
Vero	12	89	25	0
Vero	12	80	24	0
Vero	24	81	93	2×10^2
Vero	24	78	77	10^2
Vero	48	62	$>10^4$	1.6×10^6
Vero	48	81	$>10^4$	1.4×10^6
NRK	12	84	21	0
NRK	12	87	20	0
NRK	24	81	45	10
NRK	24	89	53	15
NRK	48	81	42	10^3
NRK	48	74	52	8×10^3

[a] Each cell was injected with 0.5 pl of an aquaous solution of 10 μg/ml genomic RNA

[b] Indirect immunofluorescence technique

[c] PFU/ml of culture supernatant

The data given represent the each value of independent injection experiments using the same batch of isolated viral genomic RNA

Importantly, at 12 h post injection, the number of cells expressing viral antigens was about the same (20–25) between Vero and NRK cells.

Binding of JEV to cellular proteins

To identify cellular proteins involved in JEV binding, we examined the binding of virus to electroblotted cellular proteins in cellular membrane fractions employing radiolabeled JEV as a prove. Because JEV-binding molecules present in the total crude membrane extracts could only be faintly detected, extracts of Vero and NRK cells were further processed using an anion exchange column (Fig. 3a). Unbound materials were removed by washing the column with 20 mM Tris-HCl pH 8.0. Elusion was performed with a linear NaCl gradient from 0 to 0.5 M, and then the concentration of NaCl was increased to 1 M. In Vero cells, a JEV-binding molecule of 74 K which was eluted with 1 M NaCl was found to be predominant. However, in NRK cells, radiolabeled JEV bound to a 39 K molecule which was present in the unbound fraction. In NRK cells the JEV-binding molecule of 74 K was not detected. In Vero cells the JEV-binding molecule of 39 K was not detected. The JEV-binding molecules detected in peak fractions are illustrated in Fig. 3b. Figure 3b also shows the specificity of the binding reaction, because the binding of radiolabeled JEV to the 74 K and 39 K molecules was diminished in the presence of excess of unlabeled JEV but, in contrast, use of an unrelated virus, Sindbis virus, did not cause such competition.

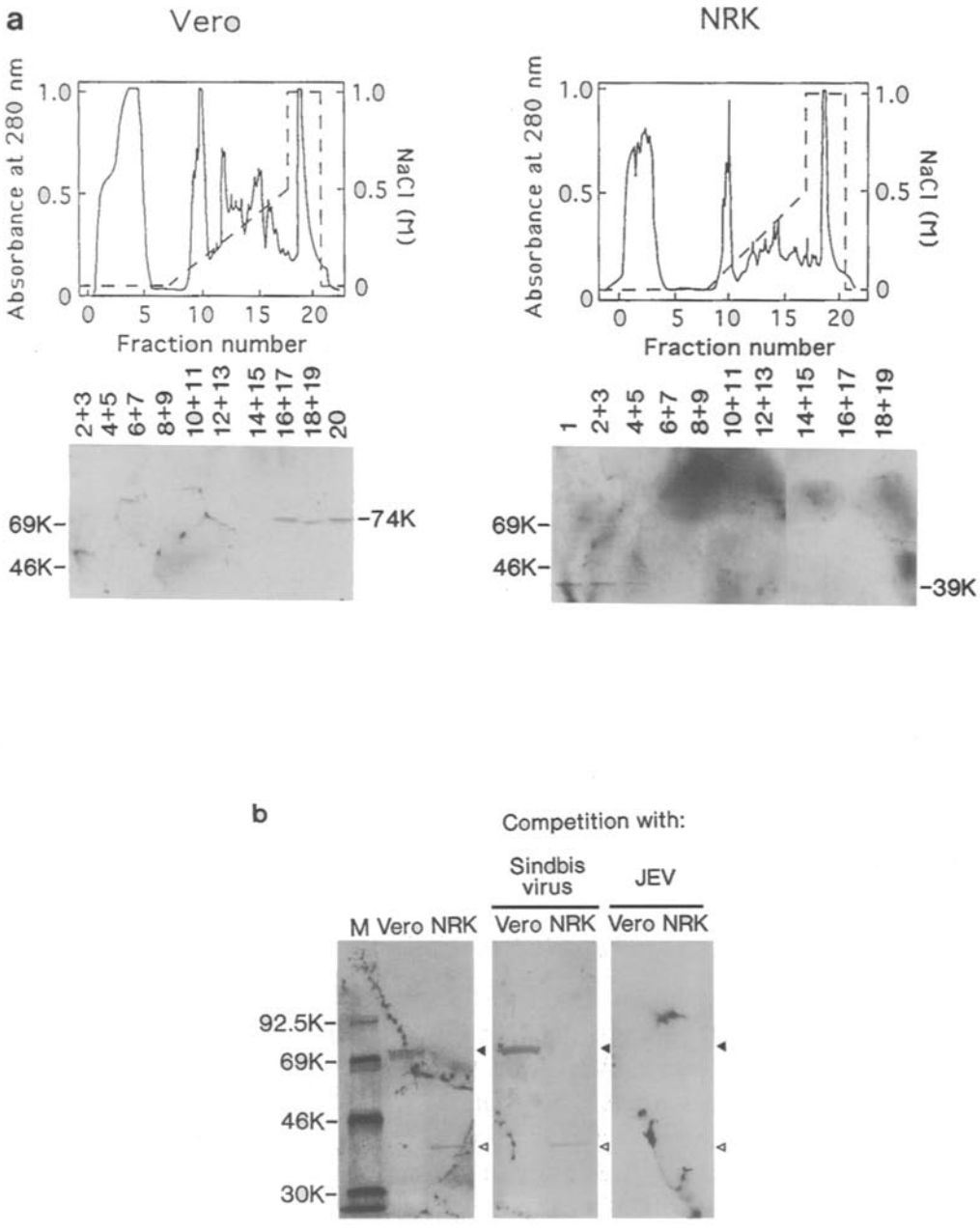

Fig. 3. a Separation of the crude membrane extracts by the anion exchange column. Fractions were subjected to SDS-PAGE, and then transferred to PVDF membrane, and probed with ^{35}S-labeled JEV. **b** JEV binding to the proteins in the purified membrane fractions of Vero and NRK cells. The lanes on the left show the binding of ^{35}S-labeled JEV to the fractions in which a JEV-binding molecule was detected. The lanes in the center were incubated with unlabeled Sindbis virus (5×10^{11} PFU) during treatment with ^{35}S-labeled JEV (3.5×10^{9} PFU). The lanes on the right were incubated with unlabeled JEV (6.4×10^{11} PFU) during treatment with ^{35}S-labeled JEV (3.2×10^{9} PFU). Filled arrowheads indicate a position of 74K. Open arrowheads indicate a position of 39K. Molecular weight marker's lane is indicated by *M*. Polyacrylamide gel, 7.5%, was used for separation

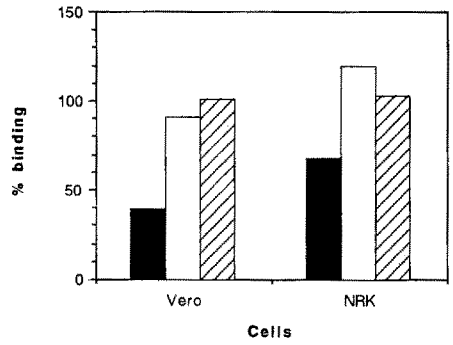

Fig. 4. Effect of anti-E- glycoprotein MAbs (Fab fragment) on JEV binding to Vero and NRK cells. [^{35}S] methionine-labeled JEV (7.0×10^7 PFU) were preincubated with 300 μ g of the Fab fragment of MAb 301 (■), MAb 503 (□), or mouse IgG (Cappel) (▨) at 4 °C for 2 h, and the JEV-MAb mixtures were added to cell monolayers (2×10^5 cells) grown in 48-well plates. After incubation at 4 °C for 90 min, the cell monolayers were washed with the binding medium and the radioactivity was determined. Vertical axis shows the counts for cells bound as percentage of bound counts without MAbs. The result represents the mean value of two independent assays

Effect of monoclonal antibodies on binding of JEV to high-susceptibility and low-susceptibility cells

To determine whether MAbs known to react with the E protein of JEV differentially affected the binding of JEV to Vero and NRK cells, we incubated radiolabeled JEV with the HI MAb 301 or the virus-neutralizing MAb 503 before adding it to the cell cultures (Fig. 4). The Fab fragments of the MAbs were used to eliminate the effect of Fc. The Fab fragment of MAb 301 blocked the binding of JEV to both cell lines, whereas the Fab fragment of MAb 503 did not inhibit the binding.

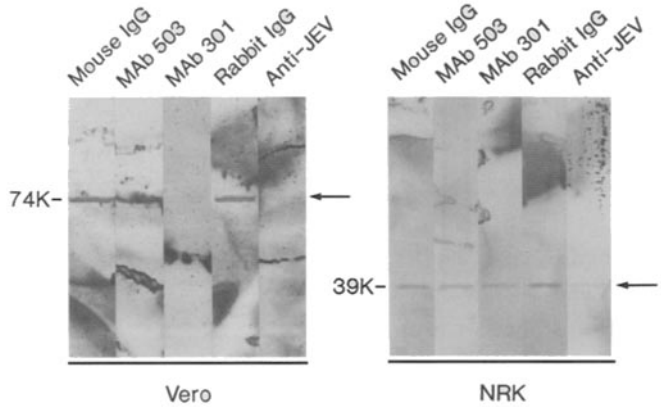

Fig. 5. Inhibitory effect of JEV binding to 74K and 39 K molecules by MAbs. [^{35}S]methionine-labeled JEV (3.5×10^9 PFU) was pre-incubated with 2 mg of MAbs or IgG at 4 °C for 2 h. Electrophoretically separated membrane fractions of Vero and NRK cells on PVDF membrane were incubated with JEV-MAbs or IgG mixture at 4 °C overnight and then washed and exposed to X-ray film. Polyacrylamide gel, 7.5%, was used for separation

Inhibitory effect of MAbs on binding of JEV to cellular proteins

The MAbs were also used to inhibit the binding of JEV to the electrophoretically separated cellular proteins on PVDF membranes (Fig. 5). The binding of JEV to the 74 K molecule of Vero cells was inhibited by incubation of JEV with MAb 301 and anti-JEV rabbit IgG, whereas MAb 503, mouse IgG and rabbit IgG did not cause such inhibition. In contrast, the binding of JEV to the 39K molecule of NRK cells was not inhibited by incubation of JEV with MAb 301, MAb 503, mouse IgG, or rabbit IgG.

Discussion

In the present study we have demonstrated that JEV attaches to and enters cells which were known to have both high-susceptibility and low-susceptibility to infection. However, it was apparent that the high-susceptibility cells adsorbed and internalized much more JEV than did the low-susceptibility cell line. When inoculations of 30 PFU/cell were employed, Vero cells internalized about six times as much JEV as did NRK cells, and it can be concluded that the susceptibility of cells to infection is determined at least to a certain extent, at the stage of binding and entry of the virus. Specifically our studies showed that JEV bound to and entered Vero cells better than NRK cells, and Scatchard analysis of this binding revealed that the binding affinity of JEV with Vero cells was higher than that occurring between JEV and NRK cells. However it is unknown if the differences between Vero and NRK cells in their capacity to adsorb JEV is due to the number of receptors, or due to the difference in binding affinity.

Susceptibility of NRK cells to JEV was significantly different between microinjected cells and naturally infected cells (52–70% in microinjection versus 0.2% in natural infection). These results also suggest that early events in viral infection, adsorption and internalization, determine the susceptibility of cell to infection. It may well be that the released virion from Vero cells easily infect again another Vero cells, because the number of Vero cells showing fluorescence at 48 h post infection was quite much more than the number of injected cells. However, the number of NRK cells showing fluorescence at 48 h post injection was lesser than the number of injected cells. Importantly, in NRK cells, the number of positive cells at 48 h post injection (45 and 53) was nearly identical to the number of positive cells at 24 h post injection (42 and 52). It is possible that the released virion from cells can not easily infect again another NRK cells, due to their low ability to adsorb and internalize JEV.

The present studies further identified a 74 K and a 39 K molecule, which were present in membrane fractions of Vero and NRK cells, respectively and which specifically bound radiolabeled JEV. The binding of JEV to the 74 K molecule of Vero cells was inhibited by MAb 301 which is known to have anti-hemagglutinating activity. Importantly, this antibody also inhibited binding of JEV to Vero cells and this raises the possibility that the 74K molecule may be one, or part of the cellular receptors for JEV.

Scatchard analysis of the specific binding of JEV to Vero cells showed a linear plot, suggesting the presence of a single class of cellular receptors for JEV on Vero cells, supporting the view that the 74 K molecule may be a cellular receptor for JEV in Vero cells. The result of Scatchard analysis also suggests the presence of a single class of cellular receptor for JEV on NRK cells. Because the 74 K molecule was not detected in the membrane fraction of NRK cells, the cellular receptor on NRK cells is likely to be different from those on Vero cells. These results suggest that JEV also employs more than one receptor for attachment to cells. This is not entirely unexpected as other viruses, e.g. Sindbis virus have the capability to use multiple receptors, which differ depending on the host cells [32]. Mouse hepatitis virus also utilize different molecules as receptors in the mouse brain and in liver [37].

In spite of the fact that MAb 301 weakly inhibited the binding of JEV to NRK cells, we could not detect a specific molecule in the cell membranes which was associated with this inhibition. Thus the role of the 39 K molecule in virus binding remains unclear. It is possible that the inhibitory effect of MAb 301 on the binding of JEV to the 39 K molecule is so weak that we could not detect the reduced binding to this molecule on the PVDF membrane, but this seems unlikely.

MAb 301 partially blocked the binding of JEV even to high-susceptibility cells. It is possible that there is a light gap between the receptor binding site and the site recognised by MAb 301 on the E protein of JEV. It is also possible that the conformational change of the protein, which is caused by the binding of MAb 301, hides incompletely the receptor binding site on the E protein.

The failure of MAb 503 to prevent JEV binding to cells suggests that the neutralization activity of MAb 503 may be associated with a post-binding step rather than binding itself. It may well be that MAb 503 inhibits the membrane fusion between the virus and cells, because we have shown that this antibody significantly inhibits JEV- induced fusion of C6/36 cells [19].

It is known that JEV has the capability to bind glycoprotein or glycolipid extracted from bovine brain, and an active fragment of the extract inhibits viral hemagglutination [24, 35]. However, it remains unclear what, if any, is the relationship between these molecules and the JEV-binding molecules which we identified in this study.

In conclusion, we have demonstrated that cells highly susceptible to JEV adsorbed and internalized much more JEV than did low-susceptibility cells and that these differences are important in the susceptibility of cells to JEV infection. Further studies on the 74 K protein detected in Vero cells including its purification and molecular cloning may provide important information on the JEV receptor, and on its in vitro and in vivo cellular tropism.

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