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SURFACE MEMBRANE REDISTRIBUTION AND STABILIZATION OF CONCANAVALIN A-SPECIFIC RECEPTORS FOLLOWING YABA TUMOR POXVIRUS INFECTION

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Summary

Monkey kidney cells productively infected with Yaba tumor poxvirus clearly exhibit plasma membrane alterations when treated with both fluorescein-labeled and unlabeled concanavalin A. The concanavalin A-mediated cytoagglutination reaction for Yaba-infected Jinet and CV-1 cells increased linearly from 12 to 16 h post-infection, reaching a maximum by 24—28 h. Treatment of either Yaba-infected CV-1 or Jinet cells with methyl-D-glucopyranoside before or after addition of concanavalin A completely blocked or reversed the cytoagglutination response. Trypsin treatment of uninfected CV-1 or Jinet cells enhanced concanavalin A-mediated cytoagglutination properties. Conversely, trypsin treatment of Yaba-infected Jinet cells resulted in a reduced cytoagglutination response. Increasing temperature and lectin concentration enhance concanavalin A-mediated cytoagglutination for uninfected, trypsin-treated and Yaba-infected CV-1 cells. Cytosine arabinoside has little or no effect on the Yaba-induced cell cytoagglutination reaction while cycloheximide blocks the cytoagglutination response if added prior to 12 h post-infection. Fluorescein-labeled concanavalin A binding studies have revealed that at 4°C, Yaba-infected CV-1 cells display a predominantly 'patchy' pattern of topological fluorescence, while trypsin-treated and uninfected CV-1 cells at 4°C display a uniform pattern of fluorescence binding. Patchy fluorescence, indicative of concanavalin A-susceptible, receptor-site clustering on the surface membrane, was reduced 50% if Yaba-infected CV-1 cells were treated with glutaraldehyde (2.5%) before addition of fluorescein-labeled concanavalin A at 4°C. Similar pre-fixation of trypsin-treated CV-1 cells resulted in uniform, fluorescent labelling patterns at all assay temperatures.

Introduction

Concanavalin A, a plant lectin derived from jack beans, is capable of binding to the surface membrane of cells. The concanavalin A molecule binds specifically with carbohydrate-containing molecules possessing α-D-glucosyl and sterically related residues [1]. Transformed cells and cells treated with proteolytic enzymes are agglutinated in the presence of concanavalin A at concentrations which do not agglutinate untreated cells [2]. When several groups demonstrated that there was essentially no difference in the number of lectin sites on normal and transformed cells [3—6], the differential binding phenomenon was explained by demonstrating a 'clustering' effect on the surface of cytoagglutinating cells [7,9]. In addition to these studies, the concanavalin A binding reaction was shown to be temperature- and lectin concentration-dependent [2, 8—12].

Since the original demonstration that SV40- and polyoma-transformed cells were more susceptible to concanavalin A-mediated cytoagglutination than non-transformed cells, a substantial number of viruses have been shown to increase their host cell's susceptibility to concanavalin A-mediated cytoagglutination [13—25]. In addition, many enveloped viruses have been shown to possess concanavalin A-susceptible sites [14,26—31] and it is possible that virus-specific sites may play a role in concanavalin A-directed host cell cytoagglutination.

In the present study we examined Yaba tumor poxvirus-infected monkey kidney cells for alterations in the surface membrane using basically two concanavalin A-associated criteria: increased tendency of Yaba-infected cells to agglutinate compared to non-infected controls in the presence of moderate concanavalin A concentrations; and treatment of infected and non-infected cells with fluorescein-conjugated concanavalin A with subsequent description of the differences in topological, fluorescent binding. In conjunction with these criteria, the effects of temperature and lectin concentration on the observed binding phenomena are also described.

Materials and Methods

Materials. Hemagglutination buffer [18] (Difco, Detroit, MI) was used in all concanavalin A-associated assays. Mg^{2+}- and Ca^{2+}-free phosphate-buffered saline [2] was used to wash cells prior to assay. Concanavalin A was purchased from Sigma Chemical Co. as a sodium salt (crystallized three times) and purified further [32]. Trypsin (five times crystallized) was purchased from Nutritional Biochemical Co., and a stock solution of 1 mg/ml in hemagglutination buffer was prepared for use.

Virus and cells. The Jinet and CV-1 cell lines of monkey kidney cells were used to propagate Yaba virus. Jinet cells [39] were used at passages 29—34. Jinet and CV-1 cell lines were judged free of mycoplasma by electron microscopy. Yaba growth, purification and assay for infectivity were performed as described previously [34]. Typically, confluent monolayers of Jinet or CV-1 cells in Blake bottles were split into Falcon plastic petri dishes at a concentration of about $7—8 \times 10^5$ cells/petri dish and allowed to reach 80% confluency

(3—4 days). Semiconfluent monolayers were infected with Yaba at a multiplicity of infection of 10 focus-forming units/cell. Following a 2 h adsorption period at 35°C, all monolayers were washed three times with serum-free Eagle's minimal essential medium and overlaid with this medium supplemented with 2% fetal calf serum.

Virus inactivation. Purified Yaba virus was rendered biologically inactive by either heat inactivation (56°C for 60 min) or by ultraviolet irradiation (3100 ergs/cm^2 at a distance of 20 cm for 20 s) [35].

Cytoagglutination assay. Yaba-infected and uninfected CV-1 or Jinet cell monolayers were prepared for assay as follows: maintenance medium was aspirated and monolayers were washed three times with buffer A at 22°C. Cells were removed from the monolayer with a solution of 0.02% disodium versenate described by Inbar and Sachs [2]. Gentle rocking greatly facilitated cell detachment. Completely dispersed cells were pelleted at 500 $\times g$ for 7 min in a refrigerated IEC PR-6 centrifuge. Specific trypsin-treatment of cells, when indicated, was conducted at this point [2]. The cell pellet was washed three times with hemagglutination buffer at 4, 22, or 35°C, depending on the assay temperature. The final cell pellet was resuspended in 0.5 ml hemagglutination buffer of the appropriate concanavalin A concentration and placed in a 35 mm plastic Petri dish (Falcon Co.). These were incubated with rocking at 4, 22, or 35°C for 30 min. The degree of cytoagglutination was assessed using a binocular microscope. The degree of cytoagglutination is, at best, a qualitative evaluation of a treated cell population unless careful manipulation, observation and enumeration are employed. Individual samples were coded and assayed on a serological scale from 0 (no agglutination) to ++++ (maximum agglutination). The basis of our standard of assessment is pictured in Fig. 1a—e, showing the complete range of cytoagglutination. Cells were photographed under phase contrast using a Leitz ortholux microscope equipped with a Leica automatic-exposure camera apparatus at a magnification of 430X.

Preparation of fluorescein-conjugated concanavalin A. In general, fluorescein-conjugated concanavalin A was prepared as described by Klein and Adams [18]. The final stock solution had a protein concentration of 1.25 mg/ml as determined by using the method of Lowry et al. [36] and a 495 nm/280 nm absorbance ratio of 1 : 2. The fluorescein-conjugated concanavalin A gave cytoagglutination responses at 250 μg/ml identical to those observed for concanavalin A alone. In addition, methyl-D-glucopyranoside completely blocked the fluorescein-conjugated concanavalin A-mediated cytoagglutination response.

Fluorescein-conjugated concanavalin A binding assay and fluorescent microscopy. Typically, Yaba-infected, uninfected and trypsin-treated CV-1 cells were dispersed and washed as described above. Certain assays were fixed with 2.5% glutaraldehyde in hemagglutination buffer at 4, 22, or 35°C for 15 min. These cells were washed three times with hemagglutination buffer at the appropriate temperature before addition of fluorescein-conjugated concanavalin A. Glutaraldehyde-treated and untreated cells were resuspended in 0.5 ml of hemagglutination buffer having a final fluorescein-conjugated concanavalin A concentration of 20 μg/10^6 cells. Each sample was incubated in a conical centrifuge tube with 30 vols. of hemagglutination buffer and in some cases fluorescein-

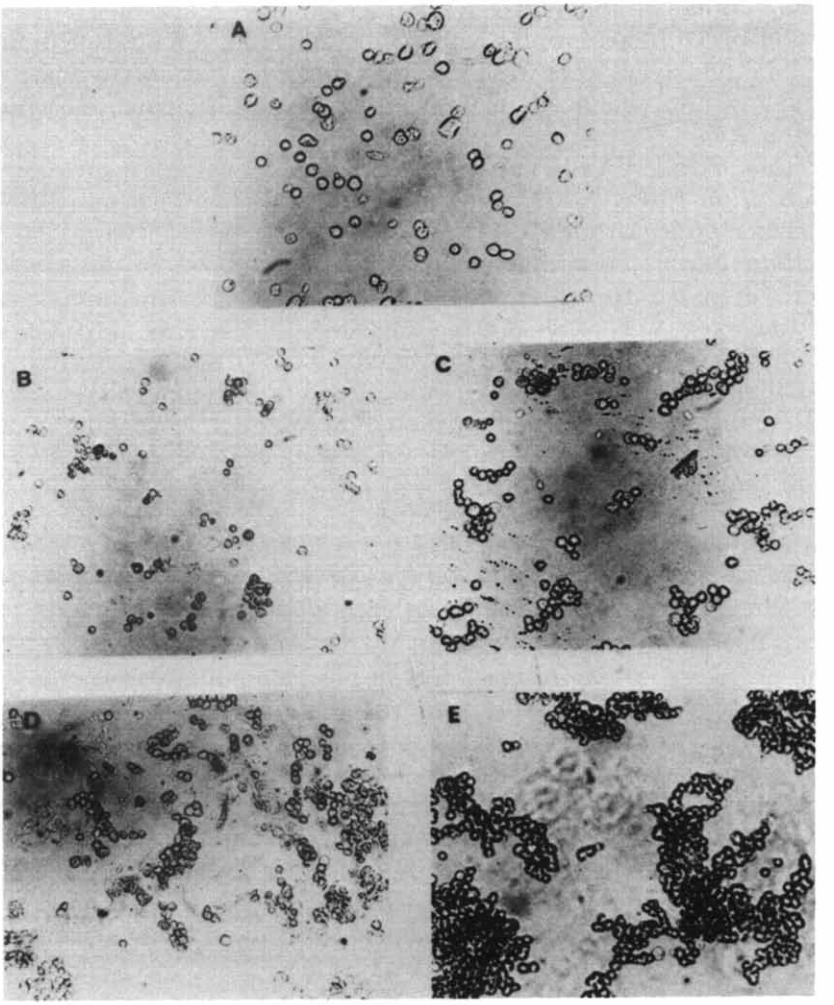

Fig. 1. Cytoagglutination evaluation based on a serological scale, 0 to +4. (A) No cytoagglutination; (B) +1, cytoagglutination; (C) +2, cytoagglutination; (D) +3, cytoagglutination; (E) +4, maximum cytoagglutination; phase contrast, magnification, 430X.

conjugated concanavalin A-treated cells were post-fixed in glutaraldehyde and processed as described above. Cell pellets were resuspended in 0.5 ml of a 50% hemagglutination buffer/50% glycerin solution and mounted for observation. The specific fluorescence-labeling patterns were observed using a Leitz ortholux fluorescence microscope employing ultraviolet illumination with a UG-1 excitation and K-430 barrier filter under oil immersion. In scoring a particular cell population (100—200 cells per assay) for the type of topological fluorescence, only single cells were evaluated. Finally, assays were coded and then scored to reduce evaluator bias.

Results

Time course of Yaba-infected cell cytoagglutination

Cultures of both CV-1 and Jinet cells in plastic petri dishes were infected as described above with parallel, uninfected cultures, and cells were harvested and tested every 4 h for their ability to agglutinate in the presence of 250 μg/ml per 10^6 cells concanavalin A; this concanavalin A concentration was found most suitable diagnostically for the cytoagglutination assay. Assays were run in duplicate and the experiment was performed twice. Fig. 2 illustrates the results of this experiment, each point being the average of four independent determinations. The linear increase in concanavalin A-mediated cytoagglutination for both Yaba-infected CV-1 and Jinet cells begins between 12 and 16 h post-infection and reaches a maximum by 24—28 h post-infection. Uninfected cells did not agglutinate above 25% or +1. The maximum cytoagglutination response observed for Yaba-infected cells remained unchanged from 28—96 h post-infection. Yaba-infected CV-1 and Jinet cells gave almost indistinguishable cyto-agglutination responses. Although not shown, Yaba-infected BSC-1 cells never gave a cytoagglutination differentiation between infected and uninfected cells comparable to that observed for Jinet and CV-1 cells unpublished observation). Treatment of Yaba-infected cells with 0.05 M methyl-D-glucopyranoside before addition of concanavalin A completely blocked the cytoagglutination response. Addition of methyl-D-glucopyranoside after formation of the cell aggregates in the presence of concanavalin A resulted in almost complete dissociation of existing cell clusters. Sucrose (0.05 M) was much less effective as an inhibitor of the concanavalin A-mediated response and *N*-acetylglucosamine (0.05 M) seemed to have no effect as an inhibitor. Jinet or CV-1 cells infected with Yaba virus which had been inactivated by ultraviolet radiation or heat failed to agglutinate in the presence of 250 μg/ml concanavalin A when assayed 48 h post-infection.

Effect of trypsin on concanavalin A-mediated cytoagglutination of Yaba-infected and uninfected cells

It was established that proteolytic enzyme treatment of normal, interphase

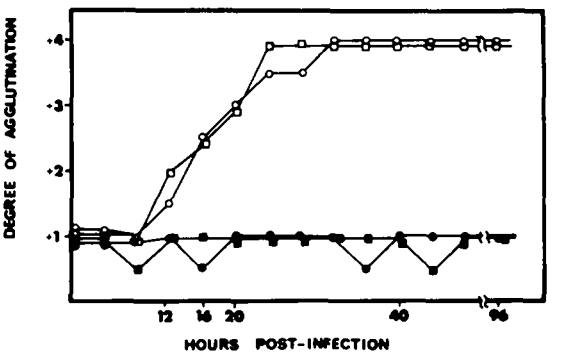

Fig. 2. Time course of Yaba tumor poxvirus infection as a fraction of concanavalin A-mediated cytoagglutination. Concanavalin A concentration, 250 μg/ml per 10^6 cells. Incubation temperature 35°C. ○, Yaba-infected Jinet cells; ●, uninfected Jinet cells; □, Yaba-infected CV-1; ■, uninfected CV-1.

TABLE I

CONCANAVALIN A-MEDIATED CYTOAGGLUTINATION OF TRYPSIN-TREATED YABA-INFECTED AND UNINFECTED CELLS

Concanavalin A treatment: 250 μg/ml per 10^6 cells. YTV, Yaba tumor poxvirus.

Cell	Trypsin treatment (μg/ml)			
	0	1.0	10.0	100.0
CV-1	+	++	++++	++++
Jinet	+	+ (+)	+++	++++
YTV-infected Jinet cells (48 h post-infection)	++++	++++	++	+ (+)

cells gives rise to enhanced cytoagglutination in the presence of concanavalin A [2]. In conjunction with our studies on Yaba-induced cytoagglutination, we tested the effects of varying trypsin treatments on CV-1, Jinet and Yaba-infected Jinet cell cytoagglutination in the presence of concanavalin A. Yaba-infected and uninfected cells were prepared as described previously. Table I demonstrates that increasing trypsin concentration response for uninfected CV-1 and Jinet cells. However, trypsin treatment of Yaba-infected Jinet cells seems to result in a reduced ability to agglutinate in the presence of 250 μg/ml per 10^6 cells concanavalin A.

Effect of varying temperature and lectin concentration

We sought to establish the effects of varyin lectin concentration and temper-

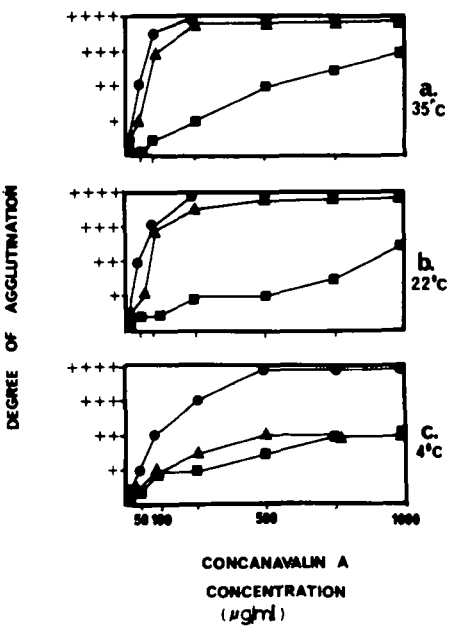

Fig. 3. Effect of varying temperature and concanavalin A concentration on the cytoagglutination of Yaba-infected, uninfected and trypsin-treated CV-1 cells. ●, Yaba-infected; ▲, trypsin-treated; ■, uninfected, untreated CV-1 cells.

ature on the ability of trypsin-treated, Yaba-infected and uninfected CV-1 cells to agglutinate. Yaba virus-infected and uninfected CV-1 cells were prepared as before. Uninfected CV-1 cells were trypsin-treated as described using 10 μg/ml trypsin. Fig. 3 shows that increasing temperature and lectin concentration enhance the cytoagglutination reaction in all cases. Maximum agglutination does not occur at 4°C, which appears to be the most significant deviation between the Yaba-infected and trypsin-treated CV-1 cell systems. At concanavalin A concentrations of about 500 μg/ml the cytoagglutination difference between uninfected and infected and trypsin-treated CV-1 cells becomes increasingly insignificant and at concanavalin A concentrations approaching 1000 μg/ml the difference is not significant. Yaba-infected CV-1 cells at 4°C are capable of achieving a maximum cytoagglutination response at concanavalin A concentrations (500—750 μg/ml) where a significant cytoagglutination difference persists between infected and uninfected cells. The results of this experiment indicated that 250 μg/ml was the most suitable concanavalin A concentration for cytoagglutination assay studies.

Effect of two metabolic inhibitors on the cytoagglutination reaction

Yaba-infected and uninfected Jinet and CV-1 cells were tested for their ability to agglutinate in the presence of 250 μg/ml concanavalin A following treatment with either cytosine arabinoside or cycloheximide. Table II shows that cytosine arabinoside, an inhibitor of DNA synthesis, apparently has little or no effect on the Yaba-induced cell cytoagglutination reaction. On the other hand, cycloheximide, a potent inhibitor of protein synthesis, effectively blocks the cytoagglutination response if added prior to 12 h post-infection, indicating that Yaba virus-specific protein synthesis between 12 and 24 h post-infection has an effect on the ability of such infected cells to be agglutinated in the

TABLE II

EFFECT OF CYCLOHEXIMIDE AND CYTOSINE ARABINOSIDE ON YABA-INFECTED AND UNINFECTED CELL AGGLUTINATION IN THE PRESENCE OF 250 μg/ml PER 10^6 CELLS CONCANAVALIN A

Cycloheximide (25 μg/ml) and cytosine arabinoside (Ara C) 100 μg/ml, were used as inhibitor treatments. 24 and 48 h post-infection cultures were dispersed, treated with concanavalin A at 250 μg/ml per 10^6 cells and assessed for degree of cytoagglutination.

Cell	Inhibitor treatment	Time added (h, post-infection)	Cytoagglutination reaction	
			24 h	48 h
Uninfected CV-1	cycloheximide or Ara C	0	+	+
Uninfected Jinet	cycloheximide or Ara C	0	+	+
Yaba-infected Jinet	Ara C	0	++++	++++
Yaba-infected Jinet	cycloheximide	0	+	+
		4	+	+
		8	+	+
		12	+ (+)	++
		16	++	++
		20	+++	+++
		24	++++	++++
		48	++++	++++

presence of concanavalin A. Addition of cycloheximide after 24 or 48 h infection appeared to have no effect on the agglutination phenomenon.

Fluorescein-conjugated concanavalin A binding studies

Yaba-infected, uninfected or uninfected trypsin-treated CV-1 cells were processed as described previously and then treated with 20 μg/ml fluorescein-conjugated concanavalin A and incubated at 4, 22, or 35°C, with agitation. Fig. 4A—I shows fluorescein-conjugated concanavalin A binding patterns representative of each cell population. Fig. 4A—C shows that uninfected CV-1 cells display a 'complete' or uniform pattern of fluorescence binding. In sharp contrast, Yaba-infected CV-1 cells display a 'patchy' incomplete ring of fluorescence (Fig. 4D—F). Trypsin-treated CV-1 cells at 4°C (Fig. 4G) show a

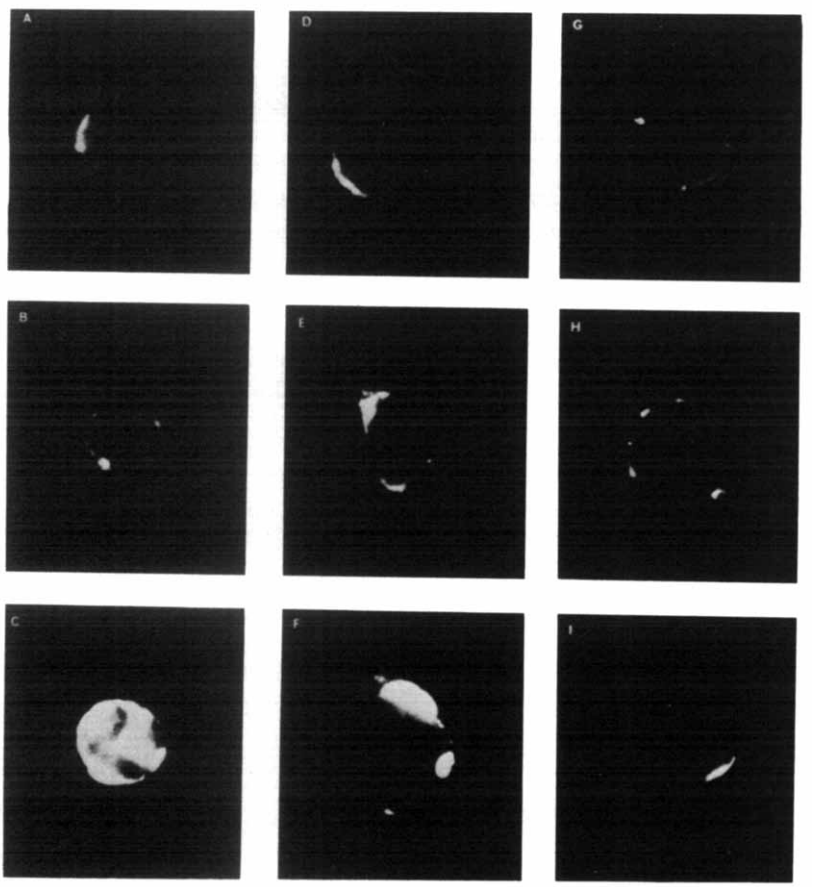

Fig. 4. Topological binding of fluorescein-conjugated concanavalin A (Fl-Con A) to Yaba-infected, uninfected and trypsin-treated CV-1 cells. Cell dispersement, Fl-Con A treatment and glutaraldehyde post-fixation as described in Materials and Methods. A, uninfected CV-1 cell, 4°C; B, uninfected CV-1 cell, 22°C; C, uninfected CV-1 cell, 35°C; D, Yaba-infected CV-1 cell, 4°C; E, Yaba-infected CV-1 cell, 22°C; F, Yaba-infected CV-1 cell, 35°C; G, trypsin-treated CV-1 cell, 4°C; H, trypsin-treated CV-1 cell, 22°C; I, trypsin-treated CV-1 cell, 35°C. Yaba-infected cells were harvested and processed at 24 h post-infection; magnification, 1000X.

TABLE III

EFFECT OF GLUTARALDEHYDE FIXATION ON FLUORESCEIN-CONJUGATED CONCANAVALIN A LABELING PATTERNS

Buffered glutaraldehyde (2.5%) was used either before and after fluorescein-conjugated concanavalin A treatment (Fl-Con A) (pre- or post-fixation, respectively) or just prior to microscopic observation; (+) treated; (—) untreated. Fluorescence labeling patterns (uniform, patchy) as noted in Fig. 4: mixed indicates a mixture of cells exhibiting both patchy and uniform patterns of topological fluorescence rather than predominantly patchy or uniform.

Cell line and treatment	Pre-fixation	Reagent	First incubation		Post-fixation	Second incubation		Fluorescent labeling pattern
			Temp (°C)	Time (min)		Temp (°C)	Time (min)	
CV-1 (untreated)	—	Fl-Con A	4	30	+	—	—	uniform
	—	Fl-Con A	22	30	+	—	—	uniform
	—	Fl-Con A	35	30	+	—	—	uniform
	+	Fl-Con A	35	30	—	35	30	uniform
CV-1 (Yaba-infected)	—	Fl-Con A	4	30	+	22	30	patchy
	—	Fl-Con A	35	30	—	4	30	patchy
	+	Fl-Con A	4	30	—	—	—	mixed
	+	Fl-Con A	22	30	—	4	30	mixed
	—	Fl-Con A	35	30	+	4	30	patchy
	+	Fl-Con A	35	30	—	—	—	mixed
CV-1 (trypsin-treated)	—	Fl-Con A	4	30	+	22	30	uniform
	—	Fl-Con A	35	30	+	4	30	patchy
	+	Fl-Con A	4	30	—	35	30	uniform
	—	Fl-Con A	4	30	—	35	30	patchy
	—	Fl-Con A	4	30	+	35	30	uniform
	+	Fl-Con A	35	30	—	—	—	uniform

uniform pattern of fluorescence, but at 22 and 35°C (Fig. 4H and I, respectively) the observed pattern is patchy and irregular like that observed for Yaba-infected cells. 'Caps' of fluorescence were often observed in both fluorescein-conjugated concanavalin A-treated Yaba-infected and trypsin-treated CV-1 cell systems. Addition of methyl-D-glucopyranoside (0.05 M) to Yaba-infected or trypsin-treated CV-1 cells at 35°C either before or after fluorescein-conjugated concanavalin A treatment resulted in a weakly fluorescing, uniform pattern of fluorescence indistinguishable from cells treated with buffered, unconjugated-fluorescein isothiocyanate. Table IV shows the population distribution for each of the treatment groups scored for the two basic patterns of topological fluorescence, patchy or uniform. At 4°C, Yaba-infected CV-1 cells exhibited a predominately patchy pattern of fluorescence while under the same conditions the uniform pattern of fluorescence dominates the trypsin-treated and uninfected CV-1 cell populations. In general, the fluorescence was more intense in fluorescein-conjugated concanavalin A treated cell populations at 22 and 35°C regardless of cell pre-treatment. Glutaraldehyde-fixation of trypsin-treated CV-1 cells prior to addition of the fluorescein-conjugated concanavalin A reagent clearly 'stabilized' the cells to the extent that concanavalin A-suscep-

TABLE IV

THE CELL POPULATION DISTRIBUTION OF FLUORESCEIN-CONJUGATED CONCANAVALIN A LABELING PATTERNS ON CV-1 CELLS DETECTED BY DIRECT FLUORESCENCE USING FLUORESCEIN-CONJUGATED CONCANAVALIN A

Clustered, uniform, ring-type fluorescence characteristic of clustered receptors. Dispersed, characteristic of randomly dispersed receptors. Values are expressed as percentage of cells in both types of cell population.

Cells	4°C		22°C		35°C	
	Clustered	Dispersed	Clustered	Dispersed	Clustered	Dispersed
Yaba-infected	70	30	85	15	92	8
Trypsin-treated	28	72	90	10	90	10
Untreated	10	90	17	83	20	80

tible surface receptors remained in a random, dispersed array regardless of subsequent temperature-shift incubation (Table III). Without gluraraldehyde pre-fixation, trypsin-treated CV-1 cells displayed the characteristic patchy, fluorescence when the fluorescein-conjugated concanavalin A was added at 35°C. Subsequent gluraraldehyde-fixation and temperature shift to 4°C for 30 min did not alter the observed patchy fluorescence binding pattern. Finally, trypsin-treated CV-1 cells incubated first at 4°C with fluorescein-conjugated concanavalin A and then incubated at 35°C for 30 min with subsequent glutaraldehyde-fixation displayed patchy, fluorescence binding patterns. This result shows that concanavalin A-susceptible, membrane-associated binding sites on trypsin-treated CV-1 cells migrate to form aggregates of fluorescein-conjugated concanavalin A when the temperature is raised to 22 or 35°C. At 4°C this migration does not take place and a uniform pattern of fluorescein-conjugated concanavalin A binding is thus detected (see Fig. 4G). Uninfected, untreated CV-1 cells appear unaffected by the application of glutaraldehyde either before or after fluorescein-conjugated concanavalin A treatment and gave a uniform pattern of fluorescence at all assay temperatures. Table III further shows that glutaraldehyde pre-fixation of Yaba-infected CV-1 cells reduces the percentage of cells displaying the patchy-type labeling pattern (usually about 50%) at all assay temperatures. These data (Tables III and IV) demonstrate that concanavalin A-susceptible, membrane-associated binding sites are at least 'partially' aggregated on the surface of Yaba-infected CV-1 cells and that, following temperature increase, they exhibit enhanced fluorescence aggregation with many fluorescein-conjugated concanavalin A treated cells displaying cap formation (see Fig. 4E and F).

Discussion

Yaba tumor poxvirus-infected CV-1 and Jinet cells agglutinate significantly more than non-infected controls in the presence of 250 μg/ml concanavalin A, starting by 12 h post-infection and reaching a maximum by 24 h. The cyto-agglutination phenomenon is completely blocked or reversed using methyl-α-D-glucopyranoside.

Protein synthesis between 12 and 24 h post-infection appears to be correlated with the increasing tendency of Yaba-infected cells to be agglutinated with concanavalin A during this time period. Treatment of uninfected CV-1 and Jinet cells with the proteolytic enzyme, trypsin, greatly enhances their ability to agglutinate in the presence of moderate concanavalin A concentrations. However, trypsin treatment of Yaba-infected Jinet cells actually reduces the degree of cellular aggregation. One possible explanation for these results would be the destruction of weakly stabilized clusters of concanavalin A-susceptible sites on the surface of Yaba-infected cells. This possibility is reinforced by the results of surface membrane labeling using fluorescein-conjugated concanavalin A. Yaba virus apparently induces changes in the host-cell plasma membrane with account for the clustering of fluorescein-conjugated concanavalin A-susceptible sites at 4°C, a temperature at which lateral movement within the cell plasma membrane is greatly reduced [7—10,37].

The data presented herein are partially consistent with the cell membrane model of Singer and Nicolson [7,37]. Their theory provides an explanation for the enhanced agglutination of cells in the presence of concanavalin A following transformation or treatment with proteolytic enzymes. In essence, such treated cells have achieved increased mobility within the membrane and thus concanavalin A-susceptible α-D-glycosyl-like residues are able to 'move' into areas of concentration dictated by their affinity to the tetravalent concanavalin A molecule [7,9]. Our data show that treatment of Yaba-infected CV-1 cells with glutaraldehyde before addition of fluorescein-conjugated concanavalin A significantly reduces the number of cells exhibiting the patchy fluorescence pattern (up to 30% at 4°C), while similar treatment of trypsin-treated CV-1 cells stabilize concanavalin A-susceptible membrane-associating receptors, thus inhibiting lateral movement within the membrane and produces the uniform 'ring' of fluorescence on all cells regardless of assay temperature. These results can be explained by assuming that Yaba infection produces alterations in the host-cell surface membrane which both increase and decrease (stabilize) molecular mobility or migration. Possibly, this difference is based on the location of Yaba-specific cytoplasmic 'factories'. Areas of host-cell surface membranes adjacent to virus-specific metabolic factories [38] would, reasonably, be more susceptible to any virus-specific influence unknown at present. The ability of membrane-associated surface markers to cluster at 4°C [14] reinforces our theory that Yaba infection greatly increases the lateral mobility of concanavalin A-susceptible moieties within the infected cell membrane.

We have found recently that mature enveloped Yaba virions possess concanavalin A-susceptible sites. Preliminary results (unpublished data) indicate that while Yaba virus is formed in intracytoplasmic vacuoles, it acquires its envelope as it passes through the cytoplasmic membrane. Therefore, Yaba envelope glycoproteins appear at the cell surface during infection. The maximum concanavalin A-mediated response for Yaba-infected cells was shown to occur at 24 h post-infection, following a linear increase from 12—16 h post-infection. This agglutination pattern coincides with virus-specific glycoprotein synthesis, the bulk of which occurs between 12 and 16 h post-infection (unpublished data). However, neither pattern coincides with the release of mature virus which occurs at approx. 72 h post-infection [34,39,40].

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