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INCREASE IN CONCANAVALIN A CAP FORMATION ON LYMPHOMA CELLS FOLLOWING INTERACTION WITH INACTIVE INFLUENZA VIRUSES

[1]G. WEIL-HILLMAN*, [3]R. LEVI, [3]Z. ZAKAY-RONES

[1]Department of Urology, Wayne State University, 550 Canfield Street, Detroit, Michigan 48201, U.S.A., and [2]Department of Virology, Hebrew University, Hadassah Medical School, Jerusalem, Israel

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Summary. - Binding of the lectin Con A to its ligand on the cell surface of normal circulating lymphocytes induces capping in 28-32 % of these cells. This Con A cap formation is markedly decreased in malignant cells from the human hematopoietic system. Among others, the human lymphoma Daudi cell line exhibit a cap formation with Con A in only 5-10 % of the cells. In this study, we found that inactivated influenza viruses induced changes in the cell surface membrane of Daudi cells resulting in an increased percentage of Con A cap forming cells (30-40 %). This phenomenon occurred independently of viral replication and was initiated by adsorption of inactivated viral particles or isolated hemagglutinin and neuraminidase viral glycoproteins. This phenomenon may be due to the binding of Con A molecules to viral receptors and to cell receptors leading to crosslinking of Con A receptors that will induce their mobility and the formation of a cap. Alternatively, experiments performed with cytochalasin B and colchicine suggest that the viral interaction with the cell membrane may have induced changes in the cytoskeleton at the level of microtubules. These changes induced increased lateral movement of the Con A receptors resulting into formation of a cap.

Key words: Daudi cells; influenza virus; Con A capping

Introduction

Cell membranes contain receptors to the lectin Concanavalin A (Con A) that are generally redistributed in the lateral plane of the membrane and can form a cap upon ligand contact (Unanue *et al.*, 1972). This cap formation has been used to demonstrate surface differences between normal and malignant

* Whom requests for reprints should be addressed to

cells of the human hematopoietic system. Cap formation was observed in 28–32 % of normal peripheral blood lymphocytes isolated from healthy individuals (Ben-Bassat *et al.*, 1974; Mintz & Sachs, 1977). A decrease in the percent of cells forming a cap with Con A (4–12 %) was noted on lymphocytes obtained from patients with chronic lymphatic leukemia (Ben-Bassat *et al.*, 1974; Ben-Bassat & Goldblum, 1978), Hodgkin's disease (Ben-Bassat & Goldblum, 1975*a*, 1978) Burkitt's lymphoma (Ben-Bassat *et al.*, 1976; Ben-Bassat & Goldblum, 1978) and other malignant lymphoma (Ben-Bassat & Goldblum, 1975*a*, 1978). Con A cap formation, therefore, might reflect changes in the cell membrane mobility associated with malignancy. Surface changes in normal and malignant cells may be induced by infection with a wide variety of DNA and RNA viruses probably due to expression of viral antigens and their mobility in the host cell membrane (Becht *et al.*, 1972; Poste & Reeve, 1972; Birdwell & Strauss, 1973). A small percentage (5–10 %) of the human lymphoma Daudi cell line forms a cap with Con A. We have previously studied the interaction of Daudi cells with active or inactivated influenza viruses and found that these viruses inhibited the growth of Daudi cells both *in vitro* and *in vivo* (Weil-Hillman & Zakay-Rones, 1985*a,b;* 1987). The aims of this present study was to monitor the Con A- cap formation of the Daudi cells interacted with influenza virus in order to detect potential alterations to the cell membrane caused by adsorption of the influenza viruses.

Materials and Methods

Cell line. The Daudi lymphoma cell line (Klen *et al.*, 1968) was maintained in suspension under tissue culture conditions (37^0C, in 5 % CO_2). Cells were transferred every 4 days in RPMI 1640 medium supplemented with 25 % Fetal Calf Serum (FCS) (GIBCO - U.S.A.) and antibiotics.

*Influenza virus.*The A/X47 (H3N2) influenza strain, recombinant of A/PR/8/34(H1N1) and A/ Victoria/3/75(H3N2) was used as active (XA), heat-inactivated (X56) (56^0C for 45 min) or formalin inactivated (XF) preparations. Growth and purification of the virus as well as determination of viral activities were carried out as described previously (Weil-Hillman & Zakay-Rones, 1985*a, b*).

Separation of viral surface glycoproteins. The viral surface glycoproteins (XFA) (consisting of hemagglutinin (HA) and neuraminidase (NA) were obtained by incubating the purified viral preparation at room temperature for 30 min with the cationic detergent Cetyl trimethyl ammonium bromide (CTAB) (Sigma) at a final concentration of 0.03 %–0.05 %. The virus core was separated from the glycoproteins using a 5–50 % w/v sucrose gradient (at 30,000 rev/min for 90 min in a cold Spinco ultracentrifuge SW41 rotor). The fractions that contained the peak activities for hemagglutinin (HA) and neuraminidase (NA) were pooled, dialyzed against PBS (PH 7.8) for 48 hrs and kept at -70^0C (Bachmayer, 1975).

Interaction between cells and viruses. Daudi cells at a concentration of 1×10^6/0.5 ml were incubated with XFA or with the complete virus at 100–1000 EID_{50}/cell for 60 min at 37^0C in 0.6 ml RPMI 1640. Following the adsorption period, medium containing 20 % FCS was added to a final volume of 2 ml. At different time points of the cell culture, samples were tested for cap formation with fluorescein-conjugated Con A (F-Con A) according to the method of Ben-Bassat (1975*b*), using Fluorescein isothiocyanate-conjugated Con A (Bio Makor, Rehovot, Israel). Fluorescent cell preparations were tested in a Zeiss Universal microscope. One hundred to 200 single cells

were counted and the percentage of caps was calculated.

In neutralization assays, viruses were preincubated with specific rabbit anti-influenza H3N2 serum for 1 hr at room temperature and then incubated with Daudi cells. To test the effect of the viral inhibitor Amantadine hydrochloride (Sigma), cells were first treated with Amantadine at 300 μg/ml for 1 hr at 37^{0}C then incubated with the viral preparations. Colchicine and cytochalasin B, drugs reacting with the cytoskeleton, were added to the cells at 48 hrs post interaction with the viral preparation. Cells were washed and treated for 30 min at 37^{0}C with colchicine (Sigma) at 10^{-4}M in PBS or with cytochalasin B (Sigma) at 10 μg/ml in DMSO, then cells were tested for F-Con A binding.

Results

Effect of influenza virus preparations on the ability of Daudi cells to form a cap following Con A binding

The human lymphoma cells Daudi have demonstrated a reduced ability to form a cap following binding of F-Con A. Only 6–9 % cells formed a cap with Con A after 2–72 hrs in culture (Table 1) while about 26 % of normal peripheral blood lymphocytes can form a cap with Con A. This decrease in the percent Daudi cells forming a cap with Con A was used to monitor potential alterations of the Daudi cell membrane following interaction with influenza viruses. Cells were incubated for 2–72 hrs with viral preparations of X47 recombinant

Table 1. Increase in the percent of Daudi cells forming a cap with Con A following interaction with inactivated influenza virus

Incubation time (hours)	Percent cells with cap			
	2 hr	24 hr	48 hr	72 hr
Cells				
Daudi	6 ± 0.8*	7 ± 0.4	9 ± 1.1	7 ± 0.7
Daudi-XA	16 ± 2.4	5 ± 1	2 ± 0.5	1 ± 1
Daudi-X56	14 ± 1.1	21 ± 0.7	23 ± 2	23 ± 1.5
Daudi-XF	18 ± 1.6	21 ± 1	30 ± 1.7	31 ± 1.2
Daudi-XFA	20	23 ± 0.3	23 ± 0.4	22 ± 0.2

Daudi cells were incubated for 2–72 hrs with influenza virus X47 at m.o.i. of 100–1000 EID_{50}/ cell. Preparations of the active virus (XA) or virus inactivated by heat (X56) or formalin (XF) were used. A preparation of isolated viral surface glycoproteins consisting of hemagglutinin and neuraminidase (XFA) was also tested. At each time point, cells were tested for Con A-cap formation as described in *Materials and Methods.* The percent of Con A-capping cells in normal lymphocytes was 26 ± 0.6.

*±S.E. The data is presented as the mean of 4 experiments ± S.E.

influenza and their ability to form a cap with Con A was tested. As shown in Table 1, an increase in Con A cap formation (14–18 %) was already noticed after 2 hrs incubation with an active preparation of the virus (XA) or with the inactivated preparations X56 or XF. Following further culture of Daudi cells with XA, a significant decrease in the percent of cells capping with Con A was observed (1–5 %). In contrast, cells incubated with inactivated X56 or XF preparations showed an additional increase in cells forming a Con A cap (up to 23 % cells with X56 and 31 % cells with XF) (Table 1).

Titration of the active and inactivated viral preparations revealed that m.o.i. of at least 10 EID_{50}/cell was necessary to detect this increase in the percent of cap-forming cells. Viral titration of all preparations was carried out as described previously (Weil-Hillman *et al.,* 1985*a,b).* Thus, a minimal number of inactivated viral particles is required to cause this effect.

Neutralization of X56 or XF by pretreatment with specific rabbit anti-H3N2 influenza sera inhibited adsorption of the viral particles on the cells (as monitored by immunofluorescence; Weil-Hillman *et al.,* 1985*a, b).* The cells interacted with X56 or XF pretreated with anti-H3N2 did not exhibit an increase in Con A cap formation indicating that this phenomenon is the result of viral adsorption and interaction with the cell membrane (data not shown).

Interestingly, whole virions were not necessary to obtain this increase in cap formation since a preparation of surface viral glycoproteins (XFA) consisting of HA and NA caused cap formation of 20–23 % cells (Table 1). This suggests that the increased mobility of Con A receptors might be due to the insertion of HA or NA in the cell membrane.

Involvement of viral replication

The decrease in Con A cap formation seen in Daudi cells interacted with XA at 24–72 hrs in culture was probably due to the viral replication of XA particles in the cell (Weil-Hillman *et al.,* 1985*a, b).* Indeed, this decrease was reversed when Daudi cells were pretreated with amantadine, an inhibitor of viral uncoating (Skehel *et al.,* 1977) and then infected with XA. At 24 hrs post XA infection, 40 % of the cells capped with Con A in contrast to 6 % in cells not treated with amantadine but infected with XA (Table 2). In contrast, amantadine had no effect on the cap formation ability of Daudi cells interacted with inactivated influenza virus (Table 2). This suggests that the increase in Con A-cap formation obtained in Daudi-XF or Daudi-X56 cells was independent of early events in the viral cycle.

Role of the cytoskeleton in the increased Con A-cap formation seen in Daudi-XF cells

Membrane structures such as the cytoskeleton, that consists of microtubules and microfilaments, have been shown to be involved in receptors mobility, precisely in the lateral movement of Con A receptors leading to a cap formation

Table 2. Con A cap formation of Daudi cells interacted with X47 (H3N2) in the presence of amantadine

Incubation time (hours)	Percent cells with cap	
	2 hr	24 hr
Daudi	7	8
Daudi + Amantadine	7	8
Daudi-XA	13	6
Daudi-XA + Amantadine	12	40
Daudi-X56	16	19
Daudi-X56 + Amantadine	15	22
Daudi-XF	16	20
Daudi-XF + Amantadine	14	21

Daudi cells were pretreated with amantadine for 1 hr at 37^{0}C and then incubated with the viral preparations XA, X56 or XF. At 2 or 24 hrs cell-virus interaction, cells were tested with F-Con A as described in *Materials and Methods.*

(Schreiner & Unanue, 1976; De Petris, 1974; Albertini *et al.*, 1977). Con A capping was inhibited by treatment of the cells with cytochalasin B that acts on microfilaments (Schreiner & Unanue, 1976; De Petris, 1974). In contrast, colchicine that causes disassembly of microtubules, increased the mobility of receptors resulting into a cap formation (Schreiner & Unanue, 1976; Albertini *et al.*, 1977). To determine the influence of the cytoskeleton on the increased Con A cap formation observed in Daudi-XF cells, colchicine or cytochalasin B were added to Daudi cells preincubated with XF for 48 hrs. Colchicine caused an increased cap formation with Con A in untreated Daudi cells (from 5 % to 31 % capping cells) while cytochalasin B did not affect Con A capping (Table 3). However, the treatment of Daudi cells with the combination of both drugs resulted in a low percentage of cell capping (10 %). Colchicine enhanced the Con A cap formation in Daudi-XF cells (from 22 % to 58 % capping cells). Cytochalasin B did not alter the Con A capping of Daudi-XF cells. Addition of both drugs reversed the effect otained by colchicine alone (Table 3) as shown previously (De Petris, 1974; Albertini *et al.*, 1977). Interestingly, preincubation of Daudi-XF cells with F-Con A followed by addition of cytochalasin B, maintained the increased percentage of cells in cap formation (21 %) (Table 3). Thus, the microfilaments do not seem to play a role in this phenomenon. It seems that XF interaction with Daudi cells causes an increase in the mobility of Con A receptors probably by interacting at the level of the microtubules.

Table 3. Effect of colchicine and cytochalasin B on Con A cap formation of Daudi cells interacted with inactivated X47 (H3N2) virus

Treatment	Percent cells with cap
Untreated Daudi	5
Daudi + Colchicine	31
Daudi + Cytochalasin B	8
Daudi + Cytochalasin B + Colchicine	10
Daudi-XF	22
Daudi-XF + Colchicine	58
Daudi-XF + Cytochalasin B	19
Daudi-XF + Cytochalasin B + Colchicine	28
Daudi-XF + Con A + Cytochalasin B	21*

Daudi cells were first incubated for 48 hrs with XF viral preparations, then cells were washed and treated for 30 min at 37^0C with colchicine and cytochalasin B and incubated with F-Con A (as described in *Materials and Methods*).

*Daudi-XF cells were preincubated with F-Con A prior to addition of cytochalasin B.

Discussion

A restricted lateral movement of Con A receptors (Ben-Bassat, 1977) as well as the high viscosity of the membrane (Schreiner & Unanue, 1976) seem to be responsible for the low level of Con A capping observed in lymphoma cells in contrast to normal lymphocytes.

The interaction of influenza viruses with Daudi lymphoma cells induced changes in the cell surface membrane resulting in an increased percentage of Con A cap forming cells (from 5–10 % prior to virus addition to 30–40 % after addition). This phenomenon was initiated by adsorption of active or inactivated viral particles on the cells.

Viral glycoproteins, which are inserted into the cell membrane as a consequence of viral replication (Maeno & Kilbourne, 1970) probably bind Con A since HA (Laver, 1971), and particularly NA (Palese *et al.*, 1973), contain mannose residues, a Con A specific sugar. During viral replication and budding of new viral particles even more Con A binding sites are uncovered. Indeed, cells treated with active XA virus showed an intense staining with F-Con A concommitant with a decrease in capping. This may be due to the binding of many Con A molecules to the cell surface resulting into inhibition of the lateral movement of the receptors required to form a cap (Edelman *et al.*, 1973; Schreiner & Unanue, 1976). Pretreatment of the cells with amantadine, an inhibitor of viral uncoating, did reverse this decreased capping ability by inhibiting

viral replication and appearance of newly synthesized viral glycoproteins on the cell surface. Nevertheless, the increased ability to form a cap with Con A, observed in cells interacted with inactivated virus, was independent of viral replication and rather due to interaction of the viral particles with the cell membrane.

Preparations of the viral glycoproteins HA and NA were sufficient to cause an increase in Con A cap formation on Daudi cells. This indicates that this phenomenon may be due to: 1) binding of Con A molecules to viral receptors (probably present on HA and NA as mentioned above) and to cell receptors leading to crosslinking of Con A receptors that will induce their mobility and the formation of a cap, or 2) adsorption of the virus or viral glycoproteins on the cell surface inducing alterations in the membrane structure at the level of the cytoskeleton. We found that incubation of Con A with viral preparations caused the formation of small clumps (flocculation). Addition of the Con A competitive inhibitor α-methyl mannoside dissolved these clumps (unpublished data). This flocculation phenomenon was previously described with Sendai and herpes viruses incubated with Con A (Okada and Kim, 1972) and indicates that Con A can bind to influenza virus particles.

Alternatively, the induction of changes in the membrane structure at the level of the cytoskeletal system by virus adsorption might explain the changes in the lateral movement of the Con A receptors by the influenza virus. Several viruses including influenza virus have been shown to interact with cytoskeletal components of various types of cells, either in initial stages of the viral multiplication cycle (adsorption, penetration) similar to our findings or in late stages of assembly and release (Križanová *et al.*, 1986; Hamaguchi *et al.*, 1985; Luftig & Weiking, 1975; Norrild *et al.*, 1986; Ruter & Mannweller, 1977). Microtubules and microfilaments are involved in the regulation of the receptors movement on the cell surface. Disruption of the cytoskeleton result into an increased mobility of receptors (Sheetz *et al.*, 1980). Cytochalasin B acts on microfilaments and inhibits capping by Con A (Schreiner & Unanue, 1976; De Petris, 1974) in contrast to colchicine that disassembles the microtubules and enhances receptor mobility to form caps (Schreiner & Unanue, 1976; Albertini *et al.*, 1977).

We have shown in this study that the increased cap formation caused by XF virus is not altered by cytochalasin B. However, colchicine did cause an additional increase in the cap formation ability of Daudi-XF cells. This suggests that the inactivated influenza virus interaction with the cell membrane may have induced changes in the cytoskeleton at the level of microtubules as indicated by the study of Križanová *et al.* (1986). Further studies are required to determine the biological significance of restoration of capping values observed in lymphoma cells interacted with influenza virus. This phenomenon is of interest and contrasts with the finding that normal circulating lymphocytes from healthy individuals do not show an increase in Con A capping following inte-

raction with influenza viruses (unpublished data) although these cells bear receptors to influenza virus (Woodruff & Woodruff, 1974).

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Abbreviations: XA, active X47 influenza virus; X56, heated X47 virus; XF, formalin inactivated virus; m.o.i., multiplicity of infection; HA, hemagglutinin; NA, neuraminidase; XFA, preparation of HA and NA; EID_{50}, 50 % endpoint egg infectious dose.

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