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Exchange of the cellular growth medium supplement from fetal bovine serum to Ultroser G increases the affinity of adenovirus for HeLa cells

Brief Report

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Summary. A comparative investigation was performed on the process of attachment of adenovirus type 2 to HeLa cells cultivated in the presence of 3.5% fetal bovine serum (FBS-cells) or 2% Ultroser G (USG-cells). The initial rates of virus attachment were markedly higher at temperatures between 10 and 35 °C for the virus binding to USG-cells than to FBS-cells. This was not caused by a higher amount of available virus-recognizing cellular receptor sites or cellular receptor units recognizing the viral fiber, but could be explained by a higher affinity of virions for USG-cells as compared to FBS-cells. Studies of virus attachment to cells, pretreated with neuraminidase and/or wheat germ agglutinin, suggested that the cellular receptor sites of FBS-cells were masked to a higher extent by sialic acid than the cellular receptor sites of USG-cells.

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Previous studies of the adenovirus-HeLa cell system indicate that a proper interaction between the virus and the cellular membranes is a prerequisite for the subsequent production of progeny virus later in the infection cycle [6, 7, 26]. There are many examples demonstrating the importance of a correct membrane constitution for optimally maintaining a number of cellular functions and in these studies modifications in the composition of membrane lipids have been introduced [22, 31]. To investigate the role of the plasma membrane composition during the early interaction between adenovirus type 2 (Ad2) and the HeLa cell, the lipid constitution of the cell membranes may be metabolically modified by altering the lipid composition of the culture medium [22, 23]. To achieve reproducible and controlled growth conditions we have chosen to exchange fetal bovine serum, the normally used medium supplement, to the com-

mercially available serum substitute Ultroser G, which has been shown to be equivalent or in some instances superior to fetal bovine serum for the propagation of a number of different cell types [15, 16, 19, 32]. However, by changing the medium supplement from fetal bovine serum to Ultroser G the constitution of the HeLa cell plasma membrane is drastically altered with regard to the protein and lipid composition [5]. In the present investigation we have studied the consequences of such plasma membrane alterations on the process of Ad2 attachment to HeLa cells. A lower affinity between virus and cells propagated in the presence of fetal bovine serum, as compared with cells cultivated with Ultroser G, is suggested to be a consequence of an interference by sialic acid residues on the surface of the former cells with the interaction between virus and cellular receptor sites.

The established HeLa cell line (ATCC CCL 2.2) was grown at 37 °C in suspension culture at a maximum density of 6×10^5 cells/ml in Eagle's minimum essential medium (EMEM) (Flow Labs., Irvine, Scotland) supplemented with gentamicin, 20 μg/ml, (Biological Industries, Beth Haemek, Israel) and 3.5% fetal bovine serum (FBS) (Flow Labs.). Cells grown with 2% Ultroser G (USG) (Réactifs IBF, Villeneuve-la-Gavenne, France) as a supplement were maintained as monolayer cultures in Eagle's minimum essential medium for monolayer culturing (MMEM). USG supplied at a concentration of 2% yielded the same total protein concentration in the medium as 3.5% FBS. Cells in monolayer cultures were treated with 0.02% EDTA in phosphate buffered saline (PBS) and the released cells were established as a suspension culture in 2% USG in EMEM. Such cells were propagated for two days and subsequently used in experiments. In the following, cells grown in the presence of FBS or USG are referred to as FBS- and USG-cells, respectively. FBS-cells cultivated in the presence of ethanolamine (EA) are designated EA-cells. EA was added to FBS-cells at a final concentration of 50 μg/ml and the cells were further cultivated for 48 h before they were harvested. All cells were routinely assayed for *Mycoplasma* infections by a Mycoplasma T. C. II kit (Gen-probe, San Diego, CA). Cell enumerations were made in a Buerker counting chamber.

Ad2 was propagated in FBS-cells maintained in suspension culture and [^{3}H]thymidine-labelled virus (10,500 cpm/10^{10} virions) was purified according to Svensson et al. [27].

The fiber antigen was isolated from the excess pool of structural proteins remaining after virus purification. The viral antigens were separated by DEAE-cellulose chromatography, and after application of a salt gradient the fiber was recovered as the first eluting antigen [29]. The fiber was extensively dialyzed against 0.1 M Na-acetate buffer, pH = 5.0, and then it was applied to a CM-Sephadex C-25 column equilibrated in the same buffer system. A gradient (0.1–0.4 M acetate buffer, pH 5.0) was connected, and the peak fractions corresponding to the fiber were after concentration further purified by gel exclusion chromatography on a column of Sepharose CL-6B. 5 μg quantities of fiber protein revealed only one stainable band upon reductive SDS-polyacrylamide

gel electrophoresis (SDS-PAGE) [13]. For concentration determination an extinction coefficient ($E_{278}^{0.1\%}$) of 0.850 was used [10] together with a molecular mass of 180,000 Da for the native protein [10, 24].

Cells to be used in the attachment experiments were harvested by centrifugation (500 × g_{max}/10 min), washed twice in ice-cold PBS and then resuspended in PBS containing 1% bovine serum albumin (BSA) and 50 mM NaN_3 to give 3×10^7 cells/ml. Prior to kinetic attachment studies the cells were temperature-equilibrated for 10 min on a shaking water-bath and then [^{3}H]thymidine-labelled adenovirus was added to give a calculated multiplicity of infection (MOI) of 400–800 virions/cell. The virus-cell mixtures were further incubated as above and at 2 min intervals, between 1 and 11 min post addition of virus, 50 µl portions of the suspensions were withdrawn and rapidly diluted in 10 volumes of cold PBS containing 1% BSA and centrifuged. The recovered supernatants and pellets were assayed for radioactivity. The relative attachment in per cent was plotted as a function of incubation time and rate constants were calculated for the linear portions of the curves.

In equilibrium binding experiments virus was added at MOIs between 100 and 2.5×10^4 virions/cell to samples of 6×10^6 cells, which were suspended in the BSA- and NaN_3-containing buffer as above. The virus-cell mixtures were incubated at 15 °C for 3 h and at the end of the incubation duplicate portions of 50 µl were removed from each sample and processed as in the kinetic studies. In the corresponding fiber binding studies [^{3}H]amino acid-labelled fiber was added at inputs between 1×10^4 and 2×10^6 fiber molecules/cell to portions of 1.6×10^7 cells. The fiber-cell mixtures were processed as above.

Cells, pretreated with wheat germ agglutinin (WGA), were used to further characterize the process of virus attachment. WGA was added to samples of 6×10^6 cells in 200 µl of PBS to give quantities of WGA ranging between 0 and 20 µg/sample. The WGA-cell suspensions were incubated at 12 °C for 1 h on a shaking water-bath, and then they were diluted 10 times in ice-cold PBS. The cells were sedimented and then resuspended in PBS containing 1% BSA to give the same sample volume as above. [^{3}H]Thymidine-labelled virus was added to give a MOI of 500 virions/cell and the virus attachment was continued for 1 h at 12 °C before duplicate portions of 50 µl were withdrawn from each virus-cell sample. The aliquots were processed as described in the kinetic studies. In some experiments cells were pretreated with WGA, and subsequently incubated at 12 °C for 1 h together with unlabelled fiber to give 3.8×10^5 fiber molecules/cell. Unattached fiber was removed from the cells before virus was added and the suspensions were further treated as described above. In studies of fiber attachment to cells pretreated with WGA, samples of 1.6×10^7 cells at a cell density of 3×10^7 cells/ml were preincubated with 40 µg of WGA. The suspensions were incubated and washed as above and [^{3}H]amino acid-labelled fiber was added to give an input of 1×10^5 fiber molecules/cell. The extent of attachment was determined as described in the equilibrium binding study.

To prepare plasma membranes cells were subjected to a homogenization

procedure according to Atkinson et al. [2]. Iodoacetate was excluded from the homogenization buffer and the cells were ruptured by 7 strokes in a Dounce homogenizer with a tight-fitting B-pestle. The nuclei were stabilized with 3 mM $MgCl_2$ and 10 mM NaCl, and the homogenates were centrifuged at $370 \times \mathbf{g}_{av}$ for 15 min. The pellets were suspended in 10% sucrose in PBS to give a concentration of 7×10^6 cell equivalents/ml. Such suspensions were subjected to a new centrifugation at $660 \times \mathbf{g}_{av}$ for 4 min, including acceleration and retardation. The supernatants were transferred to new test-tubes and the crude plasma membrane fractions were sedimented at $1630 \times \mathbf{g}_{av}$ for 30 min. The pellets were washed twice in 10 ml of a 50 mM Tris-HCl buffer, pH 8.0, containing $MgCl_2$ and NaCl as above and then this material was subjected to separation in a two-phase polymer system according to Brunette and Till [9].

Plasma membrane polypeptides from FBS-, EA-, and USG-cells were separated by SDS-PAGE on gels of 10% acrylamide and 0.27% bisacrylamide [13] and then the polypeptides were transferred to nitrocellulose blotting membranes (Trans-Blot, Bio-Rad Laboratories) by semidry electrophoresis. The blotting and alkaline phosphatase developing procedures were according to Blake et al. [4], using 5% powdered fat free milk and 0.05% Tween-20 in PBS as working buffers. The WGA-blottings were performed as described previously [5] and in the immunoblottings an antiserum from a rabbit immunized with plasma membranes isolated from HeLa cells grown with 3.5% FBS was used at a 50 times dilution. Biotin-conjugated goat anti-rabbit IgG (Sigma Chemical Co.) was diluted 1000 times and an avidin alkaline phosphatase-conjugate (Vector Laboratories, Burlingame, CA) was used at a final concentration of 0.2 U/ml. Polypeptides from Ad2 were used as molecular weight markers both in the WGA- and the immunoblottings. The polypeptides were visualized by a rabbit anti-Ad2 serum, diluted 100 times, in combination with the biotin-avidin system above.

The phospholipid and fatty acid composition of plasma membranes were determined by thin layer chromatography and gas chromatography, respectively [5]. The phospholipid content was quantified by phosphate assessment essentially according to McClare [14]. Cholesterol was determined colorimetrically using a "Kit for food analysis" (Boehringer Mannheim GmbH, Mannheim, Federal Republic of Germany).

The temperature dependence of the Ad2 attachment kinetics using FBS-, USG-, and EA-cells was investigated. The initial attachment rates were determined at different temperatures between 4 and 35 °C (Fig. 1). Ad2 attached at higher rates to USG-cells than to both FBS- and EA-cells, with the most pronounced differences observed at temperatures between 10 and 25 °C. In this temperature interval the attachment rates of virus binding to USG-cells were about 2 to 4 times higher as compared to FBS-cells. The attachment rate of Ad2 interacting with USG-cells reached a maximum at 25 °C, whereas the rate of virus binding to FBS- and EA-cells was still increasing between 25 and 35 °C.

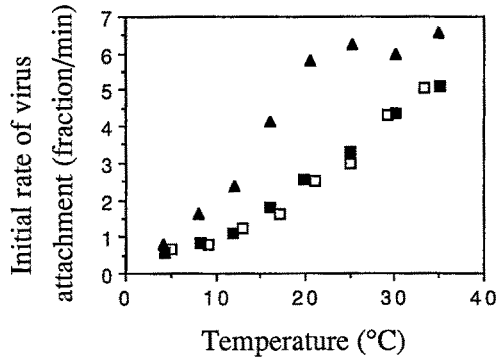

Fig. 1. Temperature dependence of the initial rates of Ad2 attachment to HeLa cells. Virus was added to FBS- (□), EA- (■) and USG-cells (▲) at a MOI of 400–800 particles/cell and incubated at temperatures between 4–35 °C. Aliquots of the virus-cell suspensions were withdrawn at intervals and the attachment efficiencies and subsequently the attachment rates were determined. The data represent one typical experiment for each cell type

Table 1. Affinity constants (K) and numbers of cellular receptor sites (CRS) and cellular receptor units (CRU) for attachment of virus and fiber to FBS-, EA-, and USG-cells

Type of cell	Ad2		Fiber	
	CRS (10^3)/cell	K ($10^{10}\,M^{-1}$)	CRU (10^4)/cell	K ($10^8\,M^{-1}$)
FBS	3.0 ± 0.6 (n = 4)	1.9 ± 1.5 (n = 4)	11.3 ± 1.2 (n = 4)	3.0 ± 0.5 (n = 4)
EA	3.5 ± 0.6 (n = 2)	2.0 ± 0.02 (n = 2)	14.4 (n = 1)	2.3 (n = 1)
USG	2.1 ± 1.3 (n = 5)	17.3 ± 9.0 (n = 5)	7.1 ± 0.9 (n = 3)	9.8 ± 0.5 (n = 3)

The values are means ± standard deviation (S.D.). *n* Number of separate determinations

The temperature dependence of the Ad2 attachment rates displayed the same profiles in the FBS- and EA-cell systems.

Attachment data from the equilibrium binding experiments were represented by Scatchard plots [20], from which the numbers of cellular receptor sites for virions (CRS), cellular receptor units for fibers (CRU) and affinity constants were determined [11] (Table 1). Using Student's t-test no difference was revealed in the numbers of CRS on FBS- and USG-cells at a 5% significance level, whereas for CRU a difference was found at a 1% significance level. Regarding the affinity constants it was shown at significance levels of 1 and 0.1%, that virions and fibers, respectively, bound with higher affinities to USG-cells than to FBS-cells. Receptor numbers and affinity constants for EA-cells were essentially the same as those for FBS-cells.

The lectin WGA was subsequently used to further characterize the mechanisms underlying the different attachment rates. Thus, pretreatment of USG-

cells with WGA resulted in an inhibition of the subsequent virus cell-binding, while the same treatment of FBS- and EA-cells caused an increase in the extent of virus attachment (Fig. 2). In dose-response studies maximal inhibition of virus binding to USG-cells was obtained at 12 μg of WGA/10^6 cells. At this concentration of WGA the degree of virus attachment to USG-cells was reduced by 60%, while the corresponding binding of virions to FBS- and EA-cells increased by 40 and 150%, respectively (Fig. 2). Virus attachment to FBS- and USG-cells, pretreated with both WGA and subsequently with fiber, was reduced to the same level as virus attachment to cells only preincubated with fiber (Fig. 2), indicating that the virus attachment to WGA-pretreated cells was specific. Pretreatment of cells with 2.5 μg of WGA/10^6 cells reduced the degree of fiber attachment to USG-cells by 50%, whereas the binding of fiber to FBS- and EA-cells increased by 35 and 76%, respectively.

In a series of experiments cells were treated with neuraminidase from *Arthrobacter ureafaciens* (10 units/ml, Boehringer Mannheim) at final concentrations of 0.001, 0.01 and 0.1 units/ml at 25 °C for 30 min. Then [^{3}H]thymidine-labelled virus was added to cell samples of 6 × 10^6 cells at a MOI of 1,700 virions/cell. The virus-cell suspensions were incubated at 12 °C for 1 h and further treated as the samples of the attachment kinetic series. The level of attachment of Ad2 to FBS-cells increased due to the enzymatic pretreatment of the cells, and the magnitude of the increase was shown to be dependent on the concentration of neuraminidase present during the period of the pretreatment (Fig. 3). The increase in the relative virus attachment following neuraminidase-pretreatment of USG-cells was less pronounced as compared with FBS-cells. The observed increase in virus attachment upon neuraminidase treatment of FBS-cells could be inhibited by incubating such cells with WGA prior to the addition of virus (Fig. 3).

The equilibrium binding of virus was studied in order to further characterize

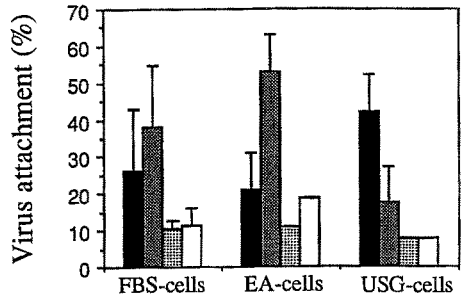

Fig. 2. Virus binding to cells pretreated with WGA. The levels of attachment were determined for virus interacting with control cells (■) and with cells pretreated with WGA (▩). The specificity of virus binding was also examined: Virus was added to cells that were pretreated with fiber (▦) and to cells that were first pretreated with WGA and then with fiber (□). The values are means ± S.D., where the number of separate determinations (n) varies between 2 and 6. Where no S.D. is indicated, n = 1

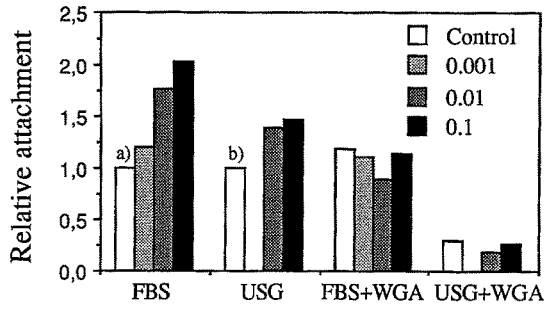

Fig. 3. Attachment of virus to cells pretreated with neuraminidase. The extent of virus attachment was determined for virus interacting with FBS- and USG-cells pretreated at a density of 3×10^7 cells/ml with 0.001, 0.01 and 0.1 units of neuraminidase per ml, as indicated within the panel, for 30 min at 25 °C. The effect of WGA on the virus binding to neuraminidase-treated cells was also determined. For each series an untreated control was included. *a* All data from FBS-cell experiments are compared with this value. *b* All data from USG-cell experiments are compared with this value

the virus attachment following neuraminidase treatment of cells. In these experiments cells were incubated with neuraminidase at a final concentration of 0.1 units/ml at 37 °C for 1 h before the binding assays were run as described above. Series of untreated cells were run in parallel as controls and the data were represented by Scatchard plots. From such plots it was concluded that the number of CRS on USG-cells and the affinity constant for the interaction between these cells and Ad2 were unaffected in spite of the pretreatment of USG-cells with neuraminidase. However, the affinity constant for the virus binding to FBS-cells increased threefold after enzyme treatment of the cells, whereas the number of CRS remained constant.

Since the step of virus attachment to cells specifically involves the cellular plasma membrane, it was important to compare the constitution of plasma membranes isolated from USG-, FBS-, and EA-cells. Thus, plasma membranes prepared from EA- and USG-cells showed essentially the same relative composition of phospholipids. FBS-cells differed mainly from USG- and EA-cells in the relative distribution of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), which were reduced by 27% and increased by 48%, respectively, in the USG-cells as compared with the FBS-cells. However, the relative fatty acid composition of the plasma membranes was the same in EA- and FBS-cells, whereas plasma membranes from USG-cells revealed a somewhat different fatty acid distribution. The most pronounced alterations were found in the relative distribution of palmitoleic acid (16:1), stearic acid (18:0), and linoleic acid (18:2), which amounted to 16.7, 7.9, and 1.8%, respectively, in FBS-cells and 5.7, 17.2, and 6.4%, respectively, in USG-cells. For the USG-cells the relative amounts of saturated and unsaturated fatty acids were 44 and 56%, respectively, and for the FBS-cells the corresponding figures were 31 and 69%. The relative proportions between the cholesterol and phosphate contents

of the plasma membranes were the same in all cells, whereas the amounts of protein in the plasma membranes were almost doubled for EA-cells compared to FBS- and USG-cells. The composition of the HeLa cell plasma membrane was investigated in a previous report, where cells were grown as suspension cultures in the presence of 3.5% FBS or 2% USG [5]. In the present study some of the plasma membrane characteristics differ from those described in the previous report. This can be explained by the fact that in the present study a modified method for the plasma membrane preparation was employed and the USG-cells were maintained as monolayer cell cultures until used.

Qualitative and quantitative comparisons between the total polypeptide content of plasma membranes prepared from FBS-, EA-, and USG-cells were made by immuno- and WGA blottings. SDS-PAGE-separated polypeptides obtained from FBS- and EA-cells showed identical WGA- and immunoblotting patterns (Fig. 4). The immunoblotting analyses revealed three plasma membrane polypeptides with molecular weights (M_r) of 152,000, 44,000 and 31,000 (bands a, g, and h in Fig. 4 B), which were unique for the plasma membranes obtained from FBS- and EA-cells. One polypeptide, with an M_r of 79,000 (band c in Fig. 4 B), was present in all immunoblottings, but was much less intensely stained

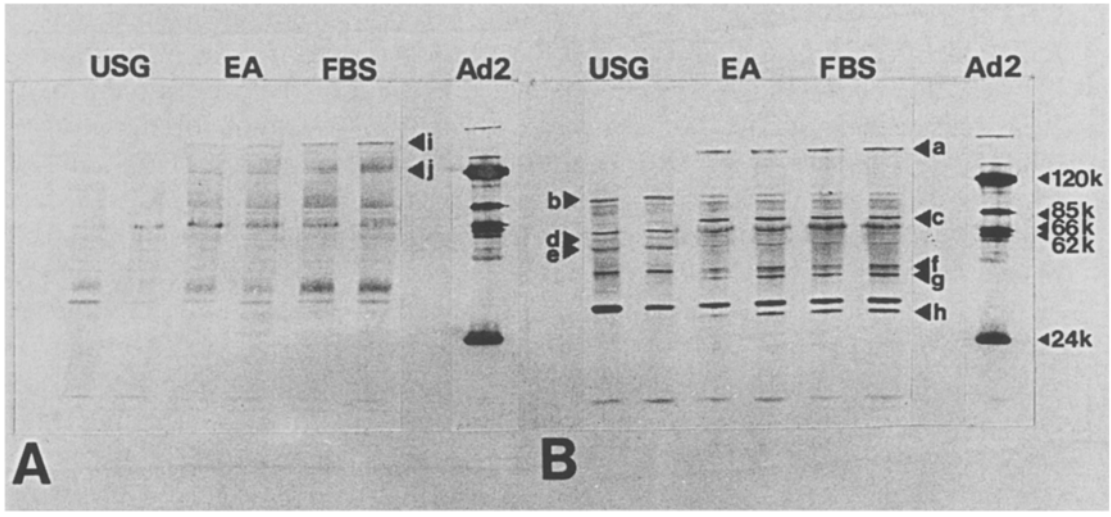

Fig. 4. Immuno- and WGA blottings of plasma membrane polypeptides derived from FBS-, EA-, and USG-cells. The polypeptides were separated by SDS-PAGE and portions of 10 μg of total protein were added to each well. After transfer of the polypeptides to nitrocellulose filters, these were subjected to incubations with WGA (**A**) or with an antiserum produced in a rabbit immunized with plasma membranes isolated from FBS-cells (**B**). Polypeptides from Ad2 were used as molecular weight markers (*Ad2*). The blottings showed different patterns for the USG-derived plasma membranes compared with plasma membranes from FBS- and EA-cells, as exemplified by the following polypeptides: *a* M_r = 152,000; *b* M_r = 99,000; *c* M_r = 79,000; *d* M_r = 67,000: *e* M_r = 60,000; *f* M_r = 49,000; *g* M_r = 44,000; *h* M_r = 31,000; *i* M_r = 152,000: *j* M_r = 113,000

in the blottings of plasma membranes obtained from USG-cells compared to those prepared from FBS- and EA-cells. The opposite situation was observed regarding three polypeptides with M_r of 99,000, 67,000, and 60,000 (bands b, d, and e in Fig. 4 B), which appeared weaker on the immunoblottings of FBS- and EA-cells than on the corresponding USG-blottings. In the 49,000 molecular weight region two polypeptides were revealed in the immunoblottings of FBS- and EA-polypeptides, whereas at the corresponding position on the USG-blottings only one polypeptide was seen (band f in Fig. 4 B). WGA blottings of plasma membranes from FBS- and EA-cells in general displayed higher intensities than the corresponding USG-blottings. Two polypeptides with M_r 152,000 and 113,000 (bands i and j in Fig. 4 A) were seen only on the FBS- and EA-blottings.

Plasma membranes prepared from HeLa cells cultivated in the presence of 3.5% FBS or 2% USG differ in their composition with regard to the phospholipid, fatty acid and protein constitution ([5] and the present investigation). In the present study our intention was to elucidate the possible influence of such alterations on the early extra cellular interaction between Ad2 and HeLa cells.

The increased initial attachment rates observed for the interaction between Ad2 and USG-cells compared to those between virions and FBS- and EA-cells were not caused by a higher number of available receptors for the virion or for the fiber. The increases could rather be explained by the higher affinity of virions and fibers for the USG-cells compared to the FBS- and EA-cells. Alterations in the plasma membrane phospholipid composition cannot explain the changes in virus-cell and fiber-cell interaction, since EA-cells showed the same relative phospholipid distribution as USG-cells, but interacted with Ad2 and fibers as FBS-cells. However, differences in the fatty acid composition might influence the Ad2- and fiber-cell interactions by the role of unique fatty acids in the lipids specifically surrounding CRS and CRU or by altering the fluidity of the plasma membrane. However, in an accompanying study it was shown that treatment of cells with benzyl alcohol as a fluidizing agent, does not affect the various aspects of Ad2 attachment to HeLa cells [6]. Therefore the differences observed in the early extra cellular interaction between Ad2 and FBS- and USG-cells in the present study could be an effect of qualitative and quantitative alterations on the protein and carbohydrate level as revealed by the immuno- and WGA blottings of plasma membrane polypeptides. Thus, alterations in the carbohydrate constitution of the plasma membrane of the two cell types were demonstrated by the different efficiencies of virus- and fiber attachment to FBS- and USG-cells that had been pretreated with WGA. Furthermore, treatment of USG-cells with neuraminidase only slightly affected the binding of virus to these cells, while the affinity constant for the interaction between virus and FBS-cells increased threefold in magnitude upon such a treatment of the latter cells. This positive effect of neuraminidase on subsequent virus attachment also has been registrated for Ad2 in the KB-cell system [8].

The substrate for neuraminidase is sialic acid residues [28], and evidently, by removing sialic acid from the cell surface, CRS on FBS-cells are modified to a conformation with a higher affinity for virions. However, incubation of FBS-cells with WGA prior to virus addition inhibited the otherwise obtained increase in virus binding upon neuraminidase treatment, which implies that the new conformation of CRS exposes a moiety that can be recognized by WGA. Sialic acid is usually located as a terminal residue on oligosaccharides of glycoproteins on the cell surface and being a bulky and negatively charged residue, sialic acid often masks other cell surface components [21]. Thus, by removing the bulky terminal residue, new sites of the glycoprotein or of a neighbouring protein may be unmasked. Therefore the limited effect of neuraminidase treatment of USG-cells on the subsequent virus binding suggests that sialic acid does not mask CRS on these cells to the same extent as on FBS-cells. The reduced masking of cellular components on USG-cells probably explains why pretreatment with WGA inhibited binding of virus to USG-cells, but not to FBS-cells, in spite of the fact that the WGA blottings of polypeptides from plasma membranes prepared from FBS- and USG-cells showed that FBS-cells in general bound more WGA than USG-cells. This also indicates that the inhibitory effect of WGA on virus binding to USG-cells was specific rather than due to a mass binding of WGA to these cells. Thus, the cell surface glycoproteins of FBS- and USG-cells revealed different oligosaccharide compositions. This property of certain glycoproteins to contain various structures of a particular oligosaccharide is referred to as microheterogeneity [3, 18]. This feature is assumed to arise following incomplete or alternative cellular synthesis of the sugar residues, and factors influencing the final structures of the oligosaccharides are suggested to be, e.g., genetic control, the relative supply of different glycosyltransferases and their substrates and the possibility of different biosynthetic pathways. How these factors in turn are controlled is not well understood. Thus, differences in the composition of Ultroser G and fetal bovine serum with regard to components that are of importance for the control mechanisms mentioned above, would explain the observed microheterogeneity of CRS and CRU obtained by cultivating HeLa cells in the presence of Ultroser G or fetal bovine serum. In fact, the composition of Ultroser G is especially designed for cells to be maintained as monolayer cultures [16] and the importance of the carbohydrate composition of glycoproteins mediating cell adhesion has been implied [17]. However, the significance of sialic acid in the adhesion process has only been shown for the neural cell adhesion molecule (NCAM) in the vertebrate central nervous system, where it has been shown that during certain stages of the development this protein is glycosylated with a polypeptide-specific polysialic acid residue, which prevents adhesion between the cells [25].

Substrates for the lectin WGA are oligosaccharides containing N-acetylglucosamine or a terminal sialic acid residue [30]. WGA contains two carbohydrate-recognizing sites, where the primary site binds either sialic acid or N-acetylglucosamine, whereas the secondary site only recognizes the latter sugar.

When WGA binds to sialic acid or N-acetylglucosamine on the cell surface, the lectin may expose different sites towards the surrounding. Thus, when WGA is attached to sialic acid on the cell surface the secondary site of WGA may be able to bind the N-acetylglucosamine residues of the fiber [12]. This could explain the increase in virus- and fiber-binding to FBS- and EA-cells observed after WGA-pretreatment of the cells. A WGA- and Ad2-recognizing polypeptide with an M_r of 40,000–42,000 has been isolated from plasma membranes of HeLa cells cultivated in the presence of FBS [27]. Thus, the least one of the oligosaccharides of this polypeptide contains either N-acetylglucosamine or sialic acid. However, in the present report we show that if sialic acid was removed from the cell surface a WGA-recognizing moiety, which was involved in the process of virus recognition of the cell, was revealed and the WGA-binding part of this moiety consequently ought to be N-acetylglucosamine. Thus, N-acetylglucosamine and sialic acid residues on the cell surface both affect the binding of virus to FBS-cells. Though, the location of these sugars to specific cellular components is not obvious. However, since the presence of WGA inhibited virus attachment to neuraminidase-treated FBS-cells it is reasonable to suggest that N-acetylglucosamine is linked directly to a component of the CRS. The masking properties of sialic acid may be performed by the N-acetylglucosamine-containing glycoprotein or another CRS-associated glycoprotein. However, the masking effect of sialic acid is probably unspecific and a consequence of its usually vast extension on the cell surface.

Based on results of the present investigation we suggest that the sialic acid composition of the cell surface is a consequence of the conditions for cell cultivation and that sialic acid, by its masking properties, interferes with the interaction between Ad2 and CRS. Our results also indicate that one of the components of the CRS is a glycoprotein containing an N-acetylglucosamine-residue, which also has been suggested previously [27].

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