Characterization of the Binding of the TC-83 Strain of Venezuelan Equine Encephalomyelitis Virus to BW-J-M, a Mouse Macrophage-like Cell Line

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SUMMARY

The first step in virus replication is attachment of the virus to the host cell surface. To investigate this process, the binding of TC-83 (the attenuated strain of Venezuelan equine encephalomyelitis virus) to BW-J-M (a macrophage-like murine cell culture line) has been characterized. The binding of radiolabelled virus can be blocked by excess unlabelled virus and has a pH optimum in the physiological range. Binding is saturable; analysis using Scatchard plots or the computerized binding data analysis program, LIGAND, yielded estimates of a single class of $4 \times 10^5$ binding sites per cell and an equilibrium binding constant of $2.0 \pm 0.7 \times 10^{12} \text{ M}^{-1}$. Virus bound to the cell could be visualized by transmission electron microscopy and was localized primarily in coated regions of the membrane. Virus, bound under optimum conditions at 0 °C, was internalized upon warming and infected cells productively. Treatment of BW-J-M cells with low concentrations (1 to 10 µg/ml) of the proteolytic enzymes trypsin, Pronase or proteinase K caused a dose-dependent reduction in binding capacity. Trypsin-treated cells, upon return to culture, progressively regained their binding capacity within 24 h. As a further characterization of the virus binding site, several lectins were studied for their ability to inhibit TC-83 binding to BW-J-M cells. *Canavalia ensiformis* agglutinin, *Glycine max* agglutinin and *Triticum vulgaris* agglutinin were potent inhibitors of virus binding. This evidence suggests that TC-83 binds to a specific receptor on the BW-J-M cell and that the receptor may be glycoprotein.

INTRODUCTION

The entry of a virus genome into a cell to initiate virus replication requires, as a first step, the binding of virus to the cell plasma membrane. Although a great deal of information is available on penetration and replication of viruses, including alphaviruses (Dales, 1973; Marker et al., 1977; Fan & Sefton, 1978; Choppin & Scheid, 1980), comparatively little is known about the initial binding of viruses to cellular plasma membranes or about the nature of the binding site involved (Lonberg-Holm & Philipson, 1975; Lonberg-Holm et al., 1976; Helenius et al., 1978, 1980a, b; Oldstone et al., 1980). Alphaherpesviruses are believed to bind to a cell by a process that involves the glycoproteins present in the virus lipid envelope (Fries & Helenius, 1979; Choppin & Scheid, 1980; Marsh & Helenius, 1980) and a specific cell membrane receptor. Several alphaherpesviruses, including Semliki Forest virus (SFV) (Helenius et al., 1978, 1980b; Oldstone et al., 1980), Eastern equine encephalitis virus (Marker et al., 1977; Marker & Jahrling, 1979), and Sindbis virus (Fan & Sefton, 1978), have been studied with respect to their binding to host cell plasma membranes. Gottlieb et al. (1980) reported that binding of Sindbis virus was significantly reduced in ricin-resistant mouse L-cells with altered glycosyltransferase activity, and suggested that loss or inaccessibility of a carbohydrate-containing receptor accounted for the defect.

The biological and biochemical properties of TC-83, the attenuated vaccine strain of Venezuelan equine encephalomyelitis virus (VEE) (Berge et al., 1961; McKinney et al., 1963),
have been studied extensively (Ehrenkranz & Ventura, 1974). VEE is a simple lipid-enveloped virus which contains two envelope glycoproteins and replicates in most cultured cells. The low virulence of certain alphaviruses, including certain VEE strains, has been postulated to involve the interaction of those viruses with cells of the reticuloendothelial system (Jahrling et al., 1974; Jahrling & Gorelkin, 1975). Studies on the Trinidad strain of VEE show a preference of the virus for the reticuloendothelial system during human infection (Ehrenkranz & Ventura, 1974). Studies by Levitt et al. (1979) have shown that VEE will replicate in macrophages from human peripheral blood. Little is known, however, about the early events in virus attachment during VEE infection.

To investigate the route of TC-83 entry into macrophages, a system was chosen which uses a stable cultured macrophage-like murine cell line and the initial binding to those cells was characterized as a first step towards understanding this process.

METHODS

Growth and preparation of virus. TC-83, the attenuated vaccine strain of VEE, was used throughout this study and has been described in detail previously (Berger et al., 1966; McKinney et al., 1963). The virus was obtained as lyophilized vaccine (Lot 4, run 2, Merrell-National Laboratories, Swiftwater, Pa., U.S.A.). Virus was grown in BHK-21 cells in 800-cm² plastic bottles (5 × 10⁶ cells) and isolated by a modification of the method of Marker & Jahrling (1979). For [³⁵S]methionine labelling, Eagle's minimum essential medium (EMEM) without methionine but with 1% foetal calf serum (FCS) and gentamicin (50 μg/ml) was used. Four h after infection, 0·5 μCi of [³⁵S]methionine was added per roller bottle (0·01 μCi/ml, final concentration).

Lactoperoxidase-catalysed iodination of TC-83. The virus (5 × 10¹⁰ p.f.u.) was labelled by lactoperoxidase-catalysed incorporation of ¹²⁵I (Hynes, 1973; Huggins et al., 1980) and virus re-isolated on a 10 to 30% (w/v) linear sucrose gradient.

Quantification of virus. TC-83 was titrated by counting p.f.u. on Vero cell monolayers grown in 10 cm² plastic plates as described previously (Jahrling et al., 1974). Virus concentration was calculated based on a plaquing efficiency of one particle per p.f.u. (Johnson et al., 1978; P. B. Jahrling & J. D. Gangemi, unpublished observation).

Growth of BW-J-M cells. BW-J-M, a murine macrophage-like continuous cell line, was obtained from Dr C. J. Peters of this Institute (Peters, 1981), and grown in EMEM containing non-essential amino acids, sodium pyruvate (1 mM) and 10% FCS (complete medium). The cells were subcultured each 7 days (split ratio 1:5) and grew loosely attached to tissue culture plasticware. For use, the cell layer was washed once with Dulbecco's phosphate-buffered saline (PBS) containing 1% (w/v) bovine serum albumin (BSA) and the cells were detached by using a gentle stream of PBS + 1% BSA. The cells were washed by centrifugation three times in PBS + 1% BSA, counted in a haemocytometer and suspended in PBS + 1% BSA.

Assay for binding of TC-83 to BW-J-M cells. BW-J-M cells grown and harvested as described above were used for 5 days after subculture. Assays were performed in triplicate. The appropriate dilution of radiolabelled virus was placed in 12 × 75 mm polypropylene tubes; both suspended cells and the virus-containing tubes were placed in a 0 °C ice-water bath and equilibrated for 5 min. The assay was initiated by the addition of cells (usually 0.5 ml) to each tube followed by incubation on a gyratory shaker in the ice-water bath for the appropriate time (0 to 3 h). To terminate the binding assay, the contents of each tube were diluted with 2.5 ml of PBS + 1% BSA and the cells were resuspended and transferred to a polypropylene counting vial for determination of disintegrations per minute (d/min) in a Beckman Gamma 8000 counter.

pH dependence of binding for TC-83. Three buffers were used for different pH ranges. For pH 5 to 6·5, 0·1 M-NaCl, 0·05 M-MES, 1 mM-MgCl₂, 1 mM-CaCl₂, 1% BSA, for pH 7·0 to 7·8, 0·1 M-NaCl, 0·05 M-MEPES, 1 mM-MgCl₂, 1 mM-CaCl₂, 1% BSA and for pH 8·0 to 8·6, 0·1 M-NaCl, 0·05 M-Tris, 1 mM-MgCl₂, 1 mM-CaCl₂, 1% BSA. Binding was assayed as described, using 10⁴ BW-J-M cells in 0·5 ml of the appropriate buffer. Cells were incubated with ¹²⁵I-labelled virus (5 × 10¹⁰ p.f.u.) at 0 °C for 2 h. Bound virus was separated from free by washing four times in the same buffer and the bound counts determined as described in the binding assay.

Time course of binding. BW-J-M cells (10⁴/0·5 ml) in PBS + 1% BSA, pH 7·4 were incubated with 5 × 10⁶ p.f.u. of ¹²⁵I-labelled virus at 0 °C. At various times (0, 30, 60, 120 and 180 min), triplicate samples were washed (four times) and bound counts determined as described.

Competition for binding of ³⁵S-TC-83 by unlabelled TC-83. [³⁵S]Methionine-labelled TC-83 (1 × 10⁸ p.f.u., 18.400 c/min) was added with unlabelled TC-83 (0 to 1·2 × 10¹⁰ p.f.u.). BW-J-M cells were added (5 × 10⁶ cells in a final volume of 0·5 ml of PBS + 1% BSA). Cells plus virus were incubated at 0 °C for 2 h and binding determined as described.

Preparation of cells for transmission electron microscopy. Virus was bound to BW-J-M cells under optimal
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conditions (10^5 cells, 7.5 × 10^7 p.f.u. TC-83 per ml, pH 7.4) at 0 °C. After the final wash, the cells were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 for 60 min at 0 °C. After washing with buffer, the pellet of fixed material was treated with 1% OsO4, dehydrated through a graded ethanol series and embedded in Epon-812. Thin sections (silver) were stained with uranyl acetate, counter-stained with lead citrate and visualized in a JEOL JEM 100B electron microscope operating at 80 kV.

Infectious centre assay. BW-J-M cells suspended at 0 °C in PBS + 1% BSA were incubated at 1 × 10^6 cells/ml with 1 × 10^8 p.f.u/ml of TC-83 (total volume 5.0 ml) for 2 h, washed four times in PBS + 1% BSA and suspended in complete medium at 37 °C for 1 h to allow virus internalization. The cells were washed in PBS, treated with neutralizing antibody (log10 neutralizing index = 5.3 at 1:10 dilution, 5 ml total volume) to TC-83, washed again in complete medium, the cell number determined with a haemocytometer, and serial dilutions (1:3) placed on Vero monolayers in 24-well plates. Plates were incubated for 48 h and wells scored for infection by cytopathic effect. The data were analysed by probit analysis to determine the minimum number of cells necessary to produce infection (TCID50).

Trypsinization of BW-J-M. BW-J-M cells were washed twice in PBS, resuspended at 10⁶ cells/ml PBS, cooled to 0 °C and subjected to proteolysis at 0, 0.01, 0.1, 1 or 10 µg/ml trypsin, Pronase or proteinase K for 15 min at 0 °C (Winzler et al., 1967; Langley & Ambrose, 1976; Huggins et al., 1976, 1980). The reaction was quenched by the addition of FCS to 10%, then incubated at 0 °C for 10 min, washed twice in PBS + 1% FCS and once in PBS + 1% BSA. The standard binding assay (5 × 10^6 cells/assay, 10^7 p.f.u. in 0.5 ml final volume incubated at 0 °C for 3 h) was performed in triplicate.

Regeneration of TC-83 binding sites following trypsin treatment. Cells were subjected to proteolysis at 0, 1 or 10 µg/ml trypsin as described above. After a third wash, they were resuspended in complete growth medium (see above) and returned to T-25 flasks. At 0, 12, 24 and 48 h in culture, triplicate flasks were removed and the standard binding assay was performed.

Lectin inhibition of binding. Washed BW-J-M cells (10^6 cells/ml) were incubated with lectins (WGA, Triticum vulgaris agglutinin; SBA, Glycine max agglutinin; or Con A, Canavalia ensiformis agglutinin) for 1 h at 0 °C. The cells were then washed twice in PBS + BSA to remove unbound lectin, resuspended and the standard binding assay performed (8.5 × 10^6 p.f.u./5 × 10^4 cells incubated at 0 °C for 3 h and washed by dilution and centrifugation as described).

RESULTS

Purified virus, labelled with 125I by lactoperoxidase-catalysed iodination and re-isolated on a sucrose density gradient, banded at the same density as unlabelled virus. The peak of binding activity corresponded both to the position of the opalescent band, the expected density of TC-83, and contained both the binding activity and virus infectivity. Alternatively, virus was metabolically labelled with [35S]methionine and purified as described. Virus labelled with 125I was used in studies requiring virus of high specific activity. Intrinsically labelled virus was used for quantitative studies to avoid possible changes in affinity caused by the extrinsic label, although both types of labelled virus gave similar results.

A suspension binding assay was used to circumvent the problem of non-specific binding of TC-83 to tissue culture vessels. Virus binding to BW-J-M cells in suspension was linear in the range of 10^2 to 10^3 p.f.u./cell (Fig. 1). Conditions within the range of linear binding were chosen for further assays. The pH optimum for binding was determined by conducting the assays in saline appropriately buffered with MES, HEPES or Tris buffer. The profile of virus binding as a function of pH showed a peak (Fig. 2) in the physiological range at pH 7.4 to 7.8. The saturability of the binding at pH 7.8 and 7.4 was determined (Fig. 3). Binding appeared to reach saturation at pH 7.8; however, the cells did not tolerate the higher pH. This was indicated by decreased viability (judged by trypan blue vital dye exclusion) (Patterson, 1979), altered morphology, and blebbing. Binding at pH 7.4 approached the maximum value obtained at pH 7.8 at the highest multiplicity tested (8 × 10^5). The time course of virus binding under optimal conditions of virus input and pH was determined and appeared to be complete by 30 min; it did not decrease appreciably during 3 h.

The equilibrium binding parameters of TC-83 to BW-J-M cells were determined in competition studies using 35S-labelled and unlabelled virus (O'Brien, 1979). The binding of labelled virus could be reduced by addition of unlabelled virus (Fig. 4). A 50% reduction in specific binding was achieved at a 1:1 ratio of labelled to unlabelled virus. Binding of labelled TC-83 to BW-J-M cells was analysed by the method of Scatchard or by using the computer program LIGAND (Munson & Rodbard, 1980) in which data are fitted to an exact
Fig. 1. Determination of linear range of virus binding. BW-J-M cells (10^4 per 0.5 ml final volume of PBS + 1% BSA) were incubated with increasing concentrations of virus (125I-labelled, 0 to 10^7 p.f.u., 3.6 x 10^{-2} d/min/p.f.u.) for 3 h at 0 °C and binding determined.

Fig. 2. pH dependence of TC-83 binding to BW-J-M. BW-J-M cells (10^4 in 0.5 ml) were suspended in buffer of the appropriate pH and incubated with 5 x 10^6 p.f.u. of 125I-labelled virus (3.1 x 10^{-2} d/min/p.f.u.). Cells were incubated at 0 °C for 3 h and binding determined.

Fig. 3. Saturation of virus binding to BW-J-M cells. BW-J-M cells (2.5 x 10^4 per 0.5 ml PBS + 1% BSA) were incubated with 0 to 2 x 10^{10} p.f.u. of 125I-labelled TC-83 (0 to 8 x 10^5 p.f.u./cell, 3.1 x 10^{-2} d/min/p.f.u.) at pH 7.4 (O) and pH 7.8 (○) for 3 h at 0 °C and binding determined.

A mathematical model of ligand binding; non-specific binding is treated as an unknown factor to be estimated (Fig. 4b). The apparent equilibrium affinity constant determined by this method was 2.0 ± 0.7 x 10^{12} M^{-1} [coefficient of variation (CV), 38%]. The estimate for binding sites per cell was 4.0 ± 0.8 x 10^5 (CV, 22%). Non-specific binding was estimated at 22% ± 11% (CV, 5%). The program fitted the data to models having 1 to 4 independent or interacting binding sites; the best fit was to a one-site model.

Virus binding to the surfaces of BW-J-M cells was visualized under the same conditions used to study radiolabelled virus binding. Fig. 5 shows virus binding to invaginated areas of the plasma membrane covered on the cytoplasmic face with a bristly coat structure. These areas are clathrin-coated pits (Helenius et al., 1980a). Virus was bound in the same relative amount in all cells, suggesting there was no significant cell-to-cell variation in binding.

A growth curve to establish that TC-83 could replicate in BW-J-M cells (m.o.i. = 1) was performed. Virus titres in the supernatant increased three log_{10} cycles in 4 days, showing that virus was replicating; however, the rate of production was much slower and the total virus yield much lower than TC-83 production in cells such as BHK-21 (C-13) or Vero. The percentage of infected cells was determined by direct immunofluorescent antibody localization (using rabbit anti-TC-83) of virus antigen on acetone-fixed spot slides prepared from the cultures. Weak
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Fig. 4. (a) Competition for binding of $^{35}$S-labelled TC-83 by unlabelled TC-83. [$^{35}$S]methionine-labelled TC-83 ($1 \times 10^9$ p.f.u., $1.8 \times 10^{-5}$ d/min/p.f.u.) was mixed with unlabelled TC-83 (0 to $1.2 \times 10^{10}$ p.f.u.). BW-J-M cells were added ($5 \times 10^6$ cells) in a final volume of 0.5 ml of PBS + 1% BSA. Cells were incubated at 0°C for 2 h and binding determined. (b) Scatchard analysis of $^{35}$S-labelled TC-83 binding to BW-J-M. BW-J-M cells ($5 \times 10^6$ in 0.5 ml PBS + 1% BSA) were incubated with increasing amounts of $^{35}$S-labelled TC-83 ($1.8 \times 10^{-5}$ d/min/p.f.u.) at 0°C for 3 h and binding determined. The data were analysed using the computer program LIGAND and plotted using the graphic system of Scatchard.

Fig. 5. Morphology of TC-83 binding to BW-J-M cells at 0°C shown by thin-section electron microscopy. Virus ($7.5 \times 10^7$ p.f.u.) was incubated with $1 \times 10^5$ cells in a total volume of 0.5 ml for 3 h at 0°C and the cells washed to remove unbound virus. Virus (arrowhead) was commonly found in close proximity to coated pits on the cell surface (arrow). Bar marker represents 0.2 μm.

fluorescent staining was seen (+1) in all inoculated cells on days 1 to 5, but not on uninoculated cells. The fluorescence intensity was, however, much fainter than that of Vero cultures infected and processed in parallel as controls. To establish conclusively that the cells were producing virus, an infectious centre assay was used. Probit analysis of these results yielded $5.5 \times 10^5$.
To characterize the nature of the TC-83 binding site on BW-J-M cells further, cell surface proteins were modified by enzymic treatments. BW-J-M cells were subjected to gentle proteolysis of their surfaces by trypsin, Pronase or proteinase K (0.01 to 10 µg/ml final concentration) and then assayed for TC-83 binding. Virus binding decreased in a dose-dependent manner following treatment with each of the three proteolytic enzymes tested (Fig. 6). Viability of the BW-J-M cells was determined at two times, immediately at the end of the proteolytic treatment and 5 h after the start of the experiment, by trypan blue vital dye exclusion (Patterson, 1979). Trypsin had little effect, but Pronase and proteinase K caused significant reduction in cell viability and some cell clumping at the highest doses used.

Trypsin, which caused no significant change in cell morphology as judged by phase-contrast microscopy or scanning microscopy, was chosen for further studies. To determine the time course for the reappearance of receptors, cells were treated at 1 or 10 µg trypsin per ml, the reaction was terminated and the cells returned to culture conditions. At the appropriate time, cells were assayed by the standard virus-binding assay. Binding decreased by 85% following trypsin treatment and returned to normal value levels within 24 h (Fig. 7). To determine whether trypsin treatment caused significant morphological changes in the cell surface, its effect on BW-
Characterization of TC-83 binding to BW-J-M cells was studied by transmission electron microscopy. No significant alterations were seen in surface morphology. In cells treated with 10 μg/ml trypsin, no virus could be seen bound to the surface of the cell, although control preparations exhibited the expected typical virus binding.

The sensitivity of the binding site to destruction by low concentrations of proteolytic enzymes was consistent with the site being an exposed surface glycoprotein (Winzler et al., 1967). If this were true, a lectin of the appropriate sugar specificity would be expected to bind to such a glycoprotein and thereby interfere with virus binding. To investigate this possibility, several lectins were screened. Maximum inhibition was observed with WGA and SBA, followed by Con A. These three lectins were studied further to determine the dose-dependence of inhibition. Cells were preincubated with each lectin at different concentrations (0 to 2 mg/ml) at 0 °C for 1 h and washed free of unbound lectin as described. Binding of 125I-labelled virus was then determined. Con A agglutinated cells at concentrations above 0.2 mg/ml and could not be used at the higher concentrations. Results are shown in Fig. 8. All three lectins inhibited virus binding; at 0.01 mg/ml, there was a 70% reduction in virus binding and with WGA binding was inhibited completely at 2 mg/ml.

**DISCUSSION**

As a first step to understanding the mechanism by which VEE virus enters cells, we have characterized the binding of the TC-83 strain of VEE to a mouse macrophage-like cell line, BW-J-M. The virus replicates more slowly in BW-J-M cells than in fibroblasts; however, all BW-J-M cells could be productively infected by being allowed to bind virus at 0 °C under the optimum conditions described here and then warmed to 37 °C to allow for virus penetration. Virus binding satisfies the criteria for specific binding in that it approaches saturation, has a physiological pH optimum and the binding of intrinsically labelled virus is reduced by unlabelled virus with a stoichiometry of 1:1. Apparent equilibrium binding parameters were determined using the computer program LIGAND (Munson & Rodbard, 1980). If non-specific binding was regarded as one of the parameters to be fitted by the program as a variable, a binding constant of 2.0 ± 0.7 × 10^{12} M^{-1} was obtained. If non-specific binding was constrained to zero and the data were fitted by the program, a value nearer to that reported for Semliki Forest virus (SFV) (Fries & Helenius, 1979) was obtained (1 × 10^{11} M^{-1}). A computer-generated Scatchard analysis yielded an estimate of 4 × 10^{5} (CV, 22%) TC-83 binding sites per BW-J-M cell. This value is comparable to the number of SFV sites (5 × 10^{4}) per BHK-21 cell. The binding data was fitted using models for 1 to 4 interacting or non-interacting sites and goodness of fit was evaluated using a statistically valid, appropriately weighted least-squares curve-fitting algorithm. The data were best accommodated by a one-site model of binding.

Although the strict applicability of a Scatchard analysis to virus binding remains to be proven, it is the most convenient tool available for these analyses and yields estimates of the number of binding sites per cell and binding affinity. It does appear that our virus binding data can be fit by the ‘m by n’ model, which is based on the mathematical theory describing any number (n) of ligands reacting with any number of classes (m) of binding sites (Feldman et al., 1972) to yield a statistically reasonable estimate of binding parameters. The ‘tight’ binding constant obtained for TC-83 and SFV (Fries & Helenius, 1979) binding to cells is not surprising given that viruses are routinely seeded at a virus to cell ratio of less than one (m.o.i. was 10^{-5} in the case of TC-83 grown in BHK-21 cells). Since it requires only one virus particle per Vero cell to initiate a productive infection, one would expect that virus binding would be of very high affinity and/or that virus internalization mechanisms would be highly efficient. The observation that unlabelled TC-83 competed for binding of 35S-labelled TC-83 on a one-to-one basis suggests strongly that: (i) labelled virus had unaltered binding affinities and (ii) the apparent binding constants obtained using labelled virus are biologically relevant. The ability of each physical particle of TC-83 to infect cells obviates a serious problem in the interpretation of many other virus-binding studies where only 1 in 10 to 1 in 100 particles is infective.

Virus binding as a function of pH showed a major pH optimum, at pH 7.4 to 7.8. Binding at pH 7.4 was rapid and essentially complete within 1 h. Binding approached saturation at pH 7.4; however, experiments were not performed at sufficiently high virus to cell ratios to achieve...
complete saturation because of the extremely large amounts of virus required. Such an experiment may in fact not be feasible due to decreased viability and cell lysis at very high multiplicities.

At the ultrastructural level, TC-83 could be seen binding to the plasma membrane of BW-J-M cells. The morphology of TC-83 binding was similar to that of SFV binding to BHK-21 cells, in that virus appeared to associate preferentially with coated pits on the plasma membrane. A major difference between the two viruses was that SFV appeared to bind to smooth portions of the microvilli prior to its association with coated pits on the plasma membrane. We found no evidence that TC-83 preferentially bound to villous or pseudopodal portions of the BW-J-M membrane.

To determine whether the binding site for TC-83 could be inactivated by proteolytic enzymes, BW-J-M cells were subjected to mild proteolysis with trypsin, Pronase, or proteinase K. Virus binding could be reduced in a dose-dependent manner to approximately 20% of control, a value equal to the estimated non-specific binding under those conditions. When trypsin-treated cells were returned to culture conditions for various times and the cells allowed to regenerate binding sites, binding activity was restored to control values within 24 h. No significant increase in binding capacity was seen; instead, binding returned to its pre-trypsin treatment values. The loss of binding following treatment of the cells with very low concentrations of trypsin (significantly less than is routinely used to subculture cells) suggested that the binding site was more exposed than bulk membrane proteins. Several cell surface glycoproteins are known to be more sensitive than bulk membrane proteins to the action of trypsin (Huggins et al., 1976). To determine whether the binding site contained key carbohydrate components, lectins were tested for their ability to inhibit virus binding. Binding could be effectively blocked in a dose-dependent manner by pre-exposure of the cells to WGA, SBA or Con A. The inhibition could be interpreted either by blockage of a carbohydrate residue on the virus-binding site or of one close enough to the binding site to cause steric interference.

The observed reduction in virus binding caused by protease or lectin treatment of the cells is consistent with the interpretation that the virus-binding site is probably a glycoprotein on the cell surface. The binding studies further suggest that TC-83 binds with high affinity to a single class of specific cell surface receptors as the first step in its infective cycle.

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