Binding of human respiratory syncytial virus to cells: implication of sulfated cell surface proteoglycans

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Binding of human respiratory syncytial virus (HRSV) to cultured cells was measured by flow cytometry. Using this assay and influenza virus as a control virus with a well-characterized receptor, a systematic search of cell surface molecules that might be implicated in HRSV binding was carried out. Treatment of cells with different enzymes or with other reagents suggested that heparin-like glycosaminoglycans (GAGs) were involved in attachment of HRSV, but not influenza virus, to host cells. This was further confirmed by a lack of binding of HRSV to CHO-K1 mutant cell lines deficient in glycosylation or GAGs biosynthesis and by an inhibition of binding after preincubation of virus with heparin and other GAGs. The degree of sulfation, more than the polysaccharide backbone of GAGs, seems to be critical for virus binding.

Introduction

Human respiratory syncytial virus (HRSV) belongs to the genus Pneumovirus, family Paramyxoviridae. This virus is the most important cause of severe lower respiratory tract infections in neonates and infants and is also an important agent of illness in immunosuppressed adults and in the elderly (Simões, 1999). The virus envelope has two major glycoproteins, G and F, which are responsible for virus attachment to the cell and for membrane fusion, respectively. While the structure of the F protein is similar to that of other paramyxoviruses, the G protein shares neither sequence nor structural features with the attachment protein (HN or H) of other viruses of the same family (Collins et al., 1996). In fact, the high serine, threonine and proline content of the HRSV G glycoprotein (Wertz et al., 1985) resembles the amino acid composition of mucins secreted by epithelial cells (Apostolopulos & McKenzie, 1994).

The G protein is a type II glycoprotein, synthesized as a polypeptide precursor of 32 kDa (Gruber & Levine, 1985), that is modified by the addition of N-linked sugars to form intermediates of 45–50 kDa. These sugar chains are converted to the complex type in the Golgi apparatus where O-linked sugars are also added to yield a mature molecule of 80–90 kDa (estimated by SDS–PAGE; Collins & Mottet, 1992; Wertz et al., 1989).

The first evidence that the G molecule is the attachment protein of HRSV was the inhibition of virus binding to cells by a polyclonal antiserum specific for the G glycoprotein (Levine et al., 1987). However, a cold-adapted attenuated HRSV mutant, lacking the G protein, is infectious and replicates in Vero cell cultures (Karron et al., 1997). This observation, together with the fact that HRSV can infect a large number of different cell types, indicates that attachment of this virus to cells might be a complex process where several cellular and/or viral components may be involved.

No specific receptor has been described for HRSV. Comparison of G protein sequences of prototype viruses of the two antigenic groups in which HRSV isolates have been subdivided identified a conserved 13 amino acid segment in the middle of the G protein ectodomain (aa 164–176). This conserved segment, which is slightly hydrophobic, was proposed as a putative receptor-binding site (Johnson et al., 1987). The structure of a peptide that included the conserved segment of G was determined by NMR spectroscopy (Doreleijers et al., 1996). This structure revealed a hydrophobic pocket with suitable dimensions to bind a small ligand or amino acid side chain.

It has been shown recently that preincubation of virus with heparin inhibits infection of cultured cells and that the G protein binds to heparin (Krusat & Streckert, 1997). These results suggested that heparin or other cell surface glycosaminoglycans (GAGs) might be involved in HRSV binding to cells through the G molecule. The heparin-binding domain (aa 184–198 in group A; aa 183–197 in group B) was mapped...
outside the conserved segment of the G ectodomain (Feldman et al., 1999). However, there has been no formal demonstration that HRSV binds to GAGs present in the surface of target cells and there is also some controversy over the mechanism by which heparin inhibits HRSV infectivity. Thus, Bourgeois et al. (1998) reported that treatment of virus preparations, but not the cells, with heparinase inhibits virus infectivity.

Certain viruses, whose infectivity is inhibited after pre-incubation with heparin, bind with low affinity to cell surface GAGs, but in addition, they use other high affinity cell surface receptors for entry into cells (Dalgleish et al., 1984; Klatzmanmann et al., 1984; Montgomery et al., 1996; Wang et al., 1992). In order to confirm the implication of proteoglycans in HRSV attachment to cells and to look for alternative receptors, we have developed a flow cytometric assay in which the effect of different enzymes or inhibitors upon virus binding can be tested. We have also used cells deficient in glycosylation or proteoglycan biosynthesis in this assay. A parallel study was carried out with influenza virus as a control virus with a well-characterized receptor. The results obtained indicate that cell surface GAGs are essential for binding of HRSV to certain cell types.

Methods

■ Cells and viruses. All cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% foetal calf serum (DMEM10), unless specified. HEP-2 cells were infected with the Long strain of HRSV in DMEM2.5. When cytopathic effect was maximal, 48 h later, cells were resuspended in the medium, vortexed and kept frozen in liquid nitrogen at low speed and stored at −80 °C until required.

Wild-type Chinese hamster ovary cells (CHO-K1), UDP–galactose/UDP–N-acetylgalactosamine 4-epimerase-deficient mutant lldD (Kingsley et al., 1986) and GAG-deficient CHO derivatives pgsA–745 (Esko et al., 1985) and psgD–677 (Lidholt et al., 1992) were obtained from the ATCC. They were grown in DMEM:Ham’s F12 (1:1) medium supplemented with 10% foetal calf serum.

■ Chemicals, enzymes and antibodies. Since the source of polysaccharides, enzymes and antibodies used in this study might be important for reproducing the results, manufacturers of these reagents are listed here. The following were obtained from Sigma: heparin (catalogue no. H3149), N-acetyltetrapyrin (A8036), N-acetyl-de-O-sulfated heparin (A6039), de-N-sulfated heparin (D4776), heparan sulfate (H7640), chondroitin sulfate A (C8529), chondroitin sulfate B (C3788), chondroitin sulfate C (C4384), heparanase I (H2519), heparanase III (heparitinase I; H8891), chondroitinase ABC (C3667), neuraminidase (from Clostridium perfringens), trypsin (TPCK-treated; T8642), D(+)-galactose (G5388) and N-acetyl-D-galactosamine (A2795). Tunicamycin was obtained from Hoechst, monensin from Boehringer Mannheim, anti-mouse Ig biotinylated species-specific whole antibody from Amersham and Streptavidin-R–phycoerythrin (streptavidin-RPE) from Southern Biotechnology Associates. Monoclonal antibodies specific for HRSV and influenza virus have been described (García-Barreno et al., 1989; López et al., 1986; Martínez et al., 1997).

■ Flow cytometric analysis of virus binding to cells. Cells growing in plastic Petri dishes were detached from the substrate with 1 mM EDTA in Ca2+- and Mg2+-free PBS. After washing with PBS, 3 × 10⁶ cells were incubated with 1–2 ml (≈1–2 × 10⁷ p.f.u.) of either clarified virus supernatant (6000 g for 5 min) or purified Long virus (as indicated in figure legends) and, successively, with mouse primary antibody, biotinylated anti-mouse Ig and streptavidin-RPE. Finally, mouse primary cells were fixed with 1% paraformaldehyde in PBS. The fluorescence of 10⁴ cells was determined in a Becton Dickinson FASCalibur cytomter with CellQuest software. All incubations were carried out at 4 °C for 30 min. PBS was used for washes carried out between steps and for dilutions of virus, antibodies and streptavidin-RPE. In some experiments, detached cells and/or virus were pretreated with reagents, as indicated in the figure legends, prior to binding of virus to cells and processing for flow cytometry.

■ Microneutralization of HRSV. The ELISA-based neutralization test developed by Anderson et al. (1988) was adapted, as described (Martínez & Melero, 1998), to assess HRSV titre reduction after preincubation of virus or cells with enzymes or other reagents.

Results

Dose-dependent binding of HRSV to HEP-2 cells

Serial dilutions of Long virus were incubated with HEP-2 cells for 30 min at 4 °C. After washing, the amount of virus bound to cells was estimated by flow cytometry using a monoclonal anti-G antibody (Fig. 1A). Background fluorescence was determined by incubating cells under the same conditions without virus. Mean fluorescence values, after subtraction of background, are represented in Fig. 1(B). A linear relationship between the amount of virus added to the assay and fluorescence intensity was observed. Saturation levels were almost reached with the lowest virus dilutions. Binding of HRSV to cells was inhibited by preincubating the virus with a pool of anti-G monoclonal antibodies (Martínez & Melero, 1998) that have been shown previously to inhibit virus infectivity (data not shown).

Effect of different treatments of HEP-2 cells upon HRSV binding

To gain information about the nature of the surface molecule(s) of HEP-2 cells required for virus binding, these cells were subjected to different treatments prior to incubation with the virus (Table 1). As it is well documented that sialic acid is the influenza virus receptor (Bachi et al., 1997), we included this virus in most experiments as a virus–cell binding control.

When HEP-2 cells were treated with 60 μM/ml neuraminidase, binding of HRSV was essentially unaffected or increased slightly. Although it cannot be excluded that a small proportion of sialic acid remained at the cell surface after neuraminidase treatment, it is important to note that the binding of influenza virus, which uses sialic acid as the receptor,
HRSV binding to cell surface proteoglycans

Fig. 1. Dose response of HRSV binding to HEp-2 cells measured by flow cytometry. (A) Fivefold dilutions of virus (Long strain) were mixed with $8 \times 10^5$ HEp-2 cells for 30 min at 4 °C and the virus bound to cells was detected by flow cytometry using a monoclonal anti-G antibody (021/19G; Martínez et al., 1997). (B) The mean fluorescence of the distributions shown in (A), after subtraction of background (no virus added), is represented. Data from an experiment representative of three are shown.

was reduced to almost background levels under the same conditions. This indicates that sialic acid is not essential for binding of HRSV to HEp-2 cells.

Table 1. Binding of HRSV to HEp-2 cells treated with different agents

<table>
<thead>
<tr>
<th>Virus</th>
<th>Neuraminidase (60 mU/ml)</th>
<th>Trypsin (0.5 μg/ml)</th>
<th>Tunicamycin (10 μg/ml)</th>
<th>Monensin (10 μM)</th>
<th>EDTA (100 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRSV</td>
<td>162</td>
<td>7</td>
<td>97</td>
<td>3</td>
<td>101</td>
</tr>
<tr>
<td>Influenza</td>
<td>5</td>
<td>87</td>
<td>106</td>
<td>100</td>
<td>ND</td>
</tr>
</tbody>
</table>

Trypsin digestion of HEp-2 cells reduced binding of Long virus to almost background levels. It should be stressed that the dose of trypsin used in the experiment described in Table 1 did not affect the viability of cells, as assessed by trypan blue exclusion (data not shown). However, HRSV infectivity was reduced more than 100-fold after trypsin treatment of HEp-2 cells under the conditions indicated in Table 1 (data not shown). In contrast, attachment of influenza A/Vic/3/75 virus was almost unaffected, indicating that non-proteinaceous molecules, probably sialic acid-containing glycolipids, could provide the binding sites for this virus under those conditions.

Since reduction of HRSV binding to HEp-2 cells by trypsin could be due to the digestion of cell surface glycoproteins or proteoglycans, we tested the effect of two different protein glycosylation inhibitors (tunicamycin and monensin) upon binding of HRSV to HEp-2 cells (Table 1). Tunicamycin blocks the formation of protein $N$-glycosidic linkages by inhibiting the transfer of $N$-acetylglucosamine 1-phosphate to dolichol monophosphate. Monensin has a more general effect on protein glycosylation and vesicle trafficking as it disrupts the Golgi apparatus, where trimming and modification of $N$-linked carbohydrates occurs and $O$-linked carbohydrates are added to serine or threonine residues.

HEp-2 cells were detached from plates with trypsin–EDTA to remove pre-existing glycoproteins and proteoglycans. Cells were then replated in the presence of either tunicamycin or monensin at inhibitory glycosylation concentrations (Martínez et al., 1997). Tunicamycin did not influence the binding of HRSV to cells. In contrast, preincubation of HEp-2 cells with monensin reduced HRSV binding almost to background levels. Binding of influenza virus to HEp-2 was unaffected by pretreatment of the cells with either drug. Although the effect of monensin has to be interpreted with caution because of its multiple effects on the biology of the cell, the results in Table 1 are compatible with cell surface components containing $O$-linked, but not $N$-linked, sugar chains being required for HRSV binding. Presumably, in monensin-treated cells, as in the case
of trypsin treatment, influenza virus was still binding to sialic acid of other cell surface molecules, probably gangliosides.

Finally, the binding of HRSV to HEP-2 cells seems independent of divalent cations, since it is not inhibited in the presence of 100 mM EDTA (Table 1), a concentration 100-fold above that utilized to detach cells from plastic Petri dishes and capable of disrupting intercellular unions.

**HRSV binding to cells deficient in either protein glycosylation or biosynthesis of GAGs**

The relevance of cell surface oligosaccharides for virus binding was also studied in a cell line (IdlD) that is deficient in protein glycosylation. IdlD cells are UDP–galactose/UDP-N-acetylgalactosamine 4-epimerase-deficient and are unable to synthesize UDP–galactose and UDP-N-acetylgalactosamine under culture conditions where glucose is the only external monosaccharide source (Kingsley et al., 1986). HRSV and influenza virus binding to IdlD cells was clearly inhibited under conditions in which a general inhibition of oligosaccharide biosynthesis, including glycolipids, occurred (Fig. 2). However, addition of galactose and N-acetylgalactosamine to IdlD cells, which restores glycosylation, restored significant levels of HRSV binding to these cells. In the case of influenza virus, addition of galactose and N-acetylgalactosamine to IdlD cells even stimulated threefold virus binding compared with the parental CHO-K1 cells. These results also indicated that cell surface glycoconjugates were needed for HRSV binding to cells.

There are three major forms of carbohydrates in higher-animal cell surface glycopeptides: N-acetylgalactosamine-N-asparagine-linked and N-acetylgalactosamine-O-serine/threonine-linked oligosaccharides bound to glycoproteins and xylose-O-serine-linked GAGs bound to proteoglycans. The results of tunicamycin treatment shown in Table 1 suggested that N-linked oligosaccharide chains were not essential for HRSV binding to HEP-2 cells. Proteoglycans are abundant components of the cell surface, which would be affected by trypsin treatment and by inhibition of O-glycosylation. Thus, we tested whether HRSV and influenza virus binding to mutant CHO-K1 cells deficient in GAGs biosynthesis was altered. As seen in Fig. 2, influenza virus binding was similar to that seen in CHO-K1 cells in pgsA-745 and pgsD-677 cells, whereas the binding of HRSV was considerably reduced in the latter two cell lines. pgsA-745 cells have a defect in xylooligosaccharide biosynthesis and do not produce GAGs before being added to cells. As shown in Fig. 3(A), purified virus was incubated with various forms of desulfated heparin and did not produce GAGs (Esko et al., 1985). pgsD-677 cells produce chondroitin sulfate A (CS-A) but not heparan sulfate (HS) because they lack both N-acetylgalactosaminyltransferase and glucuronosyltransferase activities, which are required for synthesis of HS (Lidholt et al., 1992). Together, the results with CHO-K1 cell mutants indicate that GAGs similar to HS are involved in binding of HRSV.

**GAGs are involved in binding of HRSV to HEP-2 cells**

To confirm the implication of GAGs on binding HRSV to HEP-2 cells, which are commonly used for growing the virus in tissue culture, purified HRSV was preincubated with different GAGs before being added to cells. As shown in Fig. 3(A), heparin was the most efficient at inhibiting binding, followed by chondroitin sulfate B (CS-B). Neither chondroitin sulfate C (CS-C) nor HS blocked the binding. CS-A had some effect only at the highest concentration, in agreement with the reduced...
binding of HRSV to pgsD-677 cells that produce CS-A but not CS-B. The inhibitory effect of heparin was abolished when desulfated forms of this molecule were utilized (Fig. 3B), suggesting that sulfation is important for HRSV attachment.

Heparin is the most highly sulfated GAG. Commercial preparations of heparin contain, on average, 2-4 sulfate groups per disaccharide. CS-B also contains more sulfate than other forms of CS. The superior inhibitory capacity of heparin and CS-B may also be due to the presence of \( \alpha \)-iduronic acid (IdUA) units, which are believed to confer conformational flexibility to the polymers (Kjellén & Lindahl, 1991). Both heparin and CS-B contain normally sulfated IdUA, while CS-A and CS-C contain non-sulfated \( \beta \)-glucoronic acid (GlCUA). Heparin and HS have similar structure, but HS has less IdUA and sulfates than heparin (Rostand & Esko, 1997).

When HEp-2 cells, instead of the virus, were preincubated with the same concentrations of GAGs as those shown in Fig. 3, only heparin had a marginal inhibitory effect at the highest concentration of 200 µg/ml (data not shown). Thus, the inhibition of HRSV binding to cells is mediated by interaction of GAGs with viral components (presumably G glycoprotein) and not by masking a potential viral receptor in the cell surface.

As an alternative way of testing the relevance of cell surface GAGs for HRSV attachment, HEp-2 cells were treated with heparinase I and heparinase III. Both enzymes inhibited binding of HRSV, but not binding of influenza virus. In contrast, binding of neither virus was affected by chondroitinase ABC treatment (Fig. 4), confirming the fact that heparin-like GAGs, but not CS, are involved in HRV binding to cells. The higher inhibitory effect of heparinase I treatment may be related to the specificity of this enzyme for di-sulfated or tri-sulfated disaccharides that contain sulfated IdUA, while heparinase III (heparitinase) degrades defined oligosaccharides at hexosaminidic linkages with GlCUA (Linhardt et al., 1990). Both structures (IdUA and GlCUA) are present in heparin and HS, but the former is more common in heparin and the latter in HS. Incubation of virus with heparin or treatment of HEp-2 cells with heparinase I inhibited HRSV infectivity in a micro-neutralization test, highlighting the relevance of the virus–GAG interaction for productive infection (data not shown).

**Discussion**

To investigate the nature of viral and cellular components involved in the initial binding of HRSV to the cell surface, we have developed a quantitative binding assay in which cells and virus are incubated at 4 °C. Bound virus is then detected by flow cytometry of cells labelled with anti-HRSV-specific antibodies.

Since the nature of the HRSV receptor is still unclear, we decided to explore a number of possibilities that could yield information about the nature of the molecules implicated in HRSV binding to cells. Inclusion of influenza virus as a control virus, for which a well-characterized receptor is known, validated the specific effects of different treatments upon HRSV binding to cells.

Several paramyxovirus and influenza viruses bind to cell surface sialic acid through HN or H (Lamb & Kolakofsky, 1996). Although HRSV G protein has neither haemagglutination nor neuraminidase activities there was no formal demonstration that sialic acid is not required for HRSV binding to cells. In addition, the size of the hydrophobic pocket identified in the structure of the conserved segment of the G ectodomain (Doreleijers et al., 1996) was sufficient to accommodate a small receptor, such as sialic acid. However, the results of HEp-2 cells treated with neuraminidase suggest that sialic acid is not required for HRSV binding.

It has been speculated that, owing to the highly glycosylated nature of the G protein, cell surface lectins could serve as HRSV receptors. In fact, the G protein is recognized by several plant lectins (García-Beato et al., 1996) and lung surfactant protein A (SP-A). SP-A is a mammalian C-type lectin that enhances HRSV clearance in vivo, presumably by cross-linking the virus to specific SP-A cell-surface receptors on alveolar macrophages (LeVine et al., 1999). The interaction of C-type lectin with ligands, as with many other intermolecular interactions, is Ca\(^{2+}\)-dependent. We have not found evidence for such interaction, as binding of HRSV to cells was not inhibited in the presence of 100 mM EDTA.

Our results, however, indicate that cell surface proteoglycans are important for HRSV binding. Trypsin digestion of cells, preincubation of virus with certain GAGs or enzymatic removal of GAGs from the cell surface inhibited binding of HRSV to HEp-2 cells. In addition, binding of virus to CHO cells deficient in GAG biosynthesis was greatly impaired. These results are in agreement with those of Krusat & Streckert (1997), who reported that preincubation of virus with heparin, or enzymatic digestion of cell surface GAGs, inhibited virus infectivity. However, our results do not agree with those of
Bourgeois et al. (1998), who reported that pretreatment of cells with heparinase did not affect virus infectivity. Interaction of HRSV with cell surface proteoglycans is not restricted to the Long strain. Thus, incubation of other virus isolates with heparin inhibited both virus binding to cells and virus infectivity, as in the case of Long virus (data not shown).

Heparin-like GAGs mediate attachment of many viruses, including herpesvirus (Lycke et al., 1991; WuDunn & Spear, 1989), Sindbis virus (Byrnes & Griffin, 1998), human immunodeficiency viruses (HIV; Mondor et al., 1998; Patel et al., 1993), Dengue virus (Chen et al., 1997) and foot-and-mouth disease virus (Jackson et al., 1996). Protein binding to GAGs is mediated largely by electrostatic interactions between positively charged arginine and lysine side chains and negatively charged sulfates (Gromm et al., 1995). A large variety of polyanionic substances (polysulfates, polysulfonates and polyoxometalates) are known to interfere with the binding of a number of enveloped viruses to cells (De Clercq, 1996). It is assumed that these polyanionic substances neutralize positively charged amino acid residues of the viral envelope glycoproteins and thus prevent their interaction with the cell surface. We found that the degree of sulfation, more than the polysaccharide backbone of GAGs, is critical for inhibition of HRSV binding to cells.

GAGs exhibit tremendous structural heterogeneity in the length of the sugar chains, in the extent and pattern of sulfation and in the degree of epimerization of GlcUA to IdUA. In fact, a preparation of GAGs consists of hundreds of thousands of different molecular species, or glycosoars (Kjellén & Lindahl, 1991). The fact that heparin inhibited binding of HRSV in our assay more than HS may reflect the specific origin of the commercial preparations of both substances (commercial preparations of heparin are more sulfated than those of HS). Nevertheless, under physiological conditions in vivo, highly sulfated forms of HS, rather than heparin (which is generally confined to the interior of mast cells), are likely to provide the binding sites for HRSV. HS is abundant in the lung, the site of HRSV replication, where it contributes to the organ structure and physiology by attracting large amounts of water (Kjellén & Lindahl, 1991; Rostand & Esko, 1997).

It has been shown that HRSV G protein binds specifically to heparin (Krusat & Streckert, 1997) and a heparin-binding domain has been identified in the G protein primary structure (Feldman et al., 1999). Thus, it is tempting to postulate that the G protein mediates HRSV binding to cellular HS. Inhibition of virus binding by a pool of anti-G antibodies further supports this conclusion (data not shown), although it is still unknown whether the inhibitory effect of the antibodies is related to inhibition of HRSV binding to HS. Nevertheless, alternative routes for HRSV attachment and entry into cells may operate in different cell types. As mentioned in the introduction, a HRSV mutant that lacks glycoprotein G can replicate efficiently in Vero cells (Karron et al., 1997), although its growth is greatly impaired in vivo and in other cell lines. Alternative routes for virus entry have been described for other viruses. For instance, Sendai virus normally binds to sialic acid through HN, although Sendai virus lacking HN can still infect cells that express the asialglycoprotein receptor on their surface by interacting directly with the F protein (Leyrer et al., 1998).

Viruses that bind to GAGs normally use other cell surface components as high-affinity receptors. For instance, CD4 is the primary receptor for HIV-1 (Dalgleish et al., 1984; Klatzmann et al., 1984), entry of herpes simplex virus type 1 into cells is mediated by a member of the TNF/NGF receptor family (Montgomery et al., 1996) and the 67 kDa high-affinity laminin receptor has been identified as the Sindbis virus receptor in some mammalian cells (Wang et al., 1992). Nonetheless, HIV-1, herpes simplex virus and Sindbis virus bind to cell surface GAGs before interacting with their high-affinity receptors. The conserved G protein segment (aa 164–176) postulated originally as the receptor binding site (Johnson et al., 1987) lies near the heparin binding site (aa 184–198 in group A; aa 183–197 in group B) in the primary structure of the G molecule (Feldman et al., 1999). It is therefore possible that the interaction of HRSV with cell surface GAGs may promote attachment of G to an as yet unidentified receptor through the conserved central region. The virus-binding assay described here may be a useful test for evaluating a such hypothesis.

Note added in proof. Hallak et al. (Virology 271, 264–275, 2000) reported recently that GAGs are required for efficient infection of HEp-2 and CHO cell lines by a recombinant HRSV (A2 strain) that expresses the green fluorescent protein. Their results, which are likely to reflect virus entry into cells, are in good agreement with our results obtained by the direct virus-binding assay. In both studies, heparin-like GAGs and to a lesser extent CS-B at the cell surface, but not in the virion, are implicated in virus binding and penetration into cells.

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References


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