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Author(s): Makkonen, K.-E.; Turkki, Paula; Laakkonen, J. P.; Ylä-Herttuala, S.; Marjomäki, Varpu; Airene, K. J.

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Reply to “Heparan Sulfate in Baculovirus Binding and Entry of Mammalian Cells”

K.-E. Makkonen,^a P. Turkki,^b J. P. Laakkonen,^a S. Ylä-Herttuala,^{a,c,d} V. Marjomäki,^b K. J. Airene^a

A. I. Virtanen Institute, Department of Biotechnology and Molecular Medicine, University of Eastern Finland, Kuopio, Finland^a; Department of Biological and Environmental Science, Division of Cell and Molecular Biology/Nanoscience Center, University of Jyväskylä, Jyväskylä, Finland^b; Gene Therapy Unit, Kuopio University Hospital, Kuopio, Finland^c; Research Unit, Kuopio University Hospital, Kuopio, Finland^d

In our article “6-O- and N-Sulfated Syndecan-1 Promotes Baculovirus Binding and Entry into Mammalian Cells” by Makkonen et al. in *Journal of Virology* (1), we investigated the interaction of baculovirus and mammalian cell surface heparan sulfate proteoglycans (HSPG). The data show that baculovirus requires HSPG sulfation, particularly N- and 6-O-sulfation, to bind and transduce mammalian cells. We also show that baculovirus associates specifically with syndecan-1 (SDC-1) but not with other syndecans or glypicans.

As discussed in the article, HS has previously been shown to be involved in glycoprotein 64 (gp64)-mediated baculovirus binding onto mammalian cells. Heparin and heparinase I and II treatment of cells have also been shown to prevent the virus binding (2, 3). The role of HS in baculovirus entry was further studied in our article (1). Binding and baculovirus-mediated gene delivery were seen to decrease after NaClO₃ treatment, an inhibitor of sulfation. Sulfation studies also indicated that N-sulfation and 6-O-sulfation of HSPGs are important for the virus binding. Thus, it can be concluded that several studies including ours indeed support the role of HS in baculovirus interaction with mammalian cells. Since there are several HS-bearing proteins at the cell surface, we wanted to investigate further whether the virus prefers HS chains that are linked to a specific core protein. Core protein defines the type, size, amount, and orientation of the HS chains. Additionally, the core protein locates the HS chains to a specific location on the cell membrane, thus enabling, for example, the interaction with possible coreceptors. Thus, the optimal HS chain composition for virus binding can be found from specific core protein(s). The role of the core protein is equally important during virus internalization, since receptor signaling defines the entry route taken.

Decreased virus entry seen by Dr. M. Nasimuzzaman in hamster pgsD-677 cells with low or severely impaired HS expression (4) supports our data well. However, even high HSPG expression/sulfation does not guarantee efficient entry of baculovirus into cells if the virus entry and intracellular trafficking are impaired in nonpermissive cells. As stated in our article, although some cells (e.g., EA.hy926) have high HSPG/SDC-1 expression on their cell surface, the transduction rate/entry of baculovirus can be very low. This dilemma has been studied further in another one of our articles (5), in which baculovirus entry is shown to be arrested at the cell surface where the virus forms aggregates with SDC-1. Thus, the amount of HS is not necessarily directly proportional to efficient virus entry and other cellular factors such as protein kinase C phosphorylation and vimentin organization contribute to the cell susceptibility to baculovirus transduction (5).

Antibody inhibition studies were performed in our article with a polyclonal rabbit SDC-1 antibody (sc-5632; Santa Cruz Biotechnology, CA). Although the antibody is against SDC-1 ectodomain

and not HS, it was able to decrease baculovirus binding and transduction in a dose-dependent manner, in contrast to the control antibody. A similar type of protocol with the same or similar antibody (rabbit polyclonal syndecan-1; Santa Cruz Biotechnology) was reported to block herpes simplex virus 1 (HSV-1) entry into HeLa cells in a dose-dependent manner (6). This decrease can be a result of several reasons. (i) The antibody can at least partially mask the HS side chains and thus prevent the virus binding. (ii) Baculovirus is a large rod-shaped virus which most probably requires room and exact positioning to bind to SDC-1 HS chains on mammalian cells. Therefore, steric hindrance caused by the antibody binding to the SDC-1 ectodomain may well inhibit virus binding. (iii) As discussed also in the article, binding of a ligand to syndecan causes its clustering and subsequent internalization of the receptor and its cargo (7, 8). Thus, the antibody may affect baculovirus binding and entry by interfering with the clustering and/or possible conformational change of SDC-1 in response to baculovirus binding. Antibody binding may also have further consequences on syndecan-1 signaling or interaction with other molecules.

Inhibition/competition experiments, performed with increasing concentrations of recombinant SDC-1 (ab83609; Abcam, Cambridge, MA), resulted in decreased baculovirus entry and transduction efficiency in a dose-dependent manner. Although the recombinant SDC-1 protein is not glycosylated, it can still interfere with baculovirus transduction. This can happen either by SDC-1 directly binding to baculovirus and thus competing with the cell surface SDC-1 or by interfering with essential intra- and intermolecular syndecan interactions. The latter is supported by the fact that for example the SDC-1-mediated regulation of $\alpha\beta 5$ -integrin activity in cell attachment and spreading does not require the HS-glycosaminoglycan (GAG) chains of SDC-1 (9). Direct binding between nonglycosylated SDC-1 and baculovirus remains to be studied. As discussed in our article, involvement of other significant cellular factors interacting or participating in the virus entry cannot be excluded. The recombinant protein was added to cells 1 h before the addition of the virus. This offers sufficient time for interaction with cell surface molecules to take place. The exact mechanism behind this inhibition remains to be studied.

Viral uptake and cellular trafficking are complex processes,

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Address correspondence to K. J. Airene, Kari.Airene@uef.fi.

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known to require the involvement of several molecules. Although the number and size of the GAG chains can vary in a tissue- and cell type-specific manner (10), we detected colocalization of baculovirus with SDC-1 in many cells of human origin. However, we do agree that it is probable, even likely, that other molecules may also play important roles in baculovirus-mediated gene delivery.

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