Mannosyl Glycodendritic Structure Inhibits DC-SIGN-Mediated Ebola Virus Infection in *cis* and in *trans*

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We have designed a glycodendritic structure, BH30sucMan, that blocks the interaction between dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) and Ebola virus (EBOV) envelope. BH30sucMan inhibits DC-SIGN-mediated EBOV infection at nanomolar concentrations. BH30sucMan may counteract important steps of the infective process of EBOV and, potentially, of microorganisms shown to exploit DC-SIGN for cell entry and infection.

Ebola virus (EBOV) causes hemorrhagic fever and is considered to be one of the most lethal pathogens known. The risk of a widespread epidemic episode, or even its potential use in bioterrorism, has stimulated research on the agent and the development of vaccine programs (18). Due to its extraordinary virulence, EBOV is classified as a biosafety level 4 pathogen; however, the generation of pseudotyped recombinant retroviral particles has recently allowed the exploration of important aspects of EBOV biology using biosafety level 2 facilities (21). Despite these efforts, there is no specific cure for EBOV infection.

Our group and others (1, 17) have previously demonstrated that EBOV binds to C-type lectins dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) and liver/lymph node SIGN (L-SIGN) and utilizes these molecules as mediators to gain access to certain cell types. Furthermore, DC-SIGN and L-SIGN can act as *trans*-receptors to efficiently capture infective particles and transmit the infection to susceptible cells (1). DC-SIGN, originally cloned as a human immunodeficiency virus (HIV) gp120-binding protein (7), is expressed exclusively on immature dendritic cells and confers upon these cells the ability to facilitate, in *trans*, HIV infection of activated CD4$^+$ T cells (8). L-SIGN is a closely related molecule expressed on the surface of endothelial cells in the liver, lymph node sinuses, and placental villi (3). DC-SIGN and L-SIGN bind ligands containing oligomannose glycans through the C-terminal carbohydrate recognition domain. It is highly likely that EBOV, in a manner similar to that of HIV, subverts the physiological role of these C-type lectins to achieve important steps in its infectious process, these molecules being critical for the initial steps of viral infection at the mucosal level and subsequent dissemination throughout the body. Therefore, DC- and L-SIGN can be considered potential

new targets for the design of compounds acting as viral entry inhibitors. The low affinity of carbohydrate interactions, compensated for in nature by the multivalent presentation of carbohydrate units, has encouraged the design of carbohydrate multivalent structures to interfere with biological processes in which carbohydrates are involved. Among these multivalent systems, glycodendrimers are one of the most popular systems (4), and they have been used previously in several processes, including blockage of viral receptors (13, 15). Previous works with glycodendritic structures, based on the hyperbranched polymer BoltornH30 (Perstorp Specialty Chemicals), have shown a number of advantages, including low cytotoxicity, high solubility in physiological media, and relatively low cost (2).

In order to inhibit EBOV DC-SIGN interaction, we have designed a glycodendritic structure based on BoltornH30 (Fig. 1), presenting 32 mannose units linked through a succinyl spacer, BH30sucMan. We tested this compound in an experimental model using an EBOV-GP-pseudotyped lentivirus (1). Pseudotyped viral vectors have been successfully used in a wide variety of experimental applications (16), including characterization of the receptors used by EBOV (1, 5, 6, 17, 19, 21).

The lentiviral vector pNL4-3.Luc.R-E-, obtained from Nathaniel Landau (11) (through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health), was used for production of vesicular stomatitis virus G and EBOV Zaire GP pseudotypes (1) in a transient transfection protocol using 293T cells as previously described (20). The expression plasmid for the GP of the Zaire strain of EBOV (i.e., EBOV-GP) was kindly provided by A. Sanchez (Centers for Disease Control and Prevention) (21). BH30sucMan was prepared as described elsewhere (2). Jurkat cells stably transduced with DC-SIGN were infected with EBOV-GP-pseudotyped lentiviral vectors expressing luciferase using a multiplicity of infection of 0.1. A range of concentrations of BH30sucMan was added simultaneously along with the control compound BH30sucL (without mannoses) (Fig. 1). After 48 h, cells were lysed and assayed for luciferase expression. The 50% inhibitory concentrations (IC_{50}) were calculated with Graphpad Prism software version 3.0. The inhibitory activity of BH30sucMan for the in *trans* function of DC-SIGN was studied on K562 erythroleukemia cells stably expressing DC-SIGN

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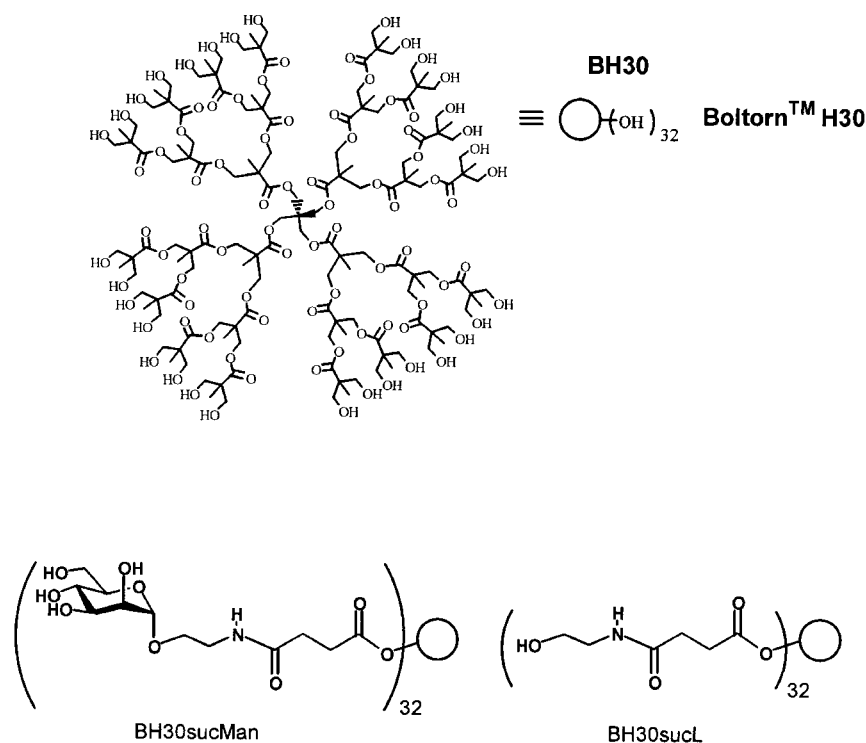
FIG. 1. Structure of BoltornH30 (BH30) (top) and schematic representation of BH30sucMan and BH30sucL (bottom).

(14). Cells were incubated by rotation for 60 min at room temperature with supernatants containing Ebola Zaire-pseudotyped retroviruses in the presence or absence of BH30sucMan (500 nM) and controls. After this step, cells were extensively washed with phosphate-buffered saline–1 mM $CaCl_2$–0.5% bovine serum albumin, resuspended in fresh medium, and plated onto HeLa cell monolayers. After 48 h, K562 cells were removed, the monolayer of HeLa cells was washed twice, and luciferase activity was measured.

Results of our experiments showed that BH30sucMan was able to selectively inhibit DC-SIGN-mediated EBOV infection in an efficient dose-dependent manner (IC_{50}, 337 nM), whereas

it did not affect infection mediated by a DC-SIGN-indepen-dent viral envelope such as vesicular stomatitis virus G (1). Infection was also inhibited by the monosaccharide α-methyl-D-mannopyranoside in a dose-dependent manner with an IC_{50} of 1.27 mM (data not shown). The control structure, BH30sucL, which does not present sugar units, only showed a limited effect at concentrations beyond 10^{-5} M, which is most likely explained by nonspecific interactions with receptors at the cell surface (Fig. 2). Similar results were obtained in parallel experiments by using L-SIGN-expressing cells (data not shown). As a further proof of the specific action of the glycodendritic structure, BH30sucMan did not show any inhibitory effect in
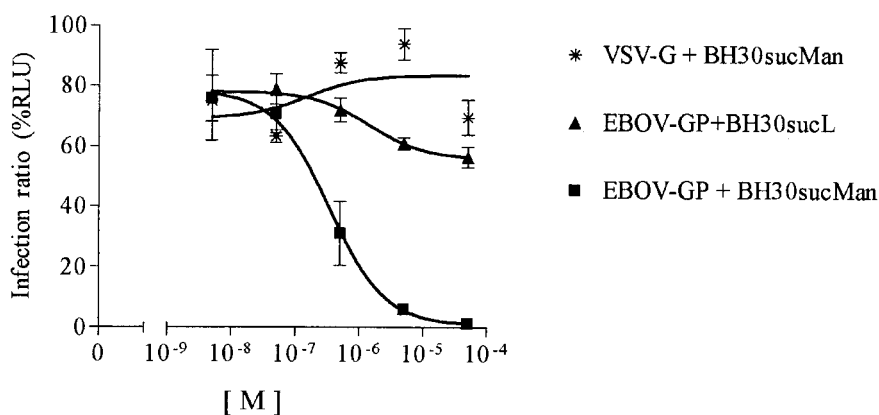

FIG. 2. Inhibition of DC-SIGN-mediated EBOV-GP pseudotyped viruses in *cis*, using Jurkat cells expressing DC-SIGN and BH30sucMan or BH30sucL. Results are shown as the proportion of inhibition in comparison to control (no compound), and the values correspond to the means of three experiments ± standard error of the mean.
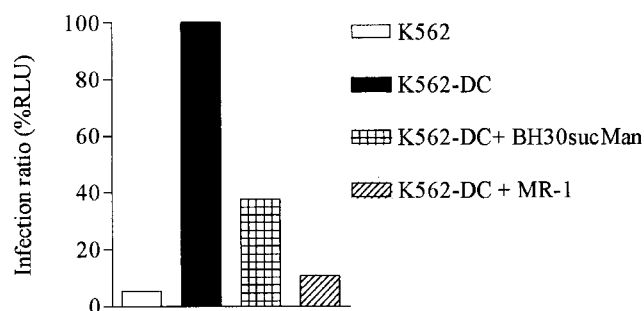
FIG. 3. Inhibition of infection of EBOV-GP pseudotyped viruses mediated in *trans* by DC-SIGN. K562 cells (negative control) and K562-expressing DC-SIGN (K562-DC) were used to bind and transfer viral particles to HeLa cells in the presence or absence of BH30sucMan and the anti-DC-SIGN monoclonal antibody MR-1 (14). One representative experiment out of three is shown.

infection experiments using DC-SIGN-negative cell lines, such as HeLa, which are known to be susceptible to EBOV infection (data not shown). In the experiments in *trans*, in which a more complex series of events such as internalization and presentation of the viral particle to susceptible cells can take place (12), BH30sucMan also showed a significant reduction of DC-SIGN-mediated infection in *trans* at levels comparable to the inhibition shown in *cis* (Fig. 3).

We have shown that BH30sucMan is a potent inhibitor of EBOV infection mediated by DC-SIGN both in *cis* (Fig. 2) and in *trans* (Fig. 3), presumably due to the same mechanisms of inhibiting the interaction between the lectin and the viral envelope. A carbohydrate-dependent inhibitory effect has been demonstrated in these experiments; also, a multivalent effect of two orders of magnitude is shown, since monovalent mannose was able to inhibit this interaction but at millimolar concentrations.

Blocking EBOV interaction with lectin receptors in the mucosal and endothelial territories is a reasonable goal and also a model for other microorganisms such as HIV (8), cytomegalovirus (10), or *Mycobacterium tuberculosis* (9), any of which could potentially exploit a similar mechanism. Additionally, the use of these glycodendritic structures will allow us to better understand the molecular basis of this interaction as well as the design of more potent and selective inhibitors.

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