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Multivalent Manno-Glyconanoparticles Inhibit DC-SIGN-Mediated HIV-1 Trans-Infection of Human T Cells

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Viral entry is a critical step in human immunodeficiency virus (HIV) infection that takes place through a series of sequential interactions between the envelope glycoprotein gp120, cellular receptor CD4, and coreceptors CCR5 or CXCR4 on T cells. Through a mechanism named trans-infection, dendritic cells (DCs) efficiently transfer the virus to T lymphocytes where viral replication occurs.[1] Interference in HIV transmission in the DC–lymphocyte synapse is a preferential but complex target in the development of new compounds displaying anti-HIV activity. HIV–DC interaction is mediated by the glycans of gp120 and the C-type lectin DC-SIGN (dendritic cell-specific ICAM 3-grabbing nonintegrin) expressed on DCs.[2] DC-SIGN is tetrameric and specifically recognizes N-linked high-mannose oligosaccharides ($Man_9GlcNAc_2$) through multivalent and Ca^{II}-dependent protein–carbohydrate interactions.[3] Mimicking the cluster presentation of the oligomannosides on the surface of the virus is a strategy for designing carbohydrate-based antiviral agents.[4] Mannose multivalent systems based on proteins,[5] peptides,[6] liposomes,[7] and dendrimers[8] as scaffolds have recently been prepared for targeting DCs and tested as inhibitors of DC-SIGN binding to gp120 in ELISA and SPR experiments. Nevertheless, none of these systems has so far been tested in cell-based trans-infection models.

Glyconanoparticles (GNPs) are polyvalent, biocompatible sugar-functionalized gold nanoclusters.[9,10] Glyconanoparticle platforms offer the unique potential for simultaneous incorporation of different ligands in variable densities on a single gold cluster. We have prepared a small library of GNPs (manno-GNPs) coated with sets of different structural motifs of the N-linked high-mannose undecasaccharide $Man_9(GlcNAc)_2$ of gp120 (or with an unnatural heptasaccharide) and have observed that GNPs that display multiple copies of the disaccharide Manα1-2Manα block DC-SIGN/gp120 binding at 120 nM in surface plasmon resonance (SPR) experiments.[11] The manno-GNPs were designed to target DC-SIGN receptors present on DCs by mimicking the clustered carbohydrate display of gp120. In this work we present the results obtained with manno-GNPs (Scheme 1) as inhibitors of DC-SIGN-mediated HIV trans-infection of human activated peripheral blood mononuclear cells (PBMCs). We show that manno-GNPs coated with the linear tetrasaccharide Manα1-2Manα1-2Manα1-3Manα inhibit HIV trans-infection of human T cells similarly to GNPs coated with more complex branched penta- and heptaoligomannosides.

Hybrid gold manno-GNPs displaying different densities of linear and branched mannose oligosaccharides were prepared (Scheme 1). The manno-conjugates **6**–**10** were synthesized by conjugation of ethylamino mannosides **1**–**5**, obtained by the protocol of Wong et al.,[12] with isothiocyanate linker **11** and subsequent removal of the acetyl group (Scheme 1 A). A shorter five-carbon atom aliphatic linker was used for the preparation of 5′-mercaptopentyl β-D-glucopyranoside ($GlcC_5S$), and was hidden internally in order to allow correct presentation of the oligomannosides. Manno-GNPs with 100, 50, or 10% densities of dimannoside (D-100, D-50, and D-10, respectively), trimannoside (T-50 and T-10), tetramannoside (Te-50 and Te-10), pentamannoside (P-50 and P-10), and heptamannoside (H-50) were prepared from mixtures of manno-conjugates **6**–**10** and gluco-conjugate $GlcC_5S$ by using a previously described methodology (Scheme 1 B).[13] GNPs were characterized by transmission electron microscopy (TEM), ^{1}H NMR, IR, UV/Visible, and elemental analysis as previously reported.[9] The average number of (oligo)mannosides per GNP and their average molecular weights (Table 1) were calculated from elemental analysis and gold cluster size (1–2 nm as determined by TEM; see the Supporting Information).

For trans-infection studies, we used Raji DC-SIGN transfected lymphoblastoid B cells, which can capture and transmit HIV with an efficiency similar to that of monocyte-derived DCs.[1] Manno-GNPs are nontoxic to Raji DC-SIGN$^+$ and to human activated PBMCs at concentrations of 100 μg mL^{-1}, as determined by the CellTiter cell viability assay (Figure S9 in the Supporting Information). The activities of manno-GNPs against R5 or X4 HIV-1 were evaluated through an original DC-SIGN transfer assay in which inhibition of HIV-1 infection by GNPs was assessed by use of recombinant viruses carrying the Renilla reporter genes in their genomes.[14] In this assay, inhibition of viral replication is proportional to Renilla-luciferase activity in cell lysates. Briefly, Raji DC-SIGN$^+$ cells were preincubated with GNPs for 1 h and were then pulsed with JR-Renilla (R5) or NL4.3-Renilla (X4) recombinant viruses for 2 h. Afterwards, the cell cultures were extensively washed and co-cultured with activated PBMCs that would be infected through transfer of the virus bound to DC-SIGN in Raji cells. Viral replication was as-

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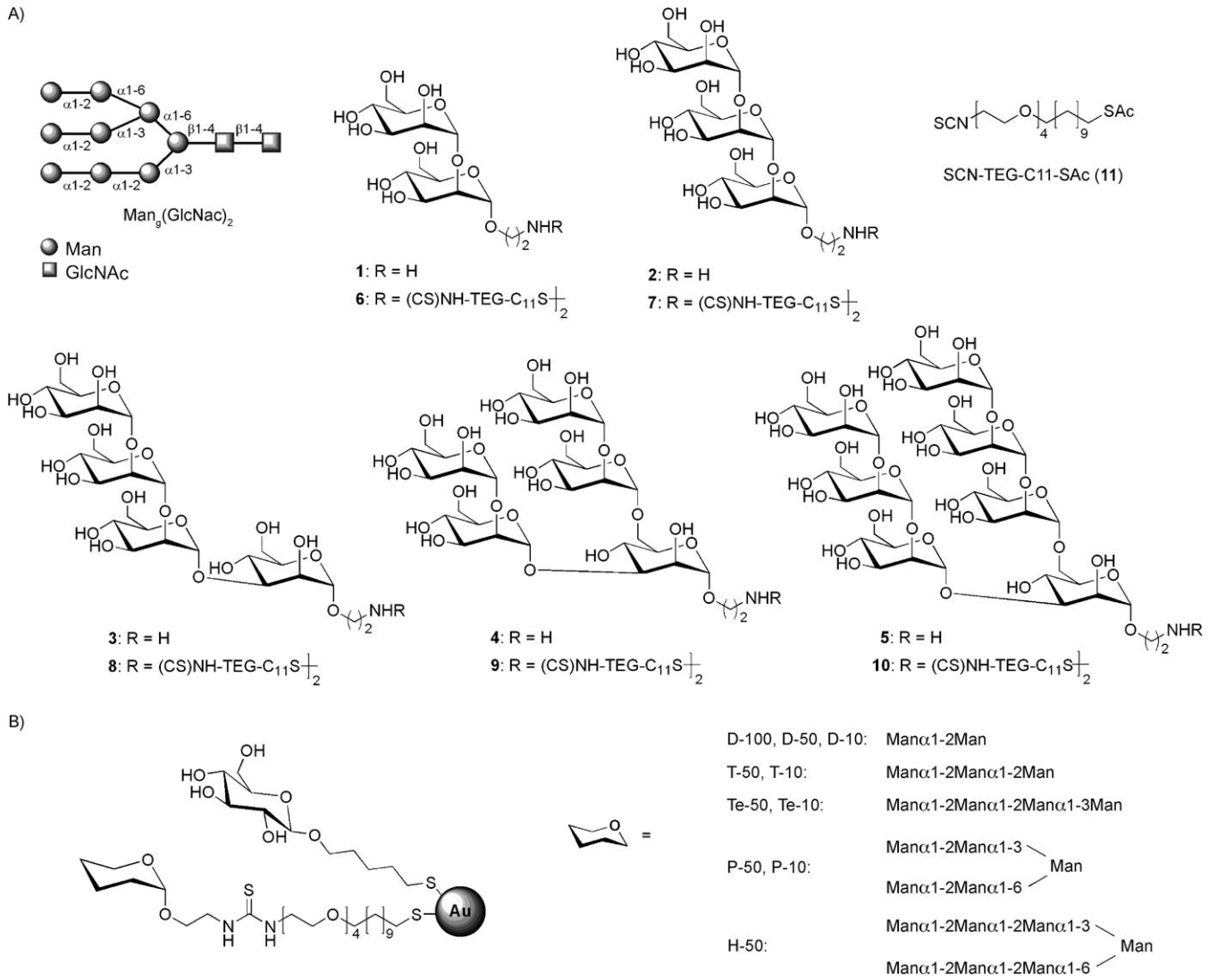

Scheme 1. A) Structures of gp120 undecasaccharide $Man_9(GlcNAc)_2$, oligomannoside conjugates **1**–**5** and **6**–**10**, and linker **11**. Compounds **6**–**10**, through their disulfide functional groups, allowed the preparation of gold manno-GNPs that were tested as inhibitors of DC-SIGN-mediated HIV trans-infection of T cells. The oligomannoside moieties were chosen on the basis of their recognition by the DC lectin, DC-SIGN. The linear oligosaccharides **6**–**8** and the branched oligosaccharide **9** are $Man_9(GlcNAc)_2$ structural motifs. The branched heptasaccharide **10** is an unnatural oligosaccharide. B) Schematic representation of manno-GNPs. D, T, Te, P, and H stand for di- tri-, tetra-, penta-, and heptamannose conjugates, respectively; the numbers indicate the percentages of mannose oligosaccharides on GNP, the rest being the stealthy $GlcC_5S$ conjugate component.

Table 1. Inhibitory activities of manno-GNPs against HIV-1-exposed Raji DC-SIGN cells co-cultivated with uninfected activated PBMCs.

GNPs	Average M_W [KDa]	Man per GNP[a]	$IC_{50\,GNP}$ [ng mL^{-1}][b]		$IC_{50\,GNP}$ [nM][b]		$IC_{50\,Man}$ [nM][b]	
			JR	NL	JR	NL	JR	NL
D-50	39	22	80.5	1469	2.04	37.2	44.9	818
T-50	105	62	166	287	1.58	2.7	198	169
Te-50	123	56	41.6	103	0.34	0.83	18.9	46.6
P-50	105	28	58.5	306	0.56	2.9	15.6	81.5
H-50	150	58	79.2	199	0.53	1.3	30.7	77.3

[a] Average mannoside copy numbers per GNP. [b] IC_{50} is the concentration in GNP ($IC_{50\,GNP}$) or in mannoside ($IC_{50\,Man}$) that reduces luciferase activity by 50%. For data analysis of the best-fit curves for each GNP, see Figure S8 in the Supporting Information.

sessed 48 h post-infection by luciferase activity (RLUs) in cell lysates. Under the conditions tested, Raji cells do not become infected and luciferase activity reflects PBMC infection and viral expression. This experimental setting tries to mimic the natural route of virus transmission from DCs to T lymphocytes. A Raji cell line not expressing DC-SIGN (Raji DC-SIGN$^-$) was used to allow for DC-SIGN-independent viral transfer. Mannan was used as positive control.[15]

The obtained results (Figure 1) indicate that the carbohydrate density on the gold surface has a notable effect on the inhibition of DC-SIGN-mediated trans-infection. At 1 μg mL^{-1}, hybrid manno-GNPs with 10% density in oligosaccharides were much less active than the corresponding GNPs with 50% density. The

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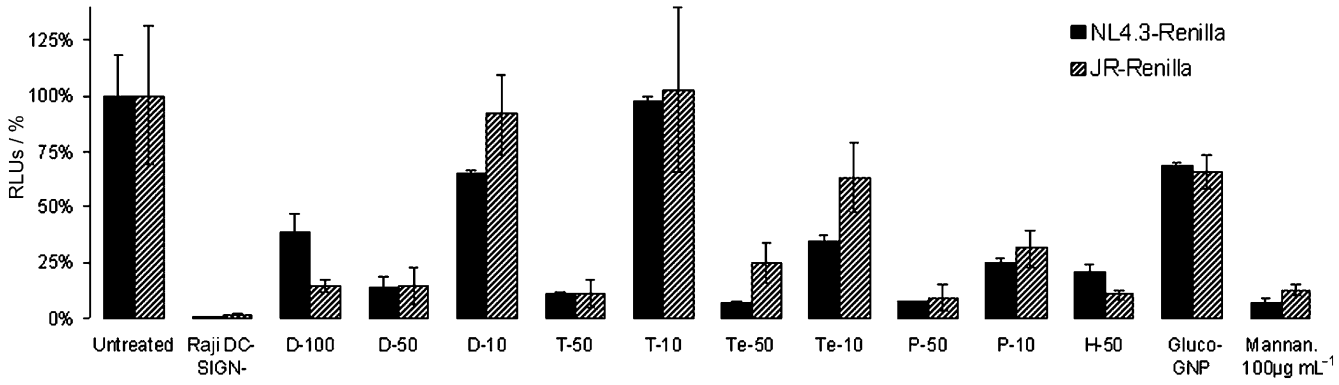

Figure 1. Anti-HIV evaluation of manno-GNPs at 1 μg mL^{-1} in DC-SIGN-mediated trans-infection of human T cells. HIV-1 recombinant viruses JR-Renilla R5 (striped) or NL4.3-Renilla X4 (black) were used. Raji cells not expressing DC-SIGN (Raji DC-SIGN$^-$) were used as control to allow for DC-SIGN-independent viral transfer. Mannan (100 μg mL^{-1}) was used as a positive control. Results are expressed as percentages of infection related to untreated control.

latter class of GNPs showed similar inhibition activities (inhibition range 69–93%), this indicates that 50% density in oligosaccharides is sufficient to achieve significant inhibition. GNPs coated with analogous mono-mannose conjugate (50% and 100% density) were less active (inhibition range below 50%). GNPs incorporating only $GlcC_5S$ (gluco-GNPs) were inactive even at 10 μg mL^{-1} (Figure S7 in the Supporting Information); this shows that neither glucose moieties nor gold contribute significantly to antiviral activities of hybrid manno-GNPs.

To evaluate the inhibitory activities of D-50, T-50, Te-50, P-50, and H-50 better, IC_{50} values were calculated from concentration–response curves (Figure S8 in the Supporting Information). All manno-GNPs proved to be potent inhibitors of R5 and X4 HIV, with IC_{50} values in the ng mL^{-1} range (Table 1). The IC_{50} values were also expressed in terms of molar concentrations of GNP ($IC_{50\,GNP}$) or mannosides ($IC_{50\,Man}$), by consideration of the average molecular weights of the GNPs and the number of mannosides per nanoparticle. Te-50, which incorporates 56 units of linear tetrasaccharide **8**, was the most potent inhibitor against both R5 and X4 tropic HIV, and similar to P-50 and H-50, which incorporate 28 and 58 units of the branched penta- and heptasaccharides **9** and **10**, respectively. All GNPs coated with 50% density showed excellent inhibition activities in the nanomolar range. The calculated $IC_{90\,Man}$ values for Te-50 were 82 nM for R5 and 361 nM for X4. At the same concentrations, the monovalent tetramannoside **3** and the corresponding divalent conjugate **8** did not show any inhibitory effects (data not shown). These results show that the multivalent display of oligomannosides on the GNP is essential to enhance their binding to DC-SIGN and subsequently to inhibit HIV-1 trans-infection.

Because of the lack of previous trans-infection assays with multivalent carbohydrate-based systems, we compared the manno-GNPs with the few examples of efficient noncarbohydrate-based inhibitors of the DC-SIGN-mediated HIV-1 trans-infection of T cells reported in the literature.[16] The diversity of methods, concentrations, virus, and cell lines used in these experiments makes an accurate comparison with our results quite difficult. However, the IC_{50} values of the manno-GNPs (Table 1) are better than those reported for most inhibitors.[16] Furthermore, it is remarkable that the activities of unbranched oligomannosides match those observed for more complex branched oligosaccharides when multivalently presented on the gold surface (Te-50 versus P-50, H-50).

In conclusion, oligomannoside-functionalized gold glyconanoparticles are able to inhibit the HIV DC-SIGN-mediated trans-infection of T cells at nanomolar concentrations. This, to the best of our knowledge, is the first example of a synthetic carbohydrate-based multivalent system that seems to function as an anti-adhesive barrier at an early stage of HIV-1 infection, prevent viral attachment to DC-SIGN-expressing cells, and subsequently inhibit trans-infection of human T lymphocytes. The multivalent presentation of mannosides on nanoparticles is essential for efficient inhibitory activity to be reached. It is noteworthy that the presentation of simple linear di-, tri-, and tetraoligosaccharides on the nanocluster results in efficiencies similar to those observed for complex branched penta- and heptamannosides. Recently, a nice example of multivalence efficiency of gold nanoparticles coated with inhibitors of HIV fusion has been reported.[17] Gold GNPs are an advantageous alternative to other multivalent scaffolds such as dendrimers, polymers, and liposomes, because they allow control over the degrees of multivalency and multifunctionality.[13] The natures of the linkers (length, flexibility, amphiphilic properties) can also be modified for each ligand as a function of the desired presentation. In addition, gold nanoparticles offer the potential to use the singular optical and electronic properties of metal nanoclusters for other biomaterial applications.

Experimental Section

Cell culture and preparation of PBMCs from blood: Raji cells were kindly provided by Dr. Alfredo Toraño (Instituto de Salud Carlos III, Madrid, Spain), and Raji DC-SIGN$^+$ cells were kindly provided by Dr. Fernando Arenzana-Seisdedos (Institut Pasteur, Paris, France). Both cell lines were cultured in RPMI 1640 medium containing fetal bovine serum (10% *v/v*), L-glutamine (2 mM), penicillin (50 IU mL^{-1}), and streptomycin (50 μg mL^{-1}; all from Whittaker M.A. Bio-Products, Walkerville, MD, USA). The 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing fetal bovine serum (10% *v/v*), L-glutamine (2 mM), penicillin (50 IU mL^{-1}),

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and streptomycin (50 μg mL^{-1}). The 293T cells were cultured at 37 °C in a humidified atmosphere with CO_2 (5 %) and split twice a week. PBMCs were obtained from buffy coats from healthy donors. Briefly, PBMCs were harvested from buffy coats by centrifugation over Lymphoprep (Sigma–Aldrich) gradient by standard procedures, stimulated with interleukin-2 (IL-2, 300 IU mL^{-1}; Chiron) and phytohaemagglutinin (PHA, 5 μg mL^{-1}), and incubated at 37 °C under humidified CO_2 (5 %) for 48 h.

Cell viability assay: The cell viability test was performed in Raji DC-SIGN$^+$ or in Raji DC-SIGN$^+$ plus preactivated PBMCs by the same protocol as described for the Raji DC-SIGN-mediated trans-infection assay, but in the absence of viral supernatants. After 48 h CellTiter-Glo reagent (CellTiter-Glo® Luminescent Cell Viability Assay, Promega) was added to cell cultures and viability was quantified in a luminometer (Figure S9 in the Supporting Information).

Trans-infection assay: Raji or Raji DC-SIGN$^+$ cells (10^5 cells per well) were incubated with GNPs for one hour prior to addition of either R5 or X4 tropic recombinant viruses (JR-Renilla or NL4.3-Renilla, respectively; both 200 ng p24 per well) and left for 2 h at 37 °C for efficient adsorption. Afterwards, cells were washed extensively with PBS, followed by the addition of preactivated PBMCs (10^5 per well). Viral replication after trans-infection was followed by measurement of RLU activity in cell lysates. Briefly, cells were harvested and lysed after 48 h and sample activity was measured with the Renilla luciferase assay system (Promega) according to the manufacturer's protocol. Relative luminescence units (RLUs) were obtained by using a luminometer (Berthold Detection Systems, Pforzheim, Germany) after the addition of substrate to cells extracts. To rule out possible interference from DC-SIGN-independent mechanisms all the experiments were performed in parallel with nonexpressing Raji cells as control. IC_{50} values were calculated with GraphPad Prism Software (Sigmoidal dose-response analysis). Results are each representative of at least three independent experiments (Figure S8 in the Supporting Information).

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