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Detection and Isolation of Dendritic Cells Using Lewis X-functionalized Magnetic Nanoparticles

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Abstract

DC-specific intracellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) is a receptor found on dendritic cells (DCs) that recognizes antigens bearing mannose-rich or fucosylated glycans, including Lewis X (Le^X). Here, we report the fabrication of magnetic nanoparticles coated with multivalent Le^X glycans using the Cu (I)-catalyzed azide-alkyne cycloaddition. The resulting nanoparticles are selective and biocompatible, serving as a highly efficient tool for DC detection and enrichment.

Keywords

click chemistry; dendritic cells; glycoconjugates; magnetic nanoparticle

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that serve as messengers between innate and adaptive immunity.^{1,2} Prior to acute infection and inflammation, DCs are in an immature state as plasmacytoid and myeloid DCs in the peripheral blood.³ Encounter and capture of antigens result in DC maturation and migration to secondary lymphoid organs, where they present processed antigens as antigenic fragments on major histocompatibility complex (MHC) class I or II molecules to naïve T cells to initiate the adaptive immune response.³ This interaction is facilitated by three signals: the binding of T cell receptor (TCR) to peptide-MHC complexes, the binding of co-stimulatory molecules on APCs to T cell receptor CD28, and release of a co-stimulatory signal from the activated DCs.⁴ Adhesion molecules such as DC-specific intracellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) on the surface of DCs also play a critical role in establishing contact between the activated DCs and the naïve T cells through interactions with intercellular adhesion molecule 3 on T cells.⁴ Upon interaction with DCs, T cells are

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Supporting Information

Additional characterization data and figures as discussed in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

directed into specific effector functions.⁵ Due to their unique roles, DCs are under active investigations as the target for antigen delivery in vaccination against human immunodeficiency virus (HIV), cancer and autoimmune diseases.^{67,89}

Many immunological studies require the use of a large quantity of DCs; however, relatively few DCs circulate in the peripheral blood of healthy adults.¹⁰ To obtain immature myeloid DCs in bulk quantities, monocytes from human peripheral blood mononuclear cells (PBMCs) can be differentiated into immature myeloid DCs, termed monocyte-derived dendritic cells (moDCs), *in vitro* using growth factors interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF).¹¹ This differentiation procedure is straightforward; however, in most cases it results in a heterogeneous population of differentiated moDCs and undifferentiated monocytes.

Conventional methods for isolating moDCs from PBMCs rely on antibody-based affinity capture, in which cells other than DCs are depleted using a cocktail of magnetic nanoparticles (MNPs) coated with antibodies against non-DC surface markers.¹² In a subsequent step, DCs are further enriched using nanoparticles that are functionalized with antibodies that target DC surface receptors. Though already commercialized, capture capacities of antibody-functionalized magnetic nanoparticles are typically low (10–30%).¹³ Additionally, immobilization of antibodies on solid support in an orientation-specific manner requires extensive genetic and chemical manipulations, which are responsible for the high cost of fabricating these nanoparticles.^{14,15} These limitations prompted us to search for alternative, cost-effective strategies that could be used for sensitive detection and facile isolation of dendritic cells from a complex cell population.

DC-SIGN is a member of the C-type lectin receptors, which is found on immature and mature DCs derived from monocytes. DC-SIGN is also present on DCs found in lymph nodes, spleen and tonsil, and on rare subpopulations of organ-restricted macrophages, making it a very specific marker for targeting the desired cell population.¹⁶ DC-SIGN recognizes antigens bearing mannose-rich or fucosylated glycans, such as those found in Lewis blood group antigens, including Lewis X (Le^X).¹⁷ Upon DC-SIGN binding, antigens are internalized, processed and presented on the surface of DCs to elicit an antigen-specific T cell response.^{18,19} While the molecular mechanism that relates DC-SIGN signaling to immunomodulatory functions of fucosylated glycans is still obscure,²⁰ these discoveries certainly raise the possibility of using these glycan epitopes (e.g. Le^X) as targeting elements for DC capture and enrichment. Inspired by recent studies that exploited glycan-functionalized MNPs for targeting the CD62 carbohydrate-binding transmembrane proteins in the brain²¹ and for *Escherichia coli* detection,²² we report here the fabrication of multivalent Le^X-functionalized MNPs using the Cu (I)-catalyzed azide-alkyne cycloaddition (CuAAC),^{23,24} a prototypical example of bioorthogonal click chemistry.²⁵ The resulting MNPs are biocompatible, serving as a powerful and highly efficient tool for moDC detection and enrichment (Scheme 1). The proposed system serves as a proof-of-concept for the use of glyco-nanoparticles as cell enrichment tool as cheaper, more consistent alternatives to traditional, antibody-based affinity capture methods.

Materials and Methods

General methods and materials

Cytosolic orange Cell Tracker[™] was purchased from Invitrogen. *Erythrina cristagalli* conjugated with fluorescein isothiocyanate (ECA-FITC) was purchased from EY-Laboratoies. Phycoerythrin (PE)-conjugated anti-SSEA-1, PE/Cy5-conjugated Anti-CD1a and PE-conjugated HLA-DR (MHC class II cell surface receptor encoded by the human leukocyte antigen complex) were purchased from BD Biosciences. FITC-conjugated Anti-

DC-SIGN was purchased from R&D Systems. The corresponding isotype control was purchased from ebiosciences. IL-4 and GM-CSF were purchased from ebiosciences.

2-Azidoethyl O - β -D-galactopyranosyl-(1 $\rightarrow$ 4)- O -[6-deoxy- α -L-galactopyranosyl-(1 $\rightarrow$ 3)]-2-acetamide-2-deoxy- β -D-glucopyranoside (azido Le^X),²⁶ 2-azidoethyl O - β -D-galactopyranosyl-(1 $\rightarrow$ 4)- O -2-acetamide-2-deoxy- β -D-glucopyranoside (azido LacNAc)²⁶ and 2-azidoethyl O - β -D-galactopyranosyl-(1 $\rightarrow$ 4)- β -D-glucopyranoside (azido Lactose)²⁷ were prepared using the previously published procedure. The CuAAC accelerating ligands BTTP²⁸ and BTAA²⁹ were prepared using the previously published procedures developed in the Wu Lab. Azido Fluor 488 was purchased from Click Chemistry Tools, AZ.

Transmission electron microscopy (TEM) images of MNPs were obtained on a Hitachi H-800 (Tokyo, Japan) transmission electron microscope. Samples were prepared by depositing suspensions onto grids. After air drying, the samples were tested at 200 kV. Scanning electron microscopy (SEM) images were obtained on a Zeiss Supra-55 (Carl Zeiss NTS GmbH, Oberkochen, Germany) field emission scanning electron microscope operating at 10 kV. Samples were prepared by depositing suspensions onto a piece of silica wafer. Powder X-ray diffraction data were taken on a D/max-Ultima III (Rigaku, Japan) using Cu K α radiation (40kV, 30mA, λ =1.5418Å). Samples were step-scanned in steps of 0.02° (2 θ) in the range 3–70° using a count time of 4s per step. The magnetization of the magnetic nanoparticles was tested on a JSM-13 vibrating-sample magnetometer at 298 K and $\pm$ 15 kOe applied magnetic field.

Tissue culture/cell growth conditions

Jurkat cells, THP-1 cells and THP-1/DC-SIGN cells were grown in RPMI 1640 Medium supplemented with 10% FBS (Sigma). All cells were incubated in a 5.0% carbon dioxide, water saturated incubator at 37 °C.

Fabrication of PAA-MNPs and alkyne-MNPs

PAA-MNPs were prepared using the previously published procedure.³⁰ Alkyne-MNPs were prepared by reacting PAA-MNPs with propargylamine. Typically, to PAA-MNPs (100 mg) in H₂O (25 mL), were added propargylamine (0.11 g, 2.0 mmol), EDC·HCl (0.38 g, 2.0 mmol), NHS (0.12 g, 1.0 mmol) and DMAP (49 mg, 0.4 mmol). The reaction was stirred at room temperature for 24 hours. The product was separated using an external magnetic field and washed repeatedly with deionized water for eight times and stored in 20 mL deionized water.

Quantification of alkynyl groups on alkyne-MNPs

To the alkyne-MNPs (500 μ g in 1 μ L PBS buffer, pH 7.4) were added azido Fluor 488 (5 mM), CuSO₄·5H₂O/BTTAA complex (1:6, 7 mM in total), and sodium ascorbate (20 mM). The reaction was sonicated using a water bath sonicator for 20 minutes, followed by incubation at room temperature for 6 hours at on a vortex shaker. The resulting Fluor 488-MNPs were then separated and washed repeatedly with deionized water for 8 times, and stored in water with a final concentration of 1 mg/mL. The fluorescence intensity of the Fluor 488-MNPs was measured and the amount of alkynyl groups was quantified using a standard curve generated by measuring the azido Fluor 488 in PBS at a series of concentrations. The quantification was based on the assumption that the ligand-accelerated CuAAC proceeded at 100% efficiency on the particle surface and the absorption coefficient of the isolated fluorophore remained unchanged upon conjugation to MNP surface. The fluorescence measurements were performed on a Hitachi F-4500 spectrophotometer (Tokyo, Japan) equipped with a Xenon lamp excitation source. The fluorescence spectra were measured at an excitation wavelength of 488 nm and emission wavelength of 525 nm.

Conjugation of glycans to MNPs via CuAAC

The alkyne-functionalized MNPs (500 μg) were sonicated for 5 minutes on a bath sonicator and the MNPs were magnetically isolated. An azido glycan (5 mM), CuSO_4 (5 mM), BTTP (5 mM), and sodium ascorbate (20 mM) were added to the alkyne functionalized MNPs in 100 mM KPB buffer, pH 7.0 at a total volume of 100 μL and sonicated for 5 minutes. The reaction mixture was incubated for 4 hours on a shaker. The resulting MNPs were then washed twice with deionized water on magnetic strip, once with 100% ethanol and once more times with deionized water. All samples were resuspended and stored 1 mg/mL in deionized H_2O at 4 $^\circ\text{C}$.

Lectin Binding Assays

Glycan-functionalized MNPs were sonicated for 5 min and added to magnetic strip to remove the storage liquid. 1 mL PBS (1% FBS) were added to the MNPs, vortexed and incubated for 30 minutes to block. After removal of the blocking buffer, 100 μL of the lectin or antibody solution (100 $\mu\text{g}/\text{mL}$ in PBS with calcium and magnesium) was added to the MNPs and incubate at room temperature overnight in the dark on a shaker. On the magnetic strip, the liquid was separated from the MNPs and washed 5 times with PBS (1% FBS) to remove nonspecific binding. We measured the increase in fluorescence on the glycan-functionalized MNPs as compared to fluorescence detected for the alkyne-functionalized MNPs. Fluorescence was recorded on a Synergy plate reader.

Cell viability assays

Jurkat cells, THP-1 cells, and THP-1/DC-SIGN cells were incubated with the glycan-functionalized MNPs (150 $\mu\text{g}/\text{mL}$) for 3 days. In control experiments, the cells were incubated with unmodified MNPs or with media alone. Each day, viable cells were counted using the Trypan blue dye exclusion method.

Glycan-functionalized MNP binding assay using cultured THP-1 cells

THP-1/DC-SIGN cells were incubated with cytosolic orange Cell TrackerTM diluted in serum free RPMI 1640 for 30 minutes at 37 $^\circ\text{C}$. The cells were washed, then resuspended in RPMI 1640 (10% FBS) and separated into tubes with equal cell counts, then mixed with equal counts of unstained THP-1 cells for a final volume of 500 μL . 50 μg of MNPs were blocked for 30 minutes in PBS (0.2 % FBS), then added to each sample. The cell and MNP mixtures were incubated for 15 minutes at 37 $^\circ\text{C}$, unless otherwise indicated. The MNPs were then isolated on a magnetic strip and the flowthrough separated. The MNPs were washed five times with PBS (0.2% FBS), then resuspended in flow cytometry buffer (Hank's Balanced Salt Solution, pH = 7.4, 1% BCS, 0.2% NaN_3 , 1 $\mu\text{g}/\text{mL}$ Bisbenzimidazole live/dead stain) Flow cytometry was performed on the iCyt Eclipse instrument, and the analysis was conducted using FlowJo software. The flowthrough samples were centrifuged at 300 g for 3 minutes, and resuspended in flow cytometry buffer. The capture efficiency range of Le^X-MNP was determined by 2 methods: (1) the total number of THP-1/DC-SIGN cells captured by the Le^X-NPS were divided by the total number of THP-1/DC-SIGN cells on the MNPs and in the flowthrough (~86%); (2) the percentage decrease of the THP-1/DC-SIGN cells in the flowthrough was subtracted from the original population (50% – 15%) and divided by the total population of THP-1 DC-SIGN cells (~70%).

TEM imaging of cells treated with Le^X-MNPs

To prepare cells for TEM imaging, THP-1/DC-SIGN cells were incubated with Le^X-MNPs at 37 $^\circ\text{C}$ for 15 minutes. The cells were washed 3X in media, then subjected to fixation, or cultured for an additional two days before fixing. Cells were fixed with 2.5% glutaraldehyde, in 0.1 M sodium cacodylate buffer and pre-embedded in 2% gelatin. The

samples were postfixed with 1% osmium tetroxide followed by 2% uranyl acetate, dehydrated through a graded series of ethanol dilutions, and embedded in LX112 resin (LADD Research Industries, Burlington VT). Ultrathin sections were cut on a Reichert Ultracut E, stained with uranyl acetate followed by lead citrate and viewed on a JEOL 1200EX transmission electron microscope at 80 kv.

Generating moDCs from PBMCs

PBMCs were differentiated into moDCs according to standard protocols. Briefly, 5×10^8 viable PBMCs were incubated in 10 mL of RPMI 1640 (10% FBS) media for 2 hours so the monocytes would adhere to the plastic surface of the plate. After 2 hours, the lymphocytes that remained floating were removed and the media were replaced with RPMI 1640 (10% FBS) supplemented with 200 IU/ml IL-4 and 100 IU/ml GM-CSF. This media was replaced again on day 3. On day 6, many cells will have characteristics of immature myeloid DCs.

Glycan-functionalized MNP binding assay using primary moDCs

PBMCs were differentiated into moDCs according to the protocol above. Le^X- or alkyne-functionalized MNPs (50 μ g) were blocked for 30 minutes in PBS (0.2 % FBS), then added to each sample. The cell and MNP mixtures were incubated for 15 minutes at 37 °C. The MNPs were then isolated on a magnetic strip and the flow-through separated. Cells collected in the flowthrough were centrifuged at 300 *g* for 3 minutes and the MNPs were washed five times with PBS (0.2% FBS) on the magnetic strip. All samples were resuspended in 97 μ L of PBS, 1 μ L of anti-CD1a-PE/Cy5, 1 μ L of anti-huDC-SIGN-FITC, and 1 μ L anti-HLA-DR, and were incubated on ice for 30 minutes in the dark. These samples were subsequently washed and resuspended in flow cytometry buffer and analyzed by flow cytometry. Flow cytometry was performed on the iCyt Eclipse instrument, and the analysis was conducted using FlowJo software.

Results and Discussion

Fabrication and Characterization of Glycan-functionalized MNPs

As the first step to fabricate glycan-conjugated MNPs, we chose to use poly(acrylic acid) (PAA)-coated magnetite (Fe₃O₄) colloidal nanocrystal clusters as the starting material based on their well-documented high magnetization, high water dispersibility, and excellent biocompatibility.³⁰ Using the method developed by Yin and coworkers,³⁰ we successfully prepared the PAA-coated magnetite colloidal nanocrystal clusters with a uniform size of approximately 200–300 nm (Table S1), which are composed of multiple-single magnetite crystallites (~10 nm in diameter) as shown by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray diffraction (XRD) characterizations (Figure 1a and Figure S1a and S1b Supporting Information). For simplicity, the magnetite colloidal nanocrystal clusters are referred to as MNPs hereafter. We confirmed the strong response of PAA-MNPs to external magnetic field by measuring the mass magnetization (Figure S1c, Supporting Information). The composition of PAA-MNPs was determined to contain approximately 84% of magnetite and 16% of PAA using thermogravimetric analysis (TGA).

The PAA-MNPs were then coupled with propargylamine to incorporate terminal alkyne groups to the surface for subsequent conjugation to glycans bearing azido linkers (Scheme 1a). The coupling reaction does not result in significant change to the MNP size (Figure 1b). To confirm the presence of alkynyl groups on MNP surfaces, the functionalized MNPs were reacted with azido Fluor 488 under the ligand-accelerated CuAAC conditions^{28,29,31} and analyzed by fluorescence spectroscopy. The propargyl amine-treated MNPs displayed a significant increase in fluorescence when compared to PAA-MNPs, confirming the incorporation of terminal alkyne groups onto the MNP surfaces (Figure S2, Supporting

Information). Quantitative analysis on the fluorescence intensity of Fluor 488 on MNP surface revealed the availability of approximately 3×10^5 alkynyl groups per alkyne-MNP, or 14 nmol/mg, for conjugation (Figure S3, Supporting Information).

Next, we synthesized Le^{X} with an azido linker for conjugation to the alkyne-functionalized MNPs. We adopted a chemoenzymatic method previously developed by our laboratory for transferring a fucose residue regio-specifically to the azido-labeled disaccharide, 2-azidoethyl α - β - N -acetylglucosamine (LacNAc), using recombinant FKP for fucose activation and α 1,3 fucosyltransferase for fucose incorporation.²⁶ Azido Le^{X} was then introduced onto the alkyne-functionalized MNPs using the ligand-accelerated CuAAC to ensure the quantitative conversion of alkynes to glycan moieties (Scheme 1a). The resulting Le^{X} -MNPs exhibited a similar diameter to that of PAA-MNPs and alkyne-MNPs (Figure 1c). Using the same approach, we also functionalized MNPs with several additional glycans, including sialyl Lewis X (sLe^{X}), LacNAc and lactose. To test the magnetic capture of MNPs, we subjected the solutions of PAA-, alkyne-, and Le^{X} -MNPs to a magnetic field induced by a small magnet (Figure 1d–f). All three MNPs were completely captured and separated from the solution within minutes. Removal of the magnetic field brings the MNPs back to solution rapidly upon slight agitation (data not shown).

Specificity and Biocompatibility Evaluation of the Glycan-functionalized MNPs

With the glycan-functionalized MNPs in hand, we first tested whether the immobilized glycans retain their binding capability for glycan-specific antibodies and lectins. To this end, MNPs coated with different glycans were incubated with a phycoerythrin (PE)-labeled anti-stage specific embryonic antigen-1 (SSEA-1, SSEA-1 = Le^{X}) antibody and a FITC-labeled *Erythrina cristagalli* (ECA, a lectin that recognizes LacNAc and lactose epitopes) overnight. After stringent washes, the remaining fluorescent signal on the MNPs was measured using fluorescence spectroscopy. Significant fluorescence was detected for SSEA-1-treated Le^{X} -MNPs, whereas only background fluorescence was detected for SSEA-1-treated sLe^{X} -, LacNAc- and lactose-MNPs (Figure 2, Figure S4, Supporting Information). By contrast, Le^{X} - and sLe^{X} -functionalized MNPs only displayed background fluorescence upon ECA-treatment, whereas significant fluorescence was observed upon incubation of LacNAc- and lactose-MNPs with ECA-FITC, with LacNAc-MNPs displaying higher levels of fluorescence than the lactose-functionalized counterparts due to the stronger affinity of ECA toward LacNAc (Figure 2).³²

To evaluate if the glycan-functionalized MNPs cause any perturbation to cells, we incubated the MNPs with three mammalian cell lines for three days. Viable cells, based on Trypan Blue assay, were counted each day. Cells incubated with the MNPs proliferated at similar rates as untreated cells, indicating that the glycan-functionalized MNPs do not interfere with cell viability (Figure 3)

Le^{X} -functionalized MNPs Allow Specific Capture of DC-SIGN-expressing Cells from Complex Cell Populations

After verifying the specificity and biocompatibility of the glycan-functionalized MNPs, we evaluated the feasibility of using the Le^{X} -MNPs for capturing DC-SIGN positive cells in a mixed cell population. A well-characterized, human, monocyte-like leukemia cell line stably transduced to express DC-SIGN, designated as THP-1/DC-SIGN,³³ and its parental cell line THP-1 were used in this study. We first labeled THP-1/DC-SIGN cells with cytosolic orange Cell Tracker™ as their identity marker. We then mixed THP-1/DC-SIGN and THP-1 in various ratios and incubated them with the Le^{X} -functionalized MNPs. Cells captured on the magnetic MNPs were isolated from the unbound ones by applying a magnetic field. The two cell populations were then analyzed using flow cytometry. The Le^{X} -functionalized

MNPs were highly efficient in selectively capturing the THP-1/DC-SIGN cells (Figure 4); when THP-1/DC-SIGN cells were mixed with THP-1 cells in a 1:1 ratio, the capturing efficiency was around 70%–86% as calculated from the flow cytometry data. Notably, as few as 5,000 THP-1/DC-SIGN cells could be isolated from a mixed cell population of 500,000 cells (data not shown). This assay was repeated in increments of incubation times up to 24 hours. It was observed that a 15-minute incubation with the MNPs was sufficient to isolate the THP-1/DC-SIGN cells from the mixed cell population of cells (Figure S6, Supporting Information).

In control experiments, we incubated the unmodified alkyne-bearing MNPs, lactose- and LacNAc-functionalized MNPs with a 1:1 mixture of THP-1/DC-SIGN and THP-1 cell to evaluate their capturing specificity. Consistent with the results obtained from the lectin binding assay, the alkyne-, lactose- and LacNAc-functionalized MNPs bound poorly to both THP-1 and THP-1/DC-SIGN cells, and were unable to capture DC-SIGN positive cells specifically (Figure 4, Figure S5 Supporting Information). Interestingly, sLe^X-functionalized MNPs bound to both THP-1 and THP-1/DC-SIGN cells due to the presence of sialoadhesin, a cell surface receptor specific for α 2,3 sialosides, in both cell lines.³⁴

To analyze if the cell-bound Le^X-MNPs are internalized, we incubated THP-1/DC-SIGN cells with the Le^X-MNPs for 15 minutes. We fixed the cells and prepared ultrathin sections for TEM imaging. As shown in Figure 5, the MNPs were clearly found in the intracellular compartments. This observation is consistent with the well-established endocytic function of DC-SIGN.

Finally, we explored the possibility of using the Le^X-functionalized MNPs to capture differentiated, immature moDCs from PBMCs derived from a mixed population of primary human cells. Briefly, human monocytes were incubated with IL-4 and GM-CSF for six days to induce their differentiation. On day six, the cytokine-treated cells were incubated with the Le^X-functionalized MNPs. The cells bound to the MNPs were separated from the unbound ones and were stained with anti-DC-SIGN-FITC and CD1a-PE/Cy5 as markers for moDCs. As shown in Figure 6, DC-SIGN⁺ and CD1a⁺ moDCs were specifically pulled down onto the Le^X-functionalized MNPs with the concomitant disappearance of this cell population in the corresponding flow-through cells.

Conclusions

In summary, we demonstrated that glycan-functionalized MNPs can be easily fabricated using ligand-accelerated CuAAC. MNPs functionalized with the Le^X epitopes are not only biocompatible, but can serve as powerful tools for rapid isolation of both cultured and primary DC-SIGN expressing cells from a heterogeneous cell population. To our knowledge, this study is the first example of using glycans as ligands for DC capture and isolation. We anticipate that these MNPs may also be used for in vivo dendritic cell imaging using magnetic resonance imaging techniques, which is currently under exploration in our laboratories.

This new method for utilizing glycans to isolate cells expressing glycan-binding proteins offer several advantages over the conventional, antibody-based methods for capturing specific cell types. The use of immobilized, synthetically prepared glycans eliminates the batch-to-batch variation associated with antibody preparation and conjugation, and significantly decreases the costs associated with preparation and manipulation of the antibodies. This method is advantageous in the isolation of immune cells, including DCs, due to the abundant presence of glycan binding cell surface receptors, e.g. DC-SIGN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Size distribution and magnetic behavior of functionalized and MNPs. Representative TEM images of PAA-MNPs (a), alkyne-MNPs (b), and Le^X-MNPs (c). The scale bars are 200 nm. Photographs of the aqueous solutions of PAA-MNPs (d), alkyne-MNPs (e), and Le^X-MNPs (f) that are with (right) or without (left) a magnetic field.

Figure 2.

Lectin binding to glycan-functionalized MNPs. Le^x- functionalized MNPs bind to anti-SSEA-1 specifically. LacNAc and lactose-coated MNPs both bind to ECA-FITC; the binding affinity of LacNAc-MNPs is stronger towards ECA. Error bars represent standard errors of three repeated experiments.

Figure 3.

Cells incubated with glycan-functionalized MNPs remain viable. Cells were co-incubated with glycan-functionalized MNPs for 3 days. Live cells assayed by trypan-blue staining were counted every day for three days and compared to control cells cultured in the absence of MNPs. Average and standard error of 5 counts are reported. (a) Viability of Jurkat cells, (b) Viability of THP-1 cells, (c) Viability of THP-1/DC-SIGN cells.

Figure 4.

Le^x-functionalized MNPs specifically bind to DC-SIGN expressing cells. THP-1/DC-SIGN cells were stained with cytosolic orange Cell Tracker™ and mixed at a 1:1 ratio with unstained THP-1 cells. Cell mixture was incubated with MNPs functionalized with various glycan epitopes for 15 minutes at 37 °C and captured on a magnetic strip. The captured cells were washed five times with PBS (1% FBS) and analyzed using flow cytometry. The numbers shown in the dot plots represent the percentages of each cell type in the MNP-captured population or in the flowthrough population. Prior to incubation with the MNPs, each cell type had ~50% of each cell population. The data shown was from one representative experiment out of three replicates.

Figure 5.

TEM images of THP-1/DC-SIGN cells incubated with Le^X-MNPs indicated the internalization of the MNPs. THP-1/DC-SIGN cells were incubated with Le^X-MNPs for 15 minutes and fixed. Ultrathin sections were prepared for TEM imaging. The MNPs were found in the intracellular compartments.

Figure 6.

Enrichment of moDCs using Le^X-functionalized MNPs. PBMCs were incubated in the presence of GM-CSF and IL-4 for six days and differentiated into moDCs. (a) Contour plot shows the total cell population prior to capture. moDCs were defined as CD1a⁺/DC-SIGN⁺ (gated in the box). (b) Cell flowthrough following incubation with alkyne-functionalized MNPs. (c) Cell flowthrough following incubation with Le^X-functionalized MNPs. moDCs were absent from this population. (d) Histogram shows that the population of cells bound to Le^X-functionalized MNPs are CD1a⁺/DC-SIGN⁺/HLA-DR⁺ when stained with FITC-conjugated anti-DC-SIGN, PE/Cy5-conjugated Anti-CD1a and PE-conjugated anti-HLA-DR. Shown is one representative experiment out of three replicates.

Scheme 1.

(a) Fabrication of Le^X-coated MNPs. (b) Selective capture of DCs from a mixed cell population using Le^X-functionalized MNPs.