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Enhanced delivery of immunoliposomes to human dendritic cells by targeting the multilectin receptor DEC-205

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Abstract

Dendritic cells (DC) are specialized white blood cells that initiate and direct immune responses. Targeting DC surface proteins to deliver liposomes carrying antigens has demonstrated potential for eliciting antigen-specific immune responses. To evaluate this strategy in preclinical studies, we prepared anti-human DEC-205 immunoliposomes (anti-hDEC-205 iLPSM) and compared their uptake by monocyte-derived DC (MoDC) and blood DC (BDC) with conventional liposomes (cLPSM). Antibody conjugation increased the number of immature MoDC taking up liposomes to 70–80%, regardless of the antibody coupled, whereas less than 20% endocytosed cLPSM. Anti-hDEC-205-IgG specifically increased cell uptake by 15% and the total iLPSM uptake six-fold. The non-specific iLPSM uptake was unlikely to be Fc receptor-mediated as excess immunoglobulins failed to block the uptake. Only a small population (7–24%) of mature MoDC took up cLPSM and control iLPSM. In contrast, ~70% of mature MoDC took up anti-hDEC-205 iLPSM, endocytosing 10-fold more iLPSM than the control iLPSM. Anti-hDEC-205 iLPSM uptake by $CD1c^+$ BDC was similar to the immature MoDC, but was five-fold increased compared to the control iLPSM. Confocal microscopy confirmed that the anti-hDEC-205 iLPSM were phagocytosed by DC and available for antigen processing. Thus, DEC-205 is an effective target for delivering liposomes to human DC.

Keywords: C-type lectins; Immunotherapy; Macrophage mannose receptor

1. Introduction

Dendritic cells (DC) include distinct populations of white blood cells, which function as professional antigen presenting cells, integrating innate and adaptive immune responses [1,2]. Derived from haematopoietic progenitors in bone marrow, DC precursors circulate in blood and reside in tissues as sentinel cells (immature DC). Immature DC take up antigens from the extracellular milieu using macropinocytosis, pinocytosis and receptor-mediated endocytosis, then process and load antigenic peptides onto major histocompatibility complexes (i.e. MHC class I and II) in the endoplasmic reticulum or late endosomes. During this process, DC migrate into lymph nodes and display the processed antigens to T lymphocytes, thereby eliciting antigen-specific immune responses or tolerance/anergy, depending on the presence or absence of additional costimulatory/tolerogenic signals. Successful delivery of antigens into DC both *in vitro* and *in vivo* is a key step for successful vaccination against infectious diseases, cancer and autoimmune diseases [3–5]. A new strategy, which targets DC cell surface proteins, particularly their specialized antigen-loading receptors, has recently been described in mouse models [6–8].

To elicit an immune response to an antigen, it is best delivered to the highly endocytic and phagocytic form of DC

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(immature DC) capable of processing the antigen for presentation to T lymphocytes prior to their differentiation in the presence of "danger signals" and/or costimulatory signals. In the absence of these stimulating signals or with alternative activation, immature DC loaded with antigen become regulatory/suppressor DC and down-regulate antigen-specific immune responses. Therefore, antigen-loading to immature DC can be used to both activate and suppress specific antigen responses, depending on the presence and type of DC-activating/modulating agents, such as Toll-like receptor (TLR) ligands and costimulatory receptor ligands or DC-activation inhibitors (e.g. BAY 11-7082) [6–9]. Inappropriately activated/matured DC may also provoke autoreactive T lymphocytes [10], resulting in autoimmune diseases or allogeneic responses, which limit transplant acceptance [11,12]. We and others are also interested in exploiting the DC antigen-loading receptors to target cytotoxic/immunomodulator reagents to DC *in vivo* to suppress the patients' disease-causing T lymphocyte reactions.

DC are equipped with an array of C-type lectin receptors, which behave as antigen uptake receptors [13]. The prototypical C-type lectin receptors on DC include, macrophage mannose receptor (MMR), dectin-1/β-glucan receptor [14,15], DC-SIGN [16], MGL [17], DCL-1 [18] and DEC-205/CD205 (DEC-205 hereafter) [19,20]. Some have been exploited as targets for DC antigen-loading. For example, the MMR on immature monocyte-derived DC (MoDC) has been targeted with mannosylated or mannnan-conjugated antigens and this elicited antigen-specific immune responses with 100-fold efficiency compared to non-receptor-mediated antigen uptake by macropinocytosis [21,22]. DEC-205 is another member of the MMR family of type I transmembrane C-type lectins expressed on DC [19,20,23–26], whose specific ligand(s) remains unknown. The DEC-205 cytoplasmic domain (CP) contains protein motifs for endocytosis (i.e. tyrosine-based and dihydrophobic amino acid-based internalization signals) and late endosome/MHC II compartment-targeting (i.e. acidic triad), which facilitates efficient antigen-loading to MHC class II molecules for antigen presentation to T lymphocytes. As a consequence, DEC-205 CP-mediated antigen presentation to T lymphocytes is ∼100-fold more efficient than that delivered via the MMR CP, which lacks an acidic triad [27].

Liposomes have emerged as a versatile carrier system to deliver biologically active molecules in humans, however, their further optimization by more specific targeting remains an important research goal [28,29]. Composed of lipid bilayers, liposomes can carry loads of soluble and insoluble antigens and have theoretical advantages for delivering complex mixtures of antigens and activators to DC *in vivo*. Mannosylation of liposomes can improve their uptake by immature MoDC by targeting the MMR [30], however, this approach cannot be used to target BDC as they do not express the MMR [25] and the expression of MMR by interstitial DC remains uncertain [24,26,31]. Furthermore, because mature MoDC decrease their MMR expression [25,32], an alternative strategy is required to target mature MoDC *in vitro*.

To enhance liposome uptake by DC, we chose human DEC-205 (hDEC-205) as a target because: (i) DEC-205 is expressed abundantly by DC in mice and humans [23–26], (ii) mouse DEC-205 (mDEC-205) behaves as an effective antigen uptake and processing receptor [27], (iii) anti-mDEC-205 monoclonal antibody–antigen conjugates injected subcutaneously in mice are taken up specifically by DC and elicit antigen-specific immune responses [6–8] and (iv) anti-mDEC-205 scFv-conjugated liposomes are taken up specifically by mouse DC and elicit immune responses against the antigen enclosed in the liposomes [33]. Although hDEC-205 expression is not restricted predominantly to DC, unlike mDEC-205 [23], both MoDC and BDC express high levels of hDEC-205 [26] and functional cellular specificity is likely to result from their unique antigen presenting cell properties.

2. Materials and methods

2.1. Reagents and antibodies

Egg phosphatidylcholine (EPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1-{8-[4(*p*-maleimidophenyl) butaroylamino]-3,6-dioxaloctyl}-2,3-distearyl glyceryl-*dl*-ether (MPB-TRIG-DSGE) were purchased from Northern Lipid (Vancouver, BC, Canada). The fluorescent lipophilic dye, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), was from Invitrogen (Melbourne, Vic., Australia). *N*-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), dithiothretol (DTT) and, Sepharose CL-4B were from Sigma (Sydney, NSW, Australia). Unconjugated anti-CD14 was from Beckman Coulter Immunotech (clone RMO52, Gladesville, NSW, Australia). Goat anti-mouse IgG MACS beads and a BDCA1$^+$ DC purification kit were from Miltenyi Biotec (North Ryde, NSW, Australia). Unless specified, all other chemicals were of at least reagent-grade obtained from Sigma (Castle Hill, NSW, Australia) or BDH (Poole, England).

Rabbit anti-hDEC-205 serum was produced in house by immunizing a mixture of hDEC-205-Ig fusion proteins covering the whole hDEC-205 extracellular domain [26] in a standard immunization protocol using Freund's adjuvant. The serum was subjected to HiTrap protein A affinity column chromatography (GE Healthcare Bio-Sciences, Rydalmere, NSW, Australia) to purify IgG and its anti-human IgG activity was absorbed by passing the antibody through a human IgG (Intragam, CSL, Parkville, Vic., Australia)-conjugated NHS-activated HiTrap column (GE Healthcare Bio-Sciences). The specificity of the antibody to hDEC-205 and complete absorption of the human IgG activity was confirmed by ELISA assay using a 96-well ELISA plate (Maxsorb, Nalge Nunc International, Rochester, NY)