Frenz, T., Grabski, E., Durán, V., Hozsa, C., Stępczyńska, A., Furch, M., Gieseler, R.K., Kalinke, U. Antigen presenting cell-selective drug delivery by glycan-decorated nanocarriers (2015) European Journal of Pharmaceutics and Biopharmaceutics, 95, pp. 13-17.

Antigen presenting cell-selective drug delivery by glycan-decorated nanocarriers

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Keywords: Dendritic cells, C-type lectin receptor, glycan-functionalized liposomes

Abstract: (237 words)

Text: (4938 words)

Abstract

Targeted drug delivery systems hold promise for selective provision of active compounds to distinct tissues or cell subsets. Thus, locally enhanced drug concentrations are obtained that would confer improved efficacy. As a consequence adverse effects should be diminished, as innocent bystander cells are less affected. Currently, several controlled drug delivery systems based on diverse materials are being developed. Some systems exhibit material-associated toxic effects and/or show low drug loading capacity. In contrast, liposomal nanocarriers are particularly favourable because they are well tolerated, poorly immunogenic, can be produced in defined sizes, and offer a reasonable payload capacity. Compared with other immune cells, professional antigen-presenting cells (APC) demonstrate enhanced liposome uptake mediated by macropinocytosis, phagocytosis and presumably also by clathrin- and caveolae-mediated endocytosis. In order to further enhance the targeting efficacy towards APC, receptor-mediated uptake appears advisable. Since APC subsets generally do not express single linage-specific receptors, members of the C-type lectin receptor (CLR) family are compelling targets. Examples of CLR expressed by APC include DEC-205 (CD205) expressed by myeloid dendritic cells (DC) and monocytes, the mannose receptor C type 1 (MR, CD206) expressed by DC, monocytes and macrophages, DC-SIGN (CD209) expressed by DC, and several others. These receptors bind glycans, which are typically displayed by pathogens and thus support pathogen uptake and endocytosis. Further research will elucidate whether glycan-decorated liposomes will not only enhance APC targeting but also enable to preferentially deliver their payload to discrete subcellular compartments.

Introduction

This review summarizes recent developments in the field of controlled drug delivery systems with special emphasis on the selective targeting of antigen presenting cells (APC). First, we describe currently available nanocarrier systems with a special emphasis on liposomes, then introduce C-type lectin receptors (CLR) as promising target structures for APC targeting, and finally point towards new research directions in the field of APC-selective drug delivery.

Nanocarriers are applied to serve two main functions, i.e., (i) to enhance the lifetime and/or to control the release of an encapsulated active agent, and (ii) to target such agents specifically to selected single cell types whereby reducing adverse effects in irrelevant cell types. Drug-delivering nanoparticles can be produced from lipids, diverse biodegradable polymers, or solid non-biodegradable materials. Non-biodegradable materials comprise metals and ceramics [1], and are generally used only for very specific applications. Polymeric nanoparticles can be composed of gelatin, chitosan, alginate, acrylate (such as polyisohexylcyanoacrylate (PIHCA) [2], polyesters (such as poly (ecaprolactone) (PCL)), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA) matrices [3], or silicon and its oxide [4]. Besides polymeric and liposome-based vectors, combinations of polymeric and liposome-based drug delivery systems are also being assessed [5], i.e., for their ability to target bacterially infected bones [6].

Liposomes are particularly promising nanoparticle carriers. Initially described by Bangham in 1965 [7], it took 30 years until a first non-targeted liposomal drug formulation was approved by the FDA [8]. This first product, Doxil, is a PEGylated liposome-encapsulated formulation of doxorubicin and is used for the treatment of various cancers [9]. Upon intravenous injection of this product, PEGylation and nano-liposomal formulation prolong the drug bioavailability and avoid clearance by the reticuloendothelial system. The enhanced permeability and the retention effect (passive targeting) support effective accumulation of the drug within a tumor [10]. Notably, adverse effects such as cardiotoxicity are reduced upon application of the liposome formulation compared to non-formulated doxorubicin treatment [11,12]. Furthermore, liposomes can be designed to deliver defined amounts of hydrophobic

or hydrophilic active agents to cells [13]. Currently, more than ten liposome-based drugs have been approved for clinical use and many liposome formulations are being scrutinized in different clinical trial stages [14]. In addition to enhanced permeability, another advantage of liposomes is that they can be produced in different, well defined sizes. This point is of particular relevance because nanoparticle size not only influences the bioavailability but also the selective delivery potential towards subcellular compartments. For example, while particles smaller than 5 nm are cleared rapidly from the blood [15], particles larger than several hundred nm may accumulate in organs such as the liver, where they can cause pharmacotoxicity. Besides size, also the shape and lipid composition influences the distribution and uptake of nanoparticles [16-18]. Depending on their physical properties, particles can be taken up by macropinocytosis, phagocytosis (reviewed in [19]) or by clathrin- and caveolae-mediated endocytosis [20]. Details of the mode of endocytosis are important since they determine the trafficking pathway through subcellular compartments [21]. For example, lysosomal compartments are targeted *via* clathrin-mediated but not caveolin-mediated endocytosis [22]. Additionally, there are still some obstacles mainly concerning the manufacturing process such as sterilization procedures and stability issues [23].

The concept of cell-selective drug delivery

Targeting of single cell subsets or of specific tissues holds promise to tremendously advance therapeutics by minimizing adverse effects while simultaneously increasing therapeutic effects. In order to therapeutically modulate immune responses, targeting APC is of particular interest. APC comprise recirculating monocytes, recirculating as well as tissue-resident dendritic cell (DC) subsets, and tissue-resident macrophage subsets [24]. Numerous *in vivo* approaches have been investigated for delivering active agents such as toxins, antigens, adjuvants, macromolecules, and nucleic acids in order to achieve immunopreventive (vaccination), immunomodulatory (tolerance), or immunotherapeutic effects. The most obvious way to target a specific APC subset would be to decorate nanocarriers with antibodies specifically binding cell type-restricted surface receptors. Unfortunately, especially DC and macrophages express only few if any lineage-specific surface markers. Promising lineage markers such as blood dendritic cell antigen 1, 2, and 4 (BDCA1-4), and XCR1 were found to be expressed also on several different tissues and rather designate highly specialized

subpopulations of DC, which need to be further characterized, than conventional DC [25-28]. Furthermore, antibody-dependent targeting approaches can either affect cell functions (as exemplified for an BDCA2-specific monoclonal antibody [26]) or alter receptor expression (as exemplified for a DC-SIGN-specific monoclonal antibody targeting [29]). Moreover, monoclonal antibodies may cause immunotoxicity, initiate anti-idiotypic antibody responses, and the immune complexes thus formed may cause vascular and renal pathologies (reviewed in [30]). Immunogenicity of antibodies may vary depending on the way and route of administration, the frequency of administration, the dosage of antibody, the patients' disease status, the patients' immune status, the patients' MHC haplotype, etc. (reviewed in [31]). Thus, there are several lines of evidence that monoclonal antibodies may cause adverse effects making it very unlikely that antibodies coupled to liposomes would not do so.

Another APC-targeting approach is based on heat-shock proteins. This approach leads to major histocompatibility complex (MHC)-restricted peptide presentation and antigen-specific T-cell priming (reviewed in [32,33]). However, the expression of heat-shock protein receptors is rather broad across cellular subsets and their immunomodulatory role is only partially understood [33], which increases the risk for undesired effects when employing heat-shock proteins as targeting moieties.

APC subsets express different combinations of Toll-like receptors (TLR). TLR are pattern recognition receptors which are triggered by diverse pathogen-associated molecular patterns. Nanocarriers decorated with anti-TLR antibodies can specifically address single APC subsets. This way, it has been shown that TLR-directed targeting can deliver antigenic peptides *via* endosomes to MHC molecules whereupon being presented in order to induce antigen-specific T cell responses [34]. However, TLR triggering is associated with the risk to also trigger undesired APC activation independent of the actual payload. Besides TLR there are other types of pattern recognition receptors that are specifically expressed by APC; these might be useful as well and may not be burdened with the potential risks associated with TLR engagement. One particularly promising group of candidates is the CLR family.

CLR function for pathogen uptake and APC triggering.

CLR are a large family of carbohydrate receptors, which are abundantly expressed by antigen-presenting cells (APC). Some CLR are expressed as transmembrane molecules (with either endocytic or non-endocytic potential), while some exist as soluble proteins that serve as opsonins, namely the collectins including the mannose-binding-protein (MBP) and surfactant protein A and B [35].

CLR are characterized by the presence of a CLR-like domain (CTLD) and can be subdivided into the 'classical' and the 'non-classical' CLR [36]. Members of the classical CLR family contain structurally conserved carbohydrate-recognition domains (CRD) that bind glycan structures in a calcium dependent manner [18,37,38]. CRD feature two highly conserved disulfide bonds, up to four calcium binding sites, and conserved amino acid residues, which directly bind to carbohydrate residues in the presence of calcium (reviewed in [39,40]). The non-classical CLR lack residues in the CTLD that are involved in calcium binding and some of these receptors, e.g. Clec9a (DNGR1), do even recognize non-sugar ligands such as actin filaments of damaged cells [41]. Independent of classical or non-classical features, type I and type II CLR are distinguished based on the protein orientation within the membrane, while all type II CLR identified so far have only one CRD [42]. Type I CLR comprise receptors such as DEC-205 and MR, whereas type II CLR include Dectin 1 and 2, DC-SIGN and others. Although different CLR share a high degree of structural homology, single CLR members typically bind different glycans with high affinity. Notably, CLR are capable of recognizing glycans displayed by microbes or by damaged cells, and they interact with oxidized lipids and other self-alterations indicative of abnormality [43].

CLR are differentially expressed by different DC subsets (reviewed in [42]). The cell subset specificity of CLR expression ranges from receptors such as DC-SIGN, which is expressed by many different DC subsets, to receptors such as BDCA-2 which is found only on plasmacytoid dendritic cells. Nevertheless, a detailed analysis of CLR expression profiles of human APC derived from blood, lymphoid organs and peripheral tissues is not yet available. The CLR expression of tissue-resident APC is of particular interest, since evidence indicates that, depending on the cellular environment, DC can adopt variable CLR expression profiles [44]. Many receptors of

the mannose receptor family contain multiple CTLDs from which some are predicted to bind sugars such as mannose, fucose, N-acetyl glucosamine, and sulphated sugars. Thus, those sugars might be suitable to target a wide range of different APC lineages by a natural ligand without affecting their function by antibody binding [45]. Moreover, DC-SIGN expressing DC could be addressed specifically using multiple viral glycoproteins such as HIV-gp120, or human herpes virus 8 (HHV8) gpB as targeting anchors [46,47]. Nevertheless, for some CLR (e.g. DEC-205) natural ligands are not identified, yet [45].

Different CLR expressed by APC either only support phagocytosis or also confer signal transduction directly *via* the Syk-Card9 pathway or by modulating TLR signaling [48-51], reviewed in [40]. CLR such as Dectin-2 and Mincle signal *via* classical immune receptor tyrosine-based activation motifs (ITAM), whereas DCIR and MICL contain immune receptor tyrosine-based inhibitory motifs (ITIM), which recruit phosphatases such as SHP-1 and SHP-2. In contrast, the ITAM/ITIM-independent CLR family members DEC-205, MR, and DC-SIGN do not feature intracellular signaling motifs and thus do not trigger Syk or SHP-dependent pathways [18].

Intriguingly, some pathogens exploit CLR for immune cell entry. For example, the APC-specific CLR DC-SIGN is recognized by HIV gp120, which leads to *trans*-infection of primary human T cells [52,53]. Furthermore, DC-SIGN acts as a capture and/or attachment molecule for avian H5N1 influenza virus and thus supports infection of macrophage cell lines [54]. For Dengue virus (DENV) it was shown that immune complexes formed by immature DENV particles and antibodies are internalized *via* the DC-specific receptor DC-SIGN [55,56]. DENV particles are immature and as such are not able to enter cells. Upon binding of anti-DENV antibodies to DENV, the immune complex is targeted to dendritic cells, and upon uptake of the immune complex DENV can initiate replication. DC-SIGN also plays a central role for the transmission of Ebola virus. B cells expressing DC-SIGN are susceptible to low levels of Ebola glycoprotein-mediated infection because DC-SIGN can promote B cell attachment of Ebola virus and thus boosts viral entry into target cells [57]. Thus, blocking of DC-SIGN is a potential strategy to inhibit uptake of various viral infectious agents by DCs [58,59], reviewed in [60]. Indeed, glycol-dendri-

protein-nanocarriers binding to DC-SIGN were recently shown to prevent Ebola virus infection of T cells and DC [61]. These findings imply that besides blocking viral entry, liposomes targeting CLR such as DC-SIGN may be suitable to explore new vaccination strategies or to deliver newly developed antiviral or antibacterial drugs to the appropriate target cells.

Exploitation of CLR for APC-selective drug delivery

CLR binding of pathogen-associated carbohydrates is important for pathogen uptake by APC, and the involvement of different CLR may differentially direct antigens to certain compartments. While MR was shown to primarily deliver antigen to the early endosome and to confer re-shuttling to the surface [62,63], DEC-205 and DC-SIGN direct antigen to late endosomes and lysosomes where they are recycled or degraded [64,65]. Thus, antigens targeted to DEC-205 and DC-SIGN are directly linked to antigen processing and presentation on MHC class II. Moreover, DC-SIGN targeting by treatment with loaded dendrimers or liposomes resulted in the induction of robust $CD8^+$ T cell responses. This result indicated that DC-SIGN targeting also shuttled the antigen to a cross-presenting pathway which resulted in peptide presentation on MHC class I molecules [59,66,67]. This ability of CLR to enhance MHC I and/or MHC II presentation of antigenic protein fragments, make them promising targets for vaccine development [68-71]. For example, the fact that CLEC9a (DNGR1) recruits Syk after ligand binding is an essential event in the induction of cross-presentation [72]. In contrast, targeting ITAM/ITIM-independent CLR by liposomal formulations holds promise to specifically deliver agents into endo-/lysosomal APC compartments without stimulating strong immune responses.

Considering the efficient uptake of non-targeted liposomes into APC subsets [73], it is tempting to speculate whether the glycan targeting-ligand confers another type and/or route of internalization when compared with non-targeted liposomes. Thus, instead of direct membrane fusion or internalization *via* phagocytosis (macrophages) or macropinocytosis (DCs), receptor-mediated uptake of functionalized liposomes might rather direct the payload to the endo-/lysosomal compartments (Figure 1). Moreover, it may be possible that targeting of different CLR will direct nanocarriers decorated with glycan targeting-ligands to different subcellular compartments. Verifying such a dependency would offer most interesting therapeutic options. For

example some infectious agents accumulate preferentially in certain intracellular compartments [22], which then could be specifically addressed [74]. Indeed, and as already mentioned above, MR, DEC-205, and DC-SIGN may deliver active agents selectively into the endo-/lysosomal pathways [62-65]. Thus, glycan-mediated liposomal targeting needs to be investigated further at the subcellular level, which should also include differential pathway analyses.

Conclusions

It is tempting to speculate that receptor-mediated uptake may direct liposomally encapsulated payloads to discrete subcellular compartments within APC. Hence, CLR-directed liposomal targeting offers two major advantages in that (i) liposomes specifically address APC and thus minimize adverse effects that may result from unspecific payload delivery, and (ii) ITAM/ITIM-independent CLR-targeting hardly activates APC and thus minimizes adverse effects which might be associated with cytokine responses derived from the target cells. Future research is needed to better determine whether indeed glycan-decorated functionalized liposomes deliver their payloads to specific subcellular compartments.

Acknowledgments

This work was supported by the “Zentrales Innovationsprogramm Mittelstand” program [Grants KF3134203AJ3 (to TF, EG and UK) and KF2531702AJ3 (to CH, AS, MF and RKG)] by the Federal Ministry of Economics and Technology (BMWi), Germany, *via* the Consortium of Industrial Research Associations.

Conflict of interest statement

Rodos BioTarget holds patents or patent applications related to targeted drug delivery (TDD) systems. MF and RKG are general managers, and CH and AS are employees of Rodos BioTarget. TF, EG, VD and UK do not have financial interests.

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Figures

Fig. 1: Potential routes of (functionalized) liposomal uptake by APC. Liposomes are efficiently internalized by macrophages (Mϕ), monocytes (M), and dendritic cells (DC). Depending on the presence of glycan targeting-ligands on the surface of liposomes, the involved internalization pathways may differ. Non-targeted liposomes could be internalized through macropinocytosis (1) or direct membrane fusion (2), whereas glycosylated liposomes may be taken up additionally and/or preferentially through CLR-mediated endocytosis (3), entering endo-/lysosomal pathways to the endoplasmic reticulum (ER). Depending on the uptake pathway, the subsequent intracellular processing may differ.