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Research Paper

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

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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 favorable 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 (APCs) 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 toward APCs, 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 APCs 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 APCs targeting but also enable preferential delivery of their payload to discrete subcellular compartments.

1. 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 (APCs). First, we describe currently available nanocarrier systems with a special emphasis on liposomes, then introduce C-type lectin receptors (CLRs) as promising target structures for APC targeting, and finally point toward 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 thus 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].

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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 influences not only the bioavailability but also the selective delivery potential toward 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 influence 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].

1.1. 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 APCs is of particular interest. APCs 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, and the patients' MHC haplotype (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 (TLRs). TLRs 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 TLRs there are other types of pattern recognition receptors that are specifically expressed by APCs; 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.

1.2. CLR function for pathogen uptake and APC triggering

CLRs are a large family of carbohydrate receptors, which are abundantly expressed by antigen-presenting cells (APCs). Some CLRs 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].

CLRs are characterized by the presence of a CLR-like domain (CTLD) and can be subdivided into the 'classical' and the 'non-classical' CLRs [36]. Members of the classical CLR family contain structurally conserved carbohydrate-recognition domains (CRDs) 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 CLRs 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 CLRs are distinguished based on the protein orientation within the membrane, while all type II CLRs identified so far have only one CRD [42]. Type I CLRs comprise receptors such as DEC-205 and MR, whereas type II CLRs include Dectin 1 and 2, DC-SIGN and others. Although different CLRs share a high degree of structural homology, single CLR members typically bind different glycans with high affinity. Notably, CLRs 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].

CLRs are differentially expressed by different DC subsets (reviewed in [42]). The cell subset specificity of CLR expression