Rational Design of Targeted Next-Generation Carriers for Drug and Vaccine Delivery

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Abstract

Pattern recognition receptors on innate immune cells play an important role in guiding how cells interact with the rest of the organism and in determining the direction of the downstream immune response. Recent advances have elucidated the structure and function of these receptors, providing new opportunities for developing targeted drugs and vaccines to treat infections, cancers, and neurological disorders. C-type lectin receptors, Toll-like receptors, and folate receptors have attracted interest for their ability to endocytose their ligands or initiate signaling pathways that influence the immune response. Several novel technologies are being developed to engage these receptors, including recombinant antibodies, adoptive immunotherapy, and chemically modified antigens and drug delivery vehicles. These active targeting technologies will help address current challenges facing drug and vaccine delivery and lead to new tools to treat human diseases.

Contents

1. INTRODUCTION

Drugs and vaccines that can direct their action to a specific target on cells can increase their efficacy while reducing their toxicity. The so-called rational design of drug and vaccine delivery systems would involve targeting specific receptors that can trigger signaling events and/or enhance cellular internalization, leading to improved efficacy. For example, pattern recognition receptors (PRRs) are excellent targets for the rational design of immunomodulatory vaccines because they play an integral role in innate host defense by recognizing conserved pathogen-associated molecular patterns (PAMPs). Similarly, folate receptor (FR) upregulation in tumor cells and activated macrophages (MΦs) provides opportunities for targeted delivery of cancer therapeutics. Targeted therapeutics offer several benefits, including cost effectiveness, dose sparing, immune modulation, and low toxicity. Drugs that target specific cells help maximize drug concentration in specific tissues while avoiding a systemic response, thereby reducing toxicity (1).

This review provides a comprehensive discussion of the interlinked roles of materials science and nanotechnology in the rational design of next-generation drug and vaccine delivery vehicles. The article reviews various target receptors and discusses how ligands and carrier vehicles could be designed to enable efficient drug or antigen delivery to cells. The review outlines the structure, functions, expression patterns, and unique properties of various PRRs. A fundamental understanding of how these receptors function is essential in order to exploit their properties to rationally design and engineer targeted therapies, which are discussed in Section 2. The article concludes by showing how the insights discussed can be harnessed to enhance the efficacy of antibiotics, antivirals, vaccines, cancer drugs, and neural drugs.

2. ACTIVELY TARGETED RECEPTORS

We begin with a summary of various PRRs, their receptor mechanisms, and their functionality in order to rationally design drugs and vaccines for desired physiological outcome(s). This section

reviews the properties and signaling mechanisms of the major types of PRRs, including C-type lectin receptors (CLRs), Toll-like receptors (TLRs), and FRs.

2.1. C-Type Lectin Receptors

Most CLRs are membrane-bound proteins that contain one or more carbohydrate recognition domains (CRDs) for binding to carbohydrates. With more than 60 types of receptors, CLRs mediate host biological events and contribute to the innate immune response (2). The structure and mechanisms of several CLRs are discussed next.

The macrophage mannose receptor (MMR) has eight CRDs located sequentially on one polypeptide chain. The MMR is most efficient at recognizing single or low-order mannose moieties present on yeast, fungi, viruses, and many bacteria, including *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* (3). The MMR is usually expressed on immature dendritic cells (DCs), tissue MΦs, and some epithelial cells, with low levels of expression in circulating monocytes (**Table 1**) (4). It is an endocytic receptor that internalizes its ligand through coated pits. An example of its role in the immune response is the induction of maturation in human peripheral blood mononuclear cells, leading to a strong T helper 17 (Th17) adaptive response by mannan derived from *Candida albicans* (5). Researchers have observed that blocking MMR expression on DCs with small interfering RNA (siRNA) inhibited T cell activation, preventing $CD4^+$ cells from producing interleukin-17 (IL-17) and interferon-γ (IFN-γ). There is increasing evidence that the MMR has a dual role both as an important PRR for the innate immune system and in homeostasis by the clearance of endogenous ligands (**Table 1**) (6).

Another soluble CLR is the mannose-binding lectin (MBL). Each single-polypeptide chain has one CRD, but three chains come together to form a collagen helix, resulting in a single structure with three CRDs at its tip. These structures can further form multimers containing different numbers of structural subunits (7). Like the MMR, the MBL has specificity for mannose, *N*-acetylglucosamine (GlcNAc), and fucose (**Table 1**). Each CRD binds with relatively low affinity; ultimately, it is the clustering of multiple CRDs that increases MBL avidity. The MBL binds to bacterial peptidoglycan and fungally derived β-glucans (8). This process induces the binding of MBL-associated serine proteases to promote the activation of the lectin pathway of the complement system (8, 9).

Another CLR used for targeting is DEC-205. It does not have a signaling motif on its cytoplasmic tail; however, it has two separate motifs that influence endocytosis and intracellular trafficking (10). These include a tyrosine-based motif and a triacidic cluster. The triacidic cluster may explain why DEC-205 transports its ligand to late endosomes, whereas MMR trafficking is mostly restricted to early endosomes (11). As a result, DEC-205 is more efficient at presenting antigen than is MMR (11). In humans, DEC-205 is readily expressed on DCs, particularly myeloid DCs; is expressed at moderate levels on B cells; and is expressed at low levels on T cells and natural killer cells (**Table 1**) (12). Unlike the MMR, where DC expression is downregulated upon maturation, DEC-205 expression is upregulated, suggesting an important function in mature DCs (13). Ligands taken up by DEC-205-mediated endocytosis present antigen on major histocompatibility class (MHC) I and MHC II, including cross-presentation (14, 15).

Dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN) is a CLR expressed on immature DCs in peripheral tissues, such as the skin, gut, and lung, and its expression is downregulated upon maturation (**Table 1**) (16). As a PRR, it recognizes high-mannose structures on surface glycoproteins of bacteria, fungi, and viruses, including gp120 on the human immunodeficiency virus (HIV) and glycoprotein E on the dengue virus (17). DC-SIGN has one CRD that is used for antigen recognition, cell transport across epithelial

Table 1 Downstream physiological responses induced by PRR ligands

PRRs	Receptor	Ligands	Cellular expression	Desired physiological response	Reference(s)
CLRs	MΦ mannose receptor	Lower-order mannoses	Immature DCs, tissue MΦs	Ligand clearance, antigen presentation, or homeostasis	2, 4
	Mannose-binding lectin	Mannose, *N*-acetylglucosamine, β-glucans	Secreted by liver	Complement activation, cellular phagocytosis	9
	DEC-205	Unknown	DCs, Langerhans cells, B cells	Antigen cross-presentation	11, 15
	DC-SIGN	Higher-order mannoses, Mac-1	Immature DCs, tissue MΦs	Ligand clearance, antigen presentation, or homeostasis	2
	Dectin-1	β-Glucans	Alveolar MΦs, neutrophils	Th17 cell differentiation	20, 22
TLRs	TLR4	Lipopolysaccharides, monophosphoryl lipid A, glucopyranosyl lipid A	Monocytes, myeloid DCs, M cells	Th1 polarization	31
	TLR2–TLR1 complex	Pam_2CSK_4, peptidoglycan, zymosan	Monocytes, myeloid DCs, T cells	High antibody titers, Th2 polarization	26, 41
	TLR2–TLR6 complex	Pam_2CSK_4, peptidoglycan, zymosan	Monocytes, myeloid DCs, T cells	High antibody titers, Th2 polarization	26, 41
	TLR5	Flagellin	DCs, epithelial cells	Activation of inflammasomes	40
	TLR3	Poly(I:C), poly(ICLC), dsRNA	DCs	DC cross-presentation	39
	TLR9	CpG DNA	Plasmacytoid DCs, B cells	B cell class switching, Th1 polarization	25, 156
	TLR7	ssRNA, imiquimod, resiquimod	Plasmacytoid DCs, B cells	B cell class switching, Th1 polarization	42, 43
	TLR8	ssRNA, resiquimod	Circulating monocytes	Acute inflammatory response	42
	TLR11	Flagellin, profilin	MΦs, DCs, epithelial cells	Th1 polarization	157
FRs	FRα	Folic acid, folate derivatives	Tumor cells	Targeting of chemotherapeutic payloads, diagnostics	53
	FRβ	Folic acid, folate derivatives	Activated MΦs	Transport across BBB, delivery of anti-inflammatory drugs	54

Abbreviations: BBB, blood–brain barrier; CLR, C-type lectin receptor; DC, dendritic cell; DC-SIGN, dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin; dsDNA, double-stranded DNA; FR, folate receptor; MΦ, macrophage; PRR, pattern recognition receptor; ssRNA, single-stranded RNA; Th1, T helper 1; TLR, Toll-like receptor.

barriers, and interaction with other cells such as neutrophils. Studies have shown that DC-SIGN single-polypeptide chains form tetramers allowing each structure to present four CRDs (18). DC-SIGN has a cytoplasmic tail consisting of three motifs, which include the tyrosine-based motif, a triacidic cluster, and a dileucine motif. By inserting point mutations into each motif individually to determine its function (19), investigators found that mutating the triacidic cluster significantly reduced the surface expression of DC-SIGN and led to a 90% reduction in the phagocytosis of mannosylated beads. Cells with the mutated dileucine motif had a small defect in phagocytosis and tended to retain antigen on the surface, suggesting a role in internalization (19). Mutations of the tyrosine motif had little effect on DC-SIGN expression or function. These studies have shown that the triacidic motifs are essential for DC-SIGN surface expression and play an important role in the uptake of antigen to the late endosome for fusion with the lysosome.

Dectin-1 is a phagocytic PRR for fungal β-glucans and is expressed predominantly on DCs, MΦs, and neutrophils (**Table 1**). In particular, it is expressed on splenic MΦs, Kupffer cells, and MΦs in the lamina propria of the gut villi (20). The cytoplasmic tail of Dectin-1 is unique to CLRs in that it has an immunoreceptor tyrosine-based activation motif (ITAM) that allows it to transduce signals into the cell and mediate internalization (21). Dectin-1 activates innate immunity through Syk kinase signaling, resulting in the production of reactive oxygen species and inflammasome formation. Additionally, Dectin-1 influences the adaptive immune response by inducing IL-1β and IL-23p19 secretion, which polarizes T cells toward a Th17 response (**Table 1**) (22).

In summary, CLRs are diverse in their structure and intracellular signaling domains, allowing them to endocytose a variety of different carbohydrates and induce a number of downstream functions that include antigen presenting, scavenging ligands, mediating homeostasis, and influencing the adaptive immune response (**Table 1**). This diversity makes the resulting response of targeting these receptors difficult to predict. It is also interesting to note that many CLRs have multiple CRDs, suggesting that cross-linking could affect the type of response generated.

2.2. Toll-Like Receptors

TLRs are essential PRRs of the innate immune system. Although they are not as diverse as CLRs, they are highly conserved across multiple species. A total of 10 TLRs have been identified in humans and 12 in mice (23). Their main function is in signal transduction to induce the release of inflammatory cytokines or type 1 IFNs that can stimulate the adaptive response. TLRs are transmembrane proteins expressed on the surface of the plasma membrane or within endosomes. TLRs that recognize PAMPs are located on the plasma membrane, whereas those that recognize nucleic acids are expressed on the endosome surface (24). Intracellularly, each TLR has a single Toll/interleukin-1 receptor (TIR) domain, similar to that of the IL-1 receptor, that allows for downstream signaling. The extracellular portion contains leucine-rich horseshoe-shaped repeats. Monocytes and myeloid DCs express TLR2 and TLR4, whereas plasmacytoid DCs express TLR7 and TLR9 in greater abundance (**Table 1**). They are expressed at very low levels in B cells; however, TLR9 and TLR10 are upregulated when the B cell receptor is triggered (25). Additionally, TLRs are expressed on memory B cells (25). There is evidence that TLR2 is highly expressed on activated human T cells and memory T cells, suggesting that it has a role both as a costimulatory receptor of T cells and in the maintenance of memory T cells (**Table 1**) (26).

The best-known TLR is TLR4 because it is triggered by gram-negative bacterial lipopolysaccharide (LPS). LPS induces a proinflammatory response through a receptor complex composed of MD-2 and TLR4, ultimately triggering the MyD88-dependent pathway, the activation of nuclear

factor κB (NF-κB) or TIR-containing adaptor molecule (TRAM), and the activation of interferon response factor 3 (IRF3) (27). TLR4 activation is important in generating a protective immune response against pathogens (28–30), but overstimulation of TLR4 can have adverse consequences and potentially lead to sepsis. For example, fever and seizures have been reported with the pertussis vaccine because it triggers TLR4 (29). Other TLR4 agonists include the vaccine adjuvant monophosphoryl lipid A and a number of heat shock proteins (31, 32).

TLR2 is important in the host response against gram-positive bacteria and yeast because it binds a wide array of peptidoglycans, zymosan, and lipopeptides (**Table 1**). It is unique in that it does not form homodimers with itself, but instead forms heterodimers with either TLR1 or TLR6 in order to initiate a signaling cascade (33). TLR2 signals through the MyD88 adaptor protein only when the Mal and MyD88 adaptor proteins bind to the TIR domains on the heterodimer.

Flagellin, originating from filaments on the flagella of proteobacteria, binds to TLR5 and triggers the assembly of a homodimer, enabling MyD88-dependent signaling through the TIR domains (34). Specifically, TLR5 binds to conserved regions on flagellin that are essential to the bacteria's ability to form a filament, allowing TLR5 to recognize the flagellin of many types of gram-negative bacteria, including common pathogenic β- and γ-proteobacteria (35).

TLR3 and TLR9 are endosomal TLRs. TLR3 recognizes double-stranded RNA (dsRNA) and plays an important role in the immune response against viruses, whereas TLR9 responds to unmethylated bacterial DNA (**Table 1**). Poly(I:C) is the standard dsRNA immunostimulant that interacts with TLR3, but it is toxic; therefore, modified versions with reduced toxicity, such as poly(ICLC), are often used (36). This receptor interacts with the sugar and phosphate backbones rather than the nucleotides of dsRNA, which is why it is not sequence specific (37, 38). Studies have utilized TLR3 and TLR9 ligands to induce balanced immune responses in order to reduce Th2-dependent pathways (39).

TLRs are unique in terms of the homogeneity of their structure and signaling. They signal through either MyD88-dependent or MyD88-independent pathways, each of which eventually activates NF-κB and mitogen-activated protein kinase (MAPK) transcription factors (40). Interestingly, each TLR elicits a unique cytokine milieu to drive a unique immune response, even though the signaling pathways are mostly the same (41, 42). For example, both TLR9 and TLR2 signal via MyD88-dependent pathways, yet TLR2 drives a more robust Th2 immune response, whereas TLR9 induces a stronger Th1 immune response (**Table 1**). This finding shows that an organism can control the prevailing immune response not only through signaling but also by receptor expression across different cell types. For example, even though TLR9 and TLR2 signal through the same pathway, TLR9 is strongly upregulated in plasmacytoid DCs, which are known to be strong inducers of type I IFNs and would more likely trigger a Th1 immune response (**Table 1**) (43). TLR2, by contrast, is expressed mostly in myeloid DCs.

Unique immune responses could also be elicited from signaling redundancy with other PRRs. For example, some Nod-like receptors (NLRs) have the same ligands as TLRs. NLRs are known to recognize flagellin as well as single-stranded RNA (ssRNA), which could contribute to different signals to drive a unique immune response (44). This observation indicates that PRRs can signal together to result in a synergistic immune response (i.e., costimulation). An example of CLR/TLR collaboration is the cooperation between DC-SIGN and TLR4 in recognizing glycolipids of *Schistosoma mansoni* (45). Blocking of DC-SIGN on the DCs or defucosylation of the lipid prohibited the cells from binding to the lipid, and blocking TLR4 interfered with DC maturation (45). Without DC-SIGN, cells struggled to induce TLR4 activation. In the CLR/TLR relationship, the CLRs are responsible for binding PAMPs, which influences TLR signaling, which in turn ultimately determines the direction of the immune response (45).

2.3. Folate Receptors

FRs are glycoproteins that recognize folic acid and its reduced derivatives. There are four types of FRs, namely α, β, γ, and δ (46). The α and β isoforms are linked to the cell surface through a glycosylphosphatidylinositol (GPI) anchor that extends out from the plasma membrane of eukaryotic cells (47). The GPI anchor has no cytoplasmic domain, but the FR can still participate in receptor-mediated endocytosis upon binding to its ligand (47). Endocytosis is essential for getting folates to the cytoplasm because they are important for the synthesis of nucleotide bases and amino acids. FRs are typically expressed in low quantities in healthy tissues but at extremely high levels in tumor cells and activated MΦs (48). In tumor cells, this high demand for folate is due to the rapid proliferation of the cells, creating excellent opportunities to design FR-targeted delivery systems for cancer. Moreover, healthy tissues that express FRs are not accessible by blood, further reducing the possibility of systemic toxicity with intravenously delivered FR-targeted drugs (49). For example, in the kidney, FRs are located only in the tubule lumen, where urine filtrate collects so that folic acid can be recovered before it is excreted. Similarly, FRs exist only on the brain side of the blood–brain barrier (BBB) (49).

Mammalian cells have two other ways to import folic acid, which cannot cross the plasma membrane by diffusion. The first uses a transmembrane protein called the reduced folate carrier (RFC), which allows folates to flow in and out of the cytoplasm, driven by a folate/organic phosphate exchange (50). The second uses the proton-coupled folate transporter (PCFT), which is most prevalent in the epithelium of the small intestine, where it absorbs folic acid at low pH (50, 51). The absorption of folic acid by the PCFT is driven by a sodium–hydrogen exchanger protein that operates most efficiently at a pH of 5.5 (48). The PCFT is also overexpressed in many solid tumor cell lines but is not expressed in leukemia cells (52).

Yet of the three folate transport mechanisms, FRs have the highest affinity for folic acid. FRα is most commonly observed on tumor cells, whereas FRβ is overexpressed on activated MΦs (**Table 1**) (53, 54). The two FRs have approximately 80% primary sequence homology and recognize the same folate derivatives. FRα is overexpressed in ovarian, kidney, and breast cancers (52). Approximately 82% of human tumors are FR positive in ovarian cancer, and 74% and 56% of human tumors are FR positive for kidney and breast cancers, respectively (52). Additionally, the density of the FR can indicate the stage of cancer, as late-stage cancers overexpress FRα (49). This overexpression provides opportunities not only for therapeutic targeting but also for diagnosis (55, 56). In contrast, FRβ is overexpressed on activated MΦs, as evidenced by MΦs extracted from fluid in the joints of patients with rheumatoid arthritis (57). FRβ targeting can deliver folates and their conjugates to activated MΦs.

FRs consist of a globular head with a deep binding pocket, as determined by crystallography (58). The aromatic moieties are buried in the binding pocket and mediate many hydrophobic interactions. The glutamate moiety protrudes from the binding pocket and can easily be conjugated to carry a payload without inhibiting binding (58). Antifolates also bind to the FR in a similar configuration as folic acid. These drugs are imported to the cytosol through the same mechanisms as folates, but they then block enzymes responsible for generating nucleotide bases for DNA synthesis. Many antifolates, including methotrexate, raltitrexed, and pemetrexed, have been approved for clinical use against tumors and as anti-inflammatory drugs (48). All three drugs can be imported via the RFC in addition to the FRs (50). Upon binding, the ligand–FR complex is taken up into an endosome, whereby the ligand undergoes pH-dependent dissociation from the FR (46, 59). Antifolates bind to FRs in a pH-dependent manner with less affinity than folic acid (59). This observation supports the idea that FRs tend to be recycled and returned to the surface

of the plasma membrane once they dissociate from their ligand, while the ligand is able to escape to the cytosol.

An understanding of how folates interact with FRs can aid in the development of targeted drugs that could potentially bind FRs with higher affinity or dissociate from the FRs and escape to the cytosol more effectively. From a rational-design standpoint, folic acid conjugates are attractive because they are small, water soluble, and nonimmunogenic and have high affinity for the target receptor (49).

3. TARGETING MECHANISMS

Targeting mechanisms can be broadly classified as passive or active. In passive targeting, the drug does not have a target ligand and its efficacy depends only on its physicochemical properties and its interactions with the circulatory system or at tissue sites (60). Active targeting employs a ligand that is conjugated to the drug, allowing that drug to bind to a specific site on a cell or tissue (**Figure 1**). Active targeting is advantageous because it increases efficacy by enabling higher accumulation of the payload in the tissue. Simultaneously, it provides dose sparing, which limits drug exposure to off-target tissue (60). Active strategies that commonly target PRRs for drug and vaccine delivery are discussed below.

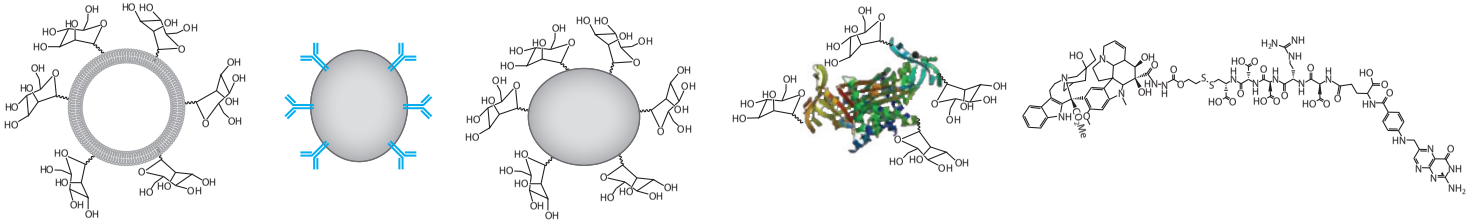
3.1. Antibodies

The strong binding and specificity of antibodies make them ideal for targeted delivery to cells. In the development of antibody-targeted vaccines, CLRs are attractive targets because of their endocytic capacity and their high expression levels on immature DCs. An antibody-conjugated vaccine that has undergone clinical trials is Lipovaxin-MM (created by Lipotek), composed of a single-domain antibody fragment (either a single V_H or a single V_L domain) that targets DC-SIGN for the delivery of a lipid-based vaccine that coencapsulates melanoma antigens and IFN-γ (60). In a separate study, ovalbumin (Ova)-conjugated antibodies were generated using a sulfo-SMCC [succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate] cross-linking agent and targeted to DC-SIGN in vivo, leading to persistent $CD4^+$ and $CD8^+$ T cell responses (**Figure 1**) (61). However, a CD40 agonist was required to protect mice from Ova-expressing *Listeria monocytogenes*. Other antibody vaccines in the literature have targeted MMR, Langerin, and DEC-205 (62, 63).

In addition to CLRs, antibodies conjugated with antigen have been targeted to other receptors on antigen-presenting cells (APCs), such as Fc receptors. The payload is usually conjugated to the Fc fragment, which is then recognized by the Fc receptor. Fc receptors are attractive targets because most of them contain ITAMs, which induce proinflammatory signals (64). Fc-conjugated drugs activate $CD4^+$ T cells and enhance antigen-specific humoral and cellular immunity (65, 66). Additionally, integrins such as CD11c have been targeted by antibody-conjugated drugs to

Figure 1

Chemical conjugation techniques to link ligands and antibodies to delivery vehicles, antigen, or drugs. Ligands and antibodies can be chemically linked to delivery vehicles, antigen, or drugs in order to achieve targeting. (*a*) The intermediates 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysulfosuccinimide (sulfo-NHS) link a carboxylic acid to an amine. (*b*) The heterobifunctional linker 4-(4-*N*-maleimidophenyl)butyric acid hydrazide (MBPH) links sulfhydryl and carbonyl moieties. (*c*) Streptavidin links noncovalently to biotin moieties. Streptavidin self-assembles into tetramers to bind up to four biotins, allowing multivalent linking. (*d*) The heterobifunctional linker succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) links amine and sulfhydryl moieties. (*e*) The homobifunctional linker glutaraldehyde links two primary amines.



Micelles	Nanoparticles	Antigen	Drugs
a Carboxylic acids to primary amines	EDC	Sulfo-NHS	
b Carbonyl to a sulfhydryl	MBPH		
c Coupling through streptavidin and biotin interactions	Streptavidin	Biotin	
d Primary amine with a sulfhydryl	SMCC		
e Two primary amines	Glutaraldehyde		

ensure that antigens reach APCs (67). CD11c targeting has been reported to induce effective T cell responses (68).

In addition to conjugating antigen directly to the antibody, there are many preclinical studies utilizing antibody-targeted nanomedicines. These antibody-targeted nanomedicines have been used for a broad range of treatments for infections, central nervous system (CNS) disorders, and inflammatory disorders (60). To date, the majority of antibody-targeted nanomedicines have been designed to treat solid tumor cancers (60). Solid tumors are characterized by having enhanced permeability and abnormal fluid transport, allowing macromolecules, such as nanomedicines, to more easily accumulate within the tumor. This process is known as the enhanced permeability and retention effect (EPR), and it has made targeted nanomedicines a particularly attractive technology for tumor treatment. These nanomedicines could be liposomes or polymeric nanoparticles, could contain targeting moieties on their surface, and could contain traditional anticancer payloads such as paclitaxel, docetaxel, or doxorubicin (60).

A number of monoclonal antibodies against cancer are not conjugated to any payload, but rather provide an immunostimulatory approach to treat cancer. For example, rituximab (Biogen) is an approved anti-CD20 antibody for use against B cell lymphomas. Antibody binding helps stimulate antibody-dependent cellular cytotoxicity (ADCC) and apoptosis. Receptors such as the transferrin receptor or epidermal growth factor receptor are often overexpressed in malignant cells, making them excellent targets for antibodies (69, 70). Many of these drugs, including cetuximab (Bristol-Myers Squibb) and panitumumab (Amgen), are already on the market. The most successful FR-targeting monoclonal antibody is farletuzumab, which is specific for FRα. Early studies in ovarian cancer mouse models showed that the drug was well tolerated and induced ADCC (71).

Given that antibodies are highly specific for their target and can easily be conjugated to deliver a payload, they are well suited for targeting tumors or for stimulating cell-mediated responses against HIV (72). Free cysteines on the protein provide flexibility to conjugate drugs through reaction with free sulfhydryls (**Figure 1**). However, their utility as a vaccine component against infections is limited. First, therapeutic antibodies currently on the market are expensive, and antibody-targeted vaccines would not be any more economical (73). The antibodies themselves are subject to breakdown if not stored properly, and once administered they are easily cleared by the mononuclear phagocytic system. A common solution is to covalently attach poly(ethylene) glycol, known as PEGylation, to increase antibody half-life (60). Second, immune responses against the targeting antibody may induce rapid clearance and render repeat immunizations infeasible (74).

3.2. Cell-Mediated Targeting

In cell-mediated targeting, immune cells are isolated from the blood of patients and exposed in vitro to an antigen or maturation stimuli, upon which they are reinjected into the patient. The process is labor intensive and costly, meaning that its use will probably be restricted to niche applications, such as cancer immunotherapy or HIV treatment. Two techniques are commonly used in cell-based targeting: DC immunotherapy and T cell adoptive transfer.

DC immunotherapy has shown promise for use against a broad range of cancers. There are several studies in humans that demonstrate the safety and efficacy of this approach. In one study, purified blood samples isolated from the peripheral blood of hepatocellular carcinoma patients were incubated with granulocyte macrophage colony–stimulating factor (GM-CSF) and IL-4 to generate immature monocyte-derived DCs (MDDCs) (75). The MDDCs were incubated with lysate from a tumor cell line to expose the cells to a number of tumor antigens, leading to maturation. The patients were then vaccinated with the mature MDDCs, whereupon a clinical response was observed with increased IFN-γ secretion and decreasing serum levels of α-fetoprotein, a

protein known to be expressed at elevated levels with this type of cancer (75). A major challenge of DC immunotherapy is ensuring that the DCs are sufficiently mature, given that immature DCs could induce anergy rather than an antitumor immune response (75). A significant recent breakthrough is the use of TLR ligands to induce MDDC maturation. Traditionally, a proinflammatory cytokine cocktail containing IL-1β, IL-6, tumor necrosis factor α (TNF-α), and prostaglandin E2 had been used to induce maturation; however, these DCs were not very good at producing IL-12p70 and thus did not induce effective Th1 responses (76). More antigen-specific T cell proliferation and higher surface expression of CD83, CD86, and MHC II were observed upon incubating MDDCs with clinical-grade poly(I:C) or monophosphoryl lipid A (76, 77). Although it is an exciting approach, DC immunotherapy has yet to live up to its promise because generating de novo immune responses in advanced cancer patients is extremely difficult (78). Because the tumor microenvironment induces immune suppression and immune dysfunction, it is unknown whether DC immunotherapy primes T cells or simply activates preexisting anergic T cells.

In T cell adoptive immunotherapy, the tumor is excised and tumor-specific T cells are extracted and proliferated through the addition of proinflammatory growth factors such as IL-2 before being reinjected into the patient (79). This approach showed some success particularly for metastatic melanomas. In one clinical trial, 20 of 93 patients showed complete tumor regression and the entire group had a 5-year survival rate of 29% (80). To overcome other types of cancers, investigators could employ combinatorial strategies by performing both DC immunotherapy and T cell adoptive immunotherapy simultaneously (81). Perhaps activating the T cells with TLR ligands in addition to cytokines could further improve their response against tumors.

3.3. Modified Antigens and Drugs

Antigen modification strategies, including chemical conjugation of either small molecules (e.g., glycans or amino acids) or full-length antibodies to antigens (82, 83), have been used to actively target APCs (**Figure 1**). Specific APC receptors are targeted, enabling the modified antigen to be internalized and presented more effectively compared with the unmodified antigen (10).

A well-studied way to modify antigens involves conjugating them with carbohydrates. Given that many antigens on pathogens are heavily glycosylated, carbohydrate ligands have been used to create "pathogen-mimicking" antigens that trigger danger signals and initiate potent antigen-specific immune responses (84). Carbohydrates such as mannose, fucose, galactose, β-glucans, and Lewis-X oligosaccharides are ligands for CLRs and are currently being exploited to modulate the immune response (85). In one study, researchers cultivated bone marrow–derived DCs (BMDCs) and splenic DCs in vitro with Ova modified with a DC-SIGN ligand, Lewis-X, by using 4-*N*-maleimidophenyl butyric acid, a bifunctional cross-linker (**Figure 1**) (85). Compared with unmodified Ova, Lewis-X–Ova enhanced antigen presentation, leading to greater T cell proliferation, particularly when OT-I T cells were added to the DCs. This result suggests that the modified antigens can be cross-presented, which is important for stimulating $CD8^+$ T cells and inducing cell-mediated immunity (83, 85, 86).

In addition to carbohydrate ligands, TLR agonists and NLR ligands have been studied (87–89). In one such study, CpG, a TLR9 agonist, was chemically coupled to siRNA to target $TLR9^+$ myeloid cells and B cells (87). The siRNA was designed to silence the immune-suppressor gene *Stat3*, which would hinder tolerogenic cells and stimulate a potent immune response in the presence of a tumor. An endosomal TLR, such as TLR9, was targeted to help the cell internalize the siRNA more efficiently. Incubation with mouse splenocytes proved that DCs, MΦs, and B cells internalized the conjugated siRNA more efficiently than the unconjugated siRNA. In vivo, local injection of the CpG–siRNA oligonucleotide inhibited growth of colon carcinoma in mice, caused

the upregulation of MHC II and the costimulatory molecules CD80 and CD40 on DCs, and led to the production of Th1 cytokines (87). In this scenario, the CpG helped target the siRNA to DCs and stimulated those cells in conjunction with the siRNA. From a translational perspective, investigators from Novartis demonstrated that a TLR7 agonist linked to a *S. pneumoniae* antigen resulted in 10-fold dose sparing and improved survival upon challenge when compared with the unconjugated antigen (90).

FRs have also been the targets of a number of therapeutics. One example is the drug vintafolide, composed of folic acid conjugated to desacetylvinblastine hydrazide (DAVLBH) through a disulfide linker (91). DAVLBH inhibits microtubule polymerization, preventing mitosis and cell division. As the conjugate is taken up into the endosome by the FR, the local environment becomes more reductive, causing the disulfide bond to become cleaved. This allows the drug to escape the endosome and carry out its biolgical activity (52). It is important that drugs escape the endosome to be efficacious. By having an improved understanding of how folates interact with their receptors, researchers can design better folate derivatives that bind to the FR with high affinity and release drugs from the endosome.

3.4. Modified Delivery Vehicles

Many of the same ligands and techniques used to modify antigens and drugs can also be used to modify delivery vehicles. Polymeric particles make excellent delivery vehicles because of their ability to stabilize encapsulated proteins, provide tailored release rates, become internalized by phagocytes, and enable high drug-loading efficiency (92).

Several recent studies have shown that targeting CLRs on BMDCs leads to an activated DC phenotype characterized by increased particle internalization; enhanced upregulation of MHC II, CD40, and CD86; and greater proinflammatory cytokine secretion (84, 93–96). In these studies, polyanhydride nanoparticles were functionalized with dimannose to target the MMR and induce a pathogen-mimicking response. Functionalization was performed with a two-step reaction using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and *N*-hydroxysulfosuccinimide to link the carboxylic acids to a diamine linker (**Figure 1**). Pathogens such as *Yersinia pestis* and *M. tuberculosis* express conserved carbohydrate structures, including a variety of mannoses, on their cell surface (93). In addition to the sugars, there is evidence that the polyanhydride particles themselves act as TLR agonists, leading to a balanced cytokine response and induction of potent $CD8^+$ T cell responses (97). As an alternative, antibodies specific for CLRs could also be used. Another recent study showed that an antibody fusion protein targeted toward DEC-205, when linked to chitosan microspheres for the intranasal delivery of plasmid DNA to mice, resulted in larger quantities of mucosal immunoglobulin A (IgA) and protein-specific IgG when administered with an anti-CD40 DC maturation stimulus (98).

Another approach is to functionalize biodegradable nanoparticles to target TLRs, for example, by using CpG nucleotides to target TLR9 (99). Immunized mice had a strong Th1-biased immune response with a high number of circulating effector T cells and showed a high degree of protection from West Nile virus infection (99). The rationale is that TLR9 is known for inducing a cell-mediated, Th1-polarized immune response (100). In this study, the CpG was chemically linked to biotin while an avidin-linked fatty acid was conjugated to the particle surface (**Figure 1**) (101). Different variations of the biospecific biotin–streptavidin interaction are often used to link ligands to nanoparticles (98). Overall, conjugating TLR ligands to nanoparticles enhances the immune response, minimizes the need for antigen, and induces cellular immunity when targeting endosomal TLRs (102). Ligands for NLRs are more commonly encapsulated into particles. NOD1 and NOD2 ligands were encapsulated into polylactic acid (PLA) nanoparticles and codelivered

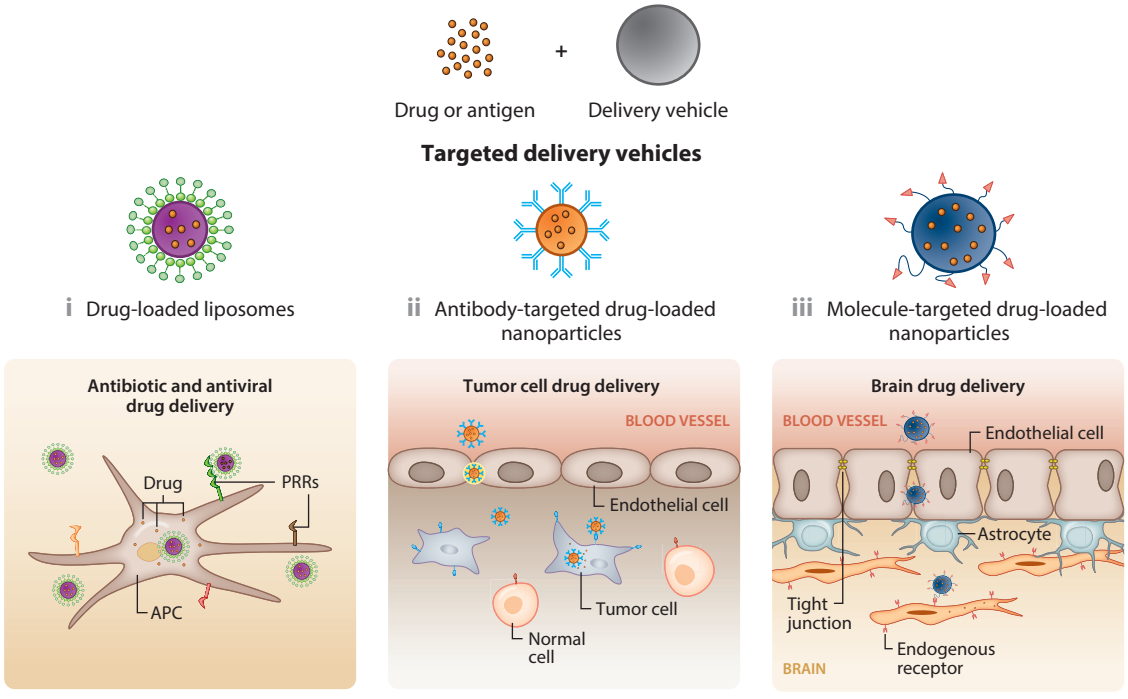


Figure 2

Targeted delivery approaches for diseases. Drug- or antigen-loaded delivery vehicles have been designed using targeting strategies based on pattern recognition receptor (PRR) agonists, antibodies, or molecules to enhance their uptake by desired cell populations. Targeted systems have been developed for antibiotic and antiviral delivery to enhance their uptake by antigen-presenting cells (APCs), selective delivery to tumor cells, and enhanced delivery across the blood–brain barrier.

with encapsulated HIV Gag p24 antigen to increase the antibody response 100-fold compared with that of alum-formulated antigen (103). Encapsulating NOD ligands into nanoparticles helps APCs endocytose the ligands, but the ligands still need to escape the endosome and reach the cytosol to trigger the receptor.

4. DISEASE PLATFORMS

Targeting strategies have helped advance prophylaxis and therapy in several diseases. Bacterial and viral infections, cancers, and neural disorders have benefited from the use of targeting and are discussed next (**Figure 2**).

4.1. Bacterial Infections

Therapeutic strategies that enhance the delivery of antimicrobial drugs against bacterial infections have been developed. These include environmentally responsive vehicles, receptor targeting, and particle- and cell-based approaches (**Figure 2**).

The use of pH-responsive materials to effectively deliver antibiotics has been previously explored (104). For example, cefdinir microspheres (a third-generation antimicrobial agent against enteric gram-negative rods) coated with Eudragit L100-55 (composed of methacrylic acid and methyl methacrylate), which dissolves at pH above 5.5, have been used to disrupt microbiota in the small intestine (~pH 6.5–7.5) of male Wistar rats following a type 2 diabetes model, since

it can protect these sensitive payloads from the acidic environment in the stomach (~1.5–3.5) (104). This study showed that gut microbiota could be modulated with pH-dependent cefdinir microspheres to reverse insulin resistance and improve their targeting to the intestinal microflora.

Another strategy to improve antibiotic therapy against intracellular infections is to actively target MΦs using PRRs (**Figure 2**). Because bacteria can survive after being ingested by phagocytes and evade the immune system, enhancement of drug delivery to these cells is desirable. An example is the use of a mannosylated nanogel containing mannosyl ligands conjugated to the shell of a degradable poly(ethylene glycol) (PEG) armed and polyphosphoester core-cross-linked nanogel (105). The gel is degraded by the phosphatase produced by a methicillin-resistant strain of *Staphylococcus aureus* and then releases the encapsulated drug vancomycin (105). The mannosylated hydrogels improved therapeutic efficacy by reducing bacterial colony-forming units (CFUs) after administration to infected MΦs. Another example is the surface decoration of polymeric nanoparticles with lectin (a protein that binds to carbohydrates present on bacterial cell walls) to treat *Helicobacter pylori*–associated infectious diseases (106).

Particle-based strategies for antibiotic delivery have focused mostly on the use of liposomes and polymeric nanoparticles. AmBisome® (NeXstar Pharmaceuticals), an amphotericin B liposomal formulation, that has been approved by the US Food and Drug Administration (FDA) for use as a therapeutic for *Candida* and *Aspergillus* infections, among others (106). Other studies have investigated ampicillin-loaded liposomes with higher antibacterial activity than soluble-drug formulations against *Salmonella typhimurium*. Liposomes loaded with benzyl and penicillin showed stronger antibiotic activity toward a strain of *S. aureus* than did free drug (106). The use of rifampicin- and azithromycin-loaded poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles for treatment of *Chlamydia trachomatis*–infected cells showed that the nanoparticle formulations reduced the bacterial load in the cells and enhanced the intracellular drug concentration (107).

Finally, cell-based targeting has been studied as an alternative to antibiotic delivery. In a *Mycobacterium avium* complex (MAC) model, which causes pulmonary disease in humans, the drug amikacin showed promising bactericidal activity in mice (108). However, the drug dose needs to be reduced to avoid ototoxicity and nephrotoxicity. One strategy to enhance cellular delivery is to use DCs as drug carriers to granulomas formed in tissues of MAC-infected mice (108). DCs from the host were administered fluorescein isocyanate–labeled amikacin, and the DCs effectively targeted granulomas throughout the body and localized drug concentrations to the site of action. In addition, rifampicin-loaded polybutylcyanoacrylate nanoparticles showed improved drug delivery to MΦs, enhancing antibiotic activity against *S. aureus* and *M. avium* (106).

4.2. Viral Infections

Innovative PRR-targeting approaches have been developed to design vaccines and therapies for respiratory viruses (**Figure 2**). Recently, there has been much interest in using flagellins as an adjuvant to stimulate the immune response against influenza (109, 110). In one study, this TLR5 ligand was genetically fused to the globular head of hemagglutinin derived from influenza virus (111). Healthy human adults immunized with this vaccine generated protective hemagglutinin-inhibiting antibody titers against the egg-grown H1N1 influenza virus (111). The vaccine was safe and generally well tolerated. However, these subjects also showed strong antibody responses against the flagellin. Therefore, adverse effects of future immunizations that contain flagellin are a concern. Overall, much research on influenza vaccines has involved coadministering TLR agonists with inactivated viruses or protein subunits. These include both TLR4 (e.g., synthetically produced glucopyranosyl lipid A) and TLR3 [e.g., poly(I:C)] agonists (112, 113).

In addition to respiratory infections, targeted delivery of antiviral drugs and vaccines has been explored for other diseases, including malaria and dengue fever. Because malaria therapies currently rely on the delivery of drugs that have little or no specificity toward infected red blood cells, the treatment regimen requires administering high doses of these compounds. Therefore, a key strategy is the design of carriers that specifically target these cells. The poly(amidoamines) AGMA1 and ISA23 have been explored for the delivery of primaquine and chloroquine (114, 115). AGMA1 showed improved specificity toward *Plasmodium falciparum*– and *Plasmodium yoelii*–infected cells and induced protection in mice after infection (114, 115). The improvement in specificity by AGMA1 was likely due to its function and the guanidine pendants that enhanced membrane interactions with the infected cells (114). Another strategy is the use of heparin as a targeting molecule for *Plasmodium*-infected cells. Heparin-coated liposomes loaded with primaquine increased the antimalarial activity of the drug threefold in cell culture and murine models (116).

4.3. Cancer

The use of targeting strategies for cancer treatment has focused on diagnostic and drug delivery vehicles that can locate cancer cells and help treat or remove tumors (**Figure 2**). Targeting approaches have focused on exploiting receptor-mediated uptake by tumor cells (117). Active targeting by enhancing cellular uptake offers more efficient delivery, which is beneficial for patients and cost effective because of dose sparing (117–121). The targets of antitumor therapies can be directed toward the surface receptors on cancer cells, or they can be used to treat and reduce angiogenesis or tumor vasculature (118, 119, 122).

Passive targeting strategies toward tumor cells have two tenets. The first is to avoid the reticuloendothelial system (RES), and the other is to exploit the enhanced permeation and retention (EPR) effect. Escape from the RES avoids the uptake of drug delivery vehicles by RES components (e.g., liver, spleen, and lungs) (118, 119). The EPR approach uses delivery vehicle design (e.g., size and charge) to deliver anticancer drugs to the tumor site by exploiting the faulty neoangiogenic vasculature created by tumor cells (118, 119).

The design of delivery vehicles for anticancer therapies or for imaging tumors for surgical removal using the RES avoidance strategy involves improving their circulation time by modifying chemistry and surface properties. Generally, less-hydrophobic surfaces avoid opsonization and uptake of these vehicles by RES components, enhancing their ability to reach delivery sites (118, 123). Glycol–chitosan nanoparticles loaded with Cy5.5 took advantage of EPR in brain, liver, and metastasis tumor models and showed enhanced tumor specificity (124). Quantum dots and magnetic nanoparticles also have potential as tumor-targeted delivery vehicles that exploit the permeability of the tumor vasculature (125).

Rational design of delivery systems depends strongly on knowledge about the specific targets and the administration route. For example, FRs are overexpressed on the surface of many types of cancer cells and are, therefore, a well-studied target for drug delivery to tumors (119). In previous studies using tumor cell lines (e.g., HeLa, HEK 293), the investigators achieved efficient cellular uptake by attaching folic acid on the surface of poly(ethylene imine)-coated mesoporous silica nanoparticles (117). Folate-modified micelles of *O*-carboxylmethylated chitosan modified with deoxycholic acid incorporating paclitaxel and verapamil (a P-glycoprotein) enhanced drug accumulation (126). Other recent studies have also identified different strategies that have greater selectivity to tumor cells, such as an adriamycin-conjugated to an NL-1 antibody (for targeting the surface of cancer cells) enhancing its delivery by using a PEG linker with cleavable peptide sequences that are degraded by enzymes in tumor microenvironments (118, 127). Use of these molecules in active targeting approaches for cancer treatment is currently under way.

Another strategy is to use antibodies that are specific for cancer types. Several of these therapies have been approved by the FDA and are becoming a viable alternative for cancer treatment using less-invasive methods than surgery; these products include Rituxan®, Herceptin®, Mylotarg®, Campath®, Zevalin®, and Iressa® for treating non-Hodgkin lymphoma, chronic lymphocytic leukemia, breast cancer, and non-small-cell lung cancer, among other cancers (118, 128–130). The use of these antibodies for targeted delivery is also ongoing.

Other methodologies for cancer therapy include targeting molecules that stimulate angiogenesis, such as vascular endothelial growth factor, basic fibroblast growth factor, platelet-derived growth factor, transferrin, or epidermal growth factor (118, 131). This strategy uses angiogenesis inhibitors such as IFN-α, -β, and -γ; thrombospondin-1 and -2; angiostatin; and endostatin. Drugs that have been widely studied for cancer therapy include doxorubicin, paclitaxel, and camptothecin (118, 131). Appropriate and specific design of these systems to target molecules present on cancer cells can help improve the delivery of drugs to tumor sites and for early diagnosis.

4.4. Neural Disorders

A major challenge in drug delivery is to improve the delivery of therapeutics to the brain. Delivering drugs to the brain is difficult because of the limited permeability of the BBB, which is formed by specialized endothelial cells that are partially covered by pericytes and basement membrane and surrounded by astrocytes. The BBB does not allow the transport of large molecules and allows very restricted quantities of small molecules (approximately 2%) that are lipophilic and <400–500 Da in size (132). Therefore, multiple strategies have been explored to enhance the transport of therapeutic drugs across the physical, metabolic, and immunological barrier that is the BBB. Among these strategies, particle-based and cell-based approaches have shown success (**Figure 2**). In addition, the use of antibody-based and receptor-targeting mechanisms has been explored to design more effective delivery systems (133, 134).

Several particle-based systems enhance drug delivery to the CNS across the BBB. These include proteins, lipids, and polymeric nanoparticles. Within protein carriers, albumin has previously been used for loperamide (analgesic) and paclitaxel (anticancer drug) penetration of the BBB in animal models (135, 136). Stearic acid has been used by itself and in combination with other components (i.e., soybean lecithin) to deliver drugs such as camptothecin (anticancer drug) and atazanavir (antiretroviral) (137). Even though multiple carriers have been studied, polymeric nanoparticles are the frontrunners for drug delivery across the BBB. Among these, poly(butyl cyanoacrylate) nanoparticles have been used to deliver drugs to the CNS such as dalargin (analgesic and modulator of the autonomic nervous system), loperamide, doxorubicin (chemotherapeutic agent), and tacrine (drug used to treat Alzheimer's disease) (137, 138).

PLGA and PLA nanoparticles have also been studied as drug carriers (139). Polyanhydride nanoparticles have been used to deliver antioxidant drugs such as mito-apocynin, and drug uptake by neurons was enhanced by use of folic acid–modified particles (140). Altogether, these therapies have shown promising results, either by enhancing drug concentrations or by improving their transport across the BBB.

Cell-based approaches are grounded in the rationale that certain APCs are able to cross the BBB, serving as Trojan horses to carry a drug and deliver it to the brain (141). MΦs have been well studied due to their phagocytic ability and capacity to deliver therapeutics to brain tissue. MΦ transport to the brain has been used to deliver antiretroviral drugs, including atzanavir, ritonavir, and indinavir, for antiretroviral therapy for HIV (142). These studies have shown that folate modification of these drugs improved their uptake by APCs and that cell-based delivery facilitated drug entry into the brain (142).

Active targeting methodologies have been developed to improve the specificity of the above-mentioned strategies. Antibodies toward the transferrin receptor (OX26) and the insulin receptor have been explored as targeting moieties for CNS drug delivery (139, 143, 144). These two receptors are expressed in brain tissues (OX26 on endothelial cells and the insulin receptor on the BBB interface and plasma membrane of glioma cells). These antibodies were designed only for mouse models, and development of these molecules for humans is ongoing.

Another strategy is the use of cell-penetrating peptides, such as TAT, which is derived from HIV-1 and leads to endocytosis, although the specific mechanism is not fully understood (137, 145), enabling greater accumulation of drugs such as ciprofloxacin (an antibiotic) and coumarin (an anticoagulant). The use of Angiopep peptides to successfully target polymeric micelles loaded with amphotericin (antibiotic) to the brain using caveolae-mediated energy-dependent endocytosis, clathrin-mediated endocytosis, and to a lesser extent macropinocytosis has also been reported (146–149). Targeting of mitochondria has been explored for drug delivery of an oxidase assembly inhibitor (mito-apocynin) for Parkinson's disease (150). Finally, molecules that are endogenous to tissue or cell surfaces (e.g., lactoferrin, transferrin, and folate) have been employed for drug transport across the BBB (137). These strategies are less likely to cause adverse effects after administration; however, they have less specificity than the above-mentioned targeting antibodies (137). Altogether, these methodologies demonstrate that targeted delivery is a key strategy for CNS therapeutics. The discovery of highly specific targets and the development of vehicles or strategies that allow efficient delivery are critical for advances in this area.

5. CONCLUSIONS AND FUTURE DIRECTIONS

Targeting strategies have significant potential to revolutionize drug and vaccine delivery. As discussed in this review, there are multiple approaches to enhance the specificity and efficacy of treatments for many diseases. An important future direction in this area is the targeting of organelles. Delivery of antibiotics like aminoglycosides, β-lactams, and fluoroquinolones is hindered by poor cellular entry and retention or confinement into endolysosomal compartments, and it can be improved if targeted to the right intracellular organelles. Mitochondria and the nucleus represent two vesicular targets that can be exploited by cell-based and particle-based systems for siRNA delivery and Parkinson's disease treatment. Other advantages of targeted delivery vehicles are that they can help overcome multidrug resistance, enhance gene treatment, and improve antibiotic stability. Further studies are necessary to evaluate the efficacy of these intracellular delivery strategies.

As discussed above, one of the main applications of targeting is the development of cancer therapies. The discovery of specific receptors and their corresponding ligands is a key step in the rational design of drug and vaccine delivery vehicles to tumor cells. Future directions in this area include (*a*) the development of theranostics that can provide both rapid diagnosis and effective treatment of cancer patients and (*b*) the development of cancer nanovaccines, especially for widespread tumors with poor prognosis and high mortality rates (e.g., pancreatic cancer).

Clinical approval for anticancer and antibacterial drugs formulated with delivery vehicles provides evidence that the pharmacological properties of drugs are often improved when using carriers (151). Future drug delivery vehicles that target specific receptors will ensure that the therapeutic effects of drugs are further improved. In this regard, targeting provides many opportunities, including design of formulations with enhanced safety and room-temperature stability, particularly for antibiotics and antivirals. Fragile proteins are often encapsulated into drug delivery vehicles to preserve stability and shelf life (152–154); however, very little information is available regarding whether encapsulating antibiotics or other small molecules will

improve their shelf life (155). Additionally, the improved biocompatibility and safety of targeted drug delivery vehicles containing antivirals or antibiotics may further the cause for targeted drugs.

Overall, as our understanding of cellular mechanisms and the functionality of specific receptors continues to mature, drugs that target specific receptors will become more prevalent. Even though antibody-mediated targeting has been largely successful (e.g., trastuzumab, bevacizumab, cetuximab), there are important limitations, as discussed above. As a result, researchers continue to search for the "magic bullet," a drug that can immediately localize to its target with high affinity and release the payload to induce a potent therapeutic effect. The development of novel targeting approaches that integrate cell and molecular biology with nanotechnology and materials science as described in this review will undoubtedly play a major role in creating advanced and personalized therapies against debilitating and life-threatening conditions.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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