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Review article

Self-assembled amphiphilic copolymers as dual delivery system for immunotherapy

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A B S T R A C T

Subunit vaccines using recombinant antigens appear as the privileged vaccination technology for safety reasons but still require the development of carriers/adjuvants ensuring optimal immunogenicity and efficacy. Micelles from self-assembled amphiphilic copolymers have recently emerged as highly relevant and promising candidates owing to their ease of preparation, low size (entering in lymphatic capillaries for reaching lymph nodes), size/surface tunability and chemical versatility enabling introduction of stimuli (e.g. pH) responsive features and biofunctionalization with dedicated molecules. In particular, research efforts have increasingly focused on dendritic cells (DCs) targeting and activation by co-delivering (with antigen) ligands of pattern recognition receptors (PRRs, e.g. toll-like receptors). Such strategy has appeared as one of the most effective for eliciting CD 8+ T-cell response, which is crucial in the eradication of tumors and numerous infectious diseases. In this short review, we highlight the recent advances in such micelle-based carriers in subunit vaccination and how their precise engineering can be a strong asset for guiding and controlling immune responses.

1. Introduction

Vaccination represents one of the most successful approaches in modern medicine and has had a huge contribution to global health [1]. It not only prevents 2–3 million deaths worldwide from infectious diseases every year, but also holds now great promise for cancer treatment. Vaccine technology however still faces major challenges, namely the failure to address numerous chronic infectious diseases (e.g., HIV-1, HCV, malaria), the hard matching with the expected safety standards, and, for vaccines which work well, the need for multiple injections, which is a practical limitation in various parts of the world.

Vaccines have traditionally consisted of attenuated or inactivated pathogens which, while efficient, raise important safety concerns [2]. Thus, much of the vaccine current efforts are focused on developing subunit vaccines based on molecularly-defined and rationally designed recombinant antigens with excellent safety profiles, but whose poor inherent immunogenicity requires use of adjuvants [3,4]. To date, aluminium salt based adjuvants remain the most widely used in licensed human vaccines [5]. Such adjuvants, though efficient for inducing humoral responses, fail in the development of T-cell mediated immunity, which is crucial in many infectious diseases and in cancer context. In addition, beside their mechanism of action which is still unknown and/or controversial, their safety remains questionable, resulting in growing negative perceptions from populations, particularly regarding infant vaccination. MF-59 squalene based emulsion, that has been licensed more recently (1997), is the other choice adjuvant [6]. But it poses as well the problem of safety [7,8], poor understanding in mechanism of action [9], and deserves further data regarding efficacy and safety for licensure in young children [10]. Therefore intensive research efforts have been devoted to safe nano/micro systems as alternative adjuvants [11,12], such as liposomes [13–15], emulsions [16,17], non-degradable/degradable polymer particles [18–22] or dendrimers [23,24], able to mimic pathogen while also providing antigen protection against premature degradation.

Beside such particulate adjuvants, molecular adjuvants based on pathogen associated molecular patterns (PAMPs) (so-called “danger signals”) have received considerable attention. These molecules can stimulate/maturate antigen presenting cells (APCs) upon interaction with various specific receptors including TLR (toll-like), NOD (nucleotide oligomerization domain) or C-type-lectin receptors [25], yielding antigen processing and cytokine production, triggering immune responses. Among APCs, dendritic cells (DCs) play a key role in the induction of cellular immune responses as they are able to present exogenous antigen through the major histocompatibility complex (MHC) class I, leading to the efficient activation of CD8+ T cells (cross-presentation) [26].

As a result, combination of danger signal molecule(s) and antigen in particulate adjuvants, typically liposomes or polymer nanoparticles, has been at the heart of vaccine design over the last decade, due to suitable pathogen mimicry and DC targeting/activation [27–33]. Furthermore,

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with continuous and spectacular advances in immunology and lymphatic biology [34–36], consensus is being made that a key requirement for vaccine efficiency is ability for targeting DCs in lymph nodes. Indeed DCs are in greater concentration in lymph nodes than in peripheral tissues and many of them are still immature, so they can still process the antigen upon targeting and promote germinal center formation [37–40]. Such targeting requires very small nanocarriers (less than 100 nm), for enabling traveling through the lymphatics directly to lymph nodes, as showed by pioneer works of Reddy et al. [41]. Finally, another key requirement increasingly considered in vaccine design is to promote efficient cytosolic delivery of antigen for eliciting MHC I presentation and further cellular immune response, which has stimulated the development of pH-sensitive vaccine carriers [42,43].

Among polymer particles, micelles are spherical nano-aggregates formed from spontaneous entropy driven self-assembly of amphiphilic copolymers in water, which have emerged as highly attractive candidates for vaccine delivery [44]: (i) they are easy to prepare, with a good batch-to-batch reproducibility; (ii) their small size (typically less than 100 nm) facilitates the antigen delivery to APCs (e.g. DCs) in the draining lymph nodes; (iii) they are chemically versatile: hydrophobic/hydrophilic blocks can be tuned to exhibit favorable features such as tailored surface charge, sensitivity to temperature or pH (endosomolytic property), and presence of reactive/ionic moieties to immobilize covalently or non-covalently protein/nucleic acid based antigens as well as immunostimulatory molecules (e.g., TLR ligands) in a controlled manner [37,38,45] (Fig. 1A). This precise engineering has particularly benefited from the progresses in controlled radical polymerization techniques (NMP, RAFT, ATRP) [46,47] and their combination with other mechanistically distinct polymerization techniques (e.g. ring opening polymerization, ROP) [48,49], that have considerably extended the range of copolymers achievable, in both architecture and functionality [50]. Such micellar nano-objects can address many of the above mentioned issues for designing efficient vaccines. In this short review, we present the recent advances in micelle-based carriers for vaccines and their decisive inputs for induction of potent and protective immune responses (Fig. 1B).

2. Antigen loaded micelle system: background and antigen loading strategies

2.1. Early works

Pioneer works on very small vaccine antigen carriers were initiated by the group of Hubbell [41,51], using Pluronic stabilized polypropylene sulfide (PPS) nanoparticles (NPs) of different sizes. They showed that 25 nm-sized particles were internalized by DCs in the lymph nodes about 10-fold more efficiently than 100 nm counterparts. These ultra-small sized NPs induced great improvements in cellular and humoral responses, using a surface conjugated ovalbumin (OVA) as antigen model through vinylsulfone-thiol chemistry. It was further showed that these NPs, when conjugated with OVA peptide antigen through disulfide bonds, i.e. sensitive to reductive environment (present in endosomes and cytosol), were more efficient to induce T cell proliferation than the ones irreversibly conjugated to the antigen (i.e. through thiol-vinylsulfone reaction) due to enhanced antigen release and processing [52]. Later works of this group were devoted to polyethylene glycol (PEG)-b-PPS micelles of about 35 nm size after surface conjugation of OVA antigen through disulfide bonds [53]. When injected intradermally in mice with CpG as co-adjuvant (a TLR 9 ligand, see following section), the OVA-coupled micelles induced a higher OVA-specific CD8+ T cells in the blood than did free OVA with CpG. More recently, this block copolymer was used in a suitable PEG/PPS composition so as to self-assemble in polymersomes (PSs), which consist in a polymeric bilayer surrounding an aqueous core, in which antigen (OVA) was encapsulated [54]. This carrier was compared with the PEG-stabilized PPS NP analog with surface coupled antigen. Both nanocarriers induced distinct biodistribution of antigen in the lymphoid organs, resulting in differences in T cell immunity, namely a

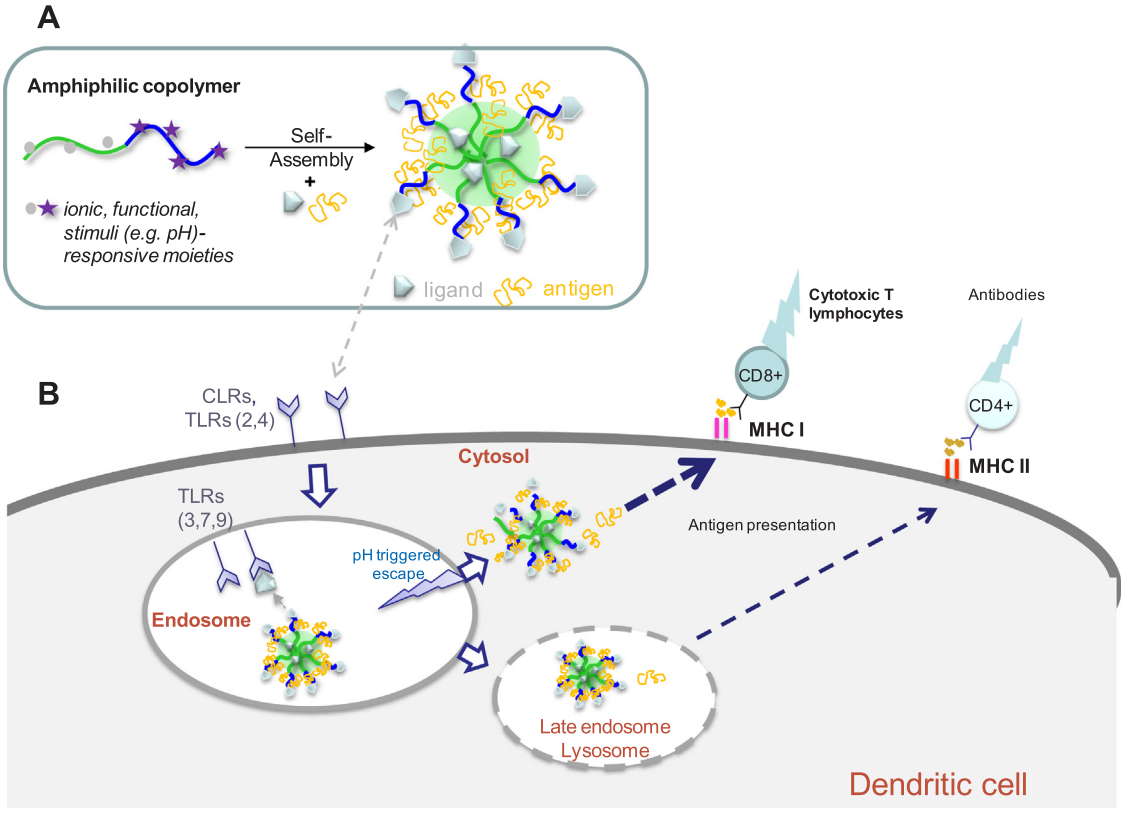

Fig. 1. The dual ligand/antigen micellar carrier (A) and its relevancy for immunotherapy through DC targeting and activation (B).

Table 1
Characteristics of antigen loaded micelles reported in the literature.

Amphiphilic copolymer (hydrophilic-*hydrophobic*)	Antigen	Association mode	Size (nm)	Adm. route (for in vivo)*	Ref.
PEG-*PPS*	OVA	coupled	30	intradermal	[53]
PDEAEMA-PEG-*POP*-PEG-PDEAEMA	OVA	mixing	n.d.	subcutaneous	[62]
PEG-*PCL*-g-PGEM	OVA	electrostatic	50	intradermal	[55]
PEG-b-*PDPAEMA*-b-PGEM	OVA	electrostatic	170	intradermal	[56,57]
Mixed PEG *PCL*/PEI-*PCL*	Cit-OVA	electrostatic	150	intranasal	[58]
DLPC/deoxycholic acid	OVA	mixing	12	intradermal	[67]
PEI-*stearic acid*	Trp-2	encaps	30	subcutaneous	[63]
Soluplus®	TTxd	encaps	68	transcutaneous	[68]
Polymersomes					
PEG-b-*PPS*	LASV	encaps	160	intradermal	[65]
PEG-b-*PPS*	OVA	encaps	150–170	intradermal	[54]
Polyphosphazene-g-*PDA*/PEG	OVA	encaps	200	subcutaneous	[66]

Abbreviations: PEG, poly(ethylene glycol); PPS, poly(propylene sulfide); PDEAEMA, poly(2-(diethylamino)ethyl methacrylate); POP, poly(propylene oxide); PCL, poly(ε-caprolactone); PGEM, poly(2-(guanidine)ethyl methacrylate); PDPAEMA, poly(2-(diisopropyl amino)ethyl methacrylate; DLPC, dilauroyl phosphatidylcholine; PEI, polyethylenimine; Soluplus®, PEG-g-polyvinylcaprolactam-polyvinyl acetate; PDA, diisopropylethylene diamine; OVA, ovalbumin; Cit-OVA, citraconic anhydride modified OVA; Trp-2, tyrosinase-related protein 2; TTxd, tetanus toxoid; LASV, Lassa virus.

* In mice for all studies, except [68] (rats).

preferential CD4 T cell response for PSs and CD8 T cell one for NPs. This showed that the nanocarrier design not only impacted but also can be tuned to guide the immune responses (MHC II vs. MHC I pathways). Overall, these results regarding PPS based nano-systems highlight how can precise tuning of the carrier physico-chemical engineering (e.g. size, antigen conjugation chemistry, core nature) positively affect the immune responses.

2.2. Antigen loading strategies

As mentioned above, initial works have mainly consisted of antigen covalent coupling on micelles. Though, other approaches can be used to load the protein antigen (i.e. ovalbumin, in most of studies) in/on the micelle nanocarriers, such as ionic interactions. Micelles from PEG-b-polymethacrylate amphiphilic copolymers with cationic guanidine pendant groups (on the methacrylate units) were for example developed to afford complexation of the OVA antigen through electrostatic interactions with the carboxylate groups of the protein [55–57] (Table 1). The guanidine moieties on the micelles were shown to improve micelle drainage into the lymph nodes and antigen cross-presentation. Well-known cationic polyethylenimine (PEI) was also used as hydrophilic block in micelles to complex OVA antigen. Li et al. prepared mixed micelles from polycaprolactone (PCL)-b-PEI and PCL-b-PEG and immobilized a citraconic anhydride modified OVA, to decrease its isoelectric point and thus reinforce the electrostatic interactions with PEI. The mixed micelles in 1/1 ratio induced strong immune responses in nasal mucosa and serum in vivo [58]. Electrostatic interactions were of particular interest when considering vaccination with DNA, due to their polyanionic nature [59]. Such an approach relies on the introduction of a plasmid containing the DNA sequence encoding the target antigen (which is thus produced in situ) [60]. Recently, vaccination with mRNA has emerged as a potent alternative with regard to safety issues, since RNA does not insert in host genome. Zhao et al. have protected anionic HIV-1 gag RNA by complexation with the cationic PEI corona of PEI-stearic acid micelles [61]. The micelle/mRNA complexes could significantly enhance anti-HIV-1 gag immune responses as compared to mRNA alone and PEI-mRNA complex. To note, whatever the antigen electrostatically complexed (i.e., protein- or nucleic acid-based), cationic micelles based on low p*K*a amino-polymers such as above-mentioned PEI or poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA) [62] have been particularly used for their endosomolytic properties based on “proton sponge” effect. They indeed favor cytosolic antigen delivery and further MHC I cross-presentation pathway for eliciting CD8+ T-cell response. Although cationic character is generally associated to some toxicity, the use of such cationic micelles did not show cytotoxicity at the used doses.

Encapsulation in the core of the micelles can be also envisioned when antigen has a sufficient hydrophobic character. Micelles from amphiphilic stearic acid-PEI have been for example used to encapsulate melanoma expressed tyrosinase-related protein 2 (Trp-2) antigen (SVYDFFVWL sequence) [63]. Yet, most of studied model antigens are hydrophilic, and to this regard their encapsulation in aqueous core of polymersomes, as an alternative to liposomes, has been receiving increasing attention [54,64–66].

3. Dual antigen/ligand loaded micelles for DC targeting

Due to chemical versatility, amphiphilic copolymer micelles are prone to decoration with molecular adjuvants in addition to the antigen. Research efforts have focused on pathogen associated molecular patterns (PAMPs) that can induce activation of DCs, through binding to pathogen recognition receptors (PRRs). Particularly, identification of toll-like receptors (TLRs) [69,70] has yielded growing interest for their related PAMP ligands as well as their incorporation in/onto antigen nano-carriers to guide and improve the immune responses [71]. Other PRRs of interest are C-type lectins (CLRs) which were typically addressed with mannose ligand-decorated micelles.

3.1. TLR ligands

Upon PAMP binding, TLRs ignite a signaling cascade mediated by intracellular adaptors that activate transcription factors such as NF-κB, leading to further dendritic cell (DC) maturation and production of pro-inflammatory cytokines, a fundamental step towards effective immune responses.

3.1.1. TLR7

TLR7, that recognizes single stranded RNA from microbial structures, has been the object of many studies. Typical ligands are synthetic nucleoside analogs, imidazoquinolines, particularly imiquimod and resiquimod. As hydrophobic, these molecules were in most cases encapsulated in the core of the micelles (Table 2). There is also a rationale for their encapsulation, because TLR7/8 receptors are not located on the surface of DCs but in the intracellular compartments (endosomes). The beneficial impact of encapsulation of TLR7 ligand (imiquimod) in micelle system on human DC stimulation/maturation was first shown by Jimenez-Sanchez et al. [72], using a polylactide-b-poly(N-vinyl pyrrolidone) (PLA-b-PNVP) based copolymer synthesized by ROP/NMP combination. Imiquimod encapsulated in the micelles was also shown much more efficient to activate the NF-κB and MAPK pathways than

Table 2
Characteristics of dual antigen/ligand loaded micelles reported in the literature.

Amphiphilic copolymer (hydrophilic-*hydrophobic*)	Ligand(s)	Association mode	antigen	Association mode	Size (nm)	Adm. route (for in vivo)*	Ref.
	TLR ligands						
PEtOxMA-PDMAEMA-*PLA* (star)	imiquimod (TLR7)	encaps	DNA	electrostatic	200–400	in vitro gene transfection	[75]
PAEM-*PCL* (star)	imiquimod	encaps	DNA	electrostatic	250	In vitro gene transfection	[74]
P(NAS-co-NVP)-*PLA*	imiquimod	encaps	HIV-1 p24	coupled	100	In vitro DC maturation	[72]
P(PEGMA-co-PDSM)-*P(LMA-co-MAA)*	imiquimod	encaps	OVA	coupled	30–80	intranasal	[76]
PHPMA-*PDEGMA* (*thermoresp.*)	resiquimod (TLR7/8)	coupled	HIV Gag	coil/coil interactions		subcutaneous	[96]
PEG-b-*PPS* (polymersome)	CL075 (TLR8)	encaps	Ag85B	encaps	120–150	subcutaneous	[97]
PEtOx-b-*PLA* + Pluronic F127	CL264 (TLR7)	encaps	OVA	coupled	50	subcutaneous	[37]
PEG-*PE*	MPLA (TLR4)	encaps	Trp-2/OVA/HPV (E7)	encaps	18	subcutaneous	[38]
PEG-*PE*/PEI-*stearic acid*	CpG (TLR9)	adsorption	Trp-2	encaps	30	subcutaneous	[63,83]
PEG-b-*PCL*/PEI-b-*PCL*	CpG	electrostatic	Trp-2	encaps	80	subcutaneous	[84]
Polyarginine-*PPS*-PEG	CpG/MPLA	electrostatic/encaps	OVA	electrostatic	15	intradermal	[88]
PEG-*ZnPP-g-PLL*	poly(I:C) (TLR3)	electrostatic	–	–	30	Intratumoral (B16-F10 melanoma)	[79]
Aminated polyglutamate-g- *cholesterol*	Poly(I:C)	electrostatic	–	–	30	subcutaneous	[39]
PEG-PLL-*PLleu*	poly(I:C)	electrostatic	OVA	encaps	120–150	intraperitoneal	[80]
PHEA-*PHEAm (acetalated)*	Amph B (TLR2/4)	encaps	RSV	mixing	30–40	subcutaneous	[87]
	CLR ligands						
PHPMA-*PLMA*	mannose/L18-MDP (NOD2)	coupled/encaps	–	–	80	In vitro DC mauration	[93]
Chitosan-g-phenylalanine	mannose	coupled	DNA (HBV)	electrostatic	200	intradermal	[91]
Chitosan-g-stearic acid	mannose/CCR7 pDNA	coupled/encaps	OVA	encaps	100–150	intradermal	[92]
PEG-*ZnPP-g-PLL*	galactose	coupled	–	–	30	intratumoral	[79]
PTEGMA-*PPFPMA*	Anti MMR nanobody	coupled	–	–	50	subcutaneous	[94]

Abbreviations: PEtOxMA, poly(oligo(2-ethyl-2-oxazoline) methacrylate); PDMAEMA, poly(2-(dimethylamino)ethyl methacrylate); PLA, polylactide; PAEM, poly(aminoethyl methacrylate; PCL, poly(ε-caprolactone); P(NAS-co-NVP), poly(N-acryloxysuccinimide-co-N-vinyl pyrrolidone); PEGMA, poly(ethylene glycol) methacrylate; PDSM, pyridyl disulfide methaccrylate; LMA, lauryl methacrylate; MAA, methacrylic acid; PHPMA, poly(2-hydroxypropyl methacrylamide); PDEGMA, poly(diethylene glycol methacrylate); PEG, poly(ethylene glycol); PPS, poly(propylene sulfide); PEtOx, poly(2-ethyl-2-oxazoline); PE, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine; PEI, polyethylenimine; ZnPP-g-PLL, zinc protoporphyrin IX grafted poly(L-lysine); PLleu, poly(L-leucine); PHEA, poly(2-hydroxyethyl acrylate); PHEAm, poly(2-hydroxyethyl acrylamide); PTEGMA, poly(triethyeneglycol methacrylate); PPFPMA, poly(pentafluorophenyl methacrylate); OVA, ovalbumin; Ag85B, *mycobacterium tuberculosis* antigen 85B; Amph B, amphotericin B; MMR, macrophage mannose receptor; SIV/HIV, simian/human immunodeficiency, HPV, human papillomavirus; Trp-2, tyrosinase-related protein 2; RSV, respiratory syncytial virus; HBV, hepatitis B virus.

* In mice for all studies.

free imiquimod in raw 264.7 macrophages. These micelles could be surface functionalized with HIV-1 p24 antigen in high density, through coupling of its amines with activated ester groups installed along the PNVP block. The surface coupling of p24 did not affect the imiquimod-induced DC activation and significantly improved its antigenicity [73]. PLA-core based micelles encapsulating imiquimod were also developed in the context of DNA vaccination, using a hydrophilic cationic corona (based on aminoethyl methacrylate (AEM) [74] or dimethyl-AEM [75] units) for electrostatic complexation of DNA encoding antigen. Imiquimod slightly enhanced gene transfection in DCs. Sevimli et al. developed micelles with fatty acid based core and pyridyldisulfide functionalized corona for both imiquimod encapsulation and OVA antigen surface conjugation, respectively, using methacrylate based amphiphilic copolymers synthesized by RAFT polymerization [76]. The micelles improved the capacity of the ligand to induce DC maturation and cytokine production, and enhanced in vitro antigen uptake and cross-presentation on MHC-I. A single intranasal immunization of mice with carriers co-loaded elicited significantly higher pulmonary and systemic CD8+ T cell responses and increased serum IgG titer relative to a soluble formulation of antigen and ligand. It is to mention that generally imiquimod release was advantageously favored at endosomal pH (i.e. mildly acidic) due to its improved solubility at this pH (p*Ka* ~ 7.3) [72,77]. This endosomal release could be optimized through use of pH sensitive moieties to either control ionic interactions with imiquimod [76], improve micelle hydrophilicity [74] or even break the micelles through hydrophobic-to-hydrophilic block transition [78]. Also, as mentioned earlier, cytosolic delivery of antigen is an important requirement for preferential processing through MHC-I pathway, yielding CD8+ T cell response particularly important for direct eradication of tumor cells. For example, incorporation of endosomolytic poly(2-ethyl-2-oxazoline) segments in the corona of Pluronic F127 based micelles co-delivering OVA antigen and TLR7 agonist (CL264) contributed to enhance cytosol delivery [37]. Both humoral and cellular responses were improved and immunization with the co-delivery system in E.G7-OVA tumor-bearing mice could not only significantly inhibit tumor growth but also markedly prolong the mice survival.

3.1.2. TLRs 3/9

TLRs 3 and 9 are also present in cell endosomes, and recognize microbial nucleic acids, i.e. RNA (double stranded, ds) and DNA, respectively. TLR3 targeting is achieved with polyinosinic:polycytidylic acid (poly(I:C)), a compound structurally similar to dsRNA, which was typically loaded in the micelle carriers through electrostatic interactions with polycations [79,80]. It is to note that inherent toxicity of poly(I:C) has limited to date its clinical application [81]. However, suitable formulation can allow to substantially decrease such toxicity, as it was shown for poly(I:C) stabilized with polylysine/carboxymethylcellulose in a recent phase II trial [82]. Liu et al. have developed PEG-b-polylysine-b-polyleucine micelles, in which the intermediate polylysine layer (between the polyleucine core and the PEG external corona) was used to complex poly(I:C) and OVA antigen (and a microRNA-148a inhibitor). The micelles induced potent anticancer immune responses

and robust tumor regression with prolonged survival [80]. Song et al prepared polyglutamic acid modified with amines, for poly(I:C) complexation, and cholesterol (hydrophobic), for generating micelles. Combination of poly(I:C) with the micelles improved intrinsic immunological effects of poly(I:C) on the production of proinflammatory cytokines (TNF-α and IL-6) and type I IFN (IFN-β). This synergistic effect was related to the enhanced delivery of poly(I:C) into endosomes, where TLR3 is located, through the help of the micelles [39]. TLR9 targeting typically uses CpG deoxynucleotide ligand which is also classically bound on micelles through electrostatic interactions. Mixed micelles self-assembled from PEG-phosphoethanolamine (PEG-PE) and PEI-stearic acid conjugate (PSA) were designed to encapsulate hydrophobic Trp-2 antigen and electrostatically bind CpG to cationic PEI. For adjusted ratio of both polymers (1/1), The Trp-2/CpG delivery system potently targeted the lymph nodes and was efficiently internalized by DCs. It also significantly expanded antigen specific cytotoxic T lymphocytes and provided a strong anti-tumor effect in a lung metastatic melanoma model [83]. The same system of mixed micelles with degradable PCL as hydrophobic block instead of PE/PSA led to similar promising results [84]. The group of Wilson was one of the first to impart endosomolytic properties in dual CpG/antigen loaded copolymer micelles using pH responsive core [85,86], resulting in antigen cross-presentation and potent CD8+ T cell response.

3.1.3. TLRs 2/4

Cell surface located TLRs, namely TLRs 2/4, have been also addressed with monophosphoryl lipid A (MPLA) ligand, a detoxified derivative of lipid A from lipopolysacharide (LPS), introduced in micellar carriers. Micelles from PEG-phosphoethanolamine (PE) were again employed to encapsulate MPLA and peptide antigens (from OVA or human papillomavirus 16 – E7) [38]. Interestingly, these micelles improved immuno-stimulatory effect of MPLA but were also able to turn the peptides into a more α-helical conformation suitable for proper cytosolic antigen release, thus enhancing process and presentation to CD8+ T cells. These both coordinated effects on the same APC led to encouraging efficacy for tumor control and memory protection. Amphotericin B (Amph B), recently identified as a ligand of TLR 2/4 was also encapsulated in micelles, which allowed to reduce its toxicity and thus to use it in higher concentrations [87]. The Amph B-loaded micelles could adjuvant antigen derived from human respiratory syncytial virus (RSV) with almost equal potency as a highly immunogenic oil-in-water benchmark adjuvant. The group of Hubbell conjugated cationic polyarginine to the abovementioned PEG-PPS micelle system, for co-loading MPLA and CpG molecular adjuvants together with OVA antigen [88]. Adjuvant coadministration did not have an additive effect in the context of the micelle platform. Singly adjuvanted micelle-antigen aggregates were sufficient to elevate cytokine expression by CD8+ T cells and support induction of humoral responses.

3.2. C-type lectin receptors (CLRs) ligands

CLRs, such as macrophage mannose receptor (MMR) and DC-SIGN, are located on APC surface and recognize PAMPs composed of carbohydrate residues [89]. CLRs utilize many of the same signaling mechanisms as TLRs in establishing the innate and adaptive immune defense. Carriers surface functionalized with mannose residues are typically used for targeting [90]. Layek *et al.* developed micelles based on chitosan modified with hydrophobic phenylalanine moieties. Mannose moieties were introduced on the polymer to trigger mannose-receptor mediated endocytosis in APCs. DNA encoding antigen (Hepatitis B virus) was immobilized through electrostatic interactions with chitosan protonated amines. The mannose improved the macrophage and DC uptake through mannose-receptor mediated endocytosis, and gene transfection. This dual vaccine system improved both serum antibody titer and T cell proliferation after intradermal administration [91]. More recently, similar chitosan-based micelles grafted with hydrophobic stearic acid and mannose residues were used to co-deliver OVA protein antigen and a plasmid DNA encoding chemokine receptor type 7 (CCR7) to improve DC migration of lymph node [92]. The micelles achieved endosomal escape and delivered antigens to the cytosol of DCs, promoting CD8+ T-cell immune responses. A dual CLR/NOD ligand micelle delivery system was also recently designed from poly(2-hydroxyproplyl methacrylamide)-poly(lauryl methacrylate) (PHPMA-PLMA) block copolymers with surface coupled mannose residues and core encapsulated hydrophobic L18-MDP (NOD2 receptor ligand). The micelles bound DC only when conjugated with mannose, and in a mannose receptor specific manner and the NOD2 ligand promoted DC activation [93]. Nanobody specific to macrophage mannose receptor was also conjugated at the surface of nanogel-like micelles for targeting [94]. A smart amphiphilic copolymer was developed very recently, based on galactose modified micelles. Galactose did not serve here as a ligand, but drove a polymersome-to-micelle transition. Indeed, upon presence of lipase enzyme (present in lysosomes), galactose deprotection occurred, provoking rearrangement of the polymer chains from polymersomes to micelles and thus release of OVA antigen for highly efficient presentation to T cells [95]. The enzyme-sensitive release appears as a potent approach as alternative or in complement to pH based ones for improving cellular response.

3.3. Additional drug loaded micelles for immunotherapy

Diverse drug loaded micellar carriers were recently developed in context of cancer immunotherapy, in complement with the vaccine carrier treatment to improve the anti-tumor efficacy. The group of Hubbell has used, in combination with their OVA/CpG nanoparticles vaccines, PEG-PPS micelles system loaded with 6-thioguanine (a cytotoxic drug used in the treatment of myelogenous leukemia) to kill monocyte myeloid-derived suppressor cells (Mo-MDSCs) in tumor-bearing mice and thus enhancing T cell-mediated anti-tumor responses [98]. Micelles of PLGA-PEG loaded in sunitinib base, a tumor inhibitor, were also applied and worked in a synergistic manner with vaccine therapy using Trp-2/CpG/mannose nanoparticle vaccine system [99] in an advanced mouse melanoma model [100]. Similarly, the combination of micelles from amphiphilic curcumin–PEG conjugate and the Trp-2 vaccine treatment resulted in a synergistic antitumor effect compared to individual treatments [101]. Micelles were also exploited for chemotherapy/immunotherapy combination: dual loading of doxorubicin and MPLA adjuvant (agonist of TLR4) in DSPE-PEG micelles generated immunogenic cell death, which further promoted maturity of DCs, antigen presentation and induced strong effector T cells in vivo [102]. A dual system of doxorubicin (chemotherapy drug) encapsulated in micelles of PEG-Fmoc-NG919, where NG919 is an hydrophobic immunostimulating drug, was also recently developed, providing a promising immunochemotherapy for lymphoma [103].

4. Conclusion and future prospects

Due to their intrinsic properties and the rapid identification of mechanism controlling immune responses, micelles from self-assembled amphiphilic polymers are increasingly considered in immunotherapy, even if, to date, none has entered clinical phase and the animal model for evaluation is still limited to mice. (i) Their size below 100 nm permits their broad diffusion to the secondary lymphoid organs (germinal centers) through the lymph capillaries, (ii) their chemical versatility allows both the binding of a large arrays of immune-modulators and an ad-hoc vaccine component ensuring their co-delivery in any antigen presenting cell, providing the temporal right signal for eliciting T cell responses. Particularly, antigen cytosolic delivery appears as a key requirement for favoring MHC I presentation and cellular response that micelles can address through careful design (i.e. introduction of polymers/moieties ensuring pH triggered escape). Furthermore, the continuous appearance of new copolymers using safe by design

polymerization process provides new clues for tailoring safer biodegradable micelles allowing a large spectrum of administration routes, from subcutaneous to nasal route, including intratumoral injection. Moreover, as the vaccine field is facing a new research paradigm with the use of mRNA as vaccine components, we could anticipate that self-assembled amphiphilic polymers will play an important role in the next generation of mRNA delivery systems [104,105]. In particular, the chemical versatility of micellar carriers, highlighted in this review, appears highly relevant to address the major issues of rapid mRNA degradation by nucleases and difficult escape from endosomes for translation, through micelle installed cationic/endosomolytic features [61]. Considering all the additional properties that micelles can display (e.g. DC targeting, degradability), they are definitely expected to represent in a near future a potent platform for mRNA vaccines, competing with lipid nanoparticles, currently considered as the gold standard in the field [106–108].

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