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Polymeric micro- and nanoparticles for immune modulation

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New advances in biomaterial-based approaches to modulate the immune system are being applied to treat cancer, infectious diseases, and autoimmunity. Particulate systems are especially well-suited to deliver immunomodulatory factors to immune cells since their small size allows them to engage cell surface receptors or deliver cargo intracellularly after internalization. Biodegradable polymeric particles are a particularly versatile platform for the delivery of signals to the immune system because they can be easily surface-modified to target specific receptors and engineered to release encapsulated cargo in a precise, sustained manner. Micro- and nanoscale systems have been used to deliver a variety of therapeutic agents including monoclonal antibodies, peptides, and small molecule drugs that function to activate the immune system against cancer or infectious disease, or suppress the immune system to combat autoimmune diseases and transplant rejection. This review provides an overview of recent advances in the development of polymeric micro- and nanoparticulate systems for the presentation and delivery of immunomodulatory agents targeted to a variety of immune cell types including APCs, T cells, B cells, and NK cells.

Introduction

Novel biomaterials approaches for immune system modulation have recently been of great interest for treatments of diseases such as cancer,[1] infectious disease,[2] autoimmune disorders,[3] and regenerative medicine.[4] Biocompatible materials such as biodegradable and bioeliminable polymers have proven well suited to deliver signals to the immune system in order to direct an activating immune response against a detrimental disease or suppress an unwanted response against one's self antigens.[5] From a reductionist standpoint, two primary approaches have been considered based on length scales as well as the specific immune cell type targeted to promote health and prevent disease. These two classes are macro-scale implantable scaffolds for controlled drug release, and regenerative medicine[6] as well as nano- to micro-scale particulate delivery systems for drug delivery.[7] Although both have been successful in immunoengineering applications, this review will highlight recent advances in particulate systems for biologic delivery (Table 1).

Micro- and nanoparticle systems are an important class of many biomaterials-based drug delivery systems. These technologies possess key advantages for modulation of immune activity. Microparticles and nanoparticles are on the same size scale as cells and subcellular components, making them an ideal vehicle for a variety of applications such as stimulation of surface receptors or internalization and intracellular cargo release.[8] In many cases, the release of therapeutics from particle cores can be controlled on a desired time scale.[9] Fragile biological cargoes such as peptide or protein antigens can be encapsulated in the cores of particulate materials and reach their targets without being exposed to harsh physiological conditions.[8] In addition, these technologies can be administered both locally and systemically for optimal pharmacokinetics. Finally, these particles can be made stealthy through incorporation of a surface feature such as polyethylene glycol (PEG) for "shielded" particles or a more natural approach such as mimicking the "do not eat me" signal of red blood cells.[10] Taken together, drug delivery at the micron and nanoscale is an important foundation for biomaterials based immune modulation.

Some of the most successful classes of existing standalone drugs that can be delivered using micro- and nano-

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Table 1 Summary of select particulate platforms for small molecule and biologic delivery

Cell type	Modulation	Material	Biologic	Size	Result	Ref.
APC	Activating	PLGA with PEGylated lipid bilayer	OVA peptide and MPLA adjuvant	200 nm *vs.* 2–3 μm	Nanoparticles induced a greater antigen-specific immune response and both sizes were effective at very low doses	34
	Activating	PBAE	IL-12	30–40 nm	Repolarized TAMs from M2 to M1 phenotype and induced significant tumor burden decrease in melanoma model compared to soluble IL-12	53
	Suppressive	Polystyrene beads, PLGA particles	MOG_{35-55} peptide	500 nm	Prevented disease onset and reduced severity of paralysis in an EAE model	64
	Suppressive	PLGA	MOG_{35-55} peptide and rapamycin	3–4 μm	Single dose administered iLN[a] at the peak of disease resulted in 75% recovery from EAE-induced paralysis	80
T-cells	Activating	PLGA	Immune signal 1 and 2 and anti-PD1	4–5 μm	Combination therapy resulted in 40% B16-F10 tumor burden decrease	90
	Activating	Iron Oxide	Anti-PDL1 and anti-41BBL	80 nm	Redirection of tumor signals to result in 50% B16-F10 tumor burden decrease	95
	Suppressive	PLGA	H2 kb, PD-L1-Fc, and CD47-Fc, TGF-β	5 μm	Induced CD4+FOXP3+ Tregs 1.5-fold band deleted myelin autoreactive CD4 and CD8+ T-cells by 2-fold	109
	Suppressive	PLGA	H2 kb dimer, anti-Fas, PD-L1, CD47-Fc, TGF-β	200 nm	Inhibited alloreactive CD8+ T-cells and exprended Tregs for doubled survival rate in alloskin transplant	118
	Suppressive	Carboxylated polystyrenebeads	HLA 02 : 01 epitopes	500 nm	Promote antigen specific tolerance for type 1 diabetes by doubling the amount of CD4+ CD25+ Tregs and 10-fold depletion of autoantigen specific T lymphocytes	116
	Suppressive	PLGA	Dby and Uty peptide, PD1	450 nm	Dby with PD1 induced tolerance to mismatched bone marrow transplantation comparable to positive control, where the level of donor cells was 49% by week 20	111
B-cells	Activating	Calcium phosphate	HEL antigen	200–400 nm	100-Fold increase in B-cell activation compared to soluble antigen	131
	Suppressive	DSPC liposomes	CD22 glycan ligands and multiple antigens (HEL, OVA, MOG, FVIII)	100 -130 ± 30 nm	10-Fold decrease in antigen specific igG antibodies for multiple antigens after treatment	139
	Suppressive	DSPC and cholesterol liposomes	CD22 glycan ligand, OVA antigen, and rapamycin	160 ± 30 nm	Coadministration of STAL-NPs with RAPA results in 5-fold decrease in OVA-specific antibody compared to STAL-NPs in naïve mice	141

[a] iLN = intra-lymph node.

particles are protein biologics and small molecule. For many conditions, these drugs have been successful in the clinic for targeted treatment in standalone and targeted treatment strategies. One example of the protein class of drugs are monoclonal antibodies (mAbs). mAbs have been applied to many diseases with a viable molecular target, such as an immune checkpoint in cancer[11] or an excess of macrophages in rheumatoid arthritis.[12] Another example of these protein biologics are peptide antigens which are used in many vaccine formulations. These antigens can be synthetically made such as in the case of hepatitis B[13] or delivered as part of an inactivated pathogen such as the common forms of the flu vaccine.[14] There also exist a plethora of small molecule drugs that can be used for immune modulation such as rapamycin for non-specific immune suppression,[15] and inhibitors of indoleamine 2,3-dioxygenase for cancer immunotherapy.[16] These immunomodulatory protein biologics and small molecules make ideal candidates for delivery using current micro- and nanoparticle strategies as they can synergize with several previously mentioned advantages of these systems to amplify their therapeutic effect for their intended purpose.

In this review, the major recent advances in the development of micro- and nanoparticulate materials for the delivery and presentation of immunomodulatory protein biologics and small molecules will be covered. The review will not cover other important areas in drug delivery for immunomodulation such as gene delivery (to which the reader is directed to a comprehensive review on the topic).[17] Both activation of the immune system, such as against cancer cells or infectious diseases, and suppression of the immune system, such as in the case of autoimmune disorders and transplant tolerance, will be described. As each particulate system is designed based on the specific immune cell type that it modulates, this review is structured based on these cell types: antigen presenting cells (APCs) including dendritic cells and macrophages, T cells, B cells, and NK cells. Continued research into the development of these biomaterials based biologic delivery strategies will unlock the full potential of immunomodulatory proteins and peptides for next generation immunotherapies.

Antigen presenting cells (APC)

Dendritic cells and macrophages are the major professional APCs of the immune system that have the potential to process and present antigen to activate T cells against cancer and infectious diseases. APCs can also adopt a tolerogenic phenotype and suppress T cells that are reactive to self-antigen, as is the case in autoimmune disease. APCs present antigen in the context of the major histocompatibility complex (MHC) to T cells, specifically using MHC I for presentation to CD8+ T cells or MHC II for presentation to CD4+ T cells. APCs also display a costimulatory signal, referred to as signal 2, that directs the T cell response. Positive costimulatory signals stimulate T cells to mount a response against cells displaying the antigen of interest. On the other hand, a lack of positive costimulation and negative costimulatory signals result in the suppression of effector T cells and direct T cells to adopt a regulatory phenotype.

APCs for immunostimulation

While APCs can be tuned broadly for immunostimulation against diverse antigens, utilizing particles to engineer APCs for cancer immunotherapy is an area of particularly rapidly growing interest. For further information on polymeric particle-based vaccines to fight infectious diseases, a reader is directed elsewhere.[18,19] Antigen processing and presentation is often dysregulated or inefficient during cancer.[20] As a result, extensive research has been performed to engineer particle-based cancer vaccines that direct APCs to present tumor antigen to T cells in an immunostimulatory manner. Additionally, tumor-associated macrophages (TAMs) play an important role in the tumor microenvironment. Tumor cells stimulate TAMs to adopt an immunosuppressive phenotype, which impairs their ability to present antigen and perform other immunostimulatory anti-tumor activities.[21] Researchers are investigating methods to modulate TAMs to make them more immunostimulatory or deplete them altogether.

Particle-based vaccine delivery. Particle-based vaccines typically deliver two components: tumor antigen and an adjuvant. Particle carriers for vaccine delivery are highly advantageous compared to delivery of free antigen and adjuvant for a number of reasons. Particles protect the antigen and adjuvant from degradation and enable control over pharmacokinetics and uptake of the vaccine by APCs.[22] Soluble antigen rapidly diffuses in an out of the lymph nodes, the major location of antigen presentation to T cells, whereas a particle can be retained for longer in the lymph nodes allowing more time for the APCs to engulf the vaccine.[23,24] Additionally, polymeric particles have the capability to co-deliver antigen and adjuvant to the same APC. This ensures that APCs are exposed to tumor antigen in the context of a danger signal, thereby preventing tolerization, anergy, or non-specific activation.[25,26]

Many polymeric particulate systems have been engineered to deliver antigen and adjuvant to APCs, namely dendritic cells, for cancer vaccination. Both micro- and nanoparticles synthesized from poly(lactic-*co*-glycolic acid (PLGA), a biodegradable polymer that has been used for multiple FDA-approved devices, have been extensively studied for cancer vaccine delivery by encapsulating peptide antigen and adjuvant.[27–32] PLGA particles encapsulating peptide antigen and adjuvant have been found to induce a much stronger anti-tumor immune response compared to soluble antigen and adjuvant.[30,33] For example, PLGA nanoparticles encapsulating a melanoma-associated antigen and the adjuvant monophosphoryl lipid A (MPLA) stimulated dendritic cells to induce approximately 10-fold higher CD8+ T cell proliferation compared to free antigen and adjuvant.[30] To overcome obstacles associated with this conventional approach, such as low antigen encapsulation efficiency, Bershteyn *et al.* engineered PLGA particles coated with a PEGylated phospholipid bilayer with incorporated lipophilic adjuvants attached protein antigen.[34] Conventional polymeric particles for cancer vaccination encapsulate a single peptide antigen. Fang *et al.* engineered PLGA nanoparticles coated with melanoma cancer cell membranes in order to deliver the full range of cancer antigens to antigen presenting cells, as opposed to vaccinating against just one or a few tumor-associated antigens, and found that the particles successfully delivered tumor antigen to dendritic cells.[35] In addition to PLGA, other biodegradable polymers, such as polylactide (PLA), poly (amino acids) and polysaccharides, have been explored for cancer vaccine delivery.[36,37]

Passive targeting to APC. In order to optimize cancer vaccine delivery, approaches for targeted delivery of particles to dendritic cells have been investigated. One such approach is passive targeting of particles to the lymph nodes, a site with a high frequency of immature dendritic cells and the location of antigen presentation to and activation of T cells. Particle size has been investigated for its effect on lymph node targeting of vaccines as nanoparticles are able to enter lymphatic vessels and be retained in the lymph nodes.[38–40] Particles less than 5 μm in diameter are efficiently phagocytosed by APC and thus do not require an active targeting strategy to reach these innate immune cells.[5] Particles can be targeted to DC in peripheral tissues through subcutaneous administration,[6] or DC in the lymph nodes through intranodal administration.[7] Reddy *et al.* modulated the size of Pluronic-stabilized polypropylene sulfide (PPS) nanoparticles encapsulating the adjuvant lipopolysaccharide (LPS) and peptide ovalbumin (OVA), a commonly used model antigen.[23] They found that, compared to 100 nm particles, 25 nm particles migrated to the lymph node and were internalized by dendritic cells at a much higher rate and induced a much stronger anti-OVA immune response (Fig. 1A). Furthermore, 30 nm PPS nanoparticles conjugated with adjuvant have been found to effectively target dendritic cells in the tumor draining lymph node and significantly reduce tumor volume in a mouse melanoma model.[41] PLGA particle size has been similarly investigated and it was found by one group that 300 nm PLGA particles encapsulating OVA and CpG more efficiently targeted dendritic cells and led to a greater antigen specific immune response compared to particles of 1 μm, 7 μm, 17 μm.[38]

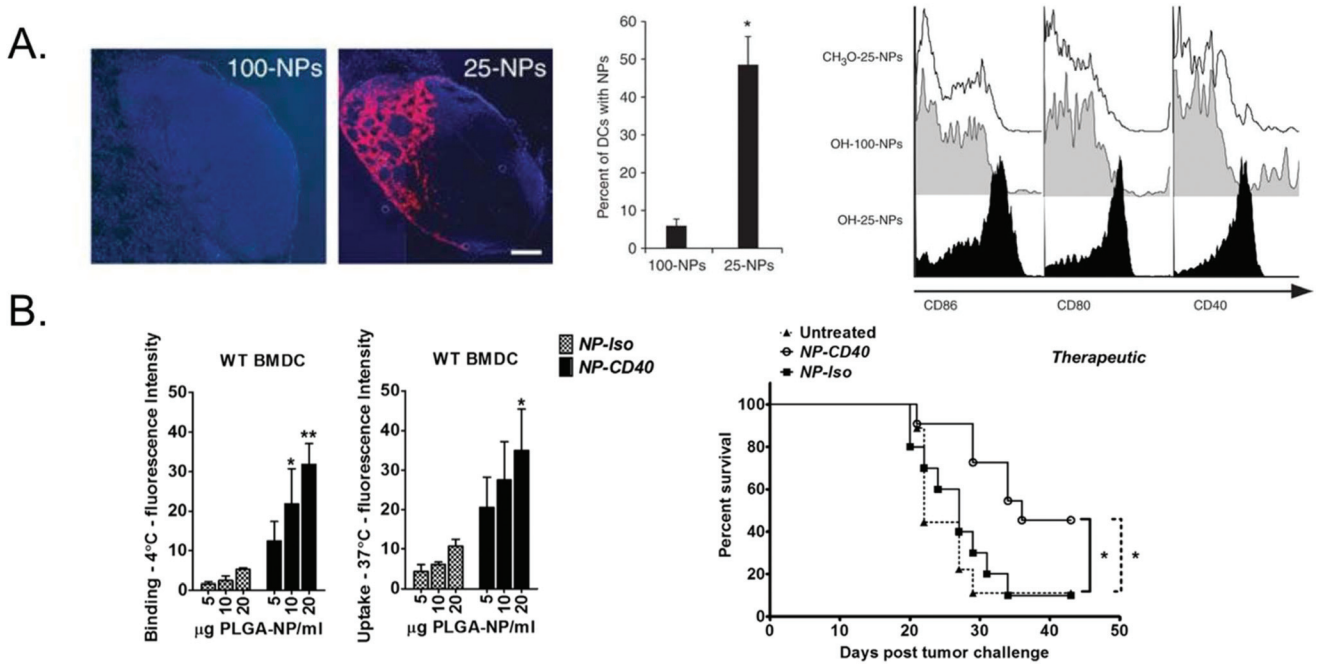

Fig. 1 Passive *vs.* active targeting of polymeric particle vaccines. (A) 25 or 100 nm PPS nanoparticles encapsulating OVA and LPS were injected intradermally and 25 nm were more efficiently transported to and retained in the draining lymph node and taken up by dendritic cells. 25 nm particles also more effectively activated dendritic cells, as measured by upregulation in maturation markers. Subfigure A adapted with permission from *Nature Biotechnology,* Copyright Nature Publishing Group 2007.[23] (B) PLGA nanoparticles encapsulating OVA peptide antigen and adjuvants were surface functionalized with anti-CD40. Dendritic cells had significantly higher binding to and uptake of CD40-targeted NPs (NP-CD40) compared to an isotype control (NP-iso) and induced prolonged survival in a therapeutic *in vivo* melanoma model compared to the non-targeted nanoparticles. Subfigure B adapted with permission from *Biomaterials,* Copyright Elsevier 2015.[45]

Active targeting to APC. In addition to passive targeting, particles can be actively targeted to APCs by coupling targeting molecules to the particle surface. One particle approach utilized red blood cell membrane-coated PLGA nanoparticles functionalized with tumor antigen peptide and mannose to actively target APCs.[42] These particles were found to extend tumor survival in a melanoma model over uncoated PLGA nanoparticles. A PLGA nanoparticle vaccine was also effectively targeted to dendritic cells by coating the particles with a PEG-lipid layer that was conjugated to an antibody targeting the DC-SIGN receptor that is present on dendritic cells and macrophages.[43] A PLGA nanoparticle melanoma vaccine was targeted to dendritic cells by functionalization with an antibody that binds to DEC-205, a dendritic cell surface ligand.[44] PLGA nanoparticles have also been targeted to CD40, a receptor highly expressed on dendritic cells that is involved in dendritic cell maturation, by conjugating the particle surface with an agonistic antibody against CD40.[45] This antibody increased dendritic cell binding and internalization of the particles and enhanced dendritic cell activation and the subsequent anti-tumor immune response, leading to prolonged survival *in vivo* in a therapeutic melanoma model (Fig. 1B). Cruz *et al.* compared PLGA nanoparticle cancer vaccines conjugated with monoclonal antibodies targeting CD40, DEC-205, or CD11c.[46] The authors found that all three targeting strategies led to significant enhancement of vaccine efficacy in comparison to non-targeted PLGA nanoparticles and that the specific targeting molecule would likely need to be tailored to each tumor type.

Modulation of tumor-associated macrophages. Macrophages are highly plastic and can transform into classically activated (M1) macrophages, which are pro-inflammatory, or alternatively activated (M2) macrophages, which are anti-inflammatory and protumorigenic.[47] In response to the highly immunosuppressive tumor microenvironment, TAMs typically adopt the M2 phenotype and, as a result, TAM tumor infiltration is correlated with a poor prognosis.[48] As such, they are a promising target for cancer immunotherapies.

One strategy that has been investigated for TAM-targeted cancer therapies is depletion of TAMs. Ernsting *et al.* synthesized a nanoparticle composed of PEG and carboxymethylcellulose to deliver docetaxel, a chemotherapy agent.[49] These nanoparticles selectively killed stromal cells and macrophages in the tumor microenvironment of a pancreatic cancer mouse model. However, this depletion of TAMs was transient, and the TAMs had fully repopulated within two weeks. Niu *et al.* fabricated an acid-sensitive sheddable PEGylated PLGA nanoparticle encapsulating doxorubicin and functionalized with mannose to target the mannose receptor overexpressed on macrophages and selectively deplete TAMs.[50,51] The acid sensitive nature of the particle is designed to avoid off-target macrophages and specifically target TAMs. Under normal physiologi-

cal conditions, the PEG chains shield the mannose on the particle surface, but in the slightly acidic tumor microenvironment the PEG chains "shed" from the particle, exposing the macrophage-targeting mannose.

An alternative strategy to TAM depletion is to re-polarize TAMs from the M2 phenotype to the M1 phenotype. This approach has the added benefit of introducing the anti-tumor activity of M1 macrophages while still reducing the protumorigenic M2 phenotype. Fuchs *et al.* found that polystyrene nanoparticles functionalized with cationic or anionic surface molecules stimulated M2 macrophage re-education into M1 macrophages.[52] Wang *et al.* designed pH-sensitive poly (β-amino ester) (PBAE) nanoparticles encapsulating IL-12, a cytokine that has been shown to re-polarize macrophages towards an M1 phenotype.[53] These polymeric nanoparticles were administered in an *in vivo* melanoma model and led to macrophage re-polarization and tumor regression with minimal side-effects due to the tumor microenvironment-responsive nature of the particles. A major challenge of the TAM re-polarization approach is inducing long-term TAM re-education into M1 macrophages, as macrophages are highly plastic may return to the M2 phenotype once the drug is no longer present due to the immunosuppressive tumor microenvironment.

APC for immunosuppression

APCs for immunosuppression. Antigen presenting cells are the most common cellular target for tolerogenic particle-based drug delivery platforms. Once tolerized, APC promote antigen-specific tolerance through their interactions with T cells, suppressing antigen-specific CD4+ and CD8+ T cells and polarizing the repertoire towards regulatory T cells (Tregs). Micro and nanoparticulate systems employ a number of unique strategies for targeting and tolerizing APC toward specific antigens of interest to treat a variety of conditions, including autoimmune disease and transplant rejection.

Targeting APC for immunosuppression. Active targeting strategies have mostly been used for immune activation purposes, but certain receptors may also be exploited to promote tolerance. Dendritic cells (DC) are a desirable target for particle-based drug delivery because of their unmatched ability to home to, engage, and activate T cells. DC205 is a commonly targeted DC receptor, and particles conjugated with monoclonal antibodies against DC205 have demonstrated increased receptor-mediated uptake.[54] Interestingly, increasing ligand density of anti-CD205 on the surface of nanoparticles has been shown to induce higher levels of receptor crosslinking and increased IL-10 production.[55] This phenomenon could potentially be exploited for tolerance induction. Another receptor of interest is the CLEC9A receptor, which presents antigen from necrotic cells and has been shown to be a critical mediator for subsequent antigen cross-presentation on DC.[56] PLGA particles loaded with antigen and bearing antibodies against CLEC9A induced antigen cross presentation,[56] and delivery of antigen targeted to CLEC9A resulted in antigen presentation on MHC II and Treg expansion.[57]

The majority of particulate tolerance therapies rely on passive targeting strategies since large nanoparticles and microparticles are efficiently taken up by APC in the spleen and liver, which is often desirable for tolerance induction.[58] These particle-based platforms for antigenic delivery to APC promote tolerance by exploiting natural tolerogenic processes or by providing tolerogenic cues by co-delivering immunomodulatory molecules.

Exploitation of tolerogenic processes. Cellular apoptosis is perhaps the most commonly exploited tolerance-inducing process. Uptake of apoptotic debris by APC in the absence of inflammatory signals promotes a tolerized phenotype characterized by upregulation of regulatory costimulatory molecules, low expression of positive costimulatory molecules, and secretion of regulatory factors promoting effector T cell anergy and regulatory T cell induction and expansion.[59] Early attempts to target apoptotic clearance pathways for antigen-specific tolerance induction coupled antigenic peptides to carrier cells using chemical cross-linker 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide,[60] a process which also induces carrier cell apoptosis. This strategy has shown to induce tolerance in a variety of preclinical models and administration of encephalitogenic peptide-coupled PBMCs has shown promise in a Phase I clinical trial to treat multiple sclerosis.[61] More recent efforts use erythrocytes as carriers and directly inject targeted peptides for *in vivo* cell coupling, leading to antigen-specific depletion of CD4+ and CD8+ T cells.[62]

Particle-based platforms can be engineered to exploit apoptotic clearance pathways without the limitations of cell-based therapies. It was discovered that polystyrene beads and PLGA nano and microparticles with a zeta potential of −40 to −70 mV are preferentially taken up by a subset of macrophages in the spleen and liver expressing the Macrophage Receptor with Collagenous Structure (MARCO), a scavenger receptor involved in apoptotic debris clearance, thereby bypassing the need for codelivery of apoptotic cell debris.[58,63] Polystyrene and PLGA particle-based platforms delivering antigenic peptides have been used to induce tolerance in a variety of models including experimental autoimmune encephalomyelitis (EAE) and transplantation,[64–66] and uptake by MARCO-expressing macrophages is crucial for the tolerogenic effects of the therapies.[64]

To more closely mimic apoptotic cells, particles bearing apoptotic markers have also been engineered to induce tolerance. Liposomes containing phosphatidylserine (PS) and carrying antigenic material have been shown to promote tolerance in several models including hemophilia and Type I Diabetes (T1D).[67,68] The use of polymeric particles instead of liposomes allows for manipulation of particle shape to enhance biomimicry and the pharmacokinetic properties of the carrier. Interestingly, 80 × 320 nm rod-shaped PLGA particles decorated with PS were shown to be more effective at inducing tolerance in an EAE model compared to 1 μm cylindrical PS-decorated PLGA particles and PS-containing liposomes.[69]

In addition to MARCO-expressing macrophages, APC in other sites including the liver, Peyer's patches, and the oral

and digestive tract have been shown to mediate tolerance.[70,71] Delivery of antigen to these tolerogenic environments can be accomplished with polymeric particle-based carriers.

Codelivery of immunomodulatory agents. Particle-based tolerance induction and maintenance therapies seek to provide antigen-specific tolerance and avoid generalized immunosuppression. Particle delivery systems that rely on natural processes and environments to mediate tolerance bypass the need for immunosuppressants. However, co-localization of inflammatory signals with antigen delivery may compromise or reverse the efficacy of these therapies, as biological cues present at the time and place of delivery are critical for determining whether the antigen will be delivered in a tolerogenic or immunogenic fashion. To address these concerns, particle carriers can co-deliver antigenic material with tolerogenic agents including suppressive cytokines, small molecule ligands, or broad immunosuppressants.

Biologics. Providing suppressive cytokines such as TGF-B, IL-10, or indoleamine 2,3-dioxygenase (IDO) locally to APC helps ensure that antigen is delivered in a tolerogenic fashion while avoiding the use of stronger, broad-based immunosuppressants. In one study, Cappellano *et al.* demonstrated that PLGA nanoparticles co-loaded with IL10 and myelin oligodendrocyte glycoprotein (MOG) peptide and injected subcutaneously significantly reduced EAE severity and decreased inflammatory cytokine secretion by T cells without any cytotoxicity.[72] Lewis *et al.* and Cho *et al.* utilized an interesting dual-size microparticle platform for delivery of tolerogenic factors both intracellularly and extracellularly to dendritic cells. Their formulation consisted of equal ratios of large microparticles loaded with GM-CSF to recruit DCs and TGFB to promote tolerization, and small microparticles delivering MOG peptide and Vitamin D3 intracellularly to DCs. Subcutaneous injection of this formulation was able to prevent T1D in 40% of mice and prevent symptom onset in an EAE model.[73,74]

Small molecule immunosuppressants. Pharmacological agents with more potent immunosuppressive potential can be co-encapsulated with antigenic material in micro and nanoparticles to locally target and tolerize APC, eliminating the need for a larger systemic dose, minimizing negative systemic side effects, and preventing generalized immunosuppression. Polymeric particles loaded with antigenic material and drugs of interest can be easily synthesized using double emulsion techniques. Particle size and drug release profiles can be controlled by changing the polymer, drug loading dose, and emulsion parameters. A variety of small molecules and immunosuppressants that modulate DC function have been tested in PLGA-based formulations. One interesting example is the use of *N*-phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxamide (PHCCC) to alter glutamate metabolism in DCs to confer a tolerogenic state that promotes regulatory T cell formation. PHCCC was co-delivered with MOG in PLGA nanoparticles and demonstrated delayed onset and reduced disease severity in an EAE model.[75]

Other biodegradable polymeric particle drug delivery platforms have been designed to deliver a range of broad immunosuppressants in a localized, controlled fashion. Rapamycin, a mammalian target of rapamycin (mTOR inhibitor), is a powerful immunosuppressant, but systemic delivery is problematic due to poor solubility and bioavailability, and nonspecific suppression of immune cells.[76] When encapsulated in PLGA micro and nanoparticles, rapamycin has been shown to inhibit DC functioning following particle uptake compared to soluble rapamycin.[77,78] PLGA microcarriers co-delivering encephalitogenic peptides and rapamycin substantially reduced the onset and severity of EAE (Fig. 2A and B), and led to recovery in 75% of mice when administered at the peak of disease (Fig. 2C). PLGA nanocarriers loaded with the same contents decreased the severity and onset of paralysis when administered prophylactically (Fig. 2D) and prevented relapse in a Relapsing-Remitting EAE model when administered as a single dose at the peak of disease (Fig. 2E).[79,80] Inclusion of rapamycin was essential for enhanced tolerogenic effects, as administration of peptide-loaded particles only conferred partial disease protection. Rapamycin-loaded nanoparticles co-administered with clinically approved biologics to treat a variety of diseases including Pompe disease, hemophilia, and inflammatory arthritis reduces the formation of antidrug antibodies and improves tolerance to the biologic.[79,81–83] Antiproliferative agents such as mycophenolic acid (MPA) also have negative effects when administered systemically that can be ameliorated through delivery in particle carriers. Injections of MPA-loaded nanoparticles significantly extended graft survival compared to a 1000-fold higher dose of free MPA in a murine skin transfer model, and no drug toxicity was observed for the nanoparticle treatment.[66] The nanoparticles were found to be taken up by DC, leading to upregulation of programmed death ligand 1 (PDL-1) which helped promote graft survival. Steroids are also commonly used as immunosuppressive agents, but are associated with unpleasant side effects when administered systemically. Treatment with dexamethasone and MOG peptide-loaded acetylated dextran microparticles resulted in a significant decrease in EAE clinical score compared to soluble drug, empty particles, and particles loaded with either peptide or drug alone.[84]

T-cells

T-cells, as the major effector arm of the immune system with respect to cancer immunotherapy and infectious disease, have been the subject of extensive investigation with respect to delivery of biomedical therapeutics from particulate systems.[85] Direct delivery to T-cells can be thought of as "short-circuiting" the typical immune response, bypassing the need of an antigen presenting cell to relay the information. Broadly, two strategies that have been used in the particle-based delivery of therapeutics to T-cells are (1) The presentation of immobilized biologics from particulate surfaces, such as through the use of artificial antigen presenting cells (aAPCs) and (2) The extra-

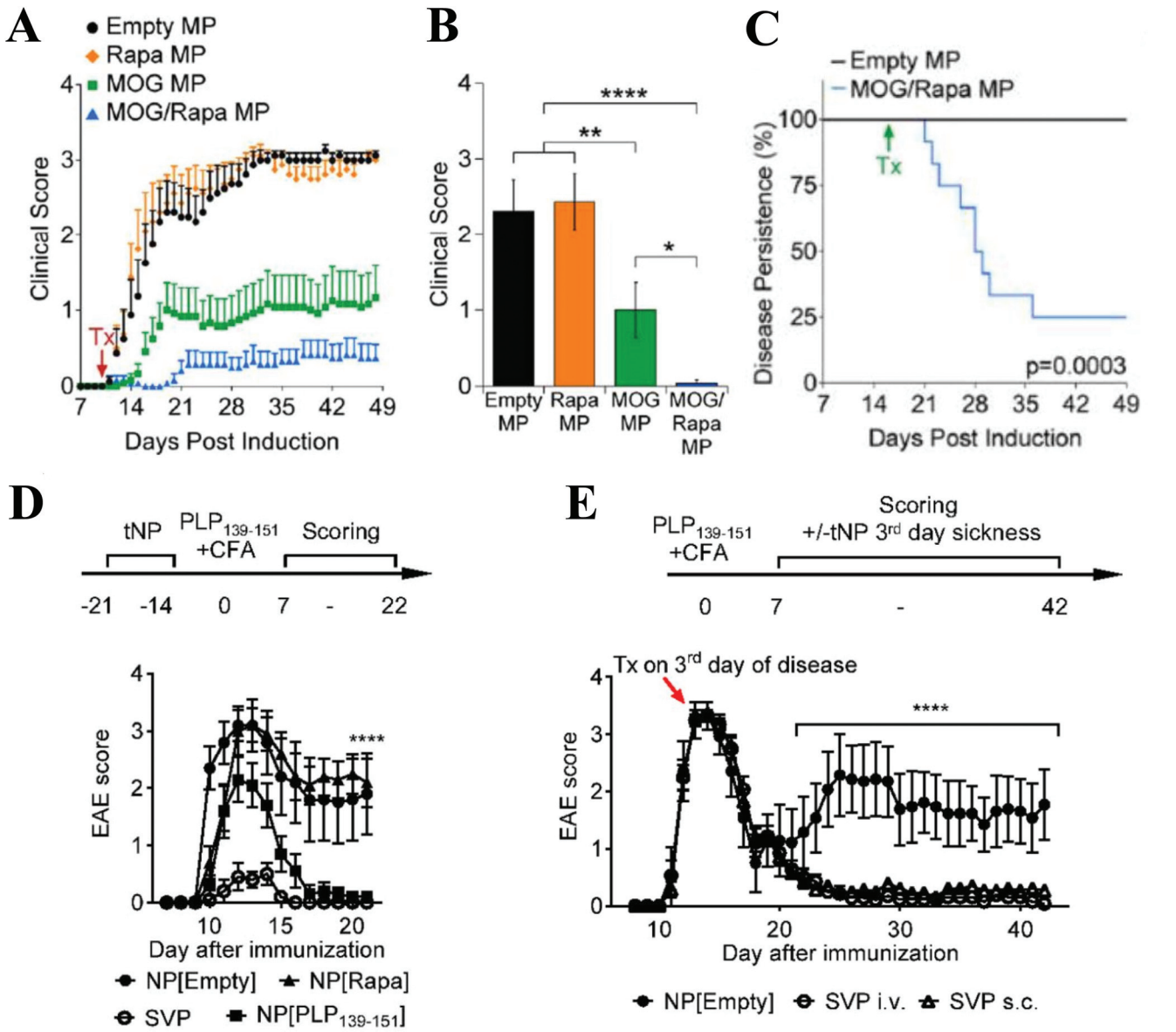

Fig. 2 (A) Prophylactic treatment with microparticles coloaded with MOG and rapamycin delayed onset and reduced severity of EAE symptoms compared to empty, MOG-loaded, and rapamycin-loaded particles. (B) MOG + rapamycin-loaded particles mediated a significant decrease in EAE clinical score on Day 19 post-EAE induction. (C) When administered at the peak of disease, MOG + rapamycin-loaded particles facilitated recovery in 75% of mice. (D) Prophylactic treatment with nanoparticles coloaded with PLP and rapamycin (SVP) delayed onset and reduced severity of symptoms in Relapsing-Remitting EAE model. Nanoparticles loaded with PLP only conferred partial protection from disease symptoms. (E) A single dose of SVP particles administered intravenously or subcutaneously at the peak of disease prevented relapse. Subfigures A–C reprinted with permission from *Cell Rep*, Copyright Elsevier 2016.[80] Subfigures D-E reprinted with permission from *PNAS*, Copyright National Academy of Sciences 2015.[79]

cellular or intracellular delivery of soluble biological factors *via* release from particles.

T cells for immunostimulation

Artificial antigen presenting cell (aAPC) based drug delivery. The delivery of biomedical therapeutics in the context of artificial antigen presenting cell technology has been recently realized as a potential stimulatory platform for cancer immunotherapy. From a reductionist standpoint, artificial antigen presenting cells attempt to recapitulate the two critical signals delivered to T-cells by antigen presenting cells. Signal 1 is typically recapitulated by an agonistic antibody for CD3 or a recombinant antigen loaded MHC protein. Signal 2 in the context of stimulation usually consists of an agonistic antibody for CD28, although other signals have been investigated with respect to this technology. These two proteins are subsequently immobilized on a particle surface to present to T-cells.[86] It has been found that aAPC perform optimally on a micron size scale[87] and a shape that allows for maximal contact with the T-cell such as an ellipsoid.[88]

aAPC particulate systems made from materials such as biodegradable polymers, iron oxide nanoparticles, and lipid based materials[85] have typically been used as a standalone therapy.[88,89] However, new evidence suggests that they would benefit from coadministration of other biologics. Recently their activity has been shown to be augmented by the presence of other immunostimulatory cytokines and monoclonal antibodies. Kosmides *et al.* investigated the use of aAPCs with systemic administration of anti-PD1 (Fig. 3A).[90] The aAPCs were found to be effective stimulators of T-cell activation and the anti-PD1 was found to protect the expanded cells from exhaustion. This combination therapy resulted in a 50% reduction in tumor burden (Fig. 3b) and an increase in median survival from day 17 to day 21 for the dual treatment strategy compared to either therapy on its own or a control.[90]

The delivery of therapeutics directly from aAPCs has also been investigated as a platform to enhance T-cell activation. Steenblock *et al.* developed an aAPC system that encapsulated the T-cell growth factor IL-2.[91] The group found that the particles encapsulating IL-2 were able to outperform empty particles with soluble IL-2. Furthermore, this was later found to be the result of the confined space between the T-cell and the aAPC, which allowed for accumulation of the cytokine for a higher apparent concentration.[92] This local release of IL-2 was subsequently appropriated in the context of a high surface carbon nanotube platform for T-cell stimulation for *ex vivo* stimulation of T-cells for cancer immunotherapy.[93] It was determined that the local release of IL-2 resulted in T-cell activity comparable to a 1000 fold increase in soluble IL-2 in an adoptive immunotherapy model. Taken together these results suggest the paracrine delivery of biologics from aAPCs is a promising therapeutic platform for cancer immunotherapy.

aAPCs can also be used to deliver surface bound signals in a targeted fashion. Schütz *et al.* investigated the use of an antigen loaded MHC Class I dimer conjugated to a particle that also had tethered to the surface an monoclonal antibody for CD19.[94] The resultant particle was found to be successful at directing antigen specific T-cells to attack CD19+ leukemia cells. The therapy was found to mediate a near 50% reduction in tumor burden for cognate redirection particles compared to the non-cognate controls.[94] Kosmides *et al.* utilized a nanoparticle loaded with an antibody bearing the Signal 2 molecule anti-41BB and the immune checkpoint blockade anti-PDL1 (Fig. 3c).[95] The resultant nanoparticle redirected the normally immunosuppressive surface of a melanoma cell to be immunostimulatory. The therapy also was able mediate a near 50% reduction in tumor burden compared to a control in a B16-F10 melanoma model (Fig. 3d).[95]

Drug loaded particles for biologic delivery. Outside of the context of artificial antigen presenting cells, other particulate systems have also been used to directly activate T-cells for

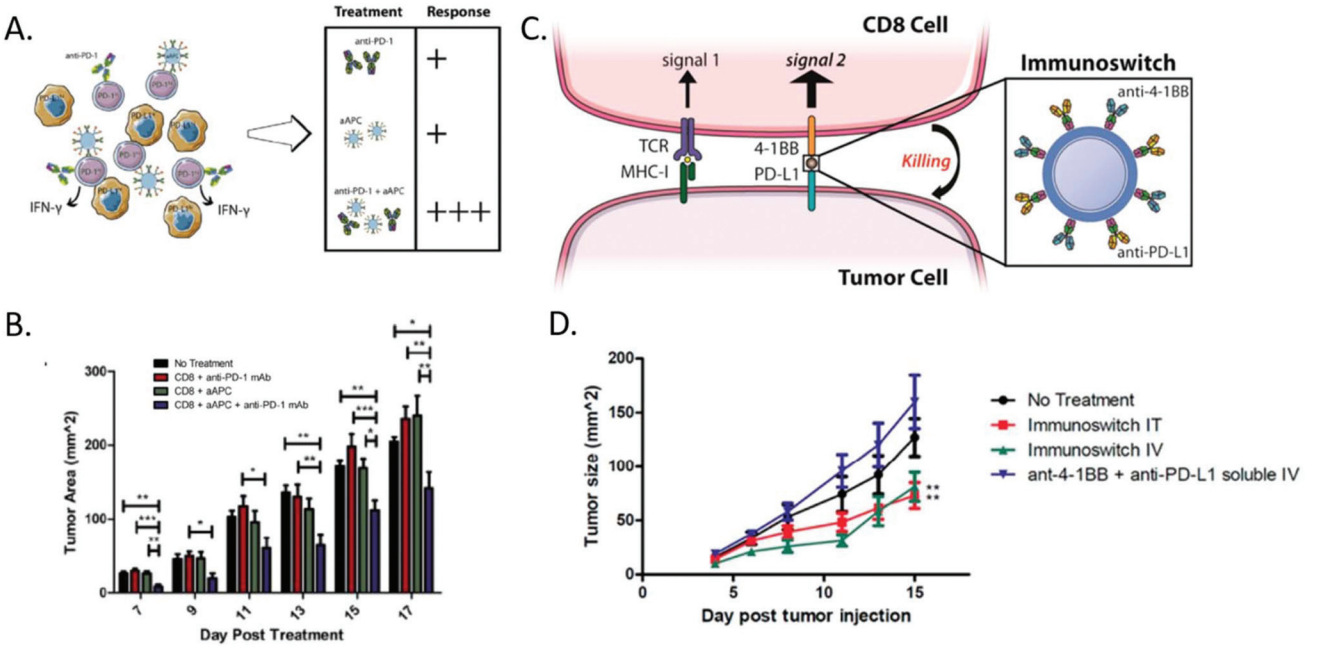

Fig. 3 Artificial antigen presenting cell strategies can be combined with biologic delivery for enhanced effect. (A) Anti-PD1 in combination with artificial antigen presenting cells were found to yield the most significant anti-tumor immune response compared to either therapy on its own. (B) The combination therapy was able to mediate a 40% reduction in tumor burden compared to the no treatment and single treatment groups. (Reprinted with permission from *Biomaterials*, Copyright Elsevier 2017).[90] (C) Signal 2 only particles can be combined with immune checkpoint blockades on the surface of a particle to act as an immunoswitch to redirect the cancer cell to activate a CD8+ T-Cell, rather than suppress it. (D) This approach led to a 50% tumor reduction in various B16 models without the need for antigen specificity. (Reprinted with permission from *ACS Nano*, Copyright American Chemical Society 2017).[95]

immunotherapies. Two categories of particles that have been used to accomplish this objective include the use of stimulus responsive materials, and the use of non-stimulus responsive materials.

With respect to stimulus responsive particles, a broad area of research that has been investigated is photothermal triggered release of cytokines or other immunomodulatory molecules. Photothermal ablation of tumor tissue, mediated by plasmonic resonation of gold nanoparticles in response to laser irradiation, has gained significant popularity as a treatment paradigm for tumors due to the creation of antigenic material in an immune stimulatory fashion. The generated heat can subsequently promote drug release from a polymeric particle. Luo *et al.* used photothermal ablation in the context of a PLGA particle releasing a peptide designed to block PD-1 on the surface of T-cells.[96] It was found that these particles could mediate a 75% reduction in tumor in a 4T1 cancer model compared to a single dose of the free peptide. Similar responses were found with another immune checkpoint blockade antibody, anti-CTLA4.[97]

Other studies have used the T-cell itself to serve as the propagator of the stimulus for drug release. Tang *et al.* used a 50 nm bioreducible protein gel to deliver the immunostimulatory cytokine IL-15.[98] Taking advantage of the increase in reduction potential of the exterior of the cell membrane upon TCR stimulation, the authors achieved TCR engagement dependent stimulation of the immune stimulatory cytokine. Utilizing CAR T-cells directed against B16-F10 melanoma, it was determined that the bioreducible responsive protein gels were able to mediate a near 20 day increase in median survival compared to the control of free IL-15.[98] The release of cytotoxic granules from CD8+ cells upon engagement with a target has also been shown to result in triggered drug release. Jones *et al.* encapsulated IL-15 in lipid nanoparticles that were then tethered to the surface of CD8+ T-cells through maleimide–thiol chemistry.[99] Upon engagement with a target, the cytotoxic compounds released from the T-cell would then cause the lipid nanoparticle to also release IL-15 to augment the T-cell activity. The nanocarriers bound to the T-cells mediated a 4-fold reduction in HIV+ CD4+ T-cells compared to an empty nanocarrier control in an HIV infection model.[99]

Non-stimulus controlled drug delivery has also resulted in augmentation of biomaterials impact on T-cell activity and remains a promising strategy for the treatment of many conditions. Many of these platforms involve the controlled release of therapeutics typically administered systemically for immune modulation. Rhines *et al.* demonstrated that IL-2 loaded gelatin based polymeric microparticles could allow for the controlled release of IL-2 at the local site a glioma in a rodent model.[100] This local release of IL-2 increased the typical survival time with BCNU treatment from 32.5 days to 45.5 days. A monoclonal antibody against OX40 has also been delivered using polymeric PLGA nanoparticles and it has been found that this resulted in a 2-fold increase in T-cell proliferation compared to soluble anti-OX40.[101]

T-cells have also been actively targeted for drug delivery in an non-antigen specific manner for the treatment of MC38 colon cancer.[102] Schmid *et al.* designed PLGA nanoparticles with a monoclonal antibody against PD-1 on the surface of an exhausted T-cell and loaded with an inhibitor of TGFβ signaling. The result was the targeted delivery of the regulatory-breaking drug to lymphocytes that were vulnerable to PD-1 suppression. This resulted in a 60% increase in median survival of mice compared to non-particle bound controls of anti-PD1 and the TGFβ inhibitor.[102]

T cells for immunosuppression

While the majority of tolerogenic, particle-based immunotherapies have been targeted at APCs to indirectly induce T-cell-mediated responses, an attractive alternative is to stimulate T-cells to induce tolerance directly, thus bypassing the need for processing and manipulating of antigen presenting cells. T-cell mediated tolerance is primarily mediated through regulatory T-cells that include natural CD4+ CD25+ FOXP3+ cells, induced Th3 CD4+ CD25+ FOXP3+ from Tr1 CD4+ CD25− FOXP3− T-cells, and CD8+ Tregs.[103,104] FOXP3+ Tregs target APCs and T effector cells with an end result of suppression of autoreactive T-cells while Tr1 Tregs target T effector cells to initiate mucosal immunity and modulate the inflammatory response.[105] CD8+ regulatory T-cells are present in lower amounts than their CD4+ counterparts and little is known about their mechanisms other than they suppress effector lymphocytes through cytokine signaling and negative receptor signaling in inflammatory environments, and they may be generated from low avidity CD8+ autoreactive cells.[104,106,107] Polymeric strategies to directly induce T-cell-mediated tolerance can be roughly separated into the following categories: 1. Artificial antigen presentation for induction of regulatory T-cells 2. Targeted Drug Delivery to T-cells 3. Depletion of effector T-cells.

Artificial antigen presentation for induction of regulatory T-cells. With the goal of inducing antigen-specific tolerance or more broadly systemic tolerance, artificial APCs seek to emulate tolerogenic APCs by presenting CD4+ or CD8+ T-cells with immunomodulatory signals 1 and 2 as well as release of tolerogenic cytokines. The majority of particle-based approaches utilize PLGA, polystyrene, or iron oxide nano or micro spheres with zeta potentials ranging from −45–60 mV and diameter scales ranging from 30 nm–5 microns.[108–111] With such a broad range of sizes and properties, it is difficult to recommend a specific size or shape for tolerogenic artificial antigen presentation, although there is an additional design dimension for particles such as those made from PLGA that have the ability to encapsulate and release antigen, cytokines, or small molecules. Likewise, it has been shown that aAPC size and shape have an effect on T-cell stimulation,[112,113] but no such shape, size, or material comparisons have been made in the current literature with respect to tolerogenic aAPCs.

Tolerogenic aAPCs have been designed to present signal 1 and antigen by a variety of methods. Most similarly to biological APCs, antigen is coupled to the surface with MHC class I or

II in the form of peptide-MHC complexes (pMHC), which gives the added advantage of CD8+ *vs.* CD4+ T-cell specificity, depending on the selection of peptide loaded MHC class I or II (Fig. 4a–c).[109,114,115] A challenge moving forward with this pMHC strategy is the diversity in human HLA haplotypes as opposed to murine MHC. In order to avoid this hurdle, Xu *et al.* conjugated 5 distinct immunodominant HLA- A*02 : 01-restricted epitopes specific for Type I diabetes to 500 nm polystyrene beads and found that certain combinations resulted in a 10-fold decrease in antigen specific CD8+ T-cell activation.[116] A simpler approach of conjugating CD47-Fc to the particle surface has been explored by Wan *et al.* and Shahzad *et al.* in response to the recent finding that CD47-Fc can act as a self-marker and prevent macrophage uptake in aAPCs[109,117,118] although this approach lacks T-cell specificity and does not classically present antigen on MHC.[109,118] Lastly, there are some strategies that aim to create antigen specificity by encapsulating antigen within particles and using coupled targeting ligands to release antigen to the T-cell in a controlled manner. Coupled surface antigen was directly compared to encapsulated antigen by Hlavaty *et al.* and found that surface coupling Dby CD4+ T-cell specific antigen was 5× more efficient than encapsulating antigen and induced tolerance to mismatched bone marrow transplantation comparable to positive control, where the level of donor cells was 49% by Week 20.[111]

In addition to signal 1 and antigen, aAPCs are often designed to present negative signal 2 costimulatory ligands and/or deliver soluble cytokines to reduce effector T-cell stimulation and promote expansion of Tregs. Negative costimulatory ligands have included anti-FAS and PD-L1, or a complete absence of signal 2, as absence of signal 2 altogether has been shown to induce T-cell anergy or induction of regulatory T-cells.[119] Costimulation was shown to have a 50% increase of cell engraftment in a model of bone marrow allotransplantation in Hlavaty *et al.* when soluble PD1 was added to the Dby CD4+ T-cell specific aAPCS compared to an anti-PD1 blockade group.[120] Likewise, in the combinatorial approach of Wan *et al.*, it was difficult to separate the impacts of costimulation of PDL1 and release of TGF-β, but both were implicated in long term maintenance of Experimental Autoimmune Encephalitus (EAE) amelioration as opposed to the short-term effects of the anti-FAS costimulatory ligand.[109]

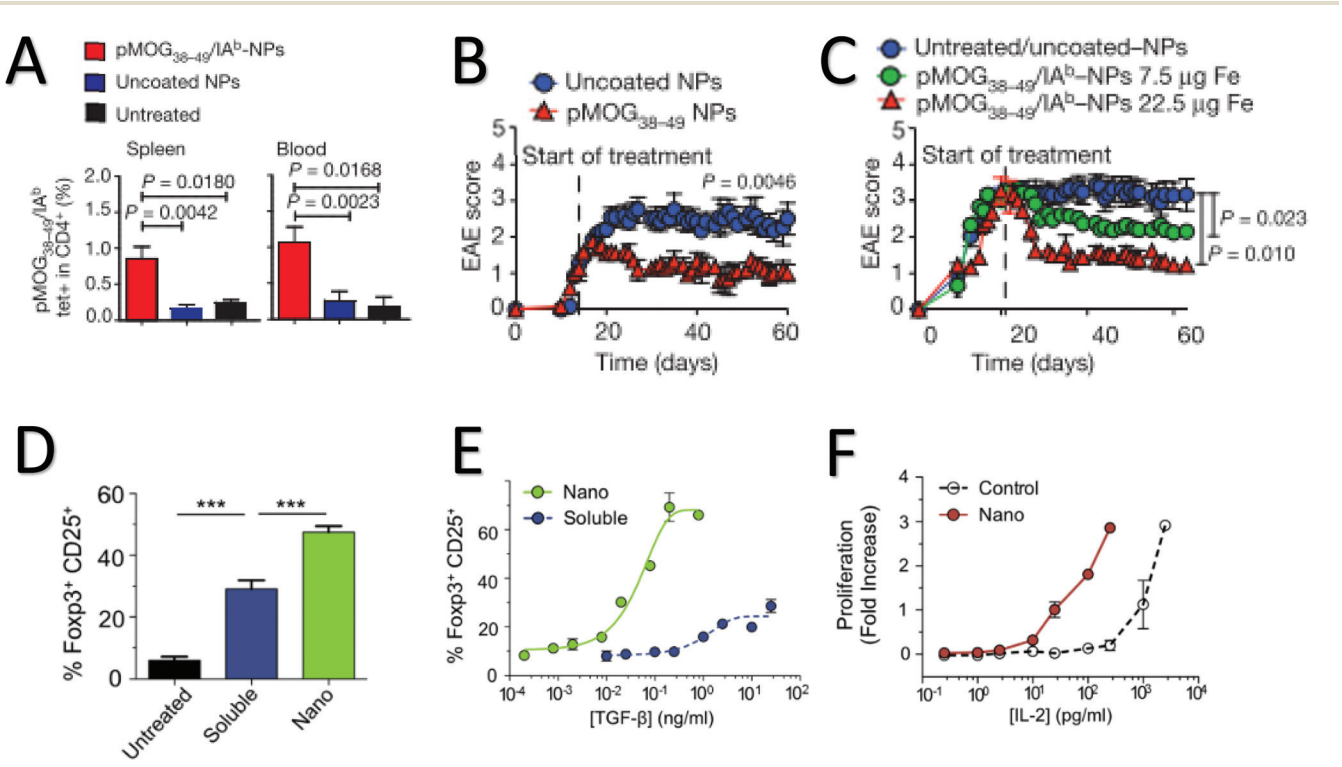

Fig. 4 Strategies for targeted expansion of CD4+ T-Cells for immunosuppression. (A) pMHCII-NP loaded with MOG antigen for Experimental Autoimmune Encephalitis (EAE) expands cognate disease-suppressing T_R1-like CD4+ T-Cells *in vivo.* Percentage of pMHCII tetramer in CD4+ cells is increased in pMOG coated PEGylated iron oxide NPs compared to uncoated NPs in both spleen and blood. (B) pMOG-NP therapy dampens disease progression when given on day 14 after immunization. Clinical EAE score of pMOG coated NP injected mice is significantly improved compared to uncoated NPs. (C) pMOG-NP therapy restores motor function in paralytic mice when given on day 21. Subfigures A, B, and C adapted with permission from *Nature*, Copyright Springer Nature 2016.[114] (D)–(F) Targeted drug delivery to CD4+ T-Cells using encapsulated TGF-β and IL-2 induces significant expansion of FOXP3+ CD25+ regulatory T-Cells compared to soluble cytokines. (D) Percentage of FOXP3+ CD25+ Regulatory T-Cells in response to targeted nanoparticle cytokine delivery is increased in nanoparticles compared to soluble cytokine (E) Percentage of Regulatory CD4+ T-Cells Dose responses of nano-encapsulated (green), and soluble (blue) cytokine. (F) Proliferation of IL-2 dependent CTRL-1842 cells after dosing with nano-encapsulated and free IL-2. Subfigures D, E and F adapted with permission from *Biomaterials*, Copyright Elsevier, 2015.[123]

Targeted drug delivery to T-cells. While co-delivering encapsulated regulatory cytokines to T-cells in tolerogenic aAPCs is a new frontier, there has been some established work in targeted immunosuppressive drug delivery to T-cells using cytokines and small molecule immunosuppressants, although differences in targeting *versus* non-targeted delivery of small molecule immunosuppressants are minimal.[121] The major tolerogenic cytokines are TGF-β, IL-10, and IL-2, although the effectiveness of IL-2 at inducing tolerance depends on the environment, as it can also expand effector T-cells if in the presence of activating costimulatory signals.[119,122] Since there is no cell binding component, delivery methods are restricted to nanoparticles ranging from 100–300 nm with particle materials including PLGA,[118,123] cyclodextrin nanogels,[121] and liposomes.[124]

Currently all targeted T-cell drug delivery for tolerance induction is directed at CD4+ T-cells for the induction of FOXP3+ regulatory T-cells through incorporation of an anti-CD4 surface ligand, although there is potential for expansion to other T-cell types. Park *et al.* showed initially that CD4 targeted, encapsulated Leukemia inhibitory factor (LIF) in PLGA nanoparticles expanded FOXP3+ CD4+ Tregs *in vitro*.[125] More recently, McHugh *et al.* demonstrated dramatic expansion of FOXP3+ Tregs and increased, lasting immune suppression after *in vivo* treatment of encapsulated IL-2 and TGF-β loaded PLGA nanoparticles compared to soluble cytokines (Fig. 4d–f).[123]

Depletion of effector T-cells. In contrast with the "positive" tolerizing strategy of inducing or expanding Tregs, there has been significant work in inducing particle-mediated tolerance through a "negative" strategy of deleting or suppressing effector T-cells directly either through targeted cytokine delivery, small molecule drug delivery, or negative receptor antigen co-presentation.[126] In this strategy, often referred to as killer aAPCs (KaAPCs), only the autoreactive T-cells are targeted allowing the remaining immune system to function unperturbed. Additionally, there has been found to be some overlap in depletion of autoreactive T-cells and aAPC expansion of Tregs, so the two strategies may be applied together for a synergistic effect.[110,119] In early studies, microscale killer aAPCs on latex beats with antigen specific $H2k^b$ monomers coupled with anti-Fas ligand were able to produce a 60% decrease in antigen-specific alloreactive T-cells while preserving general immune function.[127] In recent years, KaAPCs have progressed to be bio-degradable, target both CD4+ and CD8+ autoreactive T-cells, and able to encapsulate cytokines for controlled release *in vitro* and in vivo.[110,119,128]

B-cells

B-cells act as the major producer of antibodies that assist in the mediation of a humoral as opposed to cellular immune response. As opposed to T-cells which require antigen presented on the surface of APCs to become active, B-cells have the capability to recognize antigen that is free in solution.[113] Although B-cells can recognize free antigen in solution, multiple studies have confirmed that B-cells require a degree of crosslinking of the antigen on the surface of the membrane to achieve activation.[129] Furthermore, it has been found that *in vivo*, B-cells primarily recognize antigens that have been sequestered and presented on the surface of antigen presenting cells.[130] These physiological characteristics have driven the evolution of new biomaterials technologies that are aimed at the presentation of antigen to B-cells as well as technologies to protect antigens and deliver them in tact to B-cells *in vivo*.

Antigen presentation based stimulation

The presentation of antigen on a surface may be the most directly mimetic way to stimulate B-cells based on their aforementioned physiology. As such, many biomaterials based strategies to deliver antigen to B-cell to elicit production of antibodies, rely on the presentation of antigen on a particle surface. Temchura *et al.* developed a calcium phosphate nanoparticle with HEL antigen conjugated to the surface.[131] These nanoparticles were able to elicit an antigen specific B-cell response that was comparable to non-specific stimulation with lipopolysaccharide. Furthermore, this response was found to be 100-fold stronger than soluble antigen.[131] Similar to this platform, Ingale *et al.* developed a liposome based presentation system with well-ordered array of HIV-1 antigen on the surface.[132] This resulted in a doubling of antibody titers compared to soluble antigen in a rabbit immunization model. These antigen presentation effects can also be amplified through extensive polymerization of the antigen as shown in Bennett *et al.*, where a 300-mer antigen nanoconstruct resulted in twice the BCR stimulation of a 50-mer antigen nanoconstruct.[133]

Other antigen delivery strategies

Aside from antigen presentation, a major goal of delivery to B-cells is the protection of fragile antigens in the body prior to interaction with B-cells. Polymeric particles serve as the ideal candidate for this application due to their ability to encapsulate the antigen and protect it from a harsh exterior environment. One targeted application by Sicard *et al.* delivered antigens to B-cells using polystyrene nanoparticles that were targeted to the B-cell receptor by a monoclonal antibody.[134] Despite targeting the B-cells, the authors ultimately elicited a cellular immune response through natural B-cell antigen presentation to CD4+ cells. The activated B-cells were able to serve as antigen presenting cells to the CD4+ T-cells and expand rare antigen T-cells nearly 2-fold compared to an inactive control.[134] Other platforms for targeted delivery to B-cells take advantage of the C3 complement receptor.[135] This strategy could deliver antigen to both B-cells and antigen presenting cells that have similar receptors.

Non-targeted strategies focus on antigen protection from harsh physiological environments before delivery to B-cells. One antigen that can be difficult to deliver due to its lack of thermal stability is the polio antigen IPV. Tzeng *et al.* developed a single injection microparticle formulation that con-

sisted of a PLGA matrix with various cationic polymers in the core to stabilize the antigen.[136] The particle formulation was found to have a two burst drug release profile, consistent with an initial vaccination and a follow up booster. The particles elicited similar antibody titers in a host with a single injection of particles as the traditional dual injection strategy, thus obviating the need for a second injection. This could be especially useful in developing countries where access to regular medical care is difficult.[136] PLGA encapsulation has also been shown to protect *Salmonella typhi* antigens from long-term degradation, and provided comparable mucosal immunity to bolus injections of the antigen.[137]

B cells for immunosuppression

While delivery to APCs and T-cells for induction of tolerance may seem a more obvious approach to treat autoimmune disorders and transplant rejection, there is ongoing work harnessing the power of humoral tolerance through particle-mediated manipulation of B cells. B cells are able to directly mediate tolerance by deleting or suppressing autoreactive B cell receptors, removing autoantigen, and releasing tolerogenic cytokines.[138] Current particle-based strategies for B cell tolerance induction focus on deletion of autoreactive B cells either on its own or coupled with targeted delivery of the systemic immunosuppressant rapamycin.[139–141] B cells are targeted through the incorporation of a CD22 ligand, surface antigen, and incorporate SIGLEC ligands as costimulatory apoptotic signals. Currently, the materials for delivery have been limited to spherical DSPC nanoliposomes (100–160 nm), and the literature is sparse with respect to particle-mediated tolerizing strategies. However, there is much research in the area of B cell mediated tolerance. A unique strategy that has not been investigated using particle-based methods is to induce tolerance by engineering B cells to produce antibody to harmful self-antigens. Chakerian and Kirnbauer *et al.* have employed this strategy using virus particles as an immunogenic delivery vector of self-antigen to autoreactive B cells to induce expansion of B cells specific to TNF-α, a mediator of collagen type II arthritis.[142,143] It was demonstrated that particle delivery aliased B cell recognition of self *vs.* nonself and allowed the survival and expansion of mature autoreactive B cells. In a mouse model, vaccination with the particles prevented development of collagen type II arthritis.[143]

NK cells

Natural killer cells are similarly important effector cell of the innate immune system. These cells perform a cytotoxic role in cellular immunity, however they do not respond to antigen presented in MHC Class I as CD8+ T-cells do. In the absence of antigen specificity, NK cells can selectively eliminate cancerous cells and infected cells based on stress markers such as the downregulation of MHC Class I.[144] Furthermore it has been shown that depletion of NK cells can prevent biomaterials based anticancer therapeutics from performing at their optimal level.[145] Drug delivery to natural killer cells is a largely unexplored field in biomaterials research. Related platforms could shed some light on potential strategies for NK cell therapeutic delivery. Transfection modified cancerous cells have been used to deliver surface bound cytokines (such as IL-15 and IL-21) as well as activating signals (41BBL) to natural killer cells to enhance their activation and proliferation.[146] These approaches led to therapeutically active NK cells in clinically relevant numbers. Such a strategy could be adapted to a particulate based system for acellular activation of NK cells through biologic delivery. It is advised that these particle systems would be most effective if designed similar to artificial antigen presenting cells for T-cells in order to mimic the micron scale radius of curvature found on these cellularly based NK cell stimulators.

Sinusoidal endothelial liver cells

Liver sinusoidal endothelial cells (LSECs) are liver resident scavengers that have been shown to contribute to hepatic tolerance through induction of CD4+ FOXP3+ regulatory T-cells.[147] LSECs are able to interact with circulating T-cells and present antigen as well as signal 2 and cytokine release including immunosuppressive molecules PGE_2, PD-L1, FasL, and IL-10. To date, the tolerogenic potential of LSECs for treating autoimmune disease has remained largely untapped by tolerogenic particle-based immunomodulation strategies. The only group to investigate this tolerogenic avenue is Carambia *et al.*, who employed 10–20 nm superparamagnetic iron oxide nanocrystals or CdSe/CdS/ZnS-core–shell–shell quantum dots encapsulated into an amphiphilic polymer (poly(maleic anhydride-*alt*-1-octadecene)). They were then coupled to MBP and MOG peptides to target the SLE's to induce Tregs for Experimental Autoimmune Encephalitis (EAE). They established that administration of LSEC-targeting autoantigen peptide-loaded nanoparticles could prevent or ameliorate the onset of EAE in mice by significant expansion of antigen specific Tregs.[148]

Conclusion

Polymeric micro- and nanoparticles have been extensively explored for immune modulation *via* biologic and small molecule drug delivery. In this review, major progress in engineering such polymeric particles to suppress the immune system for autoimmune disease and transplant tolerance or to stimulate the immune system to treat cancer were described. Although suppressive and stimulatory particles have opposing effects, these particles share many design principles. Polymeric micro- and nanoparticles have been designed to target immune cells, such as T cells, dendritic cells, macrophages, B cells, and NK cells, to activate them, suppress them, or guide them toward a desired phenotype. Polymeric particles enable flexibility when designing immunotherapies as many properties, such as size, shape, biodegradability, pharmacokinetics, and targeting capability can be modulated to optimize efficacy and safety. Increasing knowledge in recent years of

these cellular subsets of the immune system combined with the development of biocompatible polymeric particles has led to a rapid expansion in the field of particles for immune modulation. Particles provide a modular platform for delivery of various small molecules and biologics and enable cell or organ targeting *via* surface functionalization or size, surface charge, and material modulation. However, many questions still remain about how polymeric particles affect the immune response. For example, there have been conflicting reports on the effect of particle size on the adjuvanticity of particles.[149] Systematic and controlled studies will help elucidate the role of various particle properties so that particles can be tuned to achieve the desired therapeutic immune response. Future research into particles engineered for immune suppression and activation will likely yield more intelligently designed particle-based immunotherapies.

Conflicts of interest

There are no conflicts of interest to declare.

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