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In vivo reprogramming of immune cells: Technologies for induction of antigen-specific tolerance

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Abstract

Technologies that induce antigen-specific immune tolerance by mimicking naturally occurring mechanisms have the potential to revolutionize the treatment of many immune-mediated pathologies such as autoimmunity, allograft rejection, and allergy. The immune system intrinsically has central and peripheral tolerance pathways for eliminating or modulating antigen-specific responses, which are being exploited through emerging technologies. Antigen-specific tolerogenic responses have been achieved through the functional reprogramming of antigen-presenting cells or lymphocytes. Alternatively, immune privileged sites have been mimicked using biomaterial scaffolds to locally suppress immune responses and promote long-term allograft survival. This review describes natural mechanisms of peripheral tolerance induction and the various technologies being developed to achieve antigen-specific immune tolerance *in vivo*. As currently approved therapies are non-specific and carry significant associated risks, these therapies offer significant progress towards replacing systemic immune suppression with antigen-specific therapies to curb aberrant immune responses.

Graphical abstract

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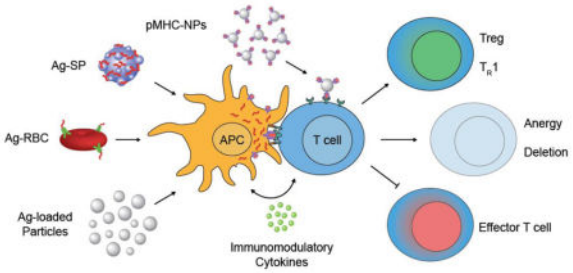

Keywords

Immune tolerance; nanoparticle; autoimmune disease; transplantation; allergy; drug delivery; regulatory T cells

1. Introduction

Induction of antigen (Ag)-specific immune tolerance is a complex process that requires the collaboration of multiple immunological pathways. Aberrant activation of T cells *in vivo* results in cellular damage against specific tissues and is responsible for the development of autoimmune diseases. To minimize the occurrence of undesirable immune responses to self-Ags, most self-reactive lymphocytes are eliminated in the thymus and bone marrow by a mechanism known as central tolerance. Unfortunately, this process is only 60-75% effective and potentially harmful Ag-specific cells with possible effector activity are released into the blood and tissues [1, 2]. To suppress potentially autoreactive cells that have avoided elimination by central tolerance, peripheral tolerance mechanisms exist. Intrinsic peripheral tolerance mechanisms are sometimes insufficient to curb inappropriate immune activation, necessitating therapeutic intervention to enable the body to limit responses to “self.” Common therapies used to subdue abnormal immune activation are not Ag-specific and involve systemic immune suppression or immunodepletion therapies that target the T cell receptor (TCR), co-signaling molecules, cytokines, or inhibit leukocyte trafficking, among other mechanisms [3, 4]. However, administration of these non-specific treatments over a prolonged period of time is associated with numerous adverse effects, including increased patient susceptibility to opportunistic infections [5], viral reactivation [6], and neoplasia [7].

Ag-specific tolerance approaches are needed to restore immune homeostasis in the cases of autoimmune disease as indicated above, and can be extended to establish selective Ag tolerance in the cases of allogeneic transplant and allergy. In Ag-specific tolerance, undesired immune activation is suppressed while the activity of the remaining immune system is maintained. Thus, the desirability of therapies to address these conditions has gained significant traction over several decades as the incidence of immune-mediated diseases has steadily risen [8, 9]. T cell-mediated autoimmune diseases are driven by the continued presentation of self-Ag by Ag-presenting cells (APCs) to autoreactive T cells. Conversely, allograft rejection involves a combination of allorecognition by T cells and alloantibody production by B cells [10]. Allergic reactions involve the activation of granulocytes such as mast cells, basophils, and eosinophils by allergen binding to antibodies [11]. Important immune elements of these diseases are the development of Ag-specific

effector T-helper type 1 (Th1) and Th17, or Th2 responses that are associated with the clinical features of disease progression [12]. The acquired phenotype of a T cell that differentiates from a naïve T cell is determined by its type of interaction with an APC as well as other factors that include the microenvironment, co-signaling molecule expression, type and load of Ag, and the intramolecular signals transduced [12]. A thorough discussion of the molecular mechanisms of these conditions is beyond the scope of this review and readers are directed towards several excellent reviews [10, 13-18].

Peripheral tolerance can be induced *in vivo* using a variety of technologies (Figure 1). For Ag-specific tolerance, the Ag is presented by APCs in the presence of low levels of co-stimulatory molecule expression and in the absence of other activating stimuli (i.e. absence of inflammation, infectious agents, and other pathologies) [3, 19]. These specific interactions aid in driving Ag-specific effector T cells towards an unreactive state (anergy or deletion) or induce regulatory T cells (Tregs) that can modify the activity of other T cells [4]. To drive immune responses towards tolerance, the Ag must be delivered to the appropriate cell types and initiate a cascade of tolerogenic signaling pathways. Other technologies, such as biomaterial scaffolds, mimic immune privileged sites in the body and can bolster tolerogenic responses through modulation of the local microenvironment. In this review, we will briefly introduce natural mechanisms of peripheral tolerance that will serve as a backdrop for an in-depth discussion of the state-of-the-art technologies available to reprogram immune cells to induce Ag-specific immune tolerance. Systematically, we will discuss technologies that promote tolerogenic responses by acting on APCs, lymphocytes, and by the creation of immune privileged sites using examples for the treatment autoimmune disease, allograft transplantation, and allergy as each of these therapies has unique immunological characteristics that motivate/influence the design of new technologies.

2. Peripheral tolerance mechanisms

The development of T cell tolerance to self-Ags begins in the thymus and is refined in the periphery. As mentioned earlier, negative selection is not completely effective and autoreactive T cells that escape to the periphery are potentially harmful since they may provoke immune responses towards common Ags such as those in food or organ tissues [20]. T cells evade negative selection through multiple mechanisms that include low TCR avidity, TCR crossreactivity, and incomplete self-Ag representation by thymocytes [21]. When autoreactive T cells reach the periphery, their activity is modulated by anergy and deletion mechanisms. However, if either of these mechanisms fails, T cells can become activated due to the presence of activating stimuli such as inflammation, infection, or other pathologies that lead to the enhanced expression of co-signaling molecules that aid in the development of effector T cell responses. This autoreactivity can propagate the development of autoimmunity, however, the body has acquired mechanisms to restrain self-Ag-reactive T cells.

Activation of naïve T helper (Th) cells has been generally assumed to be the result of 2 signals: (1) TCR stimulation (signal 1) and (2) co-signaling molecules (signal 2). However, data that has been recently accumulated provided evidence for a 3 signal model for T cell activation, where signal 3 is provided by inflammatory cytokines such as interferon-gamma

and interleukin-12 [22]. To enable TCR stimulation, proteolytically processed antigenic peptides are presented on the surface of APCs on major histocompatibility (MHC) complexes and recognized by cognate TCRs on T cells [3, 18]. A diverse repertoire of co-signaling molecules exists that function as either co-stimulatory or co-inhibitory receptors that influence the outcome of TCR signaling [23, 24]. The effects of co-signaling molecules are Ag-independent and act to modify signal 1. T cell co-signaling pathways have diverse immune regulation functions that can control effector, memory, and Treg functions [25]. Furthermore, the presence of inflammatory cytokines in the microenvironment aid in Th1 polarization. This complex interplay between co-stimulatory molecules, co-inhibitory molecules, and cytokines directs downstream immunity or tolerogenic signaling pathways and is critical to understand the type and strength of immune responses generated. These findings have given rise to the concept of the tidal model of co-signaling, where changing environmental conditions can lead to dynamic cell-surface interactions and intracellular signaling [23]. Self-tolerance and immune homeostasis are naturally maintained as self-Ags are presented to T cells in the absence of inflammation through steady-state processes such as clearance of apoptotic cells, exosomes, and by other cell death mechanisms involved in peripheral T cell tolerance.

2.1. Apoptotic cells

The clearance of apoptotic cells is a natural process that acts to maintain homeostasis and promotes the maintenance of peripheral tolerance through noninflammatory pathways. This process is unlike another cell death process, pyroptosis, which results in the activation and release of pro-inflammatory cytokines such as IL-1p and IL-18 that may hinder tolerance induction [26, 27]. For apoptosis, a combination of signals regulate dead cell clearance (efferocytosis) and aids to ensure that immunity is not generated towards self-Ags [28]. Dysfunction of these natural clearance mechanisms has been shown to result in autoimmunity [29]. When a cell dies, phosphatidylserine normally present on the inner leaflet of the plasma membrane is externalized and serves as an apoptosis indicator [30, 31]. In one pathway, the interaction of apoptotic membrane-associated ligands (Growth arrest specific-6 and Protein S) with Tyro-3, Axl, and Mer (TAM) receptor kinases on dendritic cells (DCs) interferes with Toll-like Receptor (TLR) signaling and prevents DC maturation [32]. Evidence supports that the TAM family interactions affect other phagocytic pathways which do not necessarily depend on phosphatidylserine binding. Integrin-based systems and scavenger receptors, for example, are implicated in TAM interaction and have been targeted by tolerogenic technologies but may not necessarily involve phosphatidylserine [33].

An important co-stimulatory pathway involved in apoptosis and maintenance of peripheral tolerance involves the programmed death-1 (PD-1) receptor and the corresponding ligands PD-L1 (known also as B7-H1 or CD274) and PD-L2 (known also as B7-DC or CD273) [34]. PD-1 is part of the cluster of differentiation 28 (CD28)/cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) superfamily and is expressed on a variety of cells, including T cells, B cells, monocytes, natural killer cells, and myeloid cells. PD-L1 is commonly expressed on non-activated T cells, B cells, dendritic cells, and macrophages while PD-L2 is primarily expressed by macrophages and dendritic cells [35]. The PD-1PD-L1/2 pathway has been implicated in immune suppression in models of autoimmunity, transplantation, and

allergy through a variety of mechanisms, including promotion of T cell apoptosis [34]. The complex mechanisms of PD-1-dependent immune suppression are discussed more fully in other reviews, but binding of PD-1 by PD-L1/2 typically results in cross-linkage between PD-1 and the TCR, leading to tyrosine phosphorylation of the tyrosine-based inhibitory or switch motifs (ITIM and ITSM, respectively) of PD-1 [36, 37]. The binding of Sarcoma Homology 2 domain Phosphatase 1 or 2 (SHP-1 or SHP-2) to these phosphorylated regions limits activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway by dephosphorylation of relevant signaling molecules, eventually resulting in increased apoptosis, decreased cell proliferation, and decreased IL-2 production, among other suppressive outcomes [36, 37].

2.2. Naturally occurring exosomes

Exosomes are 30-100 nm cell-derived extracellular vesicles that are produced during intraluminal vesicle formation of endosomal membranes [38, 39]. The mechanism of exosome formation is not entirely clear, yet likely involves the endosomal sorting complex or ceramide-dependent lipid raft formation [40]. Exosomes are frequent carriers of micro/messengerRNA (mi/mRNA), proteins/lipids, and other cellular material. Evidence suggests that Tregs can also produce exosomes and frequently utilize this mechanism of gene transfer to direct T cells towards tolerogenic phenotypes [41]. Additionally, recent studies support that Treg exosomes may target and influence cells other than T cells towards stable tolerance induction through a variety of pathways, including antibody inhibition, cytokine modulation, or presentation of exosome-delivered Ags [40, 42].

2.3. Non-specific deletion

In peripheral tolerance, T cell deletion occurs through apoptotic cell death via apoptosis stimulating fragment (Fas)- and Bcl-2-interacting mediator of cell death (Bim)-regulated pathways. T cells express Fas (CD95) and, following Ag and IL-2 signaling, FasL (CD178). Activation of Fas by FasL or certain antibodies initiates activation-induced cell death (AICD), involving the formation of the death-inducing signaling complex (DISC) and downstream activation of apoptotic caspases. Alternatively, Bim may act as antagonist survival protein Bcl-2 to induce apoptosis through mitochondrial membrane permeabilization [20].

3. APC Reprogramming

APCs are skilled phagocytes that are well-adapted to process and present Ag derived from native and foreign sources. This makes them a natural target for immunotherapies. APCs efficiently scavenge and traffic to T cell compartments, so carrier-based tolerance strategies rely less on targeted delivery, and more on Ag (signal 1) and co-signaling (signal 2), and cytokine (signal 3) modulation. This section discusses strategies for the delivery of Ag to APCs to promote tolerogenic phenotypes in the context of autoimmunity, allograft transplantation, and allergy.

3.1. Cell-based therapeutics

Cell-based technologies have been developed to induce Ag-specific tolerance by exploiting naturally-occurring peripheral tolerance processes responsible for maintaining immune system homeostasis. Skewing APCs toward tolerogenic phenotypes *ex vivo* has demonstrated Ag-specific tolerance and has been extensively reviewed elsewhere [43-45]. Using cells as tolerogenic agents typically utilizes *ex vivo* biochemical modification, however *in situ* modification has been successfully demonstrated [46-48]. Cell-based carriers have the advantage of closely recapitulating the body's natural clearance mechanisms such as apoptotic cell clearance resulting in secretion of inhibitory cytokines, increased expression of co-inhibitory molecules, and expansion of Ag-specific Tregs [49]. However, *ex vivo* manipulation of autologous patient or donor cells requires extreme care and must be conducted using good laboratory practices [50, 51]. The high costs and standards for procedural care may limit the translational potential for cell-based therapies that require *ex vivo* handling. Cell-based therapies that utilize *in situ* targeting strategies (e.g. Ag targeting to red blood cells) represent an “off-the-shelf” option for harnessing autologous cells destined for clearance by APCs [52]. Together, technologies that recapitulate the natural clearance of apoptotic cells represent promising cell-based strategies for inducing Ag-specific tolerance.

3.2. Particle-based therapeutics

Nanotechnology has demonstrated great potential to revolutionize the treatment of disorders with an underlying immunological pathogenesis. Nano/microparticles comprised of safe and widely-available biocompatible materials have gained traction as relevant surrogates for cell-based carriers to induce Ag-specific tolerance [53]. Synthetic materials offer the ability to achieve fine control over the structure, surface properties, and various interactions between particles and biological systems (i.e. nanobio interactions) [54], that is not always possible using cell-based platforms. Modulation of various physicochemical properties such as composition, size, and surface charge can direct biological responses [53, 55-58].

Tolerogenic responses have been achieved *in vivo* using Ag-associated particles or particles engineered to deliver non-specific immune modulators such as small molecule immune suppressants, anti-sense oligonucleotides, short-interfering RNA, and others [59-62]. The carrier itself has also been shown to modulate immune responses for some applications [63]. Particles are combined with disease-relevant peptides or proteins, either through surface-coupling or encapsulation-based approaches, to obtain Ag-specificity. Importantly, some particle platforms induce tolerance by delivering Ag alone [64], while others require the co-incorporation of immune modifiers such as rapamycin, (2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester) (IDO), IL-10, or others [65-67]. This requirement for an immune modifier is attributed to the many differences in particle physicochemical properties and differences in biological mechanisms that have not been directly compared.

Particles have been fabricated with a variety of natural and synthetic materials, yet the most common composition of particles developed to modulate immune responses have been comprised of biocompatible and biodegradable polymers such as poly(lactide-co-glycolide) (PLGA), polylactide (PLA), and co-polymers thereof such as PLA-poly(ethylene glycol)

(PLA-PEG) [53, 65]. Particles comprised of inorganic materials such as gold [66, 68], or iron oxide and quantum dots [69] have also shown success to induce tolerance. Regardless of the particle composition, to be effective, the particle must deliver its therapeutic payload at a sufficient level and distribute to the appropriate site of action in the body.

For tolerance induction, delivery of tolerogenic Ags using particles provides numerous advantages over delivery of soluble Ags. A summary of particles that have been demonstrated to promote Ag-specific immune regulation are detailed in Table 1. Through fine-tuning of the particle size (approximately 500 nm), the biodistribution can be tailored to target tolerogenic organs such as the liver and spleen [64, 70-72]. Furthermore, in contrast to soluble Ag, particles deliver a bolus of Ag to APCs which may result in lower therapeutic doses and safer Ag profiles. Directing the Ag distribution in the body is especially important for antibody-mediated immune reactions such as allergy, where encapsulation of Ags within particles alleviates potentially deadly side effects such as IgE-mediated anaphylaxis (Figure 2) [73]. Lastly, the surface properties of particles such as charge can be modulated to mimic naturally occurring apoptotic bodies (i.e. highly negative) by coating the particle surface with synthetic (poly(ethylenealt-maleic anhydride)) or natural materials (phosphatidylserine) to mediate uptake by cell debris-clearance pathways [74, 75].

3.3. Autoantigen delivery

There are at least 81 types of autoimmune diseases that are heterogeneously observed in humans and their estimated worldwide prevalence is 4.5% [76] .These diseases are associated with complex pathologies in which the immune cells of the body attack healthy cells and tissues resulting in a state of persistent inflammation and chronic tissue destruction. Many immunotherapies are not Ag-specific and target signaling pathways that curb pathogenic T cells in active autoimmune disease, however they are not always effective [77]. Notably, the difficulty in developing therapies for autoimmune diseases, such as multiple sclerosis (MS), is that the primary disease-specific Ags involved in occurrence and progression are numerous and not always well-defined. However, proteins found in the tissues targeted by autoimmune diseases are good starting targets when designing Ag-specific therapies. For example, in MS, known autoAgs are associated with the myelin sheath proteolipid protein (PLP), myelin basic protein (MBP), and myelin oligodendrocyte protein (MOG). Delivery of disease-relevant Ags using cell- and particle-based carriers to APCs results in the initiation of tolerogenic signaling cascades that can reprogram autoreactive responses. In this section, we describe the various cell- and particle-based approaches to induce Ag-specific immune tolerance to treat autoimmunity.

3.3.1. Cell-based approaches—For the past three decades, autoAg-coupled syngeneic splenocytes (Ag-SPs) have been investigated for their ability to induce tolerance in models of autoimmunity (Figure 1) based on direct and indirect T cell interaction pathways [78, 79]. These Ag carriers have demonstrated tolerance to autoAg in Th1/17-mediated autoimmune models of experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, and the non-obese diabetic (NOD) model of type 1 diabetes (T1D) [80, 81]. After intravenous infusion, Ag-SPs accumulate in the marginal zone of the spleen where they are phagocytosed by marginal zone macrophages. Ag-SP uptake is followed by IL-10

production and upregulation of PD-L1 by macrophages [82]. Tolerance was not attained in IL-10 knockout mice, or mice treated with anti-PD-L1 antibody, suggesting that IL-10 and PD-L1 are necessary for Ag-SP-mediated tolerance. Direct presentation of Ag-SPs to T cells has been demonstrated to result in unresponsiveness, but studies coupling whole protein to splenocytes or using MHC-deficient or MHC-mismatched splenocytes have shown that the indirect presentation pathway is sufficient for tolerance [80]. Furthermore, the accumulation of Ag-SPs in the spleen was necessary, as autoAg tolerance was not achieved in models of splenectomy or subcutaneous administration.

The reality of human MS is that treatment needs to be effective in individuals with pre-existing disease and that the variety of autoAgs is not well-characterized. Due to epitope spreading during chronic autoimmunity, a person may have several Ags causing the pathogenesis of their disease [83]. A study by Smith et al. evaluated the feasibility of preventing EAE using Ag-SP coupled with an array of autoAgs (i.e. $PLP_{139\text{-}151}$, $PLP_{178–191}$, $MBP_{84–104}$, and $MOG_{92–106}$) [84]. EAE clinical scores and delayed type-hypersensitivity (DTH) responses indicated that disease suppression in the therapeutic tolerance model was different than in the prophylactic model where an increase in anti-inflammatory cytokine production (IL-10 and TGF-β) was observed. This study, in addition to the years of experimental success by Ag-SPs, provided justification for a first-in-man clinical trial using Ag-coupled cells to treat MS.

In 2013, a clinical trial was published that built on the work of Miller et al. using Ag-coupled cells for the treatment of autoimmune disease. In this study, autologous peripheral blood mononuclear cells (PBMCs) were coupled with seven known myelin peptides described to be potentially antigenic in MS. This trial demonstrated that the treatment was safe, and patients that received doses of Ag-PBMCs higher than 1×10^9 cells showed a decrease in Ag-specific T cell responses following therapy [51]. This study provided evidence that autologous cells could be used to induce tolerogenic responses in humans, although further studies using larger cohorts of patients will be required to demonstrate its wide-scale applicability and versatility.

Another tolerance therapy that takes advantage of the body's natural apoptotic clearance mechanisms targets autoAgs to circulating erythrocytes (Ag-RBC) (Figure 1). Kontos et al. have utilized Ag-conjugates that targeted murine glycophorin A-binding (TER119) on erythrocytes [48]. After intravenous infusion, the Ags become associated with erythrocytes and are cleared by naturally tolerogenic pathways. This treatment resulted in cross-presentation of Ag, and proliferation of $CD8^+$ T cells with upregulated PD-1 and annexin-V indicating potential exhaustion and deletion outcomes. In the NOD T1D model, complete prevention of hyperglycemia was demonstrated with injections of erythrocyte targeting fusion peptides containing the diabetogenic mimetope peptide p31 (Figure 3). By utilizing *in situ* association to cells bound for apoptotic clearance, *ex vivo* manipulation of cells has been avoided, making this cell-based strategy a strong candidate for clinical translation.

Expanding on the work of immunoglobulin G-mediated tolerance, B cells have been retrovirally transduced to produce conjugates of the IgG heavy chain and Ags of interest (peptide-IgG) [85]. In one example of this technology, the HIV TAT protein was fused with

a peptide and IgG to form TAT-Ag-IgG, with the TAT protein functioning to mediate cell entry of the relevant Ag, as shown in previous studies [86, 87]. B cells activated with LPS and reprogrammed by TAT-MOG_{35-55}-IgG displayed the ability to reduce EAE disease score when injected 10 days after immunization with MOG_{35-55}, but interestingly did not reduce EAE disease score when injected one week prior to disease induction. In NOD T1D, B cells incubated with a fusion protein incorporating islet Ag B_{9-23} (TAT-B_{9-23}-IgG) displayed a delayed onset of diabetes when administered prophylactically [85]. In each of these studies, delivery of irrelevant peptide-transduced B cells were unable to reduce or delay disease progression, suggesting Ag-specificity of the platform [85]. Preliminary mechanistic studies indicate that the tolerance induction by transduced B cells required $CD4^+CD25^+$ Tregs as well as CTLA-4/B7-dependent interaction between B and T cells [88]. Similar successes were observed using this platform in murine models of hemophilia, experimental autoimmune uveitis, and arthritis, and represent a promising method of tolerance induction [89-93].

3.3.2. Particle-based approaches—Peripheral tolerance induction by recapitulating natural tolerance maintenance in the hematopoietic compartment has offered insight into potential avenues to develop particulate delivery systems that follow a similar mechanism of action. Intravenously injected 500 nm Ag-coupled carboxylated polystyrene particles or PLGA particles were found to localize in similar regions of the splenic marginal zone and liver as Ag-SPs and target specific scavenger receptors on APCs that play an active role in tolerance induction (Figure 1) [64]. These particles efficiently prevented the onset of EAE, prevented epitope spreading, and reversed the progression of EAE in a therapeutic disease model. Importantly, tolerance was dependent on the highly-negative charge (less than -30 mV) and size (500 nm) of the particles, which targeted their distribution to macrophages expressing macrophage receptor with collagenous structures (MARCO) in the spleen. In a follow up study, Ag-coupled PLGA particles induced tolerance in EAE as demonstrated by significantly reduced mean clinical scores, reduced DTH responses, and reduced central nervous system (CNS) immune infiltration of Th1/17 cells [74].

The previous examples have demonstrated that tolerance can be induced by particles with surface-coupled Ag. However, the covalent modification of the particle surface with Ags affects physicochemical properties of particles such as their size, charge, and solution stability [73, 74]. Therefore, encapsulation of Ag within particles represents a more advantageous method to deliver Ag *in vivo.* McCarthy et al. recently described the encapsulation of peptide Ags (OVA323-339, PLP139-151, and PLP178-191) into PLGA particles (PLGA(Ag)) to treat EAE [70]. PLGA(Ag) particles significantly abrogated EAE induction *in vivo* and inhibited Th1/17 Ag recall responses (proliferation, IFN-y, and IL-17a) *in vitro.* Corroborating previous studies using peptide-coupled PLGA particles, the biodistribution of intravenously injected PLGA(Ag) particles was found primarily in liver and to a lesser extent in the spleen and lungs. Previous studies using Ag-SP demonstrated the dependence of the spleen for tolerance induction [82], however, prophylactic tolerance induction by PLGA(Ag) particles was not solely dependent on the spleen [70]. Pearson et al. developed Ag-polymer conjugate PLGA (acNP) particles that displayed modular Ag loading (up to 150 μg peptide per mg particle), low burst release, and minimally exposed surface Ag

[94]. *In vitro,* acNPs were effective at inducing Tregs in a co-culture model of BMDCs, naïve OTII T cells, and TGF-p1. Treg induction was dependent on Ag loading and particle concentration, where the highest Ag loading in acNPs enabled a 10-fold lower concentration to be used. Furthermore, acNPs were effective at treating EAE that was induced by single of multiple peptides (Figure 4). This approach holds promise to deliver several therapeutic antigens that can tolerize multiple disease relevant epitopes.

Additional *in vivo* tolerance mechanisms have implicated the liver as critical for tolerance induction since it is exposed to numerous self and non-self Ags regularly and therefore must balance immunity and tolerance [95]. Many APCs in the liver have a low abundance of MHC and co-stimulatory molecule expression, which can reduce immune cell activation. Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs) internalize a majority of particles administered intravenously [96]. Heymann et al. utilized carboxylated latex particles to study the mechanism of action of tolerance induction using particle-delivered Ags. The liver was specifically identified as important for tolerance induction where Kupffer cells were demonstrated to be associated with Ag-presentation that induced $CD4^+$ T cell arrest and expansion of naturally occurring Tregs [97]. In a model of Ag-specific glomerular nephritis, particle delivery protected against kidney damage by reducing T cell infiltration, reduced glomerular damage, and reduced periglomerular infiltration. However, in models of liver injury, Kupffer cells lost expression of their tolerogenic phenotype, and tolerance was abrogated due to the redistribution of Ag to infiltrating monocyte-derived macrophages away from the hepatic phagocyte compartment. Similarly, the liver was determined to play an important role in tolerance induction by Carambia et al. [69]. Small poly(maleic anhydride-alt-1-octadecene)-coated particles induced tolerance though LSECs. Mice treated with particles displayed higher frequencies of Tregs and tolerance induction was abrogated when Tregs were inactivated using anti-CD25 antibody. These studies provided support that the liver plays an important role in tolerance induction by particles, however, it should be noted that due to distinct differences in particle physicochemical properties (size, charge, composition), the mechanism of action for each particle platform must be determined experimentally and cannot be assumed.

Co-delivery of immune modulating agents such as immune suppressants, cytokines, or other immune modifiers with Ag by particles is sometimes necessary to induce Ag-specific tolerance. One of the most commonly delivered agents for immune modulation is rapamycin, a mammalian target of rapamycin (mTOR) inhibitor. Encapsulation of rapamycin within PLGA nanoparticles induced a tolerizing phenotype *in vitro* where particles decreased the expression of maturation markers MHCII, CD86, and CD40 expression and increased levels of TGF-β using bone-marrow derived dendritic cells (BMDCs) [98]. Maldonado et al. developed tolerizing nanoparticles (tNPs) comprised of a mixture of PLGA and polyethylene glycol)-polylactide (PEG-PLA) that co-encapsulated Ag and rapamycin. These tNPs delivered antigenic peptides or whole proteins and could tolerize against both cellular and humoral immune responses. The co-encapsulation of rapamycin with Ag into tNPs was necessary to induce tolerance, whereas co-delivery of soluble rapamycin with Ag-encapsulated particles did not elicit tolerogenic effects but rather propagated humoral immunity [65]. In another example, immune modifying agent 2-(1-indole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), an activator of the aryl

hydrocarbon receptor transcription factor (AhR), was loaded into 60 nm gold particles with MOG35-55 peptide [66]. In the EAE model, particle treated mice displayed expanded populations of Tregs and abrogated EAE clinical disease symptoms. In a subsequent study, the same gold particles delivering **(3** cell proinsulin and ITE were found to induce a tolerogenic phenotype in dendritic cells by inducing *Socs2*-mediated inhibition of NF-κB and suppression of inflammatory cytokines as well as the promotion of Treg generation *in vivo* [68]. MOG35-55 peptide and IL-10 were separately encapsulated into 200 nm PLGA particles for the treatment of EAE by the subcutaneous route of administration. Co-administration of IL-10 containing NPs was required to significantly mitigate EAE clinical scores, while IFN-γ and IL-17 were significantly decreased [67].

Immune polyelectrolyte multilayers (iPEMs) have been built on calcium carbonate templates to promote immunological tolerance [99]. Delivery of a regulatory antagonist ligand of TLR9, GpG oligonucleotide, along with a MOG-triarginine peptide was hypothesized as able to restrain the pro-inflammatory signaling and redirect T cell differentiation from inflammatory populations and towards regulatory phenotypes such as Tregs. Interestingly, iPEMs reduced TLR9 signaling, reduced dendritic cell activation, and polarized myelin-specific T cells towards a tolerogenic phenotype and function. In an EAE model, iPEMs abrogated disease, which coincided with Treg expansion and reduced IL-17, IL-6, and IFN-γ production, with no effect on T cell proliferation.

3.4. Alloantigen Delivery

Achieving allogeneic tolerance to multiple foreign Ags represents a significant challenge to the field of transplantation. Transplantation of allogeneic or ‘non-self’ tissues between genetically different individuals of the same species leads to a T cell-mediated immune response resulting in rejection and graft destruction. Current methodologies for improving transplant tolerance are similar to those of treating autoimmunity (such as the chronic use of immunosuppressive agents) that are associated with numerous risks [18]. The severity of the immune response against the transplanted tissue depends on the differences in Ag between the donor and recipient as well as the intragraft expression of inflammatory cytokines following transplantation [100]. There are three pathways through which allograft recognition and rejection can occur: 1) direct, 2) indirect, and 3) semi-direct. In the direct pathway, recipient T cells recognize MHC molecules present on the surface of donor APCs. In the indirect pathway, recipient APCs internalize allogeneic proteins and present it to recipient T cells on recipient MHC molecules. It has been observed that $CD4^+$ T cells with indirect specificity can provide “unlinked” T cell help to $CD8^+$ T cells with direct specificity, which does not agree with the commonly-accepted “linked” model of immune response in which the same APCs activate both $CD4^+$ and $CD8^+$ T cells. An explanation for this phenomenon is the acquisition of MHC molecules from donor APCs by recipient APCs and subsequent direct presentation to $CD8^+$ T cells. This is known as the semi-direct pathway and is under investigation [101].

The wide breadth of Ags necessary to tolerize against in allogeneic transplants and the multiplicity of allorejection pathways pose significant challenges to the development of new tolerance therapies. The primary Ags that influence rejection in mice are MHCs, and in

humans are called human leukocyte Ags (HLAs). These HLA proteins are of two types, Class I and Class II, and are involved in Ag presentation and recognition, such as between APCs and T cells. Both major and minor mismatches in HLAs between the donor and recipient are highly deterministic of the success of a transplant. In addition to the major Ags, mismatches in numerous other proteins of the cell, which are termed minor Ags, can also mediate rejection. Tolerance induction strategies have aimed to affect the direct or indirect pathways of allograft rejection and are the focus of many Ag carrier therapies.

3.4.1. Cell-based approaches—Immunosuppression-free allogeneic transplant tolerance has been achieved in mice by pretreatment with ethylene carbodiimide (ECDI)-fixed splenocytes (ECDI-SP) that mimicked donor apoptotic cells. Long-term tolerance to allogeneic islets was achieved by two infusions of ECDI-SP from the donor mouse strain, 1-week prior to, and 1-day post, kidney-capsule transplant [47]. The effect was shown to be strain-specific and dependent on ECDI treatment. The durability of this tolerance was challenged with a second islet transplant 60 days after the initial treatment. Donor strain-matched islets were protected by the tolerance, whereas third party islets led to rejection as indicated by hyperglycemia. The authors attributed the allograft tolerance induction, but not maintenance, to an increase in Tregs in the spleen as Treg ablation early (day -9), but not late (day 15), prevented engraftment. Furthermore, graft rejection dominated in PD-L1 deficient mouse suggesting a potential role for the PD-1 pathway in mediating ECDI-SP allotolerance. Since the kidney capsule is not considered a translatable transplantation site, this tolerance protocol was combined with islet transplantation on a PLGA microporous scaffolds into the mouse peritoneal fat, an analogue to human omentum [102]. Tolerance for islets transplanted on the PLGA scaffold was as effective as the kidney capsule, with both providing a site for successful long-term allogeneic islet engraftment. Combined with tolerance directed toward islet Ag $InsB_{9\text{-}23}$, allotolerance by ECDI-SP offers a potential solution for islet replacement therapy for T1D patients.

Further investigation of ECDI-SP induced allotolerance revealed contribution of both direct and indirect tolerance mechanisms [46]. *In vivo* selective depletion experiments showed that DCs, but not B cells or macrophages, were necessary for ECDI-SP induced allotolerance. Furthermore, there were T cell responses to APC-processed EDCI-SPs (indirect) as well as presentation of un-phagocytosed ECDI-SP alloAg to T cells (direct). After uptake of ECDI-SPs, $CD11c^+$ DCs upregulated co-inhibitory molecules PD-L1 and PD-L2 and caused the rapid expansion of T cells, followed by clonal deletion and hindered migration to the site of engraftment. The directly stimulated T cells experienced weak proliferation and became unresponsive to subsequent stimulatory signals. Thus, in addition to the expansion of Tregs in the spleen, graft, and graft draining lymph nodes, ECDI-SPs induced tolerance by causing clonal deletion and anergy of alloreactive T cells. In other disease models, ECDI-SPs have been used to prolong cardiac allografts (indefinitely if combined with a short course of rapamycin) [103], and have induced long-lasting xenograft tolerance to rat islets transplanted into mouse kidney capsules when combined with transient B cell depletion [104].

3.4.2. Particle-based approaches—The induction of donor-specific tolerance to transplanted cells and organs remains of utmost importance to mitigate allograft rejection. The first uses of particles to improve allotransplant acceptance involved the delivery of small molecule immune suppressants such as rapamycin or calcineurin inhibitors [60]. Achieving Ag-specific suppression of anti-donor immune responses is complicated due to the wide array of major and minor Ags. PLGA particles have been used to induce donor-specific tolerance and mediate long-term acceptance of full MHC-mismatched allografts using an allogeneic islet transplant model (BALB/c to C57BL/6). Donor Ags were obtained from the lysate of BALB/c splenocytes, with the lysate Ag conjugated to the surface of 500 nm PLGA particles using ECDI chemistry. Delivery of PLGA particles with surface-coupled donor Ags to transplanted C57BL/6 mice led to tolerance in 20% of recipients. In combination with a short course of low-dose rapamycin at the time of transplant, tolerance was greatly improved to 60% (Figure 5) [71]. In another study, PLGA particles were used to induce tolerance in minor histocompatibility Ag sex-mismatched C57BL/6 model of bone marrow transplantation. Peptide Ags Dby and Uty are respective $CD4^+$ and $CD8^+$ T cell Ags that mediate male bone marrow transplant rejection in females. Interestingly, delivery of Dby peptide either by conjugation to the surface or encapsulation promoted transplant tolerance. However, delivery of Uty peptide using particles did not induce tolerance. In this model, depletion of Tregs using anti-CD25 antibody did not alter tolerance induction, suggesting other potential tolerance induction mechanisms [49].

Exosomes have also been used to induce tolerance in allogeneic transplant models. Exosomes derived from PBMCs have been used to prolong cardiac transplants in a C57BL/6 to BALB/c model [105]. Song et al. could extend cardiac allografts 40 days using 2 doses of exosomes from donor C57BL/6 mice. The authors attributed the tolerance to a skewing of Th2 T cells toward a regulatory phenotype. While they ruled out the possibility of direct induction of Tregs by exosomes, they concluded that MMP1 carried by the donor exosomes was necessary for Treg induction in a Th2:DC co-culture system.

In the same C57BL/6 to BALB/c cardiac transplant scheme, another group of investigators used immature dendritic cell exosomes (imDex) to prolong cardiac graft survival [106]. Cardiac allografts were extended 25 days using 3 doses of imDex from C57BL/6 donor mice. Interestingly, infusion of more or less than 10 μg resulted in minimal graft protection. When rapamycin was administered for 11 days, half of the grafts were maintained indefinitely. The tolerogenic effects were donor-specific and dependent on rapamycin. The same group also demonstrated liver transplant tolerance using imDex [107]. In a rat model, imDex from donor rats (Brown Norway) prolonged liver graft survival about 25 days in recipient Lewis rats. Instead of administering rapamycin to extend graft survival, recipients were infused with donor-specific recipient Tregs, which resulted in an indefinite survival of 70% of liver grafts. The mechanism by which DC-derived exosomes induce tolerance is unknown. Like ECDI-SP and PLGA NPs mentioned earlier, intravenously delivered DC-derived exosomes have accumulated in liver Kupffer cells as well as marginal zone macrophages and DCs [108]. Like their immature DC source, these exosomes express low levels of MHC class I and II as well as subimmunogenic levels of co-stimulatory molecules. In the cardiac allograft model, the data suggested that splenic T cells become

hyporesponsive to alloAg challenge, and there was an observed increase in $Foxp3^+$ splenic T cells following a co-treatment with imDex and rapamycin [106]. Co-administration and *in vivo* proliferation of Tregs seems to support their role in exosome induced allotolerance [107]. It is unknown whether the T cell interactions result from direct presentation of exosome Ags by fusion with host APCs, or indirect presentation of exosome Ags following APC phagocytosis [107].

3.5. Allergen Delivery

Allergic diseases such as asthma and food allergies are becoming increasingly common in developed nations. Immediate allergic reactions (Type 1), involve an overreaction of the immune system and the formation of IgE antibodies. The development of allergic responses is reliant on $CD4^+$ Th2 cells as they produce cytokines that induce immunoglobulin class switching to IgE. IgE binds with high affinity to mast cells and basophils that release pro-inflammatory mediators once they encounter the allergen [11]. At current, the most effective treatment regimen consists of avoidance or other specific immune tolerance (SIT) strategies that deliver soluble Ag at increasing dosages to mitigate symptoms. SIT is usually carried out by repetitive subcutaneous injections or sublingual delivery of increasing doses of the allergen [109]. However, SITs feature numerous potential issues especially for treating food allergies that pose a risk of developing adverse reactions including life-threatening anaphylaxis. Additionally, SIT by subcutaneous injections often requires 3-5 years of treatment, involving multiple sessions per week in the build-up phase [110]. Recent work has focused the delivery of antigen to the lymphatic system by ultrasound guided intranodal injections which show promise in decreasing the injection frequency and duration of SIT regimes. A study comparing a 3 year subcutaneous SIT regime (54 injections) to a 2 month intralymphatic regime (3 injections) demonstrated equivalent tolerance to pollen as measured by hay fever symptoms, skin reactivity, and decreased allergen-specific serum IgE [111]. A similar study of 3 intralymphatic inguinal resulted in a decrease in seasonal allergic symptoms and nasal inflammatory leukocytes compared to adjuvant delivery without pollen (aluminum hydroxide only) [112]. Other allergen-specific tolerance strategies include using Tregs, anti-IgE, or by blocking Th2 cytokines. SIT to allergens leads to a shift in Th2 and Th17 towards a Th1 response and Treg induction [113]. This results in reduced production of IgE production, IL-4, and IL-13 and an increased production of IFN-γ and IgG subtypes that can act as blocking antibodies and capture the allergen before activating effector cells [114].

B cells express a variety of B cell receptor (BCR) inhibitory co-receptors that aid in setting a threshold for B cell activation. Among them are CD22 and SIGLEC-G (SIGLEC-10 in humans), and members of the SIGLEC (sialic-acid binding Ig-like lectin) immunoglobulin family that recognize sialic acid-containing glycans of glycoproteins and glycolipids and ligands [115]. Targeting Ag-specific B cells is a mechanism to induce systemic humoral tolerance. Favorable approaches to induce B cell tolerance involve taking advantage of mechanisms to suppress B cell activation.

3.5.1. Cell-based approaches—One of the methods currently available to induce Ag-specific immune tolerance is the use of Ag-SP. As described earlier in this review, Ag-SP

have been prophylactically and therapeutically tolerogenic in models of Th1/17-mediated autoimmune disease. An early demonstration of Ag-SP to treat Th2-associated models was performed by Smarr et al. [85]. This study utilized two models of allergy: peanut hypersensitivity as well as ovalbumin (OVA)-induced allergic airway inflammation. Whole peanut extract (WPE) or whole OVA protein was coupled to splenocytes using ECDI, and the Ag-SPs were intravenously infused resulting in decreased local and systemic Th2-related disease. In the peanut hypersensitivity model, characterized by mast cell-mediated anaphylaxis, prophylactic WPE-SP administration diminished the levels of Ag-specific IgE (but not IgG), eosinophil numbers, and IL-3, IL-4, and IL-13 in recall assays that resulted in a prevention of anaphylactic symptoms in an oral WPE challenge. The authors observed anaphylactic symptoms when Tregs were inactivated by anti-CD25 antibody indicating a partial role for Tregs in the tolerance mechanism. In the OVA-induced allergic airway model, prophylactic OVA-SP treatment similarly reduced IgE, eosinophil numbers, and Th2-associated cytokines (*in vivo* and *ex vivo*). IgG levels were also reduced and the tolerogenic effects were Treg independent. Regardless of allergy model and Treg activity, treating with Ag-specific Ag-SPs decreased IgE levels indicating inactivation of B cell class switching which the authors posit is a result of previously observed decreased CD40L levels on helper T cells (previously observed) [85, 116]. Though IgE levels were decreased compared to controls, the levels were increased compared to Ag-inexperienced animals, however, this did not result in anaphylaxis.

The increased use of biologics in medicine has led to the development of antidrug antibodies (ADAs) in patients. This undesired immune recognition has hindered the effective use of biologics and has created a demand for drug-specific tolerance. The usage of erythrocyte-targeted Ags has recently been applied to prevent development of ADAs against acute lymphoblastic leukemia (ALL) therapeutic enzyme Escherichia coli L-asparaginase (ASNase) [117]. An infusion of ASNase conjugated to glycophorin A-binding peptide (ERY1-ASNase) significantly reduced anti-ASNase IgG titers against 6 weekly challenges, compared to infusion of untargeted ASNase. This reduction in anti-ASNase titer was observed in all IgG subclasses. The treatment showed no effect on the development of humoral responses to OVA indicating that this therapy is both effective and Ag-specific in mice.

3.5.2. Particle-based approaches—PLG particles have been used to tolerize against OVA in a Th2-mediated allergic airway inflammation model both pre- and post-sensitization [73]. Since activation of mast cells is APC independent, avoiding Ag recognition by antibodies is desirable. Encapsulation of allergens into particles shielded their detection by IgE auto-antibodies on mast cells and basophils thus preventing any undesired side effects associated with SIT and Ag-coupled particles (Figure 2). Encapsulation of OVA protein within PLGA particles (PLGA(OVA)) eliminated the presence of particle surface-associated protein that could lead to deleterious biological effects such as anaphylaxis. In a prophylactic disease model, PLGA(OVA) particles inhibited the production of anti-OVA IgE and suppressed the production of Th2-mediated cytokines IL-4, IL-5, IL-13, IL-10, and IL-17. In a therapeutic model of allergic airway inflammation, PLGA(OVA) particles inhibited the Th2 responses and airway inflammation but led to increases in OVA-specific

IgE (Figure 6) [73]. Importantly, the encapsulation of OVA into PLG particles eliminated OVA-specific IgG and IgE binding to the surface of the particle preventing anaphylaxis.

Particles loaded with rapamycin that co-delivered PEGylated uricase or adalimumab demonstrated suppression of ADAs. Interestingly, the ability to suppress ADA production was dependent on the timing of rapamycin-encapsulating particle administration. Significant suppression of anti-keyhole limpet hemocyanin (KLH) antibodies was only achieved if the protein was administered within 1 day of particle administration [118]. Macauley et al. demonstrated that SIGLEC-engaging tolerance-inducing antigenic liposomes (STALs) (liposomes that displayed Ag as well as glycan ligands for CD22) could induce Ag-specific B cell apoptosis. CD22-dependent tolerance induction was demonstrated for both T cell-independent (nitrophenol) and T cell-dependent (hen egg lysozyme) Ags as Ag-specific antibody titers were significantly reduced. Furthermore, STALs induced tolerance to FVIII using a hemophilia mouse model. STALs significantly reduced anti-FVIII titers and prevented bleeding [119].

3.6. Carrier-free therapeutics

Although much recent focus has been on cell-based and particle-based technologies for bulk delivery of Ag, molecular-scale carrier-free platforms (less than 200 kDa) offer distinct advantages. The unique properties of carrier-free technologies such as their small size enable enhanced lymphatic drainage and bioavailability from the subcutaneous route of administration. For example, subcutaneously administered Ag-graft polymers (less than 100 kDa) showed improved tolerogenic properties compared to similarly functionalized particles (500 nm) due to their decreased size and increased solubility that improved interstitial drainage and bioavailability of the Ag-grafted polymer [120]. Furthermore, soluble carrier-free platforms often incorporate specific binding motifs that target specific cell types or interrupt inflammatory signaling pathways associated with cell activation. Fusion peptides comprised of Ag-antibody bioconjugates have demonstrated improved outcomes by targeting delivery of autoAg to tolerogenic APC subtypes [121]. Alternatively, polymer platforms co-grafted with autoAg and peptide inhibitors of co-stimulation have demonstrated Ag-specific tolerance that is dependent on interrupting APC:T cell interactions [120]. Soluble carrier-free systems deliver similar payloads as particle and cell-based systems, but their small scale, solubility, and engineered specificity make these systems unique.

3.6.1. Fusion proteins—Fusion proteins are a class of biologics that have seen remarkable success in research and clinical applications. Many design variations exist, but most are composed of a protein/peptide of interest linked to an antibody (often an IgG subclass) at the fragment crystallizable (Fc) region. Conjugation to IgG provides several benefits to therapeutics, including increased serum half-life (through increased size and, consequently, reduced clearance) and recycling (through increased interaction with the neonatal Fc receptor, which protects IgG from degradation) [122, 123].

Multiple tolerogenic fusion proteins have demonstrated the ability to induce Ag-specific tolerance. Recently, blood clotting protein FVIII conjugated to human IgG1 (rFVIIIFc)

demonstrated the ability to diminish anti-FVIII antibody responses associated with FVIII protein replacement therapy. In mice, this treatment was accompanied by development of Tregs and a general shift towards a tolerogenic phenotype, as indicated by up-regulation of IL-10, TGF-β, IL-35, and IDO-1, as well as down-regulation of IL-17 measured by real time polymerase chain reaction [124]. Importantly, mice pretreated with rFVIIIFc retained the ability to mount an antibody response to both dinitrophenol and OVA, demonstrating the Ag-specificity of the platform. The mechanisms behind these observations are being investigated, and studies support a role of the Fcy class of Fc receptors, which are particularly important in phagocytosis, and the neonatal Fc receptor, which the authors suggests implicate B cells and dendritic cells [124].

Antibody-Ag fusion proteins combine solubility and specificity to achieve efficient delivery of autoAg to specific cell types. Coupling of autoAg to anti-DEC205 antibody resulted in *in vivo* targeting of the endocytic receptor DEC205 on tolerogenic dendritic cells and demonstrated tolerance in models of EAE, NOD T1D, and experimental autoimmune arthritis (EAA) [125-127]. In the EAE model, intraperitoneal injection of anti-DEC205 antibody linked with PLP, but not isotype fusion protein or anti-DEC205 with irrelevant peptide, ameliorated disease onset. The protective effects in this context were attributed to hindered proliferation of IL-17-producing T cells and anergy in the remaining Ag-specific T cell population [125]. In the NOD T1D mouse model, it was shown that delivery of β cell peptide mimotope on anti-DEC205 antibody results in cross-presentation of peptide to $CD8^+$ T cells and subsequent clonal deletion [126]. In EAA, decreased disease scores were associated with lower B cell counts and IgG1 and IgG2a serum levels resulting from insufficient T follicular helper cell differentiation. Mechanistic studies showed that targeting autoAg to migratory DC subsets using anti-DEC205-Ag and anti-Langerin-Ag prevented EAE onset and caused an increase in $Foxp3^+$ T cells, whereas targeting lymphoid-resident DCs with anti-DCIR2-Ag and anti-Treml4-Ag only mitigated symptoms [121]. These outcomes demonstrate the specificity and utility of using antibodies to deliver autoAg to tolerogenic APC subsets.

3.6.2. Soluble Ag arrays—AutoAg are being coupled to targeting proteins and peptides to deliver Ags to specific cells and internalization pathways to dictate tolerogenic Ag presentation. Soluble Ag arrays (SAgAs) are composed of hyaluronic acid polymers with grafted Ag (e.g., PLP139-151) and a co-stimulatory molecule inhibitor (B7 inhibiting peptide). Hyaluronic acid is employed as it is hydrophilic, and its relatively large molecular weight ($\approx 10^6$ Da) provides the opportunity to conjugate many Ags/inhibitor molecules. SAgAs that co-delivered B7-inhibiting peptides with $PLP_{13}9$-$1_5 1$ decreased clinical disease scores in the EAE model with three subcutaneous injections. The resulting decrease in co-stimulation in the context of TCR stimulation is thought to lead to the observed tolerance, as disease was only alleviated when the inhibitor and Ag were presented on the same polymer and not when any other combination of components was administered. Specifically, it is suggested that the coincident binding of signals 1 and 2 interferes with APC interaction and dampens T and B cell clonal expansion [120, 128].

4. Lymphocyte Reprogramming

T cells play a central role in orchestrating adaptive immune responses and their phenotypes dictate the pathophysiology of immunity and tolerance [129]. As such, they have been the target of many tolerogenic therapies. Specifically, strategies for treating autoimmunity have explored Ag-specific Tregs, and in the case of allogeneic transplant, polyclonal populations [130]. Tregs have been extensively studied for their ability to suppress Ag-specific effector T cells, and even dampen the inflammatory effects associated with inflammatory environments that lead to epitope spreading [83]. Reprogrammed T cells with chimeric Ag receptors (CAR T cells) have been studied extensively in the field of cancer immunotherapy, but have recently been applied to immune tolerance. These engineered cells have been used as effector cells to delete autoreactive cells, and more commonly as regulatory cells that specifically target autoAg and alloAg [107, 131, 132]. Particle-based systems, though common for implementing tolerance through APCs, have also been designed to target T cells with specific Ag-reactivity resulting in a regulatory T_R1-like phenotype [133]. These T cell-targeted approaches for Ag-specific tolerance are discussed in the following section.

4.1. Regulatory T cell induction

$CD4^+CD25^+Foxp3^+$ Tregs are a critical component of peripheral tolerance mechanisms, and deficiency of Tregs is associated with severe autoimmune diseases and chronic inflammation [134]. Regulatory T cells suppress immune responses to a broad range Ags and indirectly limit immune inflammation-mediated tissue damage through multiple mechanisms [135]. Tregs actively suppress activated T cells through the production of anti-inflammatory cytokines (IL-10, IL-35, and TGF-β), cytolysis, metabolic disruption, and by targeting the maturation or function of DCs [134]. There are three main subclasses of $Foxp3^+$ Tregs that are classified by the location of their differentiation: thymus-derived Tregs (tTregs), peripherally-derived Tregs (pTregs), and *in vitro*-induced Tregs (iTregs) [136]. tTregs are effectors of central tolerance and have been shown to permit differentiation of Th1 and Th17 cells in the lymphatic system, but prevent circulation of these effector T cells into the tissues containing cognate antigens [137, 138]. In contrast, iTregs, which are generated *in vitro* by stimulating T cells in the presence of TGF-β, are thought to suppress APCs by local release of anti-inflammatory cytokines such as IL-10 [137, 139, 140]. As a result, the APCs become less potent primers of effector T cells. iTregs have shown adept regulatory behavior, but have a phenotype that is considered incomplete compared to *in vivo* derived tTregs and pTregs. The role of pTregs is thought to be complementary to tTregs in maintaining tolerance especially by transiently bolster tolerance in the peripheral compartment [141]. Strategies to increase Tregs and restore a healthy T cell balance have been extensively researched and reached clinical trials.

A method to generate Ag-specific Tregs *in vivo* has been developed that was effective at treating EAE and NOD diabetes in mice. Mice were treated with a systemic sublethal irradiation or depletion of B and $CD8^+$ T cells followed by administration of autoAg peptides. The irradiation of cells induced apoptosis which triggered professional phagocytes to produce TGF-β, under which the autoAg peptides directed naïve $CD4^+$ T cells to differentiate into $Foxp3^+$ Treg cells instead of effector T cells [142].

4.2. CAR T cells

The development of chimeric Ag receptor (CAR) technology more than 25 years ago for enhanced immunity by Eshher et al., has also been employed to reverse autoimmunity [132, 143]. *In vitro* expanded $CD4^+CD25^+$ Tregs were engineered with CARs directed toward the carcinoembryonic Ag (CEA), which is overexpressed in inflamed colon tissue and colon cancer. In colitis-induced by adoptive transfer of CEA-specific effector CAR T cells, adoptive transfer of CEA-specific CAR Tregs (1:1 TregTeff) increased the 4-wk survival to 75% compared to 25% by non-specific CAR Tregs. Tolerance was also tested in the azoxymethane-dextran sodium sulfate (AOM-DSS) induced model that combines the pathogenesis of colitis and colon cancer. Here, treatment by CEA-specific CAR Tregs halved the average colitis score compared to non-specific CAR Tregs. Interestingly and paradoxically, treatment with CEA-specific Tregs also significantly decreased tumor burden. Whereas the anti-inflammatory effects of Tregs are often implicated in the unchecked progression of tumors, here they acted to reduce the tumor burden. The authors postulated that the CAR Tregs acted to reduce the inflammatory mediators associated with intestinal polyps resulting in a reduction in neoplastic exacerbation.

The usage of $CD19^+$ B cell depleting CAR T cells has demonstrated effective treatment of acute lymphoblastic leukemia (ALL), and has been recently adapted to target and eliminate autoreactive B cells responsible for causing pemphigus vulgaris (PV) [131, 144-146]. PV is currently treated with systemic corticosteroids, but the off-label use of rituximab (combined with IVIG therapy) has shown effectiveness in creating long-term remission. Unfortunately, the resulting B cell depletion by anti-CD20 treatment resulted in cases of patient infection and septicemia [147]. These chimeric autoantibody receptor (CAAR) T cells were used to selectively deplete B lymphocytes that produce antibodies against keratinocyte desmosome adhesion protein desmoglein 3 (Dsg3). The CAAR-T cell strategy utilizes human T cells transduced with lentiviral vectors to express transmembrane receptors with extracellular chimeric autoAg Dsg3 with an intracellular signaling CD173-CD3ζ domain [131]. The investigators demonstrated *in vitro* that these CAAR-T cells induce lysis of hybridomas producing antibodies against the extracellular cadherin domains on Dsg3, but not control hybridomas producing irrelevant antibodies. *In vivo,* the CAAR-T cells were evaluated in NSG mice infused with B cell hybridomas secreting antibodies against Dsg3 domains or with hybridomas cloned to express antibodies that target Dsg3 in human PV. Compared to control CAR treatment, Dsg3 CAARs depleted circulating autoAg-secreting B cells, reduced the anti-Dsg3 IgG levels in serum, and prevented mucosal blistering. Interestingly, the presence of soluble anti-Dsg3 IgG did not prohibit effectiveness of the CAAR-T cells *in vitro* or *in vivo.* This successful experimental treatment of PV shows the promise of using CAAR T cells to treat B cell-mediated autoimmune disorders in an Ag-specific manner.

In contrast to CAR T cells expressing autoAgs, others have used the chimeric receptor to home toward sites containing autoAg. Loskog et al. used a lentiviral vector system to express both a CAR single-chain variable fragment (scFv) for MOG as well as to stably express Foxp3 in $CD4^+$ T cells [148]. After intranasal administration, the MOG-specific CAR Tregs, also transduced with GFP, were histologically observed in several regions of the brain and cerebellum. In the MOG-induced EAE model, MOG-specific CAR Tregs

delivered at peak of disease resulted in complete remission of disease symptoms in ten days. Importantly, this treatment resulted in astrogliosis and remyelination. However, administration of mock-transduced T cells also resulted in a decrease in symptoms (but to a lesser extent). The therapeutic effect of these non-CAR T cells may have been affected by the naturally occurring Treg population, yet questions about specificity remain.

While most uses of CAR T cells for tolerance have focused on targeting specific autoAg epitopes, Levings et al. have developed a regulatory CAR T cell for treating alloreactivity by targeting HLA-A2 (A2-CAR Tregs), a major histocompatibility complex often implicated in transplant rejection [107]. The human $CD25^{hi}CD45RA^{+}$ A2-CAR Tregs expressed CD25, Foxp3, LAP, GARP, CTLA-4 expression. Compared to irrelevant control CAR T cells, injection of A2-CAR T cells with $HLA\text{-}A2^{+}$ xenogeneic PMBCs resulted in a prolongation of survival and a 2-fold increase in the time to graft versus host disease (GVHD) onset. Additionally, the A2-CAR T cells maintained their Foxp3 expression for twice as long. When A2-CAR Tregs were infused into $HLA\text{-}A2^{+}$ mice with HLA-A2- PBMCs, the mice experienced no observed tissue cytotoxicity (in contrast to infusion with $CD25^{-}CD45RA^{+}$ A2-CAR T cells).

4.3. Peptide-MHC complexes on particles

Peptide-MHC (pMHC) complexes attached to the surface of crosslinked dextran-coated or PEGylated iron oxide particles (pMHC-NPs) offer a new therapeutic strategy, targeted directly at specific T cells, to treat autoimmune diseases in a disease- and organ-specific manner. To date, pMHC-NPs have demonstrated efficacy in multiple mouse models of autoimmunity (diabetes, MS, collagen-induced arthritis) using MHC class I or II complexes (Figure 1) [133, 149]. Importantly, as the Ag diversity in autoimmune diseases is highly complex, it has been demonstrated that pMHC-NPs that carry subdominant disease-relevant Ags show similar biological effects towards inducing tolerogenic responses as dominant Ags. In the first study, it was initially hypothesized that the delivery of multiple Ags within MHC molecules was necessary to abrogate disease in a mouse model of diabetes [149]. However, it was found that functionalization of NPs with monospecific pMHC complexes suppressed T1D progression in pre-diabetic mice as well as restored normoglycemia in recently diagnosed diabetic mice. pMHC-NPs expanded autoAg-experienced $CD8^{+}$ T cells that suppressed the activation and recruitment of cells with non-cognate specificities to islets. Furthermore, in a humanized mouse model of diabetes, particles coated with disease-relevant pHLA complexes restored normoglycemia. Studies showed that cognate T cells internalize these NPs without accumulating in $CD11b^{+}$, $CD11c^{+}$, or B cells. The ability to blunt disease progression was demonstrated to be due to the expansion of subsets of $CD8^{+}$ T cells with regulatory potential but a conventional memory-like phenotype [133, 149].

More recently [133], Clemente-Casares et al. demonstrated that pMHCII-NPs could induce Ag-specific regulatory $CD4^{+}$ T cell type 1 (T_R1)-like cells in various mouse models of autoimmunity as well as in humanized mice (Figure 7). By using ten human or mouse autoimmune-disease-relevant pMHC-NPs, the reversion of pathology was shown to be independent of genetic background or type of disease. Note that peptide-functionalized particles in NP-Ag discussed earlier did not induce T_R1 responses suggesting significantly

different mechanisms of action. To induce the Ag-specific suppressive effects, pMHC-NPs rely on previous autoAg experience in the T cell repertoire whereas without previous experience, favorable outcomes were not observed. Disease suppression was dependent on IFN-γ and IL-10 as transgenic mice deficient in IFN-γ or IL-10 abrogated disease suppression. Taken together, the data from both studies support that the attachment of a single disease-related pMHC to NP could be used to suppress autoimmunity.

5. Immune privileged sites

Biomaterial scaffolds have been employed for several regenerative medicine and cell transplantation applications, and more recently, these scaffolds have been designed to modulate the local environment [4, 150]. The strategy aims to mimic natural sites in the body, such as testes or eye, that intrinsically have local immunomodulation [151]. Scaffolds have been implanted that release cytokines, co-transplant cells, or locally present T-cell apoptosis inducing factor FasL [152]. While these strategies have had some efficacy in delaying rejection or promoting long-term engraftment, these strategies are not typically Ag specific, which represents an opportunity for the field.

Microporous PLG scaffolds have been used to co-transplant islets along with Tregs specific for islet Ag to enhance graft survival [153]. Islet Ag (BDC2.5)-specific Tregs were cultured *in vitro* and were co-incorporated with 300 NOD scid gamma (NSG) islets before implantation within the abdominal cavity. In control scaffolds lacking Tregs, no insulin-producing islet cells were detected at day 25 of implant, while islets from scaffolds containing Tregs survived significantly longer which was associated with a significant survival benefit. Interestingly, a second implant possessing islets but lacking Tregs into the kidney capsule, 97 days after the scaffold implant, did not result in rejection of those islet cells, even after removal of the original scaffold. This protection of the second islet-only transplant demonstrated a systemic tolerance. Following removal of the scaffold and kidney capsule transplant, mice became hyperglycemic, confirming that the transplanted islets were responsible for euglycemia.

6. Conclusion and future directions

Ag-specific therapies offer numerous advantages to improve the treatment of diseases with immune-mediated pathology. However, significant challenges remain to be addressed regarding the heterogeneity and breadth of disease-relevant Ags, understanding the molecular mechanisms of tolerance induction, the refinement of the production and characterization of therapeutics, and identifying the most favorable applications to apply Ag-specific tolerance therapies.

The wide-scale application of Ag-specific immune tolerance technologies is complex, as disease progression can be mediated by a single or many Ags and multiple immune pathways are often involved. The wide-breadth of Ags implicated in clinical disease makes it difficult to produce Ag-specific therapies especially for autoimmunity, allograft transplantation, and allergy. In diseases such as MS, additional layers of complexity exist due to processes such as epitope spreading and the relative reactivity of a patient's cells to

particular autoAgs [3]. In a small cohort of MS patients, PBMCs coupled with a library of seven relevant antigenic peptides (Ag-PBMCs) demonstrated promising results [51]. It is possible that the use of cell lysates or protein extracts containing a plethora of immunogenic epitopes may be more effective in these disorders, however, as the complexity of these Ag mixtures increases, the ease of formulation and characterization of the Ag carriers is likely to decrease. Furthermore, the low concentrations of individual Ags present in the extracts may not yield significant tolerogenic responses *in vivo* as it is likely that there is an Ag loading requirement necessary for tolerance induction. Future research targeted towards the discovery of additional epitopes will aid in the development of Ag-specific tolerogenic therapies.

Molecular mechanisms described for Ag-specific therapies are complex and not completely understood. The majority of efforts for the development of therapeutics have focused on inducing tolerogenic responses through APC or lymphocyte reprogramming (Figure 1). Modulation of signal 1 and signal 2 of T cell activation through APCs represents an important pathway to alter T cell activation and induce tolerance. Other mechanisms such as direct interactions of therapeutics and CAR T cells with lymphocytes to induce tolerogenic phenotypes such as Tregs and deletion represent additional methods to curb aberrant immune activation. However, as each technology is different, individual mechanisms of action need to be identified and should not be extrapolated from one to another.

Developing improved strategies to produce therapeutics that deliver peptide and protein Ags will be required to enable the large-scale progression of these technologies to the clinic. Cell-based technologies have shown promise in clinical trials, but may have limited translatability due to limited sources of cells, complicated *ex vivo* manipulations, and poor shelf-life. Particle-based therapeutics may address these issues and are expected to gain significant traction in these applications, however, difficulties regarding scale up and controlling physicochemical properties such as size, charge, and Ag release as well as methods to characterize the presence of relevant epitopes within the particle will need to be overcome.

There is an excellent opportunity to develop novel Ag-specific therapies to improve the efficacy and long-term usability of therapeutic proteins and antibodies for which the formation of anti-drug antibodies limits their clinical applicability. ADA-specific tolerance strategies may revitalize the use of previously discovered therapies. Since the Ags are well described when tolerizing against therapeutic proteins and limiting ADAs, the use of Ag-specific tolerance therapies for this purpose is highly promising.

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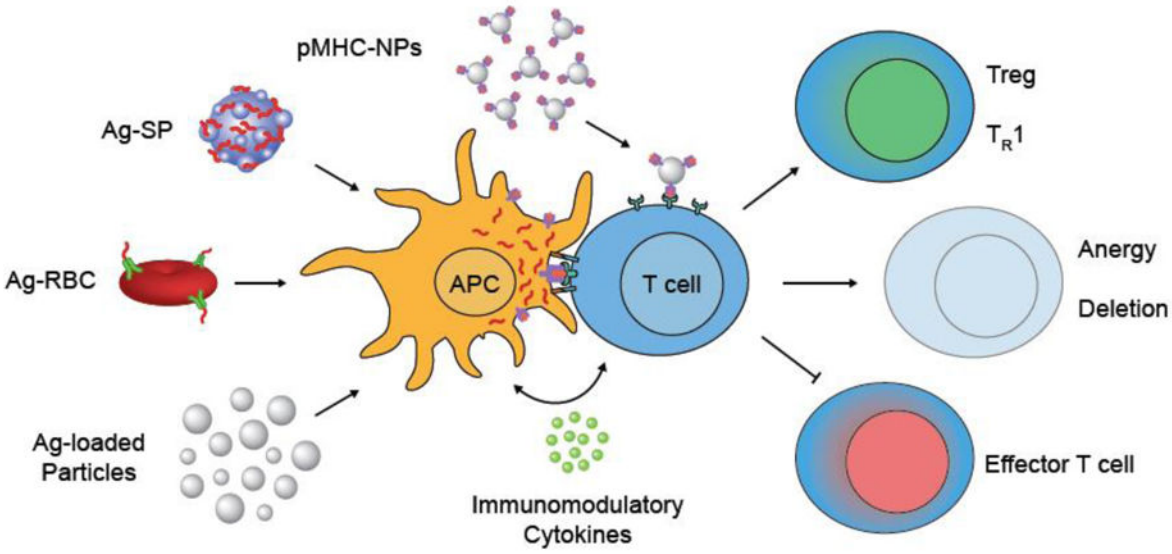

Figure 1.

Highlighted approaches of technologies implemented for antigen-specific tolerance induction. Most antigen-specific tolerance strategies result in reprogramming lymphocytes through antigen presenting cells (APCs), however, there are platforms that target T cells and specifically recognize their autoreactive T cell receptors. Inspired by the natural clearance of apoptotic cells which results in peripheral tolerance maintenance, antigen has been delivered by various platforms including antigen-coupled splenocytes (Ag-SP), erythrocyte-targeted peptides (Ag-RBC), and antigen-loaded synthetic particles. These carriers are internalized, processed by APCs, and induce tolerogenic costimulation and soluble signaling pathways that direct T cell phenotypes away from immunogenic effector T cell activation and toward regulatory T cells (Tregs), anergy, or deletion. Direct interaction of particle-bound peptide-major histocompatibility complexes (pMHC-NPs) with antigen-experienced T cells can induce a tolerogenic regulatory-like T_R1 phenotype that can mitigate immune-mediated disease progression.

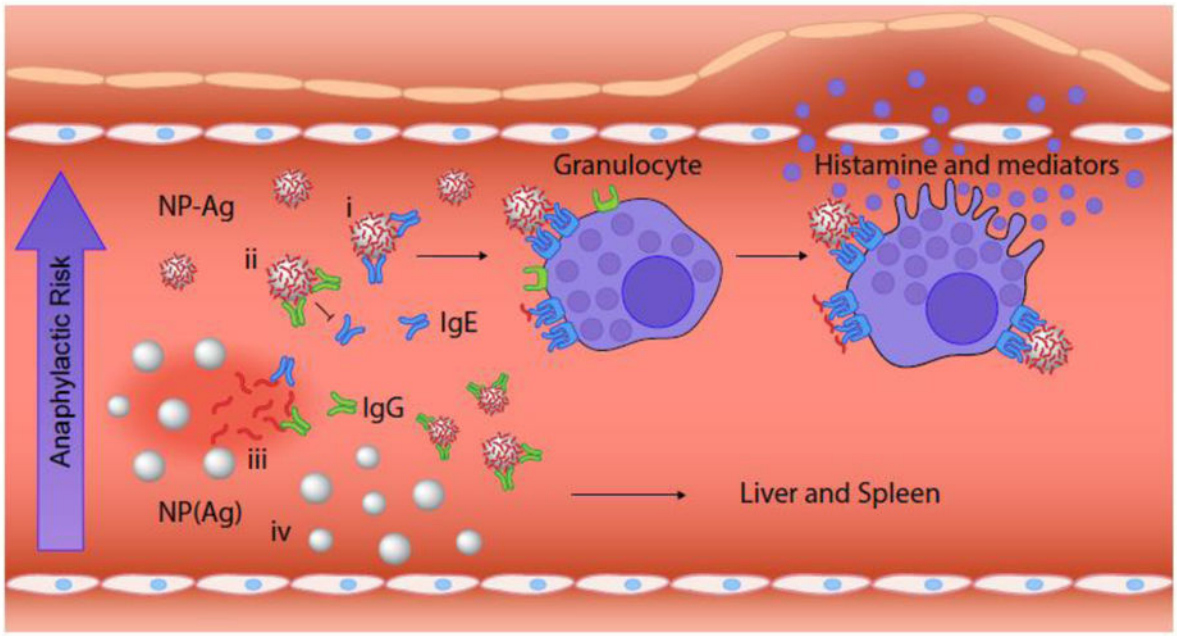

Figure 2.

Mode of antigen-association with particles affects the risk of anaphylaxis following intravenous administration in individuals with prior antigen sensitization. Antigen is associated with particles by surface-coupling (NP-Ag) or by encapsulation (NP(Ag)) methods. *In vivo,* granulocyte activation occurs when NP-Ag or soluble antigen is recognized by circulating IgE antibodies or binds to pre-bound IgE on granulocytes. Cross-linking of IgE on granulocytes triggers degranulation and subsequent release of histamine and other inflammatory mediators that cause increased permeability, distension of blood capillaries, and anaphylaxis. (i) Binding of antigen-specific IgE to NP-Ag can trigger granulocyte activation. (ii) Binding of antigen-specific IgG to NP-Ag reduces potential granulocyte activation but can result in off-targeted biodistribution and reduce tolerance induction. (iii) Pre-mature antigen release from NP(Ag) can result in granulocyte activation but to a lesser extent than NP-Ag. (iv) NP(Ag) with negligible rate of antigen release reduces the risk of granulocyte activation and enables unaffected distribution to the liver and spleen to induce tolerogenic responses.

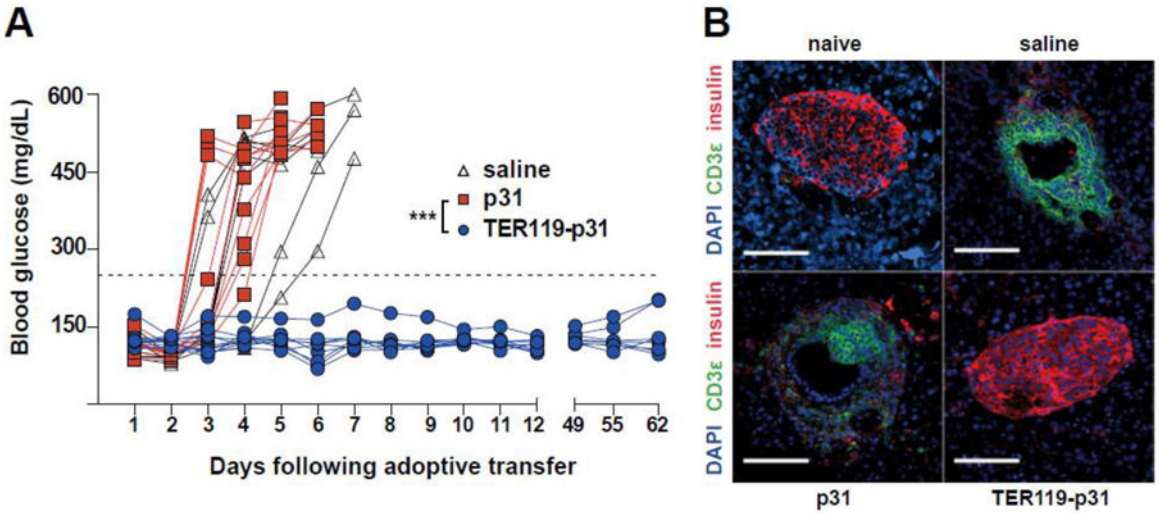

Figure 3.

Erythrocyte-binding TER119 scFv antibodies fused with autoantigen (p31) specifically target red blood cells *in situ* after intravenous administration and induce antigen-specific tolerance in a type 1 diabetes model. (A) TER119-p31 induces tolerance and results in normoglycemia. Normoglycemic NOD/ShiLtJ mice received adoptive transfer of diabetogenic BDC2.5 $CD4^+$ T cells and 3 intravenous treatments of saline, p31, or TER119-p31 (n =8, n = 9, and n = 9, respectively) over the first week. *** $P < 0.0001$. (B) Immunohistochemistry of pancreatic islets excised 4 days after treatment and stained for CD3ε T cells (green), insulin (red), and nuclei (blue). Saline-treated and untargeted autoantigen mimetope p31 resulted in T cell infiltration and islet destruction in contrast to mice treated with red blood cell-binding autoantigen (TER119-p31) which prevented T cell infiltration and preserved insulin production. (Scale bar = 100 μm). Reproduced from [48] with permission.

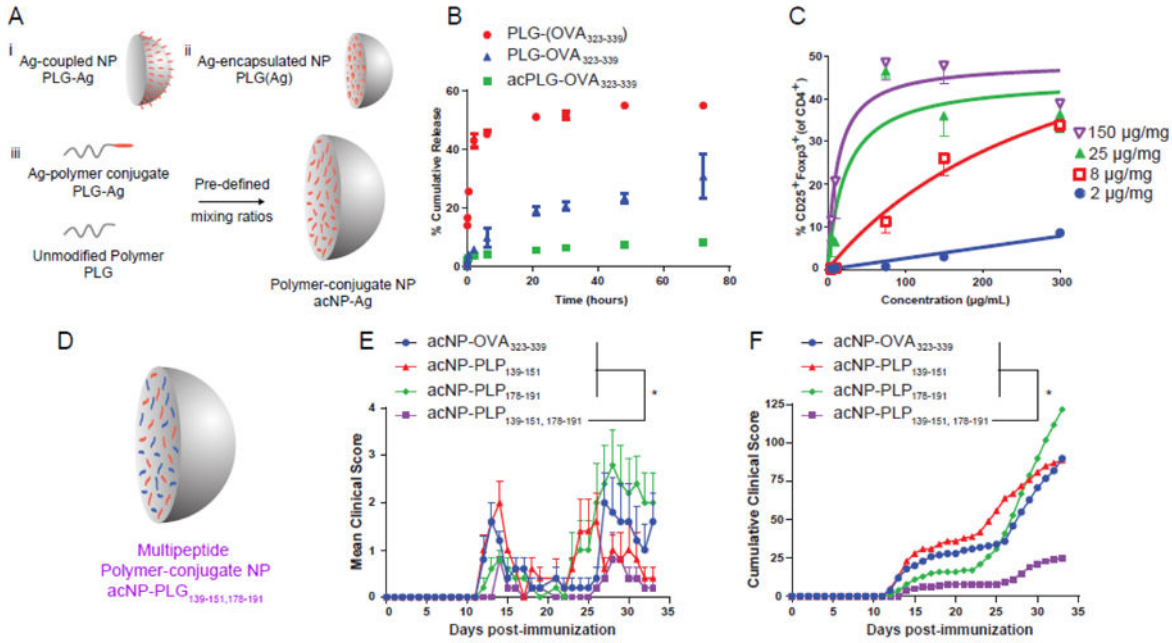

Figure 4.

Antigen-polymer conjugate nanoparticles display favorable physicochemical and biological properties for tolerance induction. (A) Schematic representation of Ag-coupled, Ag-encapsulated, and polymer-conjugate nanoparticles. (B) Release profile of NP(OVA$_{323}$-339), NP-OVA$_{323-339}$, and acNP-OVA$_{323-339}$. (C) Regulatory T cell induction is dependent on nanoparticle concentration. BMDCs were treated for 3 hr with various concentrations of acNP-OVA$_{323-339}$ (2, 8, 25, 150 µg/mg loading). Excess acNP-OVA$_{323-339}$ particles were subsequently washed from the cell surface prior to addition of OT-II T cells and 2 ng/mL of TGF-β1. (D) Schematic representation of antigen-polymer conjugate nanoparticles delivering multiple Ags. (E) Clinical scores of SJL/J mice treated with 1.25 mg of acNP-OVA$_{323-339}$ (8 µg/mg OVA$_{323-339}$), acNP-PLP$_{139-151}$ (8 µg/mg PLP$_{139-151}$), acNP-PLP1$_{78-191}$ (8 µg/mg PLP$_{178-191}$), or acNP-PLP$_{139-151,178-191}$ (8 µg/mg PLP1$_{39-151}$ and 8 µg/mg PLP$_{178-191}$) and immunized with PLP$_{139-151}$ and PLP$_{178-191}$ in CFA to induce R-EAE 7 days later. (F) Corresponding cumulative clinical score for mice treated with particles (n = 5). Differences between disease courses of different treatment groups were analyzed for statistical significance using the Kruskal-Wallis test (one-way ANOVA non-parametric test) with Dunn's multiple comparisons test ($p < 0.05$) [94].

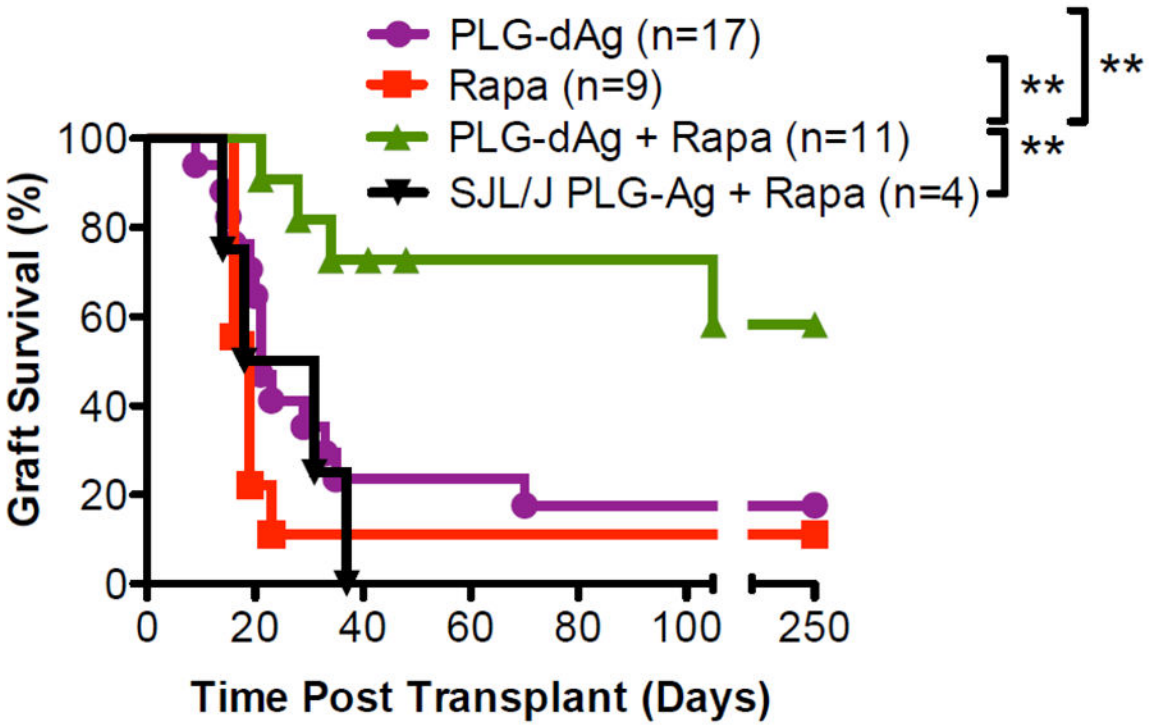

Figure 5.

A short course of low dose rapamycin synergizes with PLG-donorAg (dAg) to enhance tolerance efficacy. Recipient mice (C57BL/6) receiving donor (BALB/c) PLG-dAg injections at days 7 and +1 in combination with a 4-day course (days 1, 0. +1, and +2) of low dose (0.1 mg/kg) rapamycin demonstrated significantly greater islet allograft survival (n=11) compared with mice treated with rapamycin alone (n=9), BALB/c PLG-dAg alone (n=17), or PLG particles coupled with lysate proteins from a third party donor SJL/J (n=4). **$p < 0.01$. Reproduced from [71] with permission from Elsevier.

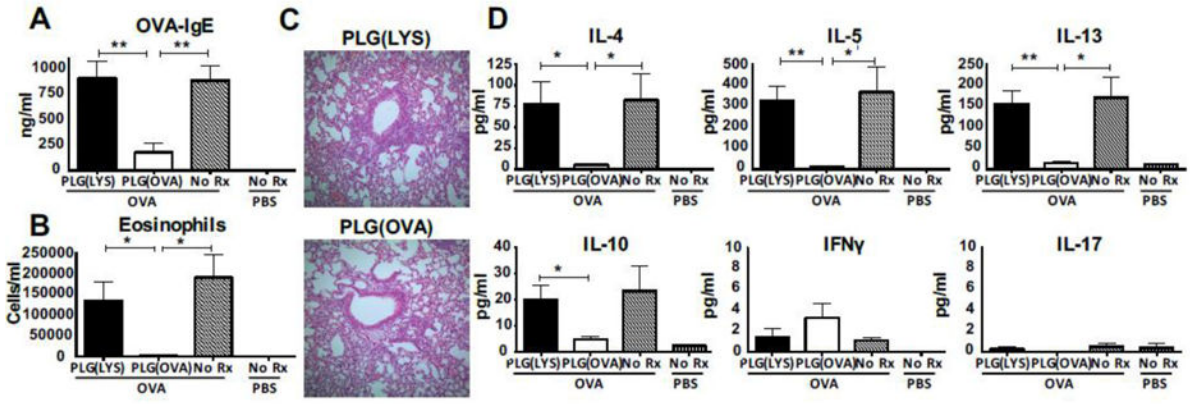

Figure 6.

Prophylactic treatment with PLG(OVA) inhibits Th2-induced airway inflammation. Naive female BALB/c mice (n = 5) were treated i.v. with 2.5 mg PLG(OVA) or control PLG(LYS) on days −7 and +7 relative to i.p. immunization with 10 μg of OVA in 3 mg of alum or alum alone on days 0 and +14 before aerosol challenge with 10 mg/mL OVA for 20 min on days +28–30 and sample collection on day +31. LYS, lysozyme. (A) Concentration of serum OVA-IgE was determined by sandwich ELISA. (B) Lungs were flushed with BALF, total cell counts were determined, and samples were cytospun onto slides before DiffQuik staining for differential cell counts of bronchoalveolar lavage eosinophils. (C) Lungs were fixed in formalin and stained with H&E. (D) Cytokines from BALF supernatant were analyzed by Milliplex. Results are mean ± SEM and are representative of three separate experiments. *P < 0.05; **P < 0.01. Reproduced with permission from [73].

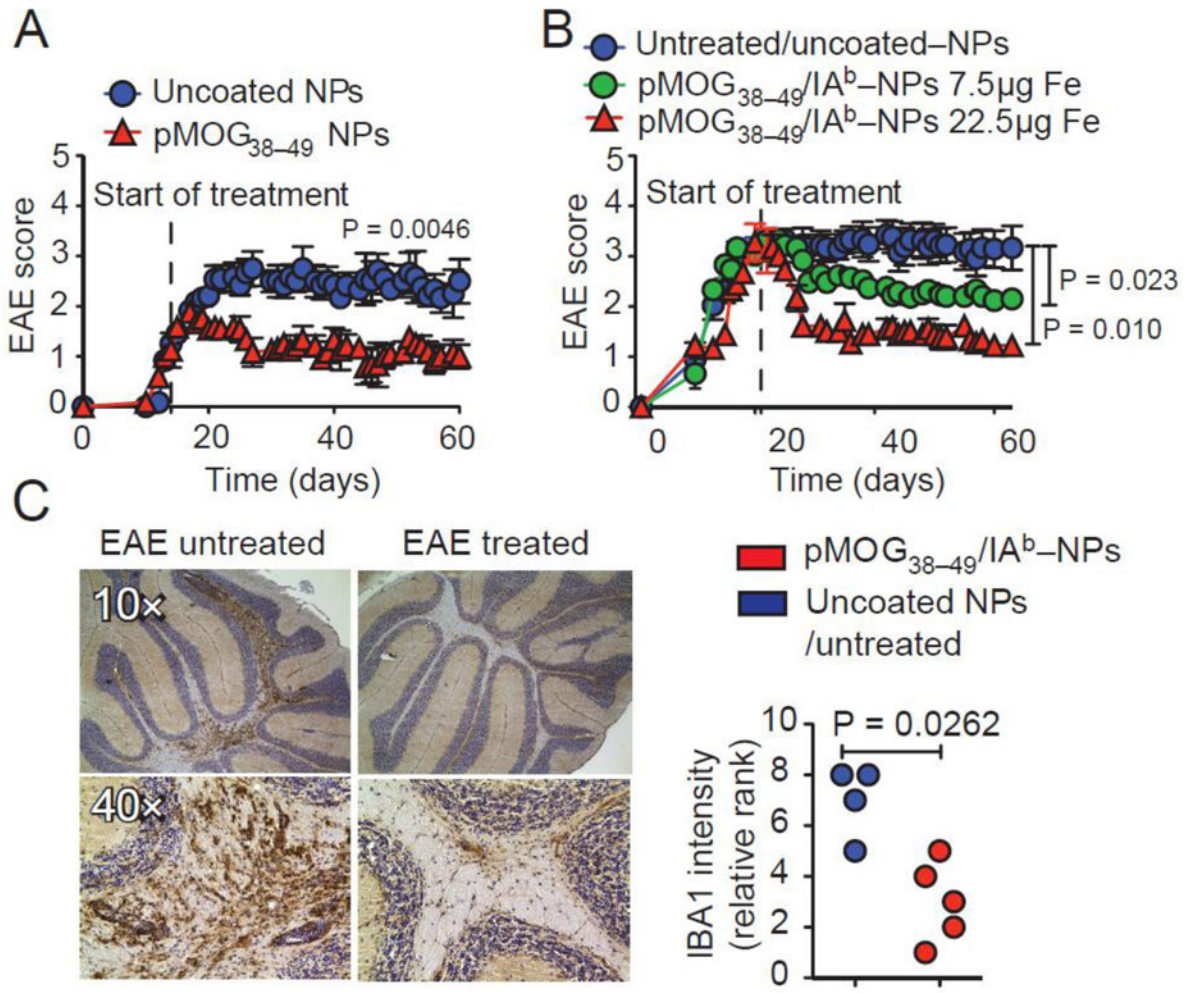

Figure 7.

pMHC-NPs suppress MOG-induced EAE *in vivo.* C57BL/6 mice were immunized with $pMOG_{35-55}$. (A) EAE scores of mice treated from day 14 (n=4 each). (B) EAE scores of mice treated from day 21 (n=10, 7 and 3 from top). (C) Representative microglial IBA1 stainings and relative rank scores in the cerebellum of mice from B (n=4–5). Reproduced with permission from [133]. ©2016 Macmillan Publishers Ltd.

Table 1

Nanoparticles investigated for antigen-specific immune regulation delivering protein or peptide antigens

Indication	Particle type	Antigen	Important finding	Refere nce
EAE	Ag-coupled PLGA and PS	$PLP_{139-151}$; $PLP^{178-191}$; MBP_{35-55}	MARCO scavenger receptor mediated tolerance induction	[64]
EAE	Ag-coupled PLGA	$PLP_{139-151}$; $PLP_{178-191}$	Lower negative charge on particles resulted in improved efficacy	[74]
EAE	Ag-coupled PLGA	$OVA_{323-339}$; $PLP_{139-151}$	Increased Ag conjugation and particle concentration enhanced Ag presentation and reduced co-stimulatory expression	[154]
EAE	Ag-encapsulated PLGA	$OVA_{323-339}$; $PLP_{139-151}$; $PLP_{178-191}$	Tolerance induction was not completely dependent on the spleen	[70]
EAE	Ag-polymer conjugate PLGA	$OVA_{323-339}$; $PLP_{139-151}$; $PLP_{178-191}$	Modular Ag loading, negligible burst release, tolerance induction to multiple epitopes	[94]
EAE	Ag-encapsulated PLGA and IL-10 encapsulated PLGA	MOG_{35-55}	Subcutaneous prophylactic administration reduced clinical disease. Co-administration of IL-10 PLGA was necessary to suppress disease	[67]
EAE	Ag and rapamycin co-encapsulated PLGA/PLA-PEG	$PLP_{139-151}$	Significantly reduced clinical disease score when Ag co-encapsulated with rapamycin.	[65]
EAE	Poly(maleic anhydride-alt-1-octadecene)-coated superparamagnet ic iron oxide nanocrystals	$MBP_{AC-1-9\ (4Tyr)}$; MOG_{35-55}	Ag delivery to LSECs by particles induced Ag-specific Tregs and suppressed clinical disease	[69]
EAE	Ag and ITE-loaded	MOG_{35-55}; $PLP_{139-151}$;	Co-encapsulation of ITE with particles expanded Tregs	[66]
	PEGylated gold	$PLP_{178-191}$	and suppressed clinical disease	
EAE	Peptide-MHCII complex-conjugated iron oxide	N/A	Tolerance mediated by expansion of Ag-specific TR1-like cells and suppressive regulatory B cells	[133]
Diabetes	Peptide-MHCI complex-conjugated iron oxide	N/A	Expanded CD8+ T cells with regulatory potential but conventional memory-like phenotype	[149]
Diabetes	Peptide-MHCII complex-conjugated iron oxide	N/A	Tolerance mediated by expansion of Ag-specific TR1-like cells and suppressive regulatory B cells	[133]
Glomerular	Ag-coupled latex	OVA	Tolerance in the liver is	[97]
nephritis			dependent on KCs in a noninflammatory microenvironment	

Indication	Particle type	Antigen	Important finding	Refere nce
Collagen	Ag-encapsulated	CII	Oral administration of	[155]
induced	PLGA		particles suppressed arthritis	
arthritis			symptoms	
Collagen	Peptide-MHCII	N/A	Tolerance mediated by	[133]
induced	complex-		expansion of Ag-specific	
arthritis	conjugated iron oxide		TR1-like cells and suppressive regulatory B cells	
Proteoglyc	Ag-encapsulated	Hsp 70-	Intranasal delivery of	[156]
an induced	PLGA and	peptide	particles suppressed arthritis	
arthritis	PLGA-TMC	mB29a	symptoms	
Islet	Ag-coupled	Donor cell	Full MHC-mismatched	[71]
transplant	PLGA	lysate	murine allogeneic transplantation was achieved in 20% of recipients and improved to 60% with short course rapamycin	
Bone	Ag-encapsulated	Dby, Uty	Delivery of CD4 Dby epitope	[49]
marrow	PLGA		prevented transplant	
transplant			rejection and delivery of CD8 epitope Uty did not induce tolerance	
Hemophilia	Ag-encapsulated PLGA/PLA-PEG	Factor VIII	Significantly reduced antibody formation when Ag co-encapsulated with rapamycin.	[65]
Hemophilia	Ag-conjugated liposomes	Factor VIII	Suppression of antibody responses and prevented bleeding when delivered with CD22 ligand	[119]
Anti-drug antibody	Ag-conjugated liposomes	OVA; MOG_{1-120}	Suppression of antibody responses when delivered with CD22 ligand	[119]
Anti-drug antibody	Soluble Ag and rapamycin-encapsulated PLGA/PLA-PEG	OVA; $OVA_{323-339}$; adalimumab; pegsiticase	Delivery of rapamycin in particles and Ag within 1 day of particle administration was necessary to suppress antibody responses	[118]
Allergy	Ag-encapsulated PLGA/PLA-PEG	OVA; $OVA_{323-339}$	Significantly reduced antibody formation when Ag co-encapsulated with rapamycin.	[65]
Allergy	Liposomes	OVA	Suppression of antibody responses when delivered with CD22 ligand	[119]
Allergy	Ag-encapsulated PLGA	Bet v 1	Subcutaneous administration of particles modulated Th2 response	[157]
Allergy	Ag-encapsulated PLGA	$OE_{109-130}$	Intranasal administration of particles suppressed IgE and IgG1 production but increased IgG2a	[158]

Indication	Particle type	Antigen	Important finding	Refere nce
Allergy	Ag-encapsulated PLGA	OVA	Inhibited Th2 responses in models of allergic airway inflammation	[73]

PLGA (poly(lactide-co-glycolide)); PS (polystyrene); PLA-PEG (polylactide-poly(ethylene glycol)); TMC (trimethyl chitosan); LSEC (liver sinusoidal endothelial cell); KC (Kupffer cell); OVA (Ovalbumin); PLP (proteolipid protein); MOG (myelin oligodendrocyte protein); MBP (Myelin basic protein); CII (type II collagen); Bet v 1 (Birch pollen allergen); OE (Olive allergen); ITE (2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester)