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Therapeutic induction of antigen-specific immune tolerance

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Abstract

The development of therapeutic approaches for the induction of robust, long-lasting and antigen-specific immune tolerance remains an important unmet clinical need for the management of autoimmunity, allergy, organ transplantation and gene therapy. Recent breakthroughs in our understanding of immune tolerance mechanisms have opened new research avenues and therapeutic opportunities in this area. Here, we review mechanisms of immune tolerance and novel methods for its therapeutic induction.

Introduction

Immune system activation is vital to the control of pathogens and cancer, but regulatory mechanisms are needed to prevent immunopathology resulting from excessive immune activity. Perturbations of this balance result in infections, cancer, inflammatory diseases or allergy. Indeed, autoimmune diseases affect as much as 5–10% of the population and are on the rise[1]. Similarly, inefficacious immune modulation results in graft rejection and graft-versus-host disease (GVHD) in 20–70% of transplant recipients, and pre-existing immunity to viral vectors limits gene therapy efficacy. The development of antigen-specific immunotherapies is an important unmet clinical need.

Key advances have been made in our understanding of immune tolerance and its regulation. Indeed, new technologies for antigen discovery, drug delivery and cell targeting have opened new avenues for the development of therapies for the induction of antigen-specific tolerance. Here we review mechanisms of immune tolerance and discuss strategies for its therapeutic modulation.

Mechanisms of immune tolerance

Immune tolerance is an active state of unresponsiveness towards a specific antigen, which involves both innate and adaptive immune cells. The breakdown of self-tolerance can result

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in the development of autoimmune disorders, whereas dysregulated immune responses to foreign antigens may lead to hypersensitivity and allergic disease. Thus it is important to define the multiple mechanisms involved in its establishment and maintenance.

Central tolerance

Central tolerance is established during T and B cell development in the thymus and bone marrow, respectively. Bone marrow-derived $CD34^+$ T cell progenitors home to the thymus, where they acquire T cell receptor (TCR) expression. Random V(D)J rearrangements generate a diverse TCR repertoire that is reactive against a wide array of antigens. T cells harbouring TCRs that do not recognize MHC-presented self-peptides die by neglect, whereas those with low affinity for peptide–MHC complexes differentiate into $CD4^+$ or $CD8^+$ single-positive T cells. The randomness of V(D)J rearrangements inevitably generates some TCR clones with high affinity for self-antigen–MHC complexes. High-affinity TCR clones are controlled by various mechanisms of central tolerance including clonal deletion and receptor editing. Some self-reactive T cells escape deletion and leave the thymus but show functional impairment and/or expression of molecules associated with tolerance[2], whereas others develop into self-reactive thymus-differentiated regulatory T cells (tT_{reg} cells), which migrate to peripheral lymphoid and nonlymphoid tissues[3].

Self-antigen–MHC complexes are expressed by thymic antigen-presenting cells (APCs) including specialized medullary thymic epithelial cells (mTECs), dendritic cells (DCs) and B cells. The transcriptional factor autoimmune regulator (AIRE) promotes the expression of peripheral tissue antigens by mTECs[4]; mutations in AIRE are linked to autoimmune pathology. However not all tissue-specific antigens expressed by mTECs are controlled by AIRE. Indeed recent studies identified mTECs that express transcription factors such as FEZF2 (ref. 5) or that co-opt lineage-defining transcription factors from peripheral cell types, termed mimetic cells[6]. These $AIRE^+$, $FEZF2^+$ and mimetic mTECs collaborate with thymic B cells and DCs to promote central tolerance through clonal T cell deletion and T_{reg} cell induction. This process is further aided by the transfer of tissue-specific antigens from mTECs to DCs through a process termed cooperative antigen transfer[7]. Of note, it was recently reported that intestinal DCs travel to the thymus to present microbiota-derived antigens, highlighting the contribution of peripheral DCs to central tolerance[8].

In the bone marrow, developing B cells acquire the expression of a B cell antigen receptor (BCR) that randomly rearranges its V, D and J gene regions to generate a diverse BCR repertoire. Up to 75% of early immature B cells are self-reactive[9], but a third of them undergo immunoglobulin gene rearrangements that reduce autoantigen reactivity[10]. Additional self-reactive B cells are removed by clonal deletion[11]. However central tolerance does not eliminate all self-reactive clones, for example those reactive to developmentally restricted or inducible antigens that are not expressed by the thymus or the bone marrow. Thus, self-reactive lymphocytes escape central tolerance and are actively controlled by peripheral tolerance mechanisms.

Peripheral tolerance

About 25–40% of self-reactive T cells[12] and approximately 40% of autoreactive B cells[9] escape central tolerance. Thus peripheral tolerance mechanisms, including anergy, deletion and suppression by T_{reg} cells, are crucial for the prevention of autoimmune diseases or hypersensitivity to antigens first encountered outside the thymus or bone marrow, including food allergens or antigens displayed during infection or pregnancy.

Three signals are required for T cell activation. Signal 1 involves the interaction of the TCR with peptide–MHC molecules. Signal 2 involves the binding of co-stimulatory receptors to their ligands on APCs, most commonly CD28 on T cells and CD80 or CD86 on APCs, but also other co-stimulatory molecules, including inducible T cell co-stimulator (ICOS) and CD40 (ref. 13). Signal 3 involves the activation of cytokine receptors. The activation of TCR signalling (signal 1) in the absence of co-stimulation (signal 2), or strong pre-exposure to cytokines (signal 3) before signals 1 and 2, induces T cell anergy, a state in which the T cell is functionally inactivated, incapable of proliferating or producing IL-2 (ref. 14). T cell anergy can also be induced by repeated antigen stimulation[15], exposure to anti-inflammatory cytokines such as IL-10 (ref. 16), or signalling via co-inhibitory receptors such as programmed cell death 1 (PD1) and cytotoxic T lymphocyte associated protein 4 (CTLA4)[17]. Similarly, B cells require BCR engagement concomitant with Toll-like receptor (TLR) signalling or interactions with T helper cells to be fully activated. High avidity BCR interactions with antigens in the absence of TLRs or T helper cell co-stimulation induce clonal deletion or anergy, inhibiting B cell proliferation and differentiation into antibody-secreting cells and overall shortening B cell lifespan[18].

Long-term T cell anergy is associated with epigenetic modifications that render cells more sensitive to inhibitory signals[19], while altering gene and surface marker expression and inducing functional changes similar to those observed in exhausted T cells induced during chronic infection or cancer[15]. However T and B cell anergy is a dynamic process, and the removal of antigen exposure can restore T or B cell functionality[15,20]. Furthermore, a subset of naturally occurring anergic T cells expressing CD73 and FR4, capable of differentiating into functional $FOXP3^+$ T_{reg} cells and $FOXP3^-IL\text{-}10^+$ type 1 regulatory T (T_R1) cells, has been described[21,22], although it is not clear whether this process involves specific APC types or anatomical niches.

The peripheral deletion of T and B cells through apoptosis also controls self-reactive cells. Intrinsic T cell apoptosis largely depends on the pro-apoptotic protein BIM, upregulated during T cell deletion, which inhibits the anti-apoptotic proteins BCL-2 and BCL-x_L, activating pro-apoptotic BAX and BAK to permeabilize the mitochondrial membrane[23,24]. Extrinsic T cell apoptosis involves FAS[25] or tumour necrosis factor (TNF) receptor[26] signalling, which ultimately triggers caspase activation to induce apoptosis. Signalling through these death receptors limits self-reactive pathogenic T cell and B cell responses. For example, central nervous system (CNS)-resident astrocytes expressing the TNF receptor ligand TRAIL induce T cell apoptosis and limit autoimmune neuroinflammation[27]. Other forms of peripheral immune cell death (necroptosis, ferroptosis and pyroptosis) also contribute to peripheral immune tolerance[28-30].

The mechanisms determining whether self-reactive T or B cells undergo anergy versus cell death following TCR or BCR activation without co-stimulation are still not fully understood. Antigen levels have been postulated to control cell fate, with higher levels triggering anergy and lower levels triggering cell death[31]. In addition, checkpoint molecule signalling (for example, through PD1, TIGIT, TIM3, LAG3 and VISTA) can induce T cell death or dysfunction[32-34].

Finally T_{reg} cells play central roles in peripheral tolerance. Major T_{reg} cell subtypes include $FOXP3^+$ cells and IL-10-producing $FOXP3^-$ T_R1 cells, but additional subsets have been linked to immune tolerance, including $CD8^+$ T_{reg} cells[35], regulatory γδ T cells[36] and regulatory invariant natural killer T cells (iNKT cells)[37].

$FOXP3^+$ T_{reg} cells differentiate in the thymus ($FOXP3^+$ tT_{reg} cells) in response to self-antigen expression[38] and then migrate to peripheral lymphoid and nonlymphoid tissues to limit pathogenic autoreactivity and promote tissue repair[39]. Some $FOXP3^+$ T_{reg} cells differentiate from naive $CD4^+$ T cells in the periphery ($FOXP3^+$ pT_{reg} cells), enforcing tolerance to antigens not expressed in the thymus, including food antigens, allergens, microbial antigens or pregnancy-linked fetal antigens[40]. In addition, tissue-resident T_{reg} cells in the skin[41], muscle[42], visceral adipose tissue[43,44] and mucosal tissues, such as intestine[45,46] and lungs[39], display specialized phenotypes and functions, as recently reviewed[47,48].

T_R1 cells are IL-$10^+FOXP3^-CD4^+$ T cells that were initially described following chronic stimulation in the presence of IL-10 (ref. 49). IL-27 was later found to be a stronger T_R1 cell differentiation inducer[50], with IFNα[51], hyaluronic acid[52], ICOSL[53], CD2 (ref. 54) and CD55 (ref. 55) expression on APCs also displaying important roles (see Box 1). $FOXP3^+$ pT_{reg} and T_R1 cell differentiation and function are modulated by host and microbial metabolites, such as aryl hydrocarbon receptor (AHR) agonists[56]. T_R1 cells produce IL-10 and transforming growth factor-β (TGFβ), as well as perforin and granzyme B, which can kill APCs[57,58]. T_R1 cells also express the inhibitory molecules CTLA4 and PD1, enabling contact-dependent T cell suppression, and CD39 (ref. 59), which degrades pro-inflammatory extracellular ATP while promoting the production of anti-inflammatory adenosine.

Multiple cell types participate in central and peripheral immune tolerance. DCs play a central role because they process and present antigen, while providing cytokines and stimulatory or inhibitory molecules to modulate T cell differentiation or trigger anergy or deletion. Thus, DCs are frequently targeted for the therapeutic induction of antigen-specific immune tolerance.

DCs as the central mediators of immune tolerance

DC subsets and their functions

DCs display phenotypic and functional heterogeneity[60,61]. DCs are classified into plasmacytoid DCs (pDCs), classical (or conventional) type 1 DCs (cDC1s) and type 2 DCs (cDC2s). In addition, monocyte-derived DCs (moDCs), sometimes called TipDCs (TNF-producing and iNOS-producing DCs), adopt a DC-like phenotype under inflammatory

conditions[62], although recent works call into question their ability to migrate to lymph nodes and prime $CD4^+$ and $CD8^+$ T cells[63]. A DC3 subtype displaying cDC2 and moDC features was also identified in humans[64]. Additional heterogeneity within DC subsets has been described. For example, cDC2s are classified into cDC2As and cDC2Bs controlled by the transcription factors T-bet and RORγt, respectively[65]. In addition CD103 and CD11b distinguish functional cDC subsets in mucosal tissues[66].

pDCs are primarily located in the blood and lymphoid tissues but migrate to nonlymphoid tissues during inflammation[67]. When activated, mainly via TLR7 or TLR9 signalling, pDCs produce large amounts of type I interferons, including IFNα and IFNβ[68]. Under homeostatic conditions, pDCs are poor activators of naive $CD4^+$ and $CD8^+$ T cells. However, a subpopulation of pDCs stimulates $CD4^+$ T helper 1 (T_H1) cells during infection[69]. pDCs also promote tolerance and T_{reg} cell induction via the expression of ICOSL[70], TGFβ[71] and inhibitory indoleamine 2,3-dioxygenase (IDO)[72]. Indeed recent findings suggest that pDC deficits contribute to GVHD following organ transplantation[73] and that pDCs contribute to oral tolerance induction[74].

cDCs are present in both lymphoid and nonlymphoid tissues at the steady state. cDC1 and cDC2 distribution varies in different tissues, and although both subsets migrate between tissues and lymph nodes, cDC2s appear to have a higher migratory potential and are enriched at mucosal-associated sites such as the lungs and intestine[75]. Of note, at the steady state cDC1s, cDC2s and pDCs are detected in the CNS choroid plexus and meninges, but they are virtually undetectable in the brain parenchyma and perivascular space[76,77]. Indeed, cDC1s are the primary subtype present in the choroid plexus, whereas cDC2s are most abundant in the leptomeninges and dura mater[76,77]. Under inflammation cDC1s, cDC2s, moDCs and pDCs infiltrate the brain parenchyma and present CNS-specific antigens to T cells[76-78]. Although both cDC1s and cDC2s can present antigen to either $CD4^+$ or $CD8^+$ T cells, cDC1s are better at antigen cross-presentation[79] and type III interferon production[80]. Within the cDC2 subset, cDC2As appear to be less pro-inflammatory than cDC2Bs, expressing higher levels of amphiregulin and matrix metalloproteinase 9, whereas cDC2Bs produce higher levels of TNF and IL-6 (ref. 65). Of note, cDC2s in the intestine have been shown to promote T helper 17 (T_H17) cell differentiation[81,82]. However, both cDC1s and cDC2s are reported to promote the differentiation of $FOXP3^+$ T_{reg} cells and $IL\text{-}10^+$ T_R1 cells[83,84].

Tolerogenic DC phenotype

Activation and maturation states dictate the effects of DCs on the immune response. Before their activation via pattern recognition receptors (PRRs), DCs reside at mucosal sites, lymphoid and peripheral tissues or in the blood in an immature state. Activation by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) upregulates DC expression of MHC class I and II, co-stimulatory and adhesion molecules such as CC-chemokine receptor 7 (CCR7). These mature DCs migrate to lymphoid tissues to promote effector T cell differentiation. Immature DCs, conversely, exhibit low expression of MHC class I, MHC class II and co-stimulatory molecules and are capable of inducing T cell anergy, T_{reg} cell differentiation and effector T cell

deletion[85]. It was originally postulated that tolerogenic DCs were essentially immature DCs, but this paradigm was challenged early on[86]. It has since been proposed that specific stimuli can induce a tolerogenic DC phenotype[87] and that tolerogenic DCs undergo some level of maturation and/or activation[88]. Indeed, specific transcriptional programmes in DCs drive immunogenic versus tolerogenic states[87,89]. For example, β-catenin signalling[90] or phagocytosis of apoptotic material[91] under steady-state conditions activate tolerogenic programmes in DCs, which migrate to lymph nodes to present self-antigens and maintain peripheral tolerance. Moreover, a tolerogenic DC phenotype can also be induced in semimature and mature DCs[92]. For example, an IL-10$^+$ DC-10 subtype was identified in human peripheral blood and the spleen, displaying cDC and moDC surface markers but capable of inducing CD4$^+$ T cell hyporesponsiveness and T_R1 cell expansion[93] (see Box 1); DC-10s can be induced in vitro by monocyte differentiation in the presence of IL-10. In addition, intestinal CD103$^+$ DCs contribute to tolerance to dietary antigens and the induction of oral tolerance[94,95]. Regardless of their origin and maturation state, DCs contribute to immune regulation via multiple mechanisms, including co-stimulatory molecule downregulation (CD80, CD86 and CD40), inhibitory molecule expression (PD-L1, ICOSL and BTLA), suppression of pro-inflammatory cytokine production (IL-6, IL-12, IL-23 and TNF) and production of anti-inflammatory cytokines (IL-10, TGFβ and IL-27) and metabolites (IDO, retinoic acid and lactate) (Box 1 and Fig. 1).

Numerous stimuli induce a tolerogenic DC phenotype. For example, IL-10 reduces DC expression of MHC and co-stimulatory molecules, decreases pro-inflammatory cytokine production and promotes T cell anergy and T_{reg} cell expansion[96,97]. These anti-inflammatory effects of IL-10 on DCs are AHR dependent[98], recapitulating previous reports of the tolerogenic effects of AHR signalling in DCs[99-105]. Additional cytokines such as TGFβ[106], IL-27 (ref. 107) and IL-37 (ref. 108) also promote an anti-inflammatory DC phenotype. Similarly the exposure of monocytes or bone marrow cells to low concentrations of granulocyte-monocyte colony-stimulating factor (GM-CSF) induces the differentiation of DCs with a tolerogenic phenotype, whereas exposure to higher GM-CSF doses induces a pro-inflammatory DC phenotype[109,110]. Moreover, commensal bacteria signalling through certain PRRs such as TLR2 (ref. 111) promotes tolerogenic DC induction. Indeed, some microbial metabolites induce tolerogenic DCs, for example via AHR activation[99,100]. Indeed, AHR agonists inhibit nuclear factor-κB (NF-κB) activation in DCs and drive the expression of IL-10 and IDO, while reducing the expression of MHC molecules, co-stimulatory molecules and pro-inflammatory cytokines such as IL-6 and IL-12. These changes in DCs result in increased FOXP3$^+$ and IL-10$^+$ T_{reg} cells and the suppression of T_H1, T_H17 and CD8$^+$ effector T cells[101-104].

Additional inducers of a tolerogenic DC phenotype include vitamin A, which is metabolized into retinoic acid, a booster of FOXP3$^+$ T_{reg} cell induction[112] and vitamin D3 that increases IL-10 production while decreasing IL-12 and co-stimulatory molecule expression[113,114]. Moreover, lactate, produced by microbiota, activated DCs or other immune cells, regulates DC function via a hypoxia inducible factor 1α (HIF1α)-driven increase in the expression of NADH dehydrogenase NDUFA4L2 that ultimately limits effector T cell activation[115].

Finally the uptake of apoptotic cells induces a tolerogenic DC phenotype via mechanisms involving AHR activation[116], prostaglandin E_2 production[117] and signalling via scavenger receptors such as MARCO[118]. Indeed, both cDCs and pDCs express IL-10, reduce co-stimulatory molecule expression and promote T_{reg} cell expansion following apoptotic cell uptake[91].

These and other pathways linked to the tolerogenic DC phenotype offer opportunities for the development of therapeutic immunomodulatory strategies, as discussed below.

Antigen-specific therapeutic strategies to induce immune tolerance

Current therapies for autoimmune diseases, transplant rejection and other pathologies driven by dysregulated immune responses are mostly based on untargeted immunosuppression and consequently are linked to significant side effects. Thus novel approaches to induce antigen-specific immune tolerance are needed, targeting improperly activated T cells but not interfering with protective immunity to pathogens and cancer. Consequently, numerous technologies have been developed to induce antigen-specific tolerance (Fig. 2 and Table 1). In the next section, we discuss strategies for the induction of antigen-specific immune tolerance in autoimmunity, organ transplantation and gene therapy (Fig. 3).

Cell-based tolerogenic therapies

The identification of stimuli inducing a tolerogenic phenotype in DCs guided cellular therapeutic approaches, commonly based on DCs generated ex vivo from peripheral blood-derived monocytes and loaded with disease-relevant antigens. However, there is not yet a standardized method to generate tolerogenic DCs ex vivo, and multiple protocols and tolerogenic molecules have been explored. For example, moDCs differentiated in vitro in the presence of low GM-CSF concentrations, termed autologous tolerogenic DCs (ATDCs), display an immature phenotype with a low expression of MHC class II, CD80, CD86 and CD40 and high IL-10 and lactate production[119]. ATDCs were well tolerated in a phase I/IIA clinical trial to prevent graft rejection following kidney transplantation, and additional trials are needed to evaluate their clinical efficacy[120,121]. Similarly, IL-10-induced DC-10s loaded with disease-specific antigens induce antigen-specific immune tolerance[122]; their clinical efficacy remains to be evaluated.

Vitamin D3 also induces a tolerogenic DC phenotype ex vivo[113,114]. Autologous vitamin D3-treated tolerogenic DCs loaded with disease-specific antigens have been tested in phase I clinical trials, including studies focused on type 1 diabetes (T1D)[123,124] and multiple sclerosis (MS)[125] (Table 2). Moreover, moDCs differentiated in the presence of vitamin D3 and IL-10 were shown to be tolerogenic and induce IL-10-producing T cells in a nonhuman primate alloimmune reactivity model[126]. Similarly, moDCs treated with dexamethasone display a tolerogenic phenotype characterized by high IL-10 and TGFβ secretion and low pro-inflammatory cytokine production[127,128]. Dexamethasone-induced tolerogenic DCs loaded with disease-specific peptides were well tolerated in phase I clinical trials in RA, MS and neuromyelitis optica[129,130]. Moreover, tolerogenic DCs induced with dexamethasone and vitamin A were tested in a phase I trial in Crohn's disease[131].

Alternatively, lymphocytes and red blood cells coupled with antigens ex vivo have been used to induce antigen-specific tolerance[132,133]. This approach is thought to induce tolerance as a result of the apoptosis of the antigen-coupled cells and their subsequent uptake by APCs, which acquire a tolerogenic phenotype following apoptotic cell uptake[134]. For example, in a study by Watkins et al. antigen-conjugated erythrocytes were taken up by $BATF3^+$ cDC1s, inducing antigen-specific T cell dysfunction via PD1, CTLA4, LAG3 and TOX expression[135]. Building on these findings, Raposo et al. developed a microfluidic loading technique to produce antigen-loaded erythrocytes, which reduce effector T cell trafficking into target organs[136]. In addition, antigen-loaded erythrocytes induced bystander tolerance[136], inhibiting effector T cell responses against the antigen loaded in erythrocytes and also other antigens expressed in the same tissue. Bystander tolerance induction is critical to the success of antigen-specific immunotherapies because multiple antigens, many of them unknown, are targeted in most autoimmune disorders and different antigens may be targeted in different patients.

Because of their ability to traffic to inflamed tissues, suppress pathogenic T cells and promote tissue repair[39], multiple tolerance-inducing approaches rely on $FOXP3^+$ T_{reg} cells or T_R1 cells. Indeed, more than 25 clinical trials have tested T_{reg} cell-based therapies in T1D, systemic lupus erythematosus, Crohn's disease, organ transplantation and GVHD[120,137-140] (Table 2). These therapies usually involve autologous polyclonal T_{reg} cells isolated from peripheral blood and expanded ex vivo in the presence of IL-2 (ref. 141). T_{reg} cell therapies are well tolerated and T_{reg} cells are stable in vivo. Indeed, in one clinical trial, 25% of ex vivo-expanded autologous polyclonal T_{reg} cells could still be detected 1 year after transfer into patients, pointing to a surprisingly long half-life for these cells[141]. However, although several studies provide early indications of clinical efficacy of T_{reg} cell therapies in phase I and I/II trials, larger clinical trials are still needed[140]. Moreover, concerns regarding nonspecific immunosuppression led to the development of antigen-specific T_{reg} cell therapies.

A further development of T_{reg} cell-based approaches has been the engineering of T_{reg} cells with chimeric antigen receptors (CARs). CAR T_{reg} cells were reported to ameliorate GVHD[142] and other immune-mediated disorders[143], and myelin oligodendrocyte glycoprotein (MOG)-targeting CAR T_{reg} cells homed to the CNS in a mouse model of MS[144]. T_{reg} cells engineered to target pro-inflammatory molecules such as TNF recently showed promising results in a mouse model of GVHD and may be useful when the pathology-driving antigens are not well known or where many antigens are targeted[145]. Similarly, CAR T_{reg} cells targeting B cells suppress antibody responses in a mouse model of haemophilia A[146], pointing to the versatility of engineered T cell therapies. Importantly, CAR T_{reg} cells have been shown to remain tolerogenic in highly pro-inflammatory environments, alleviating concerns about their potential conversion into pathogenic effector T cells[147]. CAR T_{reg} cells were also shown to induce bystander tolerance[147].

The widespread use of cell-based strategies to induce antigen-specific tolerance faces important challenges, particularly related to their patient-specific production in a clinical setup. Strategies based on gene-edited stem cells may overcome some of these challenges

by enabling the production of off-the-shelf universal cell lines for tolerance induction in multiple individuals.

Synthetic particle-based delivery systems

An exciting approach for antigen-specific immunomodulation is the use of nanoparticles. Nanoparticles offer an attractive platform for antigen-specific tolerance induction as they do not rely on patient-derived cells, are made with safe biodegradable materials and can be produced at large scale with little batch-to-batch variation. In addition, nanoparticles can be targeted to specific cells of interest and deliver multiple cargos, while improving small-molecule and antigen solubility and bioavailability. Numerous types of nanoparticles have been used for immunomodulation, including metallic, polymeric, lipid-based and peptide–polymer particles, each with its own advantages and limitations (Fig. 2).

Metallic nanoparticles, including gold, silver and iron oxide particles, have been used for simultaneous diagnostic and therapeutic purposes, for example as contrast-enhancing agents and for the delivery of surface-conjugated cargo[148]. Interestingly, iron oxide nanoparticles conjugated to MHC class II-bound peptides induce T_R1 cells, which in turn induce regulatory B cells and limit inflammation in numerous preclinical mouse models[149]. In this case, T_R1 cell induction depends on the high density of MHC molecules in the nanoparticles, which induces TCR microclusters devoid of co-stimulatory molecules on antigen-specific $CD4^+$ T cells[149,150]. In addition, regulatory B and T cells in the liver induce immunosuppressive neutrophils, limiting liver autoimmunity and fibrosis[151]. Metallic nanoparticles can be modified to improve their performance, but the resulting particles may be unstable. Indeed, surface conjugation can make metallic nanoparticles prone to aggregation during production, limiting the type of loadable cargo and interfering with scale-up efforts[152]. In addition, metal particles are not easily biodegradable and their accumulation in tissues may trigger adverse effects.

Conversely, polymeric particles made from carbohydrate acids, such as poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles, are easily modifiable, relatively simple to manufacture and quickly degraded, although some by-products induce adverse effects[153]. Polymeric particles delivering disease-specific antigens showed therapeutic effects in preclinical autoimmune disease models of MS, rheumatoid arthritis (RA) and T1D mediated by the induction of $CTLA4^+PD1^+$ T_{reg} cells, the reduction of effector T cells and decreased expression of IL-12, microRNA-155 and vascular endothelial growth factor[154-158]. Moreover, phase I and phase IIa clinical trials in coeliac disease showed that PLGA particles encapsulating a gliadin antigen were well tolerated and reduced gliadin-specific IFNγ production and effector memory T cells[159]. However, additional trials are needed to fully evaluate their therapeutic effects. Of note, PLGA particles have shown context-specific anti-inflammatory and pro-inflammatory effects independent of their cargo. Indeed, one of the primary degradation products of PLA and PLGA particles is L-lactate, which inhibits DC maturation and pro-inflammatory responses via HIF1α activation and NF-κB inhibition[115,160]. Conversely, PLGA particles can activate the NBD, LRR and pyrin domain-containing protein 3 (NLRP3) inflammasome in DCs[161] and polarize macrophages towards a pro-inflammatory phenotype[162]. PLGA particles are also reported

to induce effector $CD8^+$ T cell activation and IFNγ production[163] and also act as T_H2 cell adjuvants[164].

Lipid-based nanoparticles are widely used in cosmetics[165], as well as US Food and Drug Administration (FDA)-approved cancer treatments[166] and mRNA coronavirus vaccines[167,168]. Depending on the production method and formulation physicochemical properties, lipid nanoparticles can be classified into various categories, including liposomes, lipid nanoparticles and cubosomes. Owing to the amphipathic nature of fatty acids, lipid nanoparticles can carry hydrophobic molecules intercalated in the membrane and hydrophilic substances in an aqueous core or conjugated to the surface. Furthermore, lipids can be engineered to be easily degraded[169]. Moreover, the incorporation of lipids such as dioleoylphosphatidylethanolamine or cholesterol can modulate the fusogenic properties of nanoliposomes to improve endosomal drug release[170]. Indeed, intracellular cholesterol accumulation can induce DC tolerance via liver X receptor activation[171]. Lipid nanoparticles have been successfully used to deliver autoantigens, with therapeutic effects in numerous preclinical models of T1D, MS, RA and myasthenia gravis linked to the induction of tolerogenic DCs, T_{reg} cell expansion and suppression of pathogenic effector T cells[172-175]. Moreover, in a phase Ib clinical trial in patients with RA, liposomes co-encapsulating a collagen peptide and an NF-κB inhibitor were well tolerated, inducing an increase in circulating collagen-specific $PD1^+$ T cells and a decrease in disease activity[176].

Protein-based nanoparticles offer a biodegradable, nontoxic and stable delivery platform but are rarely used for antigen-specific tolerance induction because of their highly immunogenic nature associated with their structural similarities to virus particles[177].

The physicochemical characteristics of nanoparticles, including size, charge, structure, hydrophobicity and rigidity influence their immunomodulatory effects and can be modified to alter nanoparticle circulation, cell targeting and uptake, and immunomodulatory function to maximize therapeutic activity[153,178,179]. In general, nanoparticle surface charge is an important determinant of cellular uptake and immunomodulation. Nanoparticles with a negative surface charge have been proposed to mimic tolerogenic apoptotic cells[180,181] and be preferentially taken up by phagocytic cells via scavenger receptors such as MARCO in macrophages[182]. Conversely, positively charged nanoparticles are thought to interact directly with negatively charged cell membranes and thus be taken up more rapidly by a wider variety of cell types[183], although this property is also linked to an increased potential to disrupt lipid bilayers and cause cytotoxicity[184]. Positively charged nanoparticles can also promote inflammation via CD80 and CD86 upregulation and the production of reactive oxygen species[185,186]. However, widespread consensus about the effects of particle charge on uptake, toxicity and inflammation is still lacking.

Particle size also influences particle biodistribution, targeting, uptake and toxicity. In general, particles of <200 nm are taken up by DCs and >500 nm by macrophages[187,188]. Indeed, it was suggested that the size of antigens can dictate immune responses, promoting T_H1, T_H2 or T_{reg} cell induction[189]. Moreover, particle size and rigidity affect the immune response, skewing DCs and macrophages towards pro-inflammatory or anti-inflammatory phenotypes[190,191]. Polyethylene glycol is commonly used as a shielding agent to reduce

interactions with serum proteins, decrease uptake by the reticuloendothelial system and increase circulation time and bioavailability. The attachment of polyethylene glycol chains to a protein may also be critical for subcutaneous uptake, reducing complement activation and granulocyte recruitment[192]. Finally, it is important to consider that manufacturing processes used in basic research often differ from those used in FDA-approved therapies. Consequently, charge, size and other features may be altered during nanoparticle production scale up for clinical testing, affecting immunomodulatory activity.

Targeting of specific cell types

Most untargeted nanoparticles are taken up by DCs and macrophages via scavenger receptors and complement factor binding. This passive targeting of DCs generally results in the presentation of nanoparticle-delivered antigens on MHC class II molecules[193]. $CD4^+$ T cell recognition of MHC class II–presented antigens in the absence of co-stimulatory molecules induces clonal T cell deletion and inhibition via PD-L1 and induction of $FOXP3^+$ and $IL\text{-}10^+$ T_{reg} cells[182,194].

Nanoparticles can also be targeted to specific cell types using antibodies or other molecules reactive with specific cell populations (Table 1). For example, mannosylated antigens target the mannose receptor in DCs, inducing IL-10 production and antigen-specific tolerance[195,196]. Mannosylated liposomes encapsulating myelin peptide antigens reduced pro-inflammatory cytokines in blood in a phase I clinical trial in patients with MS[197], but their therapeutic value is still unknown.

An alternative approach is to target nanoparticles based on the antigen specificity of immune receptors in the cells they aim to modulate. For example, metallic nanoparticles displaying peptides loaded in recombinant MHC class I molecules in the absence of signals 2 and 3 induce antigen-specific $CD8^+$ effector T cell anergy and a memory-like regulatory phenotype, which inhibits DCs via IFNγ, IDO and perforin[198]. Thus, targeting nanoparticles to specific immune cells, defined by their surface molecule expression or antigenic reactivity, is an attractive approach for targeted immunotherapy. However, the incorporation of additional components to the therapeutic nanoparticles (for example, surface antibodies) may interfere with their manufacturing.

Introducing immunosuppressive agents into nanoparticles

A major risk of immunomodulation is the potential exacerbation of pathogenic immune responses. Indeed, adverse effects ranging from local reactions to anaphylactic shock and lethality have been documented while testing immunomodulatory approaches[199]; clinical trials have been interrupted because of the induction of hypersensitivity reactions[200] and autoimmune disease relapses[201]. These adverse reactions suggest that safe antigen-specific immunomodulation requires the activation of tolerogenic pathways. This concept is exemplified by a recent report on the evaluation of antigen–MHC class II complexes, which triggered inflammation in one-third of treated mice; this pro-inflammatory effect was abrogated by attaching dexamethasone to the antigen–MHC class II complex at doses 200-fold lower than those used in dexamethasone-alone treatment schemes[193]. Interestingly, self-antigen administration using nanoparticles and nanoliposomes does not seem to trigger

or boost pro-inflammatory responses[102-104,202], suggesting that intrinsic properties make some platforms safer for clinical use. However, therapeutic tolerance induction in the clinic will probably require the activation of anti-inflammatory pathways to improve both safety and efficacy.

One of the first attempts to combine autoantigens and immunomodulatory drugs used liposomes to co-deliver an antigen and an NF-κB inhibitor, ameliorating experimental arthritis in a FOXP3$^+$ T_{reg} cell-dependent manner[203]. Similarly, based on the role of AHR in the suppression of NF-κB signalling and the control of adaptive and innate immunity[204], nanoparticles engineered to co-deliver the AHR agonist 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) with disease-relevant antigens re-established antigen-specific tolerance in preclinical models of MS and T1D[102-104]. Other immunomodulatory agents co-encapsulated with antigens include IL-10 (ref. 205), vitamin D3 (ref. 206) and the mTOR inhibitor rapamycin[202,207-209], with encouraging results in experimental autoimmune encephalomyelitis, allergy and the suppression of antidrug antibodies. Indeed, the co-administration of a disease-relevant antigen with multiple immunomodulators (vitamin D3, GM-CSF or TGFβ) in T1D, RA and MS models showed significant therapeutic effects linked to the induction of IL-10 and PD1, as well as of regulatory T and B cells[210-212].

Human autoimmune diseases usually target multiple autoantigens, which may differ between patients and disease stages, posing a significant challenge to immunomodulatory interventions targeting one or a few antigens or epitopes. However, approaches based on the co-delivery of self-antigens and immunomodulatory agents are reported to induce bystander suppression. Nanoparticle-based co-delivery of antigen and ITE induced bystander tolerance via the induction of FOXP3$^+$ and IL-10$^+$ T_{reg} cells that migrate to the site of inflammation, also suppressing pathology driven by local innate immune responses[104]. Similarly, lipid-coated calcium phosphate nanoparticles loaded with citrullinated autoantigen and rapamycin induced bystander tolerance in an RA model[213], and liposomal co-delivery of vitamin D3 and autoantigen induced bystander tolerance in a T1D model[214]. Collectively, these findings suggest that the co-administration of immunomodulatory molecules with self-antigens is needed not only to boost the therapeutic activity of antigen-specific tolerogenic approaches but also to prevent the unwanted exacerbation of autoimmune pathology particularly associated with some therapeutic modalities.

Nucleic acid–based and viral particle–based approaches to antigen-specific immunotherapy

Nucleic acid–based approaches, including those based on DNA and mRNA, are attractive methods for antigen-specific immunomodulation. These methods offer several advantages over peptide-based or protein-based approaches including the ease of manufacturing and cargo alteration (both antigen and immunomodulator) and the fact that the encoded antigens can be posttranslationally modified in the host and have relatively low production costs[215].

Viral particles provide an effective platform for antigen delivery[216]. Viral particles are used as gene therapy vectors and have been used to deliver autoantigen to the liver[217] and thymus[218], inducing antigen-specific T_{reg} cell expansion, effector T cell suppression and

bystander tolerance epitopes[219]. In response to safety concerns, plant virus particles have also been tested in preclinical models of T1D and RA[220]. However, risks linked to viral gene therapy, pre-existing antibodies against adeno-associated viruses and the induction of antivector antibodies by repeated treatment limit the utility of virus-based approaches for antigen-specific immunomodulation.

Nucleic acid vaccines circumvent some of the risks linked to viral-based approaches. In pioneering work, Waisman et al. used a plasmid encoding the TCR from a pathogenic T cell clone, depleting TCR-specific pathogenic $CD4^+$ T cells and ameliorating disease in a mouse model of MS[221]. Similar encouraging results were obtained with vaccines encoding other antigens in preclinical models of systemic lupus erythematosus, T1D and RA[222-225]. Following these initial findings, DNA vaccines encoding disease-associated antigens were tested in MS and T1D clinical trials[226-228]. An important feature of the DNA vectors used for tolerance induction was the removal of TLR9-activating CpG motifs in the plasmid to minimize the activation of innate immunity. Despite showing reductions in disease-associated biomarkers and evidence of some bystander tolerance, these trials did not meet clinical end points. Thus, although DNA vaccines represent a promising approach and additional clinical trials are ongoing (Table 2), further developments may be needed for the success of this approach, including the co-administration of plasmids encoding tolerogenic immunomodulators[229]. It is also possible that the intrinsic immunostimulatory properties of plasmid DNA in combination with the limited control over its half-life, biodistribution and uptake impose unsurmountable challenges for the clinical use of antigen-encoding DNA vaccines for immunomodulation.

mRNA is less stable than DNA, requiring appropriate delivery platforms and modifications to prevent the activation of innate immunity[230]. Nanoliposomes provide a unique platform for controlled mRNA delivery. In addition, unlike peptide-based vaccines, nanoliposome mRNA vaccines do not need to be extensively optimized to accommodate each nucleic acid–encoded antigen. Moreover, mRNA is quickly degraded in vivo, diminishing concerns about long-term detrimental effects and tumorigenesis previously linked to some DNA-based approaches. Furthermore, mRNA vaccines offer a safer alternative for the treatment of patients who are immunosuppressed than attenuated viral or bacterial vaccines[231].

mRNA is a potent pro-inflammatory adjuvant because of its ability to activate innate immunity via TLR3, TLR7 and other immune receptors involved in sensing viral infection[232]. Consequently, vaccination with mRNA-encoded epitopes induces potent antigen-specific $CD4^+$ and $CD8^+$ effector T cells[233]. mRNA vaccines have been developed to induce protective immunity against pathogens such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[167,168]. Similar exciting results have been described in the context of cancer immunotherapy[234,235].

Eukaryotic RNA is heavily edited, facilitating the discrimination between self and microbial mRNAs. Thus, RNA modification has been actively pursued to minimize the activation of innate immunity and develop tolerogenic vaccines[236]. For example, nanoliposome-delivered mRNA vaccines using pseudo-UTP and encoding the myelin autoantigen MOG suppressed disease development in MS models, inducing bystander tolerance against additional myelin

antigens237. Mechanistically, these therapeutic effects were linked to the PD1- and CTLA4-dependent induction of antigen-specific T_{reg} cells[237]. Of note, mRNA has also been used to transfect T cells with autoantigen-specific CARs, with promising effects in suppressing pathogenic $CD4^+$ and $CD8^+$ effector T cells in the non-obese-diabetes mouse model[238,239]. Together, these findings suggest that vaccines containing mRNA-encoded antigens may provide efficacious platforms for the treatment of inflammatory disorders.

Conclusions, challenges and outlook

The induction of antigen-specific immune tolerance is considered the “holy grail” of disease management for autoimmunity and organ transplantation. Decades of research have resulted in numerous promising advances. Yet despite the encouraging preclinical results, no truly antigen-specific immunotherapies are currently approved for the treatment of autoimmune diseases or organ transplantation, and few approaches have been tested beyond phase I or II clinical trials.

One important challenge is our limited understanding of the breadth of immune targets recognized in autoimmune diseases. Indeed, antigen targets may vary from a single autoantigen in Graves disease[240] to multiple antigens in RA and systemic lupus erythematosus[241]. Epitope spreading remains a significant challenge, suggesting that successful antigen-specific immunotherapy must either halt epitope spreading, incorporate a method for the repeated unbiased evaluation of the specificity of the autoimmune response and/or induce bystander tolerance. In addition, it should be kept in mind that most studies of the therapeutic induction of antigen-specific tolerance assume that the modulation of T cell-mediated autoimmunity results in a concomitant decrease in pathogenic B cell responses. However, it is not clear whether the magnitude, breadth and kinetics of this indirect B cell modulation are enough to result in significant clinical improvement of B cell-driven pathology. Moreover, patient-to-patient variability, stage-specific autoimmune responses and HLA allelic diversity further complicate the design of antigen-specific therapies. Still, significant advances have been made in immune repertoire analysis, including the development of antigen microarrays[241,242], high-throughput BCR and TCR sequencing[243,244], multiplexed monitoring with barcoded tetramers[245] and bioinformatic approaches for epitope prediction[246]. These methods may enable not only the identification of candidate antigens for the induction of antigen-specific tolerance but also the monitoring of response to therapy, providing personalized approaches like those being developed for cancer immunotherapy[234].

An additional challenge is that often immunotherapeutic interventions for autoimmune diseases are initiated after years of subclinical and clinical disease, resulting in the accumulation of tissue damage, immunological memory and the triggering of local mechanisms of inflammation and disease pathology. Thus, although developments in this area have been made for some diseases, such as T1D[247], the identification of effective biomarkers for patient identification and stratification remains an important need for the development of antigen-specific immunotherapy. Indeed, these limitations highlight the challenges of translating exciting findings in preclinical models into efficacious therapies for human diseases. In this context, the selection of autoimmune diseases in which to

test antigen-specific immunomodulatory approaches remains critical. Coeliac disease, for example, offers unique opportunities for clinical trial design, as patients on a gluten-free diet may receive experimental antigen-specific immunotherapies before dietary challenge.

Finally, how can we identify target signalling pathways to increase the therapeutic activity of immunomodulatory approaches while preventing adverse events? Novel platforms may guide the identification of candidate signalling pathways for the therapeutic induction of tolerance, including the use of new methods to study cell–cell interactions involved in the regulation of inflammation[248-250], CRISPR-based platforms to study immune regulation in vivo[251] and the use of experimental systems such as zebrafish in combination with artificial intelligence[252]. These approaches have already identified novel immunoregulatory mechanisms with therapeutic potential. In addition, recently identified populations of tolerogenic APCs may offer additional targets for immune tolerance induction[253-255]. Provided these important challenges are addressed, recent advances in methods for the induction of antigen-specific immune tolerance, combined with novel methods for the identification of target antigens and regulatory pathways, will probably guide the development of platforms for personalized antigen-specific immunomodulation in autoimmune diseases, allergy, transplantation and gene therapy.

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Box 1

Tolerogenic dendritic cells and the induction of peripheral regulatory T cells

Tolerogenic dendritic cells (DCs) expand the peripheral regulatory T cell (T_{reg} cell) compartment through multiple mechanisms. Inhibitory molecules on tolerogenic DCs such as programmed cell death ligand 1 (PD-L1) and PD-L2 engage programmed cell death 1 (PD1) on T cells, boosting the differentiation of $FOXP3^+$ T_{reg} cells through the downregulation of phosphorylated AKT, mTOR, S6 and ERK2 and simultaneous upregulation of the phosphatase PTEN[274]. Additionally, DC expression of inducible T cell co-stimulatory ligand (ICOSL) activates its receptor ICOS on T cells, also promoting the development of $FOXP3^+$ T_{reg} cells and type 1 regulatory T cells (T_R1 cells), although ICOS signalling is also critical for the polarization of T helper 1 and T helper 2 effector cells[53,70]. Finally, binding of the surface receptor B and T lymphocyte attenuator (BTLA) expressed on DCs to herpesvirus entry mediatory (HVEM) on $CD4^+$ T cells is reported to upregulate CD5 and induce FOXP3 expression[275,276].

Several secreted factors released by DCs promote T_{reg} cell differentiation. Transforming growth factor-β (TGFβ) induces $FOXP3^+$ T_{reg} cell differentiation but promotes T helper 17 cell development in the presence of IL-6 or IL-21 (ref. 277). In the presence of TGFβ, IL-10 promotes FOXP3 and cytotoxic T lymphocyte associated protein 4 (CTLA4) expression[278]. IL-10 was also described to induce T_R1 cell differentiation[96,97]. IL-27 is a strong inducer of T_R1 cell differentiation through the induction of MAF, aryl hydrocarbon receptor (AHR) and IL-21 (refs. 59,279,280) and has been shown to control specific transcriptional programmes in $FOXP3^+$ T_{reg} cells[281]. Moreover, IL-27 signalling in DCs and T cells induces the expression of CD39, which degrades extracellular ATP, limiting its pro-inflammatory effects[107]. Besides cytokines, metabolites produced by DCs such as kynurenine, retinoic acid and lactate have important roles in modulating T cell responses. For example, indoleamine 2,3-dioxygenase limits T cell responses via the production of anti-inflammatory tryptophan metabolites such as kynurenine, many of which activate AHR to promote $FOXP3^+$ T_{reg} cell and T_R1 cell differentiation[56]. Retinoic acid promotes the development of $FOXP3^+$ T_{reg} cells and T_R1 cells, enhancing the effects of TGFβ and IL-10 (ref. 112). Finally, lactate produced by DCs can suppress effector T cell differentiation[115].

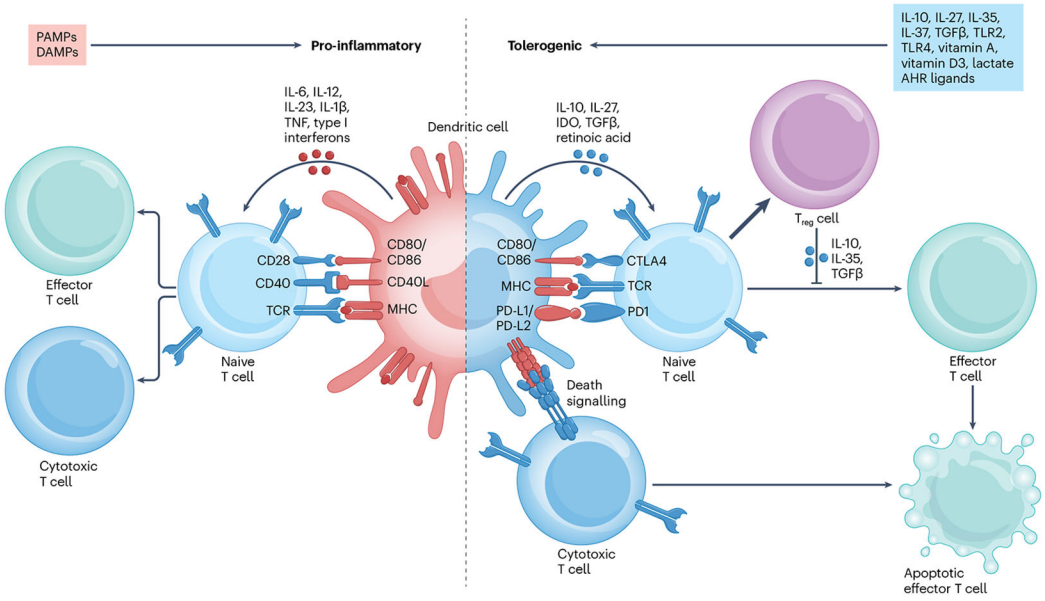

Fig. 1 |. Mechanisms and features in pro-inflammatory dendritic cells compared with tolerogenic dendritic cells.

Pro-inflammatory dendritic cells (DCs) can be induced via activation by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) and upregulate the expression of surface molecules including MHC molecules, CD80 and CD86. These surface molecules, in addition to secreted pro-inflammatory cytokines, such as IL-1β, IL-6, IL-12, IL-23, tumour necrosis factor (TNF) and type I interferons, induce the differentiation of cytotoxic and effector T cells from naive T cells. Conversely, tolerogenic DCs can be induced via several mechanisms, including exposure to cytokines such as IL-10, IL-27, IL-35, IL-37 or transforming growth factor-β (TGFβ); signalling via Toll-like receptor 2 (TLR2), TLR4 or aryl hydrocarbon receptor (AHR); or exposure to molecules such as vitamin D3, vitamin A or lactate. Tolerogenic DCs express lower levels of MHC molecules, CD80 and CD86 and secrete anti-inflammatory cytokines and molecules such as IL-10, TGFβ, IL-27, indoleamine 2,3-dioxygenase (IDO) and retinoic acid. Tolerogenic DC interactions with T cells induce the differentiation and expansion of anti-inflammatory regulatory T cells (T_{reg} cells) from naive T cells and the apoptosis of cytotoxic T cells through death receptor signalling interactions, such as between programmed cell death 1 (PD1) and PD1 ligand 1 (PD-L1) or PD-L2. CTLA4, cytotoxic T lymphocyte associated protein 4; TCR, T cell receptor.

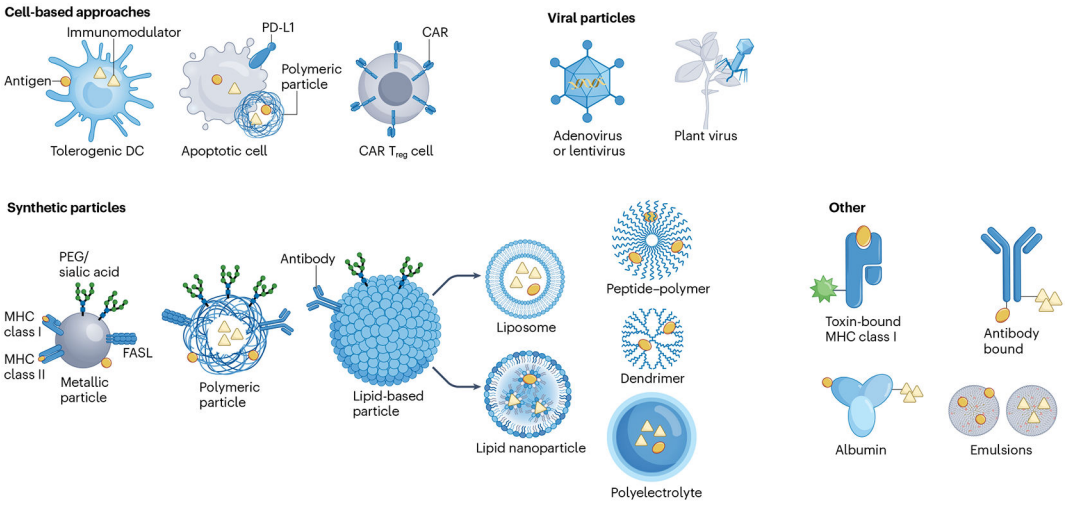

Fig. 2 |. Approaches for the induction of antigen-specific immune tolerance.

Cell-based approaches include the ex vivo induction of tolerogenic dendritic cells (DCs), apoptotic cells or regulatory T cells engineered to express chimeric antigen receptors (CAR T_{reg} cells), all of which can be designed to deliver antigen with or without an immunomodulatory signal. Viral particle approaches include the delivery of DNA-encoded or RNA-encoded antigen via adenoviruses, lentiviruses or plant viruses. Synthetic particles, including metallic, polymeric, lipid-based (including liposomes or lipid nanoparticles), peptide–polymer, dendrimer or polyelectrolyte particles, can be designed to co-deliver antigens, antibodies and immunomodulators, in various combinations. Alternatively, antigens can be delivered via toxin-bound MHC molecules to induce the death of antigen-specific cells, and albumin, antibodies or nanoemulsions can deliver antigens and immunomodulators to induce antigen-specific immune tolerance. FASL, FAS ligand; PEG, polyethylene glycol.

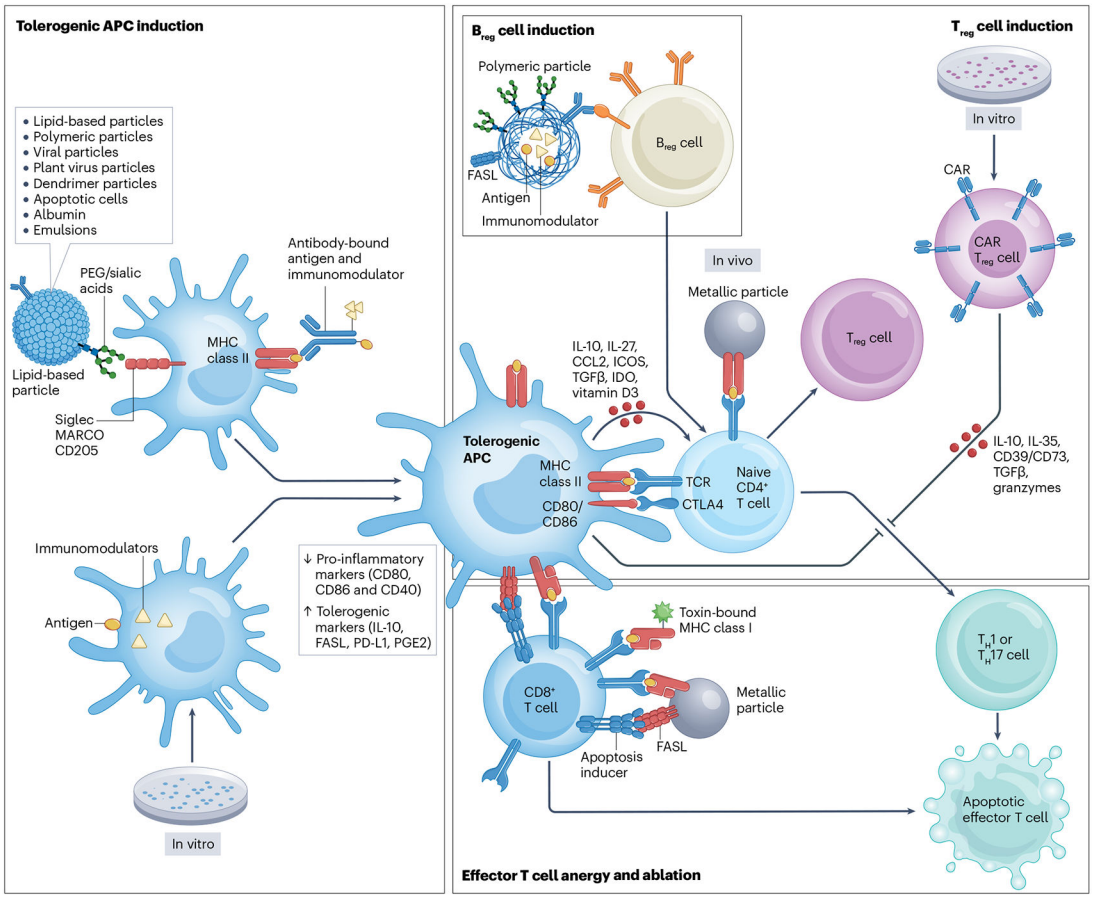

Fig. 3 |. Mechanisms for the induction of antigen-specific immune tolerance.

Tolerogenic antigen-specific antigen-presenting cells (APCs) can be induced in vivo through the delivery of synthetic particles, viral particles or cell-based approaches, or induced in vitro and engineered to express disease-specific antigens and an immunomodulatory signal. Tolerogenic APCs are characterized by reduced expression of pro-inflammatory markers including CD80, CD86 and CD40 and an increased expression or production of tolerogenic molecules such as IL-10, FAS ligand (FASL), programmed cell death ligand 1 (PD-L1) and prostaglandin E_2 (PGE2). Tolerogenic APCs can in turn induce naive $CD4^+$ T cells to differentiate into regulatory T (T_{reg}) cells or can induce effector T cell anergy and ablation. Similarly, the induction of regulatory B (B_{reg}) cells via synthetic particle administration or T_{reg} cells via in vivo delivery of particles or in vitro engineering of chimeric antigen receptor (CAR) T_{reg} cells results in the reduction of effector T cells by the induction of anergy or cell death. PEG, polyethylene glycol.

Table 1 |

Antigen-specific immunotherapy approaches

Material	Mediator of antigen specificity	Immunomodulator	Disease model	Findings	Refs.
Cell-based approaches					
IL-10-moDCs	Lentiviral vectors		NOD	Induction of antigen-specific T cell tolerance and prevention of disease	122
Schwann cells+ PEG–PLGA NPs	Natural	PD-L1–Fc, CD86–Fc, leflunomide	EAE	Inhibition of T_H1 cell responses, leflunomide loading increased myelin repair	256
Erythrocytes	Peptide mimotope		NOD	Reduced trafficking of effector T cells into organs	136
Erythrocytes	Recombinant antigen		EAE	Persistent antigen exposure via erythrocytes induced T cell exhaustion and dysfunction	135
BAR T_{reg} cells	Retroviral		ADAs	ADA suppression	146
CAR T_{reg} cells	Retroviral		EAE	Suppression of antigen-specific and other T cells even in a pro-inflammatory environment	147
CAR T_{reg} cells	Plasmid		Allograft rejection	Protection of allografts better than polyclonal Treg cells	142
CAR/FOXP3$^+$ T_{reg} cells	CAR		EAE	Intranasally administered cells accessed various brain regions and suppressed disease	144
Vitamin D3–IL-4–IL-10–GM-CSF moDCs	None	CTLA4–Ig	Alloimmune reactivity	Only in addition with CTLA4–Ig: alloreactive T_{reg} cell induction and reduction in T cell activity	126
Apoptotic cells	Peptide	CFA	Arthritis	Induction of antigen-specific tolerance via B cell-mediated T_{reg} cell induction	134
Viral-based approaches					
Cowpea mosaic virus	Plasmid		EAE	Safe and efficient gene delivery	220
Adeno-associated virus	Plasmid		EAE	Liver-targeted expression of protein induced T_{reg} cells, regardless of epitope or HLA background	219
Lentivirus	Plasmid		NOD	Transient expression of antigen with integrase-incompetent lentivirus protected from disease	217
Nanoparticles without adjuvant					
PLGA NPs			EAE, DTH	Tolerance dependent on dose and antigen loading	157
PLGA NPs	Multiple proteins		NOD	Encapsulation of multiple epitopes broadened spectrum of induced tolerance	257
PEMA-PLGA NPs	Protein		EAE	Kupffer cells and LSECs induced tolerance after antigen uptake	194
Liposome	mRNA		EAE	Bystander tolerance by induction of T_{reg} cells	237
Modified PLGA NPs	Peptide		EAE, NOD, colitis	Optimized NPs can be effectively loaded with a variety of antigens	258

Material	Mediator of antigen specificity	Immunomodulator	Disease model	Findings	Refs.
PEG–PLGA NPs	Peptide		EAE	PEGylation increased bioavailability of subcutaneously injected NPs	192
Iron oxide NPs	MHC class II-bound ubiquitous antigen		EAE	Ubiquitous liver autoantigens are involved in extrahepatic immune diseases and tolerance induction mitigates extrahepatic autoimmunity	259
PEGylated iron oxide NPs	MHC class II-bound peptide		PBC, AIH, PSC	Ubiquitous antigens are involved in autoimmune liver diseases	260
PLGA NPs	Hybrid peptide		NOD	Effector T cell anergy induced against several epitopes	154
PLGA NPs	Peptides		NOD	Antigen-coupled NPs induced T_{reg} cells	155
DSPG liposomes	Peptide		Atherosclerosis	C1q-dependent update via scavenger receptors induced tolerance	173
PLGA NPs	Peptide		R-EAE	Uptake of antigen by APCs led to PD-L1-dependent tolerance induction	182
CdSe-ZnS-quantum dots	Protein		EAE	Antigen density dictates disease suppression	158
Dextran-coated or PEGylated iron oxide	MHC class II-bound peptide		EAE, NOD	Expansion of T_R1 cells	149
PSL NPs	None		Atherosclerosis	Apoptotic cell mimicry induced IgM and reduced inflammation	181
PLGA-PEMA NPs	Protein		R-EAE	Surfactant modification increased efficacy of NPs, reduced CNS infiltration of effector T cells	156
PEI NPs	Plasmid		Arthritis	Reduction of TLR9 activation by DNA promoted IDO-mediated induction of tolerance	261
Iron oxide NPs	MHC class I-bound peptide		NOD	Induction of T_{reg} cells that suppressed APCs via IDO and perforin	198
Dendrimer branched lysine core particles	Multiple antigen peptides		EAE	Non-inflammatory presentation of antigen decreased effector T cell but not T_{reg} cell CNS infiltration	262
Peptide–polymer	Peptide		Cholangitis	LSECs presented antigen on MHC class I, decreasing liver infiltration of antigen-specific $CD8^+$ T cells	263
Particles with adjuvant					
PLGA NPs	Peptide	ICAM1 inhibitor	EAE	Dual-peptide NPs have stronger inhibitory effect	264
Dual size PLGA	Protein	GM-CSF, TGFβ, vitamin D3	NOD	Long-lasting protection in advanced disease states	210,211
Liposomes/serum-albumin–bound NPs	Protein	T_{reg} cell epitopes	NOD	Tregitopes induced tolerance via specific T_{reg} cell activation	265
Lipid-coated salt NPs	Citrullinated peptides	Rapamycin	RA	Induction of immune tolerance in advanced disease	213
PEG–PLGA NPs	Peptide	Knockdown of CD40, CD80, CD86	NOD	Expansion of antigen-specific T_{reg} cells	266
Liposomes	Mimotope	Vitamin D3	NOD	Co-encapsulation of vitamin D3 can mediate bystander tolerance	214

Material	Mediator of antigen specificity	Immunomodulator	Disease model	Findings	Refs.
Liposomes	Peptide	ITE	EAE	Co-encapsulation of immunomodulator induced T_{reg} cells and bystander tolerance	104
Liposomes	Peptide	Vitamin D3	RA, Goodpasture's vasculitis	Calcitriol-antigen loaded NPs increased T_{reg} cells and suppressed effector T cells in a PD-L1-dependent manner	206
Liposomes	Peptide	CD22 ligands, rapamycin	Hypersensitivity	Rapamycin enhanced tolerance induction in naive but not in presensitized mice	208
PEG–Gold NPs	Peptide	ITE	EAE, NOD	Co-encapsulation of immunomodulators expanded T_{reg} cells and increased efficacy	102,103
PEG–PLA NPs, PLGA NPs	Peptides/drug	Rapamycin	EAE, DTH, ADAs	Co-encapsulation of rapamycin induced durable B and T cell tolerance	202
Dextran NPs	Peptide	Dexamethasone	EAE	Immunomodulator increased effectiveness	267
PLGA NPs	Peptide	Recombinant IL-10	EAE	NPs release antigen and immunomodulator constantly for several weeks, addition of IL-10 decreased IL-17 and IFNγ	205
PEG–liposomes	Multivalent peptide	CD22 ligands	ADAs	Tolerance induction towards presented alloantigen	268
Liposomes	Protein	NF-κB inhibitors	RA	Co-encapsulation of immunomodulators increased efficacy of tolerance induction	203
Particle free					
Anti-MHC class II antibodies	Peptide	Dexamethasone	EAE, NOD, RA	Immunomodulator reduced adverse effects and increased effectiveness	207
Nanoemulsion	Citrullinated self-antigen	Rapamycin	RA	Nanoemulsion accumulated in inflamed regions and suppressed disease activity	207
Mannosylated antigen	Peptide		EAE, R-EAE	Amelioration of EAE, reduced CNS infiltration of immune cells	196

ADA, antidrug antibody; AIH, autoimmune hepatitis; APC, antigen-presenting cell; BAR, B cell-targeting antibody receptor; CAR, chimeric antigen receptor; CFA, complete Freunds adjuvant; CNS, central nervous system; CTLA4, cytotoxic T lymphocyte associated protein 4; DC, dendritic cell; DSPG, 1,2-distearoyl-sn-glycero-3-phosphoglycerol; DTH, delayed-type hypersensitivity; EAE, experimental autoimmune encephalitis; ICAM1, intercellular adhesion molecule 1; IFNγ, interferon-γ; GM-CSF, granulocyte-monocyte colony-stimulating factor; IDO, indoleamine-pyrrole 2,3-dioxygenase; LSECs, liver sinusoidal endothelial cells; moDC, monocyte-derived dendritic cell; NF-κB, nuclear factor-κB; NOD, non-obese diabetes; NP, nanoparticle; PBC, primary biliary cholangitis; PD-L1, programmed cell death ligand 1; PEG, polyethylene glycol; PEI, polyethyleneimine; PEMA, poly(ethylene-maleic acid); PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PSC, primary sclerosing cholangitis; PSL, phosphatidyl serine liposome; RA, rheumatoid arthritis; R-EAE, relapsing–remitting EAE; TGFβ, transforming growth factor-β.

Table 2 |

Antigen-specific immunotherapy approaches in phase I or II clinical trials

Study type	Vehicle or reagent	Major findings	Clinical trial ID
Cell-based approaches			
Neuroinflammation			
Tolerogenic fibroblasts	Tolerogenic fibroblasts	No significant safety concerns or side effects up to 16 weeks after infusion	NCT05080270
Tolerogenic DCs (TOLERVIT-MS)	Vitamin D3-moDCs + IFNβ	Interim report: no safety concerns	NCT02903537
"Negative" DC vaccine (MS-tolDC)	Vitamin D3-moDCs	Interim report: no safety concerns	NCT02618902
Regulatory DCs (TolDec-EM-NMO)	Dexamethasone–GM-CSF–IL-4-moDCs with seven peptides from MBP, MOG, PLP, AQP4	Safe; induction of T_R1 cells and decreased $CD8^+$ T cells, NK cells, $CD14^+CD56^+$ cells	NCT02283671
Peptide-coupled PBMCs (ETIMS)	Autologous PBMCs coupled with seven peptides	No adverse effects, reduced antigen-specific T cell response, no effects on immunoglobulins or recall antibody effect	NCT01414634
Mesenchymal stem cells (MSCIMS)	Mesenchymal stem cells	Safe; neuroprotective; met some secondary visual end points	NCT00395200
T cell vaccine	Irradiated MBP-reactive T cells against nine epitopes	Reduction in EDSS, walking time and relapses	NCT01448252
T cell vaccine	Irradiated MBP-reactive T cells	Depletion of autoreactive T cells; smaller lesion volume	269
Rheumatoid arthritis			
Autologous tolerogenic DCs (AutoDECRA)	Dexamethasone–vitamin D-moDCs with synovia	Safe, but no systemic effects detected	NCT01352858
Autologous tolerogenic DCs (TolDCfoRA)	Dexamethasone-moDCs	Well tolerated	NCT03337165
DC vaccine to suppress the immune response to citrullinated antigen (BAY11-7082; Rheumavax)	NF-κB-inhibitor-moDCs	Safe; fewer effector T cells and decreased pro-inflammatory cytokines	ACTRN12610000373077
Autologous DCs (CreaVax-RA)	moDCs pulsed with PAD4, citrullinated vimentin and fillagrin. HNRPA2/B1	Safe; reduction in autoantibody and IFNγ-producing T cells	CRiSKCT0000035
Inflammatory bowel disease			
Intralesional tolerogenic DCs (TolDecCDintra)	Dexamethasone-moDCs	Terminated (low recruitment)	NCT02622763
Antigen-specific T_{reg} cell therapy	T_{reg} cells with ovalbumin	No adverse effects; symptom reduction	2006-004712-44
Autologous tolerogenic DCs	Dexamethasone–vitamin D-moDCs	Mixed clinical response	2007-003469-42
Type 1 diabetes			
Immunotherapy vaccine (PIpepTolDC)	Autologous tolerogenic DCs loaded with proinsulin peptides	Ongoing	NCT04590872
Autologous tolerogenic DCs	Autologous moDCs primed with peptides	Ongoing	NCT05207995
AVT001	Autologous moDCs	Ongoing	NCT03895996

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Study type	Vehicle or reagent	Major findings	Clinical trial ID
Tolerogenic DCs (D-Sense)	Vitamin D3-moDCs with proinsulin	Not published	NTR5542
Polyclonal T_{reg} cells + IL-2 (TILT)	Polyclonal T_{reg} cells	No adverse effects but poor T_{reg} cell survival	NCT02772679
Tolerogenic DCs	Dexamethasone-vitamin D3-moDCs pulsed with islet antigen	Long-lasting $CD4^+$ T cell tolerance and temporary bystander tolerance	2013-005476-18
Autologous immunoregulatory DCs	Anti-CD40/CD80/CD8-moDCs with six ODNs	Not published	NCT02354911
$CD4^+CD127^{low}CD25^+$ polyclonal Treg cells	Polyclonal T_{reg} cells + IL-2	T_{reg} cell survival but also expansion of cytotoxic T cells	NCT01210664
Autologous DCs	Antisense CD40, CD80, CD86 ODNs-moDCs	Safe; induction of B220+CD11c+ B cells	NCT00445913
Treg cells	Autologous ex vivo-expanded T_{reg} cells	No adverse effects; elevated C-peptide levels and lower insulin dependence after 1 year	ISRCTN06128462
Systemic lupus erythematosus			
Autologous polyclonal Treg cells	Treg cells	Increased Treg cells in inflamed tissue	NCT02428309
Graft rejection after kidney transplantation			
DCreg	Donor-derived tolerogenic DCs	Ongoing	NCT03164265
Donor alloantigen reactive T_{reg} cells (ARTEMIS)	Donor alloantigen reactive T_{reg} cells	No adverse effects	NCT02474199
Autologous tolerogenic DCs (ONEatDC)	GM-CSF-moDCs	No adverse effects, fewer infections	NCT02252055
Regulatory macrophages (ONEmreg12)	Donor-derived regulatory macrophages induced with GM-CSF + IFNγ	No adverse effects, fewer infections	NCT02085629
Donor alloantigen-reactive T_{reg} cells (The ONE Study) (DART)	Donor-alloantigen-reactive T_{reg} cells with donor antigen	No adverse effects, fewer infections	NCT02244801
Natural T_{reg} cells (ONEnTreg13)	$CD4^+CD25^+FOXP3^+$ T_{reg} cells	No adverse effects, fewer infections	NCT02371434
T_{reg} cells (ONETreg1)	$CD4^+CD25^+FOXP3^+$ T_{reg} cells	No adverse effects, fewer infections	NCT02129881
T_{reg} cells (The ONE Study)	T_{reg} cells induced with belatacept ex vivo with donor antigen	No adverse effects, fewer infections	NCT02091232
Graft-versus-host disease			
Ex vivo expanded donor T_{reg} cells	Donor T_{reg} cells cultured with recipient DCs	Not published	NCT01795573
T cell-depleted graft with simultaneous infusion T cells	Conventional T cells and T_{reg} cells in predefined ratio	Safe, no adverse effects	NCT01660607
Treg cells	Induced T_{reg} cells	No adverse effects	NCT01634217
Treg cells	Induced T_{reg} cells	Safe, low risk of acute GVHD	NCT00602693
Free antigen approaches			
Multiple sclerosis			

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Study type	Vehicle or reagent	Major findings	Clinical trial ID
ATX-MS-1467	Synthetic peptides of four epitopes of MBP	Study 1 showed temporary lesion reduction; study 2 showed lesion reduction up to 48 weeks with higher doses	NCT01973491
Myelin peptides	Transdermal peptide mix	Reduction of gadolinium-enhancing lesions	270
Myelin peptides	Transdermal peptide mix	Induction of T_R1 cells but not $FOXP3^+$ T_{reg} cells	271
MBP8298 (MAESTRO-03)	MBP8298	No difference to placebo	NCT00468611
BHT-3009	MOG-DNA	Fewer lesions and reduction of a spectrum of antibodies	NCT00382629
Glatiramer acetate	Random peptides resembling MBP	Reduced number of lesions	272
Type 1 diabetes			
TOL-3021 in new onset disease (DAWN)	DNA-plasmid encoding proinsulin	Ongoing	NCT03794973
TOL-3021 in established disease (DAY)	DNA-plasmid encoding proinsulin	Ongoing	NCT03794960
Proinsulin peptide (MonoPepT1De)	Proinsulin	No adverse effects, reduced insulin use increase, FOXP3 and IL-10 induction	NCT01536431
BHT-3021	DNA-plasmid encoding proinsulin	No adverse effects, C-peptide level increased but no effect on insulin requirement	NCT00453375
DPT-1	Insulin, GAD65	No effect on prevention of familial disease development	
Rheumatoid arthritis			
Chicken type II collagen	Chicken type II collagen	Improvement of symptoms, albeit less than methotrexate-treated control group	ChiCTR-TRC-00000093
Altered peptide ligand approaches			
Multiple sclerosis			
RTL1000	TCR ligand	No adverse effects at <100 mg; no worsening of disease	NCT00411723
NBI-5788	Altered peptide ligand	Suspended after detection of increased brain lesions in some patients	NCT00079495
CGP77116	Altered peptide ligand	Aborted owing to exacerbation of disease in three patients	NCT00001781
NBI-5788	Altered peptide ligand	Hypersensitivity reactions, reduced lesion load	200
Type 1 diabetes			
NBI-6024	Altered peptide ligand	No effect compared with placebo	NCT00873561
Antigen and adjuvant approaches			
Multiple sclerosis			
NeuroVax	TCR peptides in IFA	Ongoing	NCT02057159
Type 1 diabetes			

Study type	Vehicle or reagent	Major findings	Clinical trial ID
Diamyd (DIAGNODE-3)	rhGAD65–Alum+vitamin D HLA-DR3-DQ2^{+} haplotype	Ongoing	NCT05018585
Diamyd booster	Booster Diamyd+vitamin D3	Ongoing	NCT05351879
MER3101: MAS-1 adjuvanted (MER3101)	β-chain + MER3101 administered into lymph nodes	Ongoing	NCT03624062
Diamyd (DIAGNODE-2)	rhGAD65–alum+vitamin D3 administered into lymph nodes	No significant improvement in overall groups but increased glycaemic control in HLA-DR3-DQ^{+} subjects	NCT03345004
Diamyd (DIAGNODE-1)	rhGAD65–alum+vitamin D	No adverse effects; T_H2 cell induction; T_H1 cell reduction; C-peptide baseline increased	NCT02352974
Islet β-chain	IFA–β-chain emulsion	Insulin-specific antibody and T cell induction; induction of long-lasting antigen-specific T_{reg} cells, no HBA1C or insulin use changes	NCT00057499
Diamyd in newly diagnosed disease (DIAPREVENT)	rhGAD65–alum	No change in C-peptide levels	NCT00751842
Diamyd in newly diagnosed disease (DIAPREVENT)	GAD–alum	No adverse effects; no difference in insulin secretion	NCT00529399
Nanoparticle-based approaches			
Multiple sclerosis			
Xemys	CD206-targeted liposomes with three MBP peptides	Cytokine normalization	#930 [FASEMS-01/01]
Rheumatoid arthritis			
DEN-181	Liposomes with collagen peptide + NF-κB inhibitor	Reduced effector T cells, increased T_{reg} cells	176
AG4263	Iron particles coated with peptide–MHC class II-bound antigen	No long-lasting effects	273
Coeliac disease			
TIMP-GLIA	PLGA nanoparticles with gliadin TAK-101	Reduction of IFNγ-producing cells on challenges	NCT03738475

AQP4, aquaporin 4; DC, dendritic cell; EDSS, expanded disability status scale; GAD, glutamic acid decarboxylase; GVHD, graft-versus-host disease; GM-CSF, granulocyte-monocyte colony-stimulating factor; IFA, incomplete Freunds adjuvant; IFNβ, interferon-β; IFNγ, interferon-γ; MBP, myelin basic protein; moDC, monocyte-derived dendritic cell; MOG, myelin oligodendrocyte glycoprotein; NF-κB, nuclear factor-κB; ODNs, oligodeoxynucleotides; PAD4, peptidylarginine deiminase 4; PLGA, poly(lactic-*co*-glycolic acid); PLP, proteolipid protein; TCR, T cell receptor; T_{reg} cell, regulatory T cell.