



Nanoparticle-based autoimmune disease therapy



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Abstract

The goal of immunotherapy against autoimmunity is to block pathogenic inflammation without impairing immunity against infections and tumours. Regulatory T-cells (Tregs) play a central role in maintaining immune homeostasis, and autoimmune inflammation is frequently associated with decreased numbers and/or function of these T-cells. Therapies harnessing Tregs to treat autoimmune inflammation remain under-developed with caveats ranging from the lack of antigenic and disease specificity to the potential phenotypic and functional instability of *in vitro*-expanded Treg cells *in vivo*. Here, we review nanotechnology-based approaches designed to promote immune tolerance through various mechanisms, ranging from systemic or local suppression of antigen-presenting cells and deletion of antigen-specific T-cells, to the systemic expansion of antigen- and disease-specific Treg cells *in vivo*.

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1. Introduction

Autoimmune diseases result from an attack of the immune system against specific tissues, compromising their structural and/or functional integrity. In most, if not all autoimmune diseases, autoimmune inflammation is orchestrated by cognate

interactions between antigen presenting cells (APCs) and autoantigen-specific T-cells [1]. Whereas recognition of self peptide-MHC (pMHC) complexes displayed by APCs in steady state (non-inflammatory) conditions tends to promote T-cell tolerance, engagement of these complexes by autoreactive T-cells in the context of inflammation tends to promote T-cell activation [2–5]. Consequently, It is not surprising that many therapeutic approaches against autoimmunity aim to promote tolerance by targeting the APC-T-cell interaction.

In general, therapies designed to induce antigen-specific tolerance include the targeting of relevant autoreactive T- and B-cell specificities via their antigen receptors. In this context, the term 'tolerance' has been used to describe a broad range of outcomes, including clonal deletion, anergy [6,7], changes in cytokine profile [8], and induction of

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regulatory T- and B-cells [9,10]. Altered peptide ligands (APLs, amino acid-substituted variants of autoantigenic peptides) [11] have been used since the early 1990s to induce antigen-specific tolerance in animal models of autoimmune disease. APLs typically function as partial agonists or antagonists of key T-cell specificities and therefore exploit the specificity of the TCR–pMHC interaction to modulate the function of those autoreactive T-cells thought to play a critical role in disease initiation and/or progression. Certain APLs induce T-cell anergy [12] or clonal deletion [13], whereas others trigger immune deviation [14–16]. In some cases, APL immunotherapy inhibited autoimmune inflammation, but in others, it accelerated disease onset [17] or triggered life-threatening anaphylactic responses [18]. The immune response to a specific APL can hardly be predicted [19]. This caveat, coupled with the antigenic complexity of most autoimmune diseases and the need to target multiple autoreactive T-cell specificities simultaneously, represents important limitations of traditional antigen-specific approaches for the treatment of autoimmune disease.

Here, we review nanotechnology-based therapeutic avenues that have sought to improve the outcomes of traditional antigen-specific therapies. Both micro- and nanoparticles have been used as vehicles for drug and/or antigen delivery to phagocytes as well as direct T-cell-targeting compounds. In some designs, the physical and chemical properties of the particles contribute to the pharmacokinetic and toxicological behaviour of the compound but not to its tolerogenic activity; in others, the nanoparticle is an integral part of the active product.

2. Harnessing the physical and chemical properties of nanomaterials for cellular targeting

Biologically relevant nanomaterials, usually nanoparticles, are defined as structures smaller than 100 nm in diameter with physical and/or chemical properties that have the potential to alter biological processes driven by specific cell types [20]. Physical and chemical parameters such as nanoparticle size, shape, and surface chemistry impact on the biodistribution, cellular tropism and toxicity of nanomaterials [21–23]. These parameters must therefore be fine-tuned to maximize its desired biological and therapeutic activities and limit the off-target toxicity of individual designs. For example, particles larger than 500 nm in diameter tend to accumulate in the spleen, and size is inversely proportional to the speed with which nanoparticles reach the lymph nodes upon intravenous delivery [22].

In the context of autoimmunity, cellular toxicity associated with certain nanomaterials can be exploited to kill or inhibit pathogenic cell types (selective block-by-toxicity). For example, inhaled multi-walled carbon nanotubes promote systemic immunosuppression without causing significant lung damage, through a mechanism dependent on the secretion of tumor growth factor-beta ($\text{TGF-}\beta$), prostaglandins, and IL-10 [24–26]. Likewise, inhaled carbon nanotubes can inhibit lung-resident dendritic cells (DCs) and promote lung immunosuppression [25]. The importance of nanoparticle chemistry on cellular tropism and biological activity is further highlighted by the description of nanotube composites for cancer immunotherapy that promote T-cell activation, rather than immunosuppression

[27]. Other nanomaterials, like citrate-coated gold [28,29] and cerium oxide nanoparticles [30–32], have been shown to have anti-oxidative and anti-inflammatory properties with limited organ toxicity.

Nanomaterials with the desired cellular tropism can be further engineered to preferentially accumulate into (or deliver cargos to) specific subcellular compartments of immune cell types, such as by using surface chemistries that promote cell entry but inhibit nuclear translocation (e.g., citrate-stabilization of gold nanomaterials) or that promote accumulation in the nucleus and mitochondria [33].

Shape and size also affect the organ and cellular tropism of nanomaterials [34] (Fig. 1). Some shapes promote and others inhibit phagocytosis by macrophages. For example, whereas sphere-shaped (vs. anisotropic) nanomaterials are easily internalized by macrophages, disk-shaped structures are not, despite readily binding to their surface. This property could be harnessed to promote the transport of the nanomaterial by macrophages to other locations like tumors or lymphoid organs [35]. In addition to shape, the size of the nanomaterial also influences its cellular tropism, such as by facilitating or inhibiting actin polymerization and wrapping of the cell membrane around the nanomaterial. Functionalization with ‘don’t eat me’ ligands [36] (like CD47) is another strategy that could be used, for instance, to inhibit the accumulation (and dumping) of certain nanomaterials into macrophage-rich organs like spleen and liver.

More complex nanoparticle designs involve the functionalization of nanomaterials with specific ligands and/or the loading of their cargo compartments with immunoregulatory molecules. By narrowing the bioavailability of these molecules to cell types capable of recognizing and internalizing these compounds, these structures have the potential to enhance the desired biological activity and limit the off-target toxicity of anti-inflammatory drugs. These nanoparticle designs typically involve the use of chemically inert materials, to reduce unwanted chemical reactivity between the nanomaterial and the anti-inflammatory cargo or components of the target cell. For example, superparamagnetic iron oxide nanoparticles (Fe_3O_4 or Fe_2O_3 SPIONs) [37], which can be rendered soluble in biological fluids by coating their surface with dextran starch or polyethylene glycol, meet these requirements. SPIONs are highly biocompatible and biodegradable; the iron core is recycled into soluble ferritin iron or hemosiderin [38]. In fact, SPIONs have been approved for clinical use in the form of Ferumoxides (dextran SPION), Ferucarbotran (carboxydextran SPION), and Ferumoxtran-10 (dextran SPION) [39,40].

The development of nanoparticles with surfaces having several different physical and chemical properties—also known as Janus nanoparticles [41]—enable the production of multi-functional compounds, hence the simultaneous modulation of various processes in target cells.

3. Nanoparticles as vehicles for delivery of anti-inflammatory compounds to phagocytes

Nanomaterials have been engineered to deliver cargos to APCs to modulate antigen presentation and downregulate innate immune signals, which collectively promote activation of adaptive autoimmune responses. Some compounds

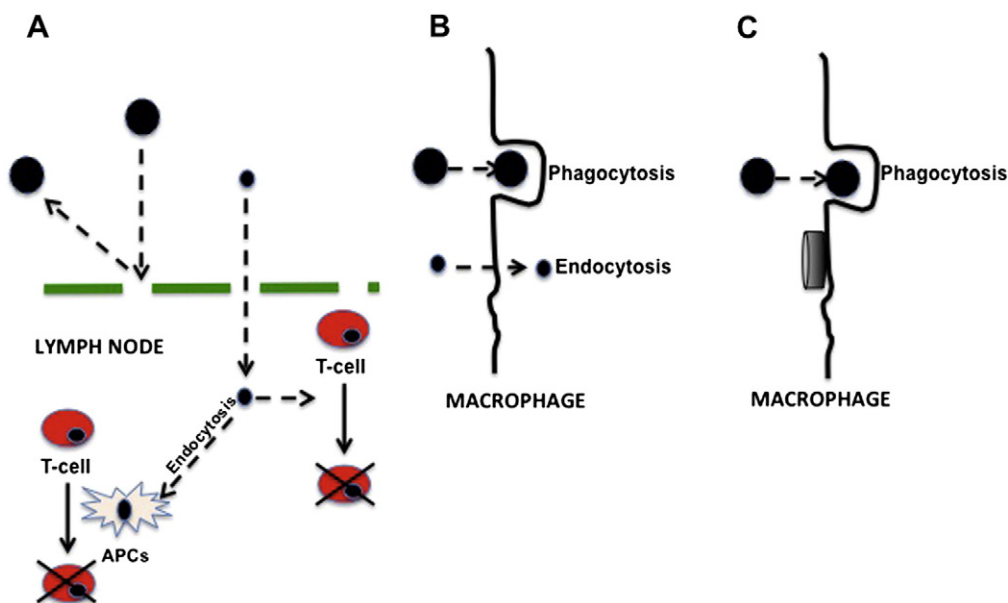


Figure 1 Particle shape and size influence the biodistribution and macrophage-targeting properties of nanomedicines. (A) Small nanoparticles can more easily traffic to lymph nodes as compared to their larger counterparts. (B) Size also influences the rate of particle uptake by phagocytes; larger structures trigger the phagocytic machinery more efficiently than their smaller versions. (C) Particle shape is another factor impacting on phagocytic activity. Disc-shaped structures, for example, inhibit phagocytic activity, favoring their transport to other anatomical locations.

are designed to deliver anti-inflammatory cargos to target tissues, leading to reductions in the systemic therapeutic dose and side effects. This is exemplified with the proposed use of polymeric polylactide nanoparticles [42] loaded with cyclosporine A, which afford decreased toxicity and promote increased uptake of the anti-inflammatory drug by DCs. This strategy also promotes sustained release of the immunosuppressant, a phenomenon that is associated with slow degradation of the hydrophobic polymer-cyclosporin A co-precipitates. Liposomes have been used for similar purposes. For example, glucocorticoid-loaded liposomes afforded a measurable therapeutic effect against experimental autoimmune encephalomyelitis (EAE) via effects on macrophages at significantly lower doses than conventional glucocorticoid therapy [43]. Of note, this approach changes the pharmacodynamics of the liposome-encapsulated glucocorticoids, promoting immunosuppression of macrophages with marginal effects on T-cells, a primary target in traditional systemic glucocorticoid treatment.

Other approaches involve the use of nanomaterials functionalized with cell-targeting ligands. For example, dendrimers covalently bound to folic acid (FA) were used to target activated macrophages (expressing folate receptor beta) in a type II collagen-induced model of arthritis in rats [44]. These FA-coated-dendrimers were loaded with methotrexate to promote arthritis amelioration by targeting activated macrophages. Likewise, azabisphosphonate (ABP)-capped dendrimers [45] were used to induce an anti-inflammatory phenotype in monocytes in two murine models of rheumatoid arthritis. These ligand-based cell-specific delivery approaches are not restricted to phagocytic cells. For example, antigenic liposomes expressing ligands for the B-cell inhibitory receptor CD22 can promote antigen specific B-cell depletion [46].

The chemistry of the nanomaterial itself and the route of administration can also be harnessed to promote its accumulation in certain tissues [47]. Nanoparticles delivered intravenously, for example, preferentially accumulate in the spleen, bone marrow, and liver. Interestingly, peptide-loaded microparticles used to promote T-cell tolerance are thought to operate by targeting marginal zone macrophages in the spleen [48].

Other authors have used microparticles or nanoparticles to deliver modulators of DC function and promote the acquisition of a regulatory phenotype. One group used 1–4 μm diameter microspheres carrying anti-sense oligonucleotides for the costimulatory molecules CD40, CD80, and CD86, to induce the formation of tolerogenic DCs *in vivo* [49] (Fig. 2). The relatively large size of the microparticles used in this study favors phagocytosis over pinocytosis and endocytosis, hence promoting the delivery of their cargo to phagocytes [50,51]. Another approach involves the delivery of small interfering RNAs (siRNA) to inflammatory monocytes/macrophages [52]. One such study used 70–80 nm diameter lipid-like nanoparticles containing a CCR2-specific siRNA, to downregulate CCR2 expression in monocytes and inhibit their migration to sites of inflammation. This compound inhibited atheroma formation in a model of atherosclerosis and prolonged the engraftment of allogeneic pancreatic islet transplants. Cationic polymers, peptides, and inorganic nanoparticles such as gold nanoparticles and quantum dots have also been used as vehicles to deliver siRNA *in vivo* [53]. Another group used magnetic nanoparticles to deliver $\beta 2$ microglobulin- or caspase-3-specific siRNAs to inhibit the rejection [54] or promote the engraftment of islet cell xenografts [55]. A related approach involves the delivery of *in vitro*-modified dendritic cells. In this case, the route of administration is a fundamental variable to consider

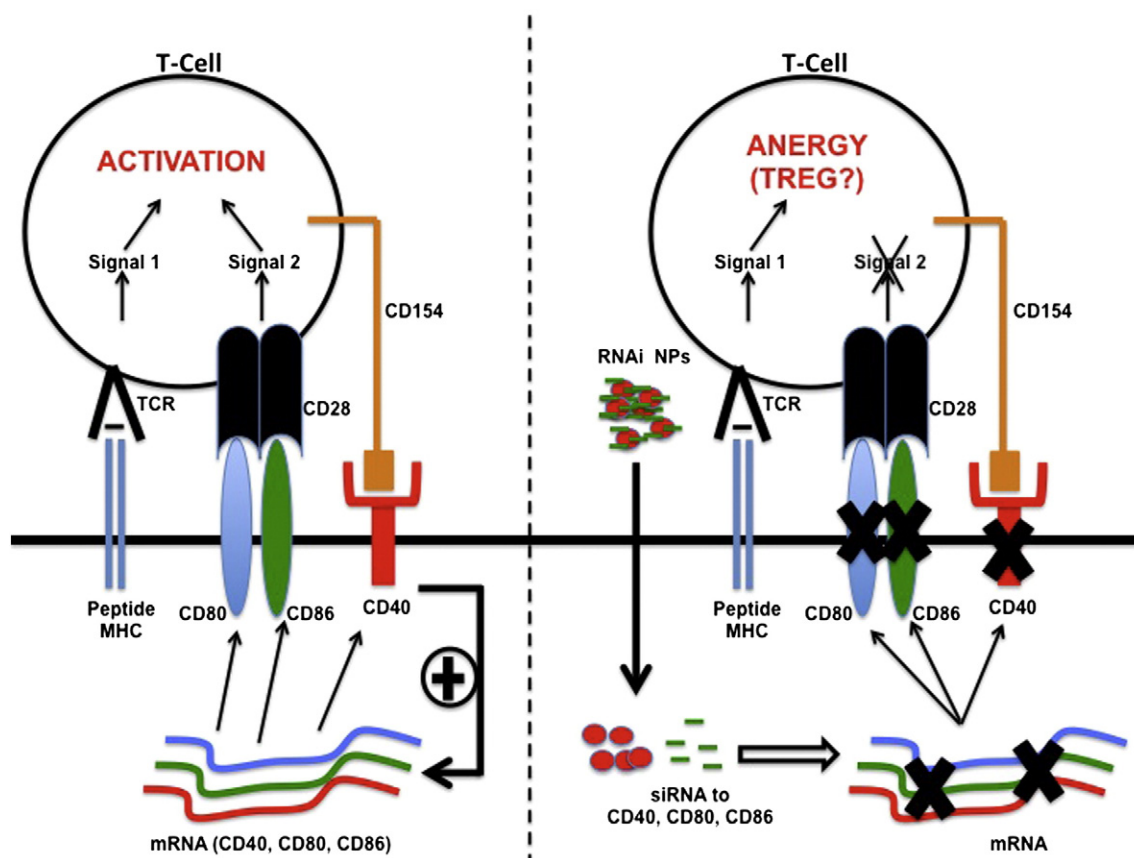


Figure 2 Particles as drug delivery vehicles. Micro- and nanoparticles can be used to deliver immunomodulatory drugs to antigen presenting cells. Here, we illustrate the effects of antisense or small interfering RNAs for CD80, CD86, and CD40 mRNAs on DC phenotype and function. Decreased levels of these co-stimulatory molecules on DCs (right) tend to promote the development of anergic (rather than activated, left) T-cells targeting cognate pMHC complexes on the DC surface. In addition, DCs expressing low levels of co-stimulatory molecules may promote the formation of CD4 + Foxp3+ regulatory T-cells.

if specific lymphoid organs are to be targeted, such as, for example, the pancreatic lymph nodes in type 1 diabetes [56,57].

4. Particles as vehicles to deliver autoantigens to tolerogenic APCs

It has been shown that transfusion of ethylenecarbodiimide (ECDI)-fixed splenocytes loaded with disease relevant antigens or APLs, protects murine models of EAE against disease [6,58–60]. These effects are thought to be mediated by uptake of apoptotic splenocytes (hence delivery of antigen/peptide) by tolerogenic DCs and macrophages, leading to functional inactivation and deletion of cognate T-cells [61,62]. Since these apoptotic cells accumulate in the splenic marginal zone and promote the activation of IL10-secreting macrophages, it has been suggested that induction of cognate CD4 + CD25 + Foxp3+ regulatory T-cells may also be at play [63]. Other approaches have investigated the use of erythrocytes, rather than splenocytes, as antigen carriers [64]. A similar approach involved the use of 500–1000 nm carboxylated polystyrene or poly(lactic-co-glycolic acid) microparticles coupled with autoimmune disease relevant peptides such as PLP_{139–151}

[48]. This approach inhibited the onset and progression of relapsing-remitting EAE in SJL/J mice immunized with PLP_{139–151}. The mechanisms of action remain unclear but are thought to include deletion or anergy of cognate T-cells, inhibition of monocyte/macrophage trafficking to inflamed tissues, and the possible induction (albeit not expansion) of CD4 + CD25 + Foxp3+ Treg cells by macrophage-derived IL-10. The relative roles of each of these mechanisms and both, the magnitude and specificity of the Treg cell-induction event remain to be elucidated. It appears, though, that the tolerogenic effect of peptide-loaded microparticles requires expression of the 'macrophage receptor with collagenous structure' (MARCO), a scavenger receptor expressed on splenic marginal zone macrophages that contributes to the uptake of particulate debris such as microparticles and apoptotic splenocytes (Fig. 3).

Another group that used nanoparticles as vehicles for peptide delivery employed 60 nm diameter gold nanoparticles that were loaded with the EAE-relevant epitope MOG_{35–55} and 2-(1Hindole-3-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) [65]. ITE is an activator of the aryl hydrocarbon receptor transcription factor (AhR) that affords DCs the potential to promote the differentiation of CD4 + CD25 + Foxp3+ regulatory T-cells. A similar approach used poly(lactic-co-glycolic) nanoparticles to co-deliver autoantigens and rapamycin to DCs.

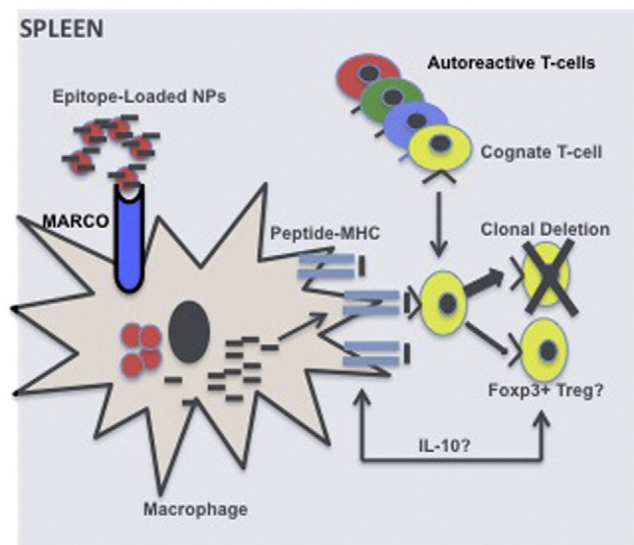


Figure 3 Particles as antigenic epitope delivery vehicles to APCs. Microparticles loaded with autoantigenic epitopes can suppress certain autoimmune responses by inducing clonal anergy, deletion, and, possibly, regulatory T-cell conversion.

This approach promoted T- and B-cell tolerance [66], via a mechanism that involved the induction of regulatory DCs by rapamycin [67–69] (Fig. 4).

Another nanostructure that has been used to deliver therapeutic cargos to DCs consists of a gel-like core containing cyclodextrins enclosed within a lipid bilayer [70]. These structures were used to deliver mycophenolic acid to an F1[NZB x NZW] murine model of lupus, modulating DC phenotype and function and promoting partial suppression of disease onset and progression.

5. T-cell-targeting nanomedicines

The approaches described above rely on the uptake of antigen-coated particles by phagocytes, and the presentation of antigenic epitopes to autoreactive T-cells. A different strategy involves direct delivery of tolerogenic molecules to T-cells. One group used anti-CD4 mAb-coated PLG nanoparticles to deliver leukemia inhibitory factor (LIF) to CD4⁺ T-cells [71], which promotes CD4⁺ CD25⁺ Foxp3⁺ T-cell development [72–74]. A caveat of this approach, however, is that the induced Treg cells are polyclonal and therefore not disease-specific.

Several authors have used monomeric or multimeric peptide-major histocompatibility complex (pMHC) molecules to selectively target (and delete) cognate autoreactive T-cell specificities. Repeated i.v. administration of relevant pMHC class I tetramers in a murine skin graft model, for example, induced graft tolerance by exhausting or anergizing cognate T-cells [75], much like APLs do. Likewise, dimers of I-A^{g7}-Fc fusions displaying peptides derived from the diabetes-relevant autoantigens GAD65, pro-insulin, chromogranin A promoted clonal deletion and IL10-expression in some T-cells [76]. Others have used toxin-coupled pMHCs to enhance the tolerogenic properties of soluble pMHC therapy. Toxin-coupled IGRP_{206–214}-H2-K^d tetramers, for example, deleted cognate CD8⁺ T-cells in pre-diabetic NOD mice, delaying the onset of spontaneous

autoimmune diabetes [77]. Another approach involved the use of microparticles co-delivering pMHC complexes with pro-apoptotic molecules (also referred to as Killer Artificial Antigen Presenting Cells (KAAPCs)). These compounds were shown to delete cognate cytotoxic T-lymphocytes (CTLs) *in vitro* [78–80] and cognate alloreactive T-cells in a murine skin graft model [81].

In an attempt to magnify the intrinsic T-cell deletional capacity of pMHC multimers delivered in the absence of co-stimulation for the treatment of autoimmunity, we produced nanoparticles displaying multiple copies of a pMHC class I complex that is targeted by a prevalent subset of diabetogenic CD8⁺ T-cells in NOD mice. This CD8⁺ T-cell subset targets the peptide spanning residues 206–214 from islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP_{206–214}) in the context of the class I molecule H2-K^d [82]. Treatment with IGRP_{206–214}-H2-K^d-NPs afforded T1D resistance to pre-diabetic NOD mice, as expected. However, T1D resistance in these mice was not associated with deletion of IGRP_{206–214}-reactive CD8⁺ T-cells, as we had expected, but rather with their systemic expansion [83]. These results suggested that T-cells respond differently to pMHC molecules delivered in solution (as monomers or multimers) than to pMHC molecules coated onto nanoparticles; whereas the former tend to trigger T-cell deletion, the latter promote the expansion of autoregulatory T-cell memory.

Detailed analyses of the pMHC-NP-expanded T-cell pools indicated that they were essentially identical to a population of anti-diabetogenic memory-like CD8⁺ T-cells that arises spontaneously in transgenic NOD mice expressing a low-affinity TCR for IGRP_{206–214} [83–86]. Additional experimentation demonstrated that treatment with IGRP_{206–214}-H2-K^d-NPs could restore normoglycemia in newly diabetic NOD mice. This ability of pMHC class I-coated NPs to expand cognate autoregulatory CD8⁺ T-cells was not a peculiarity of the prevalent IGRP_{206–214}-reactive CD8⁺ T-cell subset that exists in NOD mice, because it was also observed with NPs coated with pMHC class I complexes targeted by

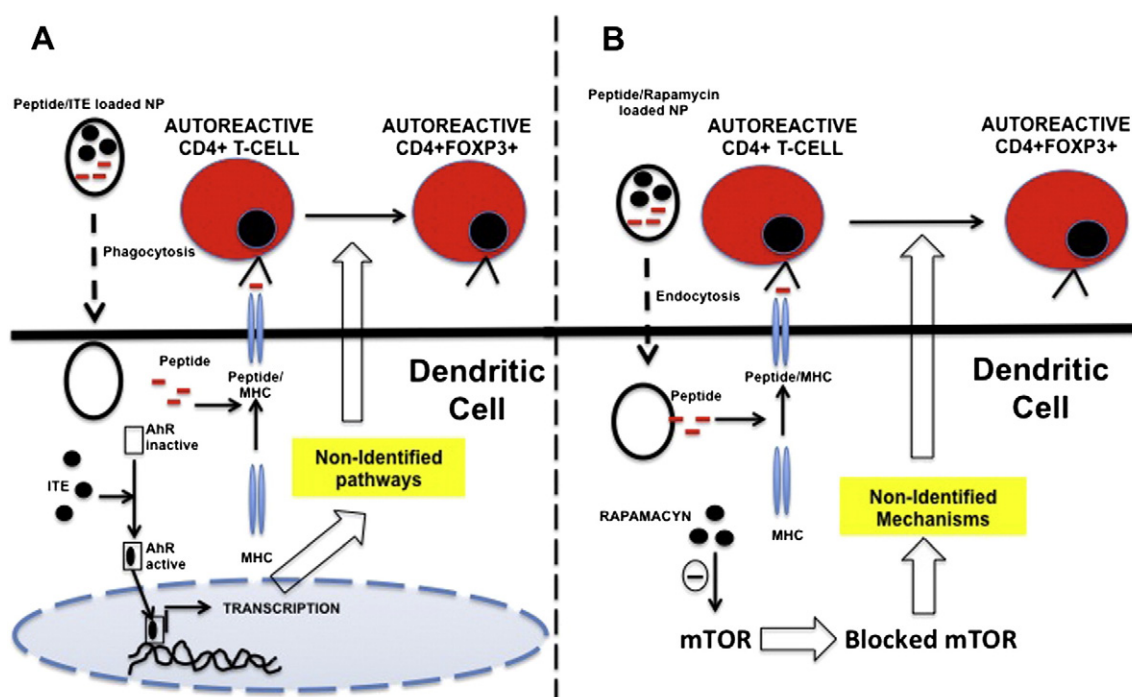


Figure 4 Nanoparticles to simultaneously deliver autoantigenic epitopes and immunomodulatory drugs to APCs. Nanoparticles loaded with ITE (A) or rapamycin (B) and antigenic peptide promote the development of cognate regulatory T-cells via poorly understood mechanisms.

subdominant autoreactive CD8+ T-cell specificities. We further demonstrated that the therapeutic effects of pMHC class I-NP therapy were mediated by the suppression and killing of local (autoantigen-loaded) APCs by the expanded CD8+ T-cells. Thus, pMHC-coated NPs trigger massive expansions of cognate low-avidity autoregulatory CD8+ T-cells that suppress highly polyclonal autoimmune responses by selectively targeting autoantigen-loaded APCs in the target tissue and draining lymph nodes [83,87–89] (Fig. 5).

These results accord with and help explain earlier observations from our group, suggesting that low-avidity autoreactive CD8+ T-cells have dominant anti-diabetogenic activity, and that complete deletion of individual high-avidity T-cell specificities cannot effectively blunt poly-antigenic autoimmunity. Briefly, to investigate the relative roles of high- versus low-avidity IGRP_{206–214} reactive CD8+ T-cells in the development of type 1 diabetes, we attempted to preferentially delete each of these cell pools by treating NOD mice with partial agonistic, agonistic, or superagonistic mimics (APLs) of IGRP_{206–214}. Whereas complete deletion of both high- and low-avidity IGRP_{206–214}-reactive T-cell pools with superagonistic APLs was inconsequential, a moderate expansion of their low-avidity counterparts by treatment with partial agonistic APLs afforded resistance to disease [86,90]. More recently, we have demonstrated that this paradigm can be harnessed for the development of nanomedicines that can expand autoreactive CD4+ Treg cells *in vivo* in various autoimmune disease models and genetic backgrounds (Clemente-Casares, our unpublished observations).

Collectively, the work described above highlights the fact that the autoantigenic complexity and T-cell repertoire diversity underlying most autoimmune disorders pose a

barrier that cannot be readily overcome by using antigen-specific deletional approaches. That is, deletional tolerance of one or a few autoreactive T-cell specificities in autoimmune diseases cannot afford tolerance against all the other specificities that partake in the disease process. Conversely, the expansion of autoreactive regulatory T-cells capable of suppressing antigen-presentation in a disease-specific manner can readily do so.

6. The future: nanotheranostics, nanorobotics, and beyond

Areas in the field of nanotechnology relevant to autoimmunity that are likely to undergo major development in coming years include the development of nanocompounds and nanorobots (nanobots) for theranostic purposes.

Several approaches have been developed to image the progression of autoimmune inflammation. To the extent that inflammation promotes vascular permeability, magnetic resonance imaging (MRI) upon administration of iron oxide nanoparticles permits non-invasive measurements of tissue inflammation in autoimmune diabetes and other autoimmune diseases [91–93] as well as evaluation of the efficacy of experimental treatments [94,95]. Changes in the endothelium of the vasculature of inflamed tissues are often associated with upregulation of adhesion molecules, a feature that can also be exploited for diagnostic imaging. For example, iron oxide microparticles functionalized with anti-VCAM-1 antibodies were used to monitor early inflammatory events in EAE [96]. Iron oxide nanoparticles can also be used to track the recruitment and accumulation of autoreactive T-cells to sites of autoimmune inflammation in

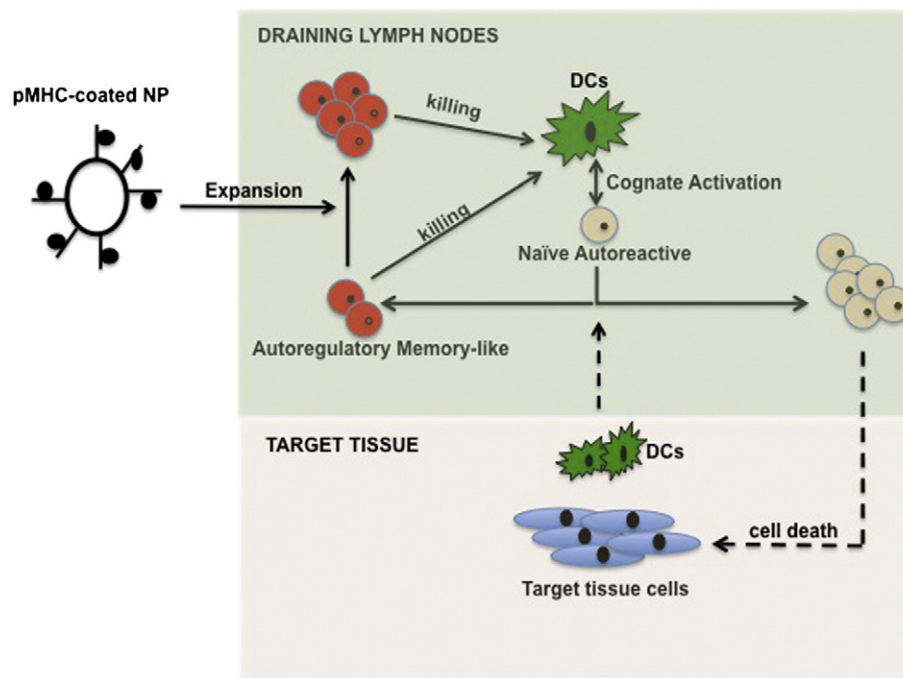


Figure 5 Direct T-cell targeting, pMHC-based nanomedicines. Nanoparticles coated with disease relevant peptide-MHC class I molecules trigger the expansion of cognate memory-like autoregulatory CD8⁺ T-cells. These cells suppress polyclonal autoimmune responses by suppressing and killing autoantigen-loaded APCs.

real time, *in vivo*. For example, we have used NRP-V7/H2-K^d-coated iron oxide nanoparticles for both therapeutic purposes, as described above, and for tracking the recruitment of cognate endogenous CD8⁺ T-cells to the pancreatic lesions, to study the progression of disease non-invasively in real-time [97,98]. Loading iron oxide nanoparticles into islet grafts also enables the monitoring of graft survival [54]. The latter approach incorporates therapeutic and diagnostic features, a combination that is defined by the term ‘theranostics’ [99,100]. The development of new nanoparticle compounds with multiple surface chemistries (janus nanoparticles [41,101]) will undoubtedly contribute to the development of next-generation theranostic nanomedicines for autoimmune disease.

Nanotechnology is also undoubtedly poised to bring cellular and molecular editing tools to medicine. DNA origami-based nanobots [102] such as those recently used to build computing robots [103] are examples of the potential of this field. It is conceivable that nanobots, in combination with CRISPR/Cas9 genome editing [104] and gene regulation [105] platforms and extremely fine-tuned cell-specific delivery strategies, will enable genome editing, therapeutic gene regulation, and molecular surgery *in vivo*, at the cellular level. It is not unreasonable to foresee the development of platforms capable of modifying cellular and subcellular structures. The application of these up-and-coming developments to immunity will change the way we categorize, address, and treat immune disorders. We envision, for example, that diseases will be defined by the cellular and molecular malfunctions that ought to be treated (corrected). Our therapeutic pMHC-based nanomedicine approach, for example, targets a regulatory T-cell pathway that can suppress diverse chronic inflammatory phenomena.

There is little doubt that the unforeseen advances lying well ahead of what we can fathom today will rise from

research conducted at the interphase among the fields of biomedicine, physics, chemistry, mathematical biology, and computational sciences and promise to bring health care to a new exciting era.

Competing financial interests statement

P. Santamaria is scientific founder of Parvus Therapeutics Inc. and has a financial interest in the company.

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