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Nano and Microparticle Emerging Strategies for Treatment of Autoimmune Diseases - Multiple Sclerosis and Type 1 Diabetes

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Abstract

Autoimmune diseases affect 10% of the world's population, and 1 in 200 people worldwide suffer from either multiple sclerosis (MS) or type 1 diabetes (T1D). While the targeted organ systems are different, MS and T1D share similarities in terms of autoreactive immune cells playing a critical role in pathogenesis. Both diseases can be managed only symptomatically without curative remission, and treatment options are limited and non-specific. Most current therapies cause some degree of systemic immune suppression, leaving the patients susceptible to opportunistic infections and other complications. Thus, there is considerable interest in the development of immunotherapies not associated with generalized immune suppression for these diseases. In this review we present current and preclinical strategies for MS and T1D treatment, emphasizing those aimed to modulate the immune response, including the most recent strategies for tolerance induction. A central focus is on the emerging approaches using nano- and microparticle (NMP) platforms, their evolution as immunotherapeutic carriers, including those incorporating specific antigens to induce tolerance and reduce unwanted generalized immune suppression.

Keywords

Multiple sclerosis; experimental autoimmune encephalomyelitis; type 1 diabetes: immunotherapy; drug delivery; immune modulation; immune engineering; microparticles; nanoparticles; antigen specific

Multiple Sclerosis Burden, Pathology and Clinical Approaches

MS is an autoimmune disease that affects the central nervous system (CNS), has onset typically in the early 30s, and a higher prevalence in women than men [1–3]. Approximately

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1 in 250 people are diagnosed with MS in the United States alone, and there has been a 5-fold increase in the prevalence of MS since 1976 [4]. According to the National Multiple Sclerosis Society currently nearly one million people are living with MS in the United States and 2.3 million in the world. MS pathology consists of autoimmune-mediated demyelination whereby immune cells destroy the myelin sheath that surrounds axons and kill myelin producing oligodendrocytes [5, 6]. This leads to clinical symptoms including numbness, tingling, fatigue, and eventually paralysis [7]. Relapsing-remitting multiple sclerosis (RRMS), where clinical disease manifests as a series of relapses and remissions with worsening from baseline subsequent to each relapse, is the most common form of MS, which is diagnosed in over 85% of patients. In primary progressive MS (PPMS), symptoms worsen rapidly following disease onset [7]. The pathogenesis of MS has been partially elucidated. Initially, the blood brain barrier becomes permeable, which is a step involved in immune cell infiltration into the CNS as blood brain barrier permeability is associated with increased trans-endothelial migration of activated immune cells [8]. Although the mechanism leading to permeability is not clear, inflammatory cytokines produced by CNS resident cells are associated with this process through the disruption of cell-cell junctions [9]. To this end, the chemokine receptors CCR2, CCR5 and CCR6 have been linked to the migration of immune cells into the CNS [7]. This infiltration of immune cells results in white matter lesions, that expand with each relapse [7]. It is known that CNS destruction is mediated by proinflammatory T cells, macrophages, activated microglia and astrocytes, with B cells playing a role as well [5]. Resident CNS astrocytes and microglia contribute to disease progression by the production of inflammatory cytokines and neurotoxic factors. The phenotype of infiltrating immune cells varies based on how far the disease has progressed, with higher levels of T cells and B cells early in disease and “a smoldering inflammation”, resulting in the development of tertiary lymphoid structures with activated microglia/macrophages in the CNS during chronic stages [7]. In the CNS microglia, recruited macrophages, dendritic cells (DCs) and B cells present autoantigen to T cells [10, 11]. The $CD4^+$ T cells are typically T helper 17 (Th17) and Th1, and react to autoantigens that are part of the CNS such as myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP). Th17 cells express the transcription factor RORγt and produce the proinflammatory cytokine interleukin-17a (IL-17a), while Th1 cells express Tbet and produce the proinflammatory cytokine IFN-γ. Numerous T helper cells express both RORγt and Tbet and produce IL17 and IFN-γ as well as GM-CSF [12–17]. T helper cell toxicity can be direct, through the release of neurotoxic cytokines or indirect by activation of macrophages [7]. CD8 T cells can secrete inflammatory cytokines such as GMCSF or directly kill oligodendrocytes through a granzyme b mediated mechanism [7]. The Th1 and Th17 response is hypothesized to be the driving force behind the preclinical mouse model of MS, experimental autoimmune encephalomyelitis (EAE), in a manner consistent with human MS [17, 18]. This is corroborated by the fact that defects in Th1 and Th17 cells prevent EAE onset [15, 16].

Therapeutic options for MS are minimal and there is no cure. Corticosteroids, such as methylprednisolone, can provide transient relief of inflammation during relapse. However, broad immunosuppressants are not viable for long-term management due to poor tolerability, ineffective disease control and susceptibility to opportunistic infections [19–22].

Other approaches seek to limit trafficking of immune cells into the CNS, either by decreasing blood brain barrier trafficking or preventing egress from secondary lymphoid organs [20, 23–26]. Treatment with interferon β, which has known immunoregulatory properties, can be given alone or in conjunction with glatiramer acetate, a random mixture of synthetic peptides highly represented in MBP. Interferon-β treatment results in decreased lymphocyte trafficking across the blood brain barrier and glatiramer acetate binds to MHC II, competing with presentation of actual myelin antigens [23, 27, 28]. However, response rate is low, with only a 30–50% reduction in relapse rate [24, 29]. Natalizumab, a monoclonal antibody targeting integrin α4 is another treatment which impedes leukocyte trafficking into the CNS and resulted in one third the number of relapsed compared to placebo control and 20% relapse rate overall [25, 30]. Conversely, Fingolimod (FTY720), a sphingosine-1-phosphate receptor inhibitor, blocks egress from lymph nodes, stopping autoreactive cells from trafficking to the CNS and treatment dropped relapse rate to 15% over a two year study [26, 31]. However, preventing trafficking with Natalizumab or Fingolimod may cause progressive multifocal leukoencephalopathy, a life-threatening opportunistic viral infection of the CNS [21, 32]. Among therapies with anti-CD20 antibodies, which deplete circulating immature and mature B cells, but not plasma cells, Ocrelizumab, a humanized monoclonal antibody, showed success, including in slowing MS disease progression as relapse rate was 46% lower than in IFN-β treatment [33]. However, Ocrelizumab increases the risk of upper respiratory infections 40% vs 33% over IFN- β treatment, oral herpes virus 2.3% vs 0.4% in placebo, and risk of breast cancer was 2.3% compared to 0.8% in placebo group [34]. Additional antibody therapies have shown promise in treatment of MS, including anti-CD52 (B and T cell depletion), and anti-CD25 (targets IL-2 receptor and Treg cells), but all have side effects related to immune suppression, such as infections in the brain [19, 35, 36]. In terms of antigen-specific treatments, a clinical trial was conducted attempting to treat with $MBP_{83–99}$, however the trial was halted after disease worsened in some patients [37].

Type 1 Diabetes Burden, Pathology and Clinical Approaches

In T1D autoimmunity often manifests in early childhood, with 490,000 children with T1D under the age of 15 worldwide. Less frequently, onset occurs later in adulthood [38, 39]. Disease incidence is increasing, with current estimates suggesting approximately 40,000 new cases annually in the United States alone [39]. T1D is characterized by hyperglycemia due to lack of insulin, which leads to clinical manifestations such as polyuria, polydipsia, mental obtundation, weight loss, nausea, vomiting, abdominal pain, and fatigue. Sequelae of chronic hyperglycemic state include diabetic neuropathy, retinopathy, nephropathy, ulcers, and vasculopathies that can ultimately end with amputation. Injection of exogenous insulin is the primary treatment used to manage T1D [40]. Although insulin supplementation therapy affords moderate disease management, T1D patients experience a number of comorbid complications, which include chronic, potentially life-threatening, kidney disease in 30% of patients, and a 10 times higher risk of developing cardiovascular diseases [41–43].

T1D is classically characterized by $CD4^+$ and $CD8^+$ T cell mediated destruction of insulin-producing β-cells in the pancreas. The innate immune system also plays a role in the pathogenesis, as macrophages and DCs have been observed surrounding the pancreatic islets

and present antigen to autoreactive T cells [40]. Stage 1 is associated with the presence of autoreactive cells and β-cell loss, stage 2, with autoreactive cells, β-cell loss and hyperglycemia, and stage 3 with autoreactive cells, β-cell loss, hyperglycemia and clinical symptoms. Stage 1 and 2 can last for years before symptomatic presentation, making it difficult to detect disease before critical β-cell loss from autoimmune attack. Awareness of genetic risk factors and advances in diagnostic procedures have made preventative treatments conceivable before the destruction of a critical mass of β-cells [44]. In particular, children with the HLA-DR4-DQ8 and HLA-DR3-DQ2 haplotypes are more likely to generate autoantibodies for insulin and glutamic acid decarboxylase 65-kilodalton isoform (GAD65) [40, 41].

Therapeutic options.

To date, there is no cure for T1D. However, a myriad of immunological therapies have been explored with limited success. Numerous clinical trials have sought to establish immune tolerance by repeated administration of T1D autoantigen, often employing the primary autoantigen insulin [45–48]. Other treatment options include monoclonal antibodies, which have been used in multiple clinical trials, including anti-CD20 [49] and anti-CD3 (T cell depletion) [50]. In the clinical trial using anti-CD20, patients were diagnosed with T1D if one circulating autoantibody was present and treatment was initiated 90 days following diagnosis with four total treatments over a one year timeframe. One year following anti-CD20 therapy, C peptide level increased to 0.56 pmol/mL compared to 0.47 pmol/mL in placebo control and patients required lower levels of exogenous insulin than placebo group [49]. The study using anti-CD3 also treated newly diabetic patients defined by the presence of autoantibodies and the need for injection of insulin, with onset of treatment began within 12 weeks of diagnosis. Treatment resulted in slower decline in C-peptide level compared to placebo control with with 40% patients who received anti-CD3 having a preservation of baseline C-peptide levels [50]. Other immunomodulatory therapies included an antibody hybrid consisting of a fusion of anti-CTLA4 (coinhibition) to the Fc region [51]. Similar to anti-CD20 and anti-CD3 studies patients were recently diagnosed with T1D via autoantibody levels and were treated at approximately 90 days post diagnosis. Results of this study showed a delayed reduction in C-peptide level with 32% of patients below the threshold of 0.2nmol/L compared to 43% in placebo control [51]. Additionally, a combination of the anti-thymocyte globulin (ATG), cyclophosphamide and granulocyte-colony stimulating factor (G-CSF) with hematopoietic stem cell transplant has shown some success in patients were recently diagnosed with T1D. Diagnosis took place no more than six weeks prior to enrollment based on anti-GAD65 antibodies and C-peptide in the serum. The treatment regimen was as follows: cyclophosphamide injection followed by ATG treatment for five days, stem cell transplant, and G-CSF injection five days following transplant. Patients became insulin independent following treatment, but after an initial rise C-peptide level eventually decreased, but levels remained higher than at diagnosis and some patients eventually progressed to an insulin-dependent state [52]. It is believed that the mechanism of treatment is ablation and reconstitution the immune system thus inhibiting β-cell destruction resulting in the return to normoglycemia [52]. In a similar study, treating patients who were diabetic for over a year, with ATG and G-CSF showed moderate success in a recent clinical trial, through increased C-peptide levels, with levels of 0.28 nmol/l/min

higher than control, indicating improved insulin production, however patients remained dependent on exogenous insulin [53]. A preservation of regulatory T (Treg) cells may be associated with greater β-cell function [53]. A continuation of this study has recently revived optimism as a new two year clinical trial with low dose ATG resulted in preservation of insulin production in patients with new onset T1D defined by the presence of at least one T1D autoantibody [54]. C-peptide levels were significantly higher two years post treatment in the ATG treated patients. Other treatments using transferred cells rather than suppressive factors, including Treg cells and tolerogenic DCs, have shown promise in clinical trials [55, 56]. The study utilizing Treg cells treated newly diabetic patients based on autoantibody level and the majority of treated patients were able to maintain elevated C-peptide levels for over two years following the initiation of the study [55]. In contrast, the clinical trial using tolerogenic DCs treated patients with insulin dependence for more than five years and had no detectable C-peptide at the onset of treatment. Following injection of 10 million DCs, C-peptide was detectable for over two years in some patients and treatment was tolerated over that timespan [56].

Commonalities in multiple sclerosis and type 1 diabetes

There are several similarities in disease progression and pathogenesis of MS and T1D. Often the diagnosis does not take place until late, as the clinical manifestations appear at stage three in T1D, and similarly, MS patients are not typically diagnosed until after their first clinical attack [7, 40]. For this reason, there is often lasting damage caused by the immune system before symptomatic presentation and diagnosis, making the burden of the disease more severe. Co-occurrence of these autoimmune diseases is also common. A nation-wide study in Denmark identified that T1D patients are at a three times greater risk for developing MS than healthy individuals [57]. Along this line, both diseases have genetic risk factors associated with the HLA, although the haplotypes vary by disease [41, 58]. There are also non-HLA associated genetic risk factors such as T-cell alleles IL-2 and protein tyrosine phosphatase, non-receptor type 22 (PTPN22) in MS and T1D, respectively. In addition to genetics, environment also plays a role in disease development, with smoking and viral exposure increasing the risk of both MS and T1D [7, 40].

In terms of pathogenesis, in both diseases autoreactive T cells play an important role, targeting autoantigens on β-cells in the pancreas in T1D, and myelin in the CNS in MS [7, 40]. Autoantibody production indicates the contribution of B cells to both diseases. The contribution of innate immune cells is evidenced by the presence of macrophages and DCs surrounding the islets of Langerhans in T1D and by the infiltrating macrophages playing a role in myelin destruction in MS.

Biomaterials and particle-based drug delivery and immune modulation

Biomaterials have been well established as an approach to mediate controlled release of drugs, peptides, and proteins in the pharmaceutical industry due to their advantages over soluble bolus drug administration [59]. The shortcomings of bolus drugs include rapid renal clearance, short half-life, and potentially fatal off-target side effects. Biomaterial systems can overcome these pitfalls by facilitating sustained release, localization of drug cargo to

cells, organs, or systems of interest to improve therapeutic response, minimize drug load required, and mitigate adverse systemic reactions.

Building on this work, biomaterial platforms have been recently extended to immunomodulatory approaches [60]. Specifically, nano- and microparticles (NMPs) based therapies have shown promise in recent years in restoring homeostatic immunity. NMPs can encapsulate or have immunomodulatory factors conjugated to their surface and can be delivered in a diverse manner including systemically or locally. Formulation of NMPs and the route of administration influence the effectiveness and the type of immunomodulation [60–62]. Intravenous and intraperitoneal injections provide better trafficking to the liver and spleen [62, 63], while subcutaneous injection may require the addition of recruitment factors, if the NMP system contains particles that are designed to be phagocytized, but are not small enough to drain passively [64]. With proper trafficking, subcutaneous delivery may be clinically favorable because of the ease and safety of administration compared to repeated bolus intravenous delivery. Furthermore, controlled-release biomaterial systems can extend the window of immunomodulation resulting in a reduced dosing frequency, however using biomaterials can decrease the sterility of the injection via in introduction of endotoxin. In addition, encapsulation allows for greater control of release kinetics. Control of release may be useful if it is desired to give a small dose over a long period of time rather than a quick acting dose, although too slow release could decrease efficacy. Additionally, synthetic biomaterials are tailorable and can be coupled with ligands or antibodies for surface receptors to target delivery of immunomodulatory agents to specific cell subsets, as it has been done with α-CD11c, α-DEC205 [65], α-CD40 for DCs [65, 66] and α-CD4 or α-CD8 for T cells [67, 68].

A specific type of treatment that has benefitted from the use of biomaterials is DC therapy. In this line, a NMP approach can alter DC phenotype *in vivo*, thus bypassing the need for *ex vivo* manipulation, which has drawbacks, including high cost, poor yield and low levels of regional lymph node homing following re-administration [69–72]. NMPs are also attractive for intracellular delivery to DCs and other antigen-presenting cells (APCs) given that they can be readily phagocytosed if less than ~ 5 μm in diameter [73].

It has been shown that poly(lactic-co-glycolic acid) (PLGA) NMPs themselves can have immunosuppressive effects in certain contexts via two distinct mechanisms. In this work, bone marrow derived DCs treated with empty PLGA MPs had reduced expression of MHC II, CD80 and CD86 due to degradation byproduct, lactic acid [74]. Furthermore, early intravenous treatment with high molecular weight PLGA NPs, without additional factors, resulted in delay in EAE scores with a concomitant shift of neutrophils away from the CNS to the liver (Table), thus altering trafficking rather than the direct suppression shown by Allen et al. [75].

Efforts to avoid off target effects associated with broad immunosuppression have recently emphasized antigen (Ag) specific approaches. The advantages of such strategies include less systemic immune suppression, the ability to modulate specific immune populations and potential for lower dosing amount and frequency [96, 97]. In this approach only the autoreactive immune cells are targeted. In the case of MS and T1D, such treatments aim to

induce tolerance or change primarily the phenotype of Ag-specific T cells. To accomplish an Ag-specific response, treatments have focused on APCs, including DCs and macrophages, given the pivotal role of these cells in promoting the immune response of T cells [61, 66, 79, 93, 98–102]. Tolerogenic DCs have been found to favor generation of Treg cells in specific environments, which further suppress autoreactive T cells, ultimately preventing or reversing disease [103]. In addition, APCs can induce anergy by presenting antigen without the proper costimulatory factors or in the context of coinhibitory receptors such as CTLA-4 and PD-1 [104, 105].

NMP Strategies for MS/EAE and T1D treatment using non-antigen-specific immune modulators

There has been significant effort in developing NMP-based strategies that seek to modulate broadly immune cells, either systemically or locally, creating a suppressive phenotype or delivering factors to block Th1 or Th17 responses, but not in an Ag-specific manner (Fig 1a). Several such strategies have been reported successful in ameliorating or inhibiting EAE, by dampening the Th1 and Th17 responses or increasing the number of Treg cells. Some NMP treatments seek to enhance delivery of immunosuppressants that are currently FDA approved such as glucocorticoids, with the goal of lowering the dose needed, which may reduce off target effects and toxicity. Such a strategy delivered the glucocorticoid betamethasone, using an inorganic-organic NP formulation consisting of a complex of zirconium dioxide, flavin mononucleotide and betamethasone phosphate (Table) [78]. *In vitro* experiments showed uptake by murine bone marrow derived macrophages and consequent downregulation of MHCII, CD86, TNFα and IL-1β [78]. When cultured with human monocytes an anti-inflammatory phenotypes with decreased IL-1β mRNA and increased arginase 1 expression was observed [78]. When injected intraperitoneally *in vivo*, betamethasone NPs similarly decreased MHCII and CD86 expression and TNFα secretion on macrophages following lipopolysaccharide challenge, and intraperitoneal injection for three consecutive days of EAE mice at a clinical score of two, halted disease progression in a macrophage-dependent manner [78].

The STING pathway has been also targeted using a polyethylenimine (PEI) NP encapsulating cyclic dinucleotide GMP (c-diGMP) administered i.v. in prophylactic and therapeutic manners in EAE-induced mice, and was found to reduce disease severity [106]. This treatment resulted in production of indoleamine 2, 3 dioxygenase (IDO) in hematopoietic cells and reduced IDO expression in neurons [106].

Another treatment employed the agonist for the metabotropic glutamate receptor-4 (mGluR4) N-phenyl-7-(hydroxyimino)cyclopropaβchromen-1a-carboxamide (PHCCC), encapsulated in PLGA. The treatment slowed EAE development (Table) [76], likely through modulating DC activity, given that mice lacking mGluR4 develop more severe EAE and mGluR4 activation was shown to restrain DC-dependent induction of pathogenic Th17 cells in EAE [107, 108]. High levels of glutamate are seen in EAE and are associated with Th17 response in disease. It is believed that mGluR4 binding limits this response by modulating metabolism, resulting in skewing of T cell phenotype towards Th2 and Treg cells.

Treatments that directly modulate T cell responses have been also explored. An example is the treatment of EAE-induced mice by i.p. administration on the day of induction with hyperforin-conjugated gold NPs. Hyperforin is an herbal compound with anti-inflammatory properties. The treatment resulted in reduced EAE severity with increased Foxp3+ Treg cells and decreased in Th1 and Th17 cells (Table) [109]. A potential drawback is the possibility for liver toxicity following gold NP treatment [110].

A cationic NP using PLGA and a polymethacrylate/dimethylaminoethyl bond has a more neutral zeta potential and improved cellular internalization or endosomal escape [77]. A single intramuscular injection of NPs containing plasmid encoding mouse IL-10, augmented plasma IL-10 levels and reduced IFN-γ levels for up to six weeks in a model of streptozotocin-induced T1D (Table) [77]. In another approach anti-sense oligonucleotides for CD40, CD80, and CD86 were encapsulated in NMPs and blocked the activation of DCs and ameliorated disease in a mouse model of recent-onset T1D (Table) [79]. Draining lymph nodes from the site of injection had increased total numbers of CD25+Foxp3+ Treg cells [79].

NMP strategies to modulate the immune response in a manner independent of the disease-specific autoantigens have shown some promise in preclinical studies both for MS and T1D. Such strategies involved immunomodulators and may cause overall dampening the immune response, but multiple factors dictate whether this is the case. The dose and route of administration can determine if there is immunosuppression as a relatively small dose delivered subcutaneously would not be expected to have systemic effect. The half-life of a therapeutic is also a factor in whether a treatment is systemically immunosuppressive as a treatment cleared rapidly may not have a lasting effect. In addition, delivering NMPs to disease-relevant tissues or lymph nodes can provide a milieu with disease specific antigen to eliminate the need for the delivery of exogenous antigen [79, 111]. However, targeting NMPs to the pancreas or CNS for the localization of treatment represents a substantial challenge. For this reason, the addition of antigen to an immunomodulatory treatment may provide benefit in some therapeutic approaches. Additionally, antigen specificity could potentially reduce the opportunity for systemically dampening the immune response, which is a concern regarding opportunistic infections and off-target effects.

Antigen-Specific NMP strategies for MS/EAE and T1D

While Ag-nonspecific immune modulation shows promise, use of disease-associated antigen may produce generalized immune suppression. The first attempts in this direction were by coupling antigens with NMPs for antigen delivery to extend its circulation time. Further studies employed NMP platforms delivering antigens in combination with immune modulatory factors.

Antigen-Specific NMP strategies for MS/EAE

Initial strategies used NMPs for delivery of antigens alone (Fig 1b), such as proteolipid protein ($PLP_{139–151}$). $PLP_{139–151}$ conjugated to PLGA NMP surface and administered i.v. prevented relapses in the relapsing-remitting EAE model in SJL mice, when administered

either prophylactically or therapeutically, with the effect being dependent on phagocytosis of peptide-conjugated MPs by MARCO+ marginal zone macrophages in the spleen (Table) [62]. Additionally, increased numbers of Treg cells, and elevated T cell anergy was observed [62]. The same group showed that when $PLP_{139–151}$ peptide surface conjugated to PLGA/ poly(ethylene-alt-maleic anhydride) (PEMA) NPs and administered intravenously, prevented EAE with prophylactic administration and attenuated disease when administered at the peak of EAE (Table) [80]. Although intravenous delivery of this formulation had success in treating relapsing/remitting EAE, subcutaneous administration of the same formulation failed to have the same success, which may be due to differences in NP trafficking [80].

Encapsulation of antigen showed superiority to surface conjugation by avoiding potential for antibody binding to exposed antigen [116]. Furthermore, encapsulation rather than surface conjugation to PLGA/ PEMA NPs was more efficient in treating EAE in prophylactic and therapeutic setups, when administered i.v. [63]. Along this line, encapsulation of recombinant human basic myelin protein (rhMBP) in poly (ε-caprolactone) (PCL) NPs and subcutaneous prophylactic administration resulted in reduction of disease scores of $MOG_{35–55}$–induced EAE (Table) [83]. The dose of encapsulated peptide can also have an effect, with the high level of encapsulated peptide $PLP_{139–151}$ having significantly higher efficacy versus low level (Table) [81]. Response to delivered antigen can be a major concern, as there is demonstrated potential to exacerbate disease, as seen in the halted phase II clinical trial treating with $MBP_{83–99}$ [37].

NMP platforms delivering antigens in combination with immunomodulatory factors have been also explored in EAE (Fig 1c). One example is the surface conjugation of the aryl hydrocarbon receptor (Ahr) ligand 2–1’H-indole-3’-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) to thio-polyethylene glycol (PEG)-coated gold NP, conjugated together with the EAE-specific antigen $MOG_{35–55}$. The treatment initiated at EAE induction and consisted of weekly intraperitoneal injections effectively suppressed EAE (Table) [94]. It was postulated that the NPs containing myelin antigen and ITE promoted tolerogenic DCs and Treg cell expansion [94], in line with the previous data showing that ITE-Ahr induces tolerogenic DCs and Treg cell differentiation, and inhibits Th1 and Th17 cell function to suppress EAE [112–115].

Other treatments co-encapsulated immunomodulators such as rapamycin with antigen for the treatment of EAE. Rapamycin is known to block the mTOR pathway causing anti-inflammatory effects and blocking B and T cell proliferation [117–121]. Subcutaneous and intravenous injection of $PLP_{139–151}$ and rapamycin co-encapsulated in PLGA NPs blocked EAE and prevented relapse in an antigen dependent manner in SJL mice (Table) [87]. In another study, a rapamycin-antigen co-encapsulated formulation was explored in $MOG_{35–55}$/ CFA-induced EAE model. Rapamycin and $MOG_{35–55}$ peptide encapsulated in PLGA MPs and injected intra-nodally into the inguinal lymph nodes prevented and treated EAE in an antigen-specific manner through increased numbers of Foxp3+ Treg cells (Table) [89]. These treatments demonstrate the strong immunomodulatory effect of rapamycin in conjunction with antigen. Another immunomodulator, dexamethasone co-encapsulated with $MOG_{35–55}$ in acetylated dextran MPs, successfully treated EAE in a therapeutic regimen with decreased splenocyte production of IL-17 [122].

Other immunomodulatory factors such as cytokines have also been explored. EAE was moderately suppressed by subcutaneous administration of PLGA NPs loaded with IL-10 and $PLP1_{139–151}$, both in prophylactic or therapeutic treatment regimens (Table) [90]. This formulation demonstrates the possibility of suppressing EAE using a subcutaneous delivery of immunomodulatory factors encapsulated in PLGA NPs for controlled release [90]. However, the antigen-specificity of the treatment was not investigated [90]. Another formulation utilized the antigen $PLP_{139–151}$ conjugated to PLGA NPs, together with cytokine TGF-β to form an antigen coupled PLG-$PLP_{139–151}$-TGF-β NP system (Table) [88]. *In vitro* treatment downregulated costimulatory molecules CD80, CD86 and MHCII *in vitro*, and *in vivo* administration intravenously or subcutaneously, in prophylactic or therapeutic regimens resulted in reduction of EAE scores [88].

We developed a dual multi-factor MP system consisting of (1) unphagocytosable 30 μm MPs separately loaded with GM-CSF and TGF-β plus (1) phagocytosable 1 μm MPs loaded with Vitamin D_3 and $MOG_{35–55}$ (Fig 1D). This treatment administered subcutaneously blocked EAE progression when administration occurred in a semi-therapeutic fashion post EAE induction (Table) [64]. In the CNS of mice treated with this dual multi-factor MP system there was a reduction in pathogenic T cells expressing both Rorγt and Tbet and lower levels of the cytokines IL-17, IFN-γ and GM-CSF compared to mice treated with unloaded MPs, as well as reduced CD80 on macrophages/microglia [64]. The number and frequency of MHC II^+ and $CD86^+$ DCs in the draining lymph nodes were reduced in mice treated with dual multi-factor MP-$MOG_{35–55}$ versus an irrelevant antigen ($OVA_{323–339}$) dual multi-factor MP, demonstrating the dependence on antigen. Localized low dose subcutaneous delivery, controlled release of specific factors, and retention at the injection site are all advantages of this dual multi-factor MP system [64], compared to soluble administration. The combination of phagocytosable antigen-MP with the phagocytosable vitamin D_3 MP allows intracellular delivery of antigen and vitamin D_3 to act on its intracellular receptor and potentially induce tolerization of DCs. Unphagocytosable GM-CSF MP allows the recruitment of DCs and precursors, and TGF-β MP allows tolerization on the recruited DCs, both assuring slow and durable release. In addition, the subcutaneous delivery assures less off-target effects compared to i.v. administration.

NMP formulations that utilize antigen in combination with low doses of tolerogenic factors show promise by avoiding systemic immune suppression. This is likely due to the low doses of immunomodulatory factors and antigen specificity. The ability to avoid rapid release or release at an undesired location is valuable because it reduces the likelihood of off-target effects, which is a concern when administering immunosuppressive factors. However, one potentially major drawback of Ag-specific treatments is difficulty in selecting the most efficacious antigen because not all patients have the same autoreactive response. As it has been shown that some patients have autoantibodies for MBP, while others react to MOG or PLP. To this end, simultaneous NP-mediated delivery of multiple antigens showed higher efficiency in EAE treatment compared to single antigens [123].

Antigen-Specific NMP strategies for T1D treatment

Antigen-Specific NMP platforms have been developed for T1D. Using an artificial antigen presenting approach, one group utilized iron oxide NMPs coated with the islet-specific peptide glucose-6-phosphatase catalytic subunit-related protein ($IGRP_{13–25}$)-MHCI or II to modulate $CD8^+$ and $CD4^+$ T cells [85, 86]. When 4 week old pre-diabetic, NOD mice were injected intravenously every two weeks for six weeks and then every third week for the remainder of the study, normoglycemia was prolonged (Table) [86]. Additionally, diabetic mice were able to return to normoglycemia following the treatment. Similarly, intravenous injection of surface conjugated $IGRP_{13–25}$-MHCII NPs in 10-week-old pre-diabetic mice prevented T1D. Mice showed more TR1 cells expressing CD49b, LAG-3, ICOS and TGF-β [85] and the treatment was dependent on the specific antigen and MHCII presentation to maintain efficacy (Table) [85]. The versatility of this NMP platform was highlighted by the multitude of immune-related conditions that can be treated by changing the antigen, including collagen-induced arthritis and EAE, which similarly demonstrated robust mitigation of diseases [85]. In contrast to $IGRP_{13–25}$, heat shock protein 65–6xP227 loaded in NPs formulated from RGD and mannose modified chitosan. These NPs were able prevent T1D when delivered orally to 4 week old NOD mice and uptake by DCs was seen in the Peyers patches (Table) [84]. In addition, treatment was concomitant with an increase in Treg cells and a decrease in Th1 cells in the pancreatic draining lymph node. This study was notable in the demonstrated efficacy of NMP-based delivery of antigen to the gut [84].

Another T1D associated antigen used in NMPs formulations was BDC2.5 mimotope peptide (p31). Mimotope peptide p31 encapsulated or conjugated to PLGA or PLGA/PEMA NMPs were used to treat NOD.SCID induced with diabetes using adoptive transfer of BDC2.5 and NY8.3 antigen-specific cells. Intravenously delivered NMPs prevented hyperglycemia for up to 50 days post transfer [82]. Intra-islet Foxp3+ Treg cells were identified, which likely tolerized $CD4^+$ and/or $CD8^+$ T cells, through an increase in expression of the coinhibitory molecules PD-1 and CTLA4 [82]. NP-mediated delivery of an insulin hybrid peptide (2.5HIP), consisting of an insulin C-peptide fragment fused to a peptide from chromogranin A (ChgA), known to be recognized by BDC-2.5 T cells, also prevented diabetes in an adoptive transfer model by decreasing IFN-γ producing T cells and increasing $FoxP3^+$ T cells [124].

In addition to administering antigen NMPs alone, combinatorial approaches have been used for T1D [60]. Some approaches have explored antigen delivery in combination with pro-tolerogenic factors to modulate antigen-specific immune tolerance. Co-delivery of the Ahr ligand ITE and proinsulin via PEG-coated gold NMPs showed efficiency in prophylactic treatment of 8-week-old NOD mice (Table) [95]. Prevention overlapped with an increase in Foxp3+ Treg cells in the pancreatic lymph node and diminished expression of Th1 and Th17 gene signatures. DCs showed anti-inflammatory phenotypes with reduced surface expression of CD40, CD80 and MHCII and expression of genes encoding the inflammatory cytokines IL-12 and IL-6 [95].

Other combinatorial treatments were engineered to augment cellular recruitment through incorporation of chemotactic factors. A macroscopic hydrogel loaded with GM-CSF and

CpG together with PLGA NMPs encapsulating denatured insulin prevented T1D in 40% of the NOD mice when injected at 8 weeks of age (Table) [93]. The efficacy was dependent on antigen. In a parallel approach, without CpG, an alginate hydrogel containing GM-CSF and PLGA NMPs encapsulating the T1D-specific peptide BDC2.5. show increased Foxp3+ Treg cells in the hydrogel, suggesting that this is a viable approach to augment antigen-specific Treg cell numbers [125]. Using the dual multi-factor MP system consisting of (1) unphagocytosable 30 μm MPs separately loaded with GM-CSF or TGF-β, plus (1) phagocytosable 1 μm MPs loaded with Vitamin D_3 or insulin $B_{9–23}$ (Table) [91], delivered agents intracellularly to the locally recruited DCs. The results demonstrated that two subcutaneous injections of the combinatorial NMP system in 4-week-old NOD mice prevented T1D in 40% of treated NOD mice [91]. A modification of this system increased the amounts of TGF-β and GM-CSF and used denatured insulin as antigen (Table) [92]. Encapsulation, antigen, tolerogenic and recruitment factors were all required, otherwise the treatment was no longer effective. When the new formulation was administered weekly via subcutaneous injection, for three weeks, followed by four monthly booster injections, it was able to delay T1D onset in 8-week-old NOD mice, with 60% of mice remaining diabetes-free [92]. This delay in onset was associated with increased PD-1 on $CD4^+$ and $CD8^+$ T cells as well as an increase in $CD11b^+$ $CD11c^+$ DCs in the draining lymph nodes. Additionally, three subcutaneous administrations during the week of onset, followed by weekly booster injections for three weeks, temporarily reversed T1D in recent onset diabetes, for up to 100 days [92].

Conclusions

The prevalence of the debilitating autoimmune diseases MS and T1D continues to rise and a cure yet to be found. There has been an uptick in exploration of biomaterial strategies for autoimmune disease, with NMP therapies having demonstrated particular promise as vehicles for drug and antigen delivery to specific cells and organs of interest. The studies discussed above suggest that a curative therapy that modulates immunity without the need for systemic immunosuppression is possible, using antigen-specific strategies both in MS and T1D. Clinical trials will be needed to validate the benefit of NMP approaches in treating MS and T1D. Looking forward, coupling immunotherapy with regeneration could reduce the failure rate of treatments. In MS for example, clemastine fumarate, a medication for allergic rhinitis and urticaria, has been shown in a double-blind crossover clinical trial to increase remyelination [126]. Similarly in EAE mice clemastine accelerate remyelination [127–129]. Regeneration may also be necessary in T1D. Along this line, reprograming of α-cells with the transcription factors, pancreas/duodenum homeobox protein 1 (PDX1) and V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA) to produce insulin is an option that has been explored. When human α-cells are transduced with these transcription factors it enabled these cells to produce insulin [130]. Thus the key to unlocking a durable cure may be through coupling antigen-specific immunotherapies with regeneration.

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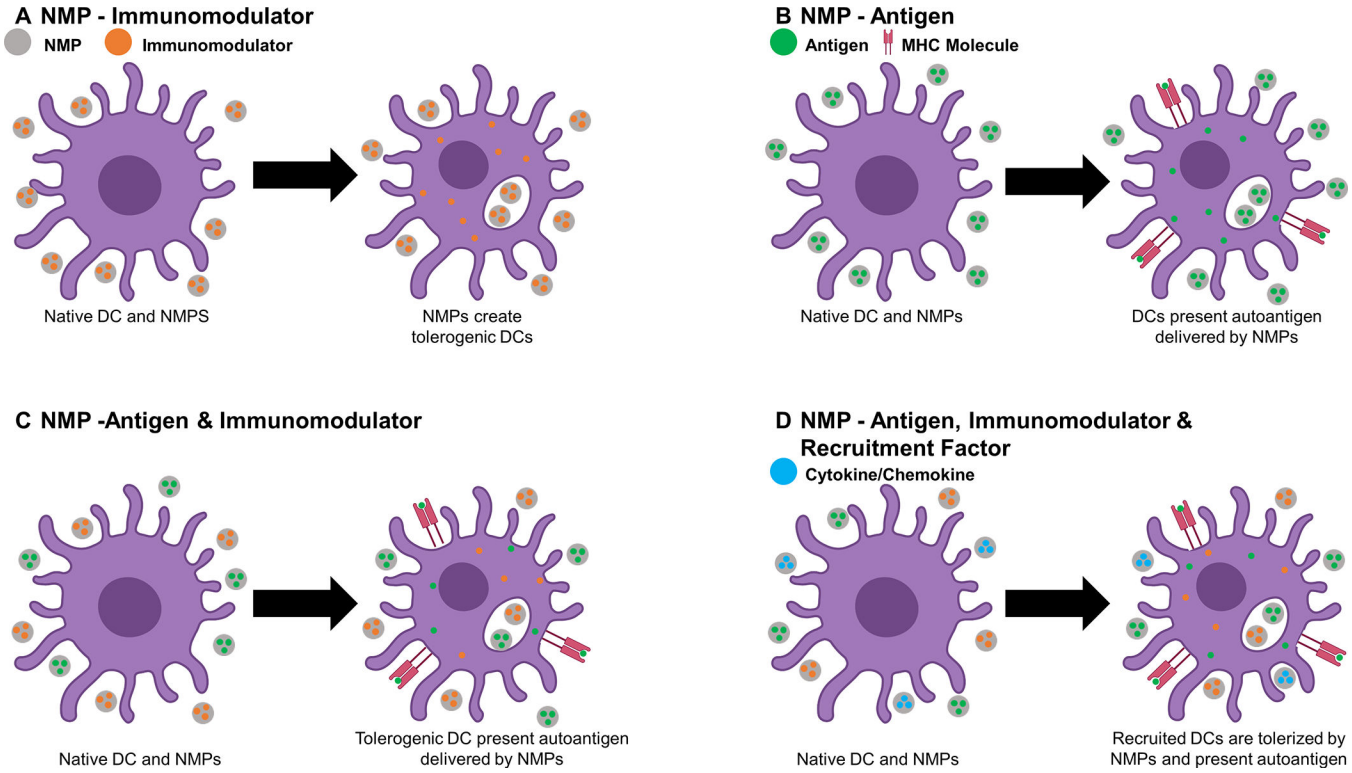

Figure. Representative methods of alleviating autoimmunity with NMPs.

A) NMPs that deliver immunomodulators to DCs in order to create a tolerized phenotype, B) delivery of autoantigen for presentation by DCs, C) delivery of both immunomodulators and antigen, to tolerize DCs which will then present the autoantigen in a tolerogenic context, and D) delivery of recruitment factors to increase DC recruitment to the injection site where autoantigen is taken up and immunomodulators induce a tolerogenic phenotype, after which DCs traffic to draining lymph nodes.

Table.

Nano and microparticle engineering strategies for treatment of autoimmune disease, multiples sclerosis and type 1 diabetes. Relapsing and remitting (RR), Primary progressive (PP), Streptozotocin induced T1D (STZ), and Mimotope (Mim)

Material		Drug	Antigen	Route	Regimen	Disease	Model	Citation
PLGA	450 nm	N/A	No	i.v.	Prophylactic	EAE	RR EAE	[75]
PLGA	150 nm	PHCCC (Encapsulated)	No	s.c.	Prophylactic	EAE	PP EAE	[76]
PLGA+ Polymethacrylate	800 nm	IL-10 Plasmid (Encapsulated)	No	i.m.	Preventative	T1D	STZ	[77]
Zirconium Dioxide	70 nm	Betamethasone phosphate (Encapsulated)	No	i.p.	Therapeutic	EAE	PP EAE	[78]
Zinc+PEG	2μm	CD40, C80, and CD86 antisense oligonucleotides	N/A	i.p. or s.c.	Therapeutic	T1D	Adoptive Transfer	[79]
PLGA	500 nm	N/A	$PLP_{139–151}$ (Surface)	i.v.	Prophylactic & Therapeutic	EAE	RR EAE	[62]
PLGA + PEMA	450 nm	N/A	$PLP_{139–151}$ (Surface)	i.v.	Prophylactic	EAE	RR EAE	[80, 81]
PLGA + PEMA	500 nm	N/A	BDC2.5 (Mim) (Encapsulated)	i.v.	Preventative	T1D	Adoptive Transfer	[82]
PLC	200–600 nm	N/A	rhMBP (Encapsulated)	s.c.	Prophylactic	EAE	PP EAE	[83]
RGD+ Mannose Modified Chitosan	320 nm	N/A	Heat shock protein 65–6xP227 (Encapulated	Oral	Preventative	T1D	NOD	[84]
Iron Oxide	70 nm	N/A	MHC-$IGRP_{13–23}$ MHC-BDC2.5 (Mim) MHC-$MOG_{35–55}$	i.v.	Preventative Reversal	T1D	NOD PP EAE	[85, 86]
PLGA + PEG	200 nm	Rapamycin (Encapsulated)	$PLP_{139–151}$ Encapsualted	s.c.	Prophylactic or Therapeutic	EAE	RR EAE	[87]
PLGA	500 nm	TGF-β (Surface)	$PLP_{139–151}$ (Encapsulated)	i.v.or s.c.	Prophylactic	EAE	RR EAE	[88]
PLGA	2 μm	Rapamycin (Encapsulated)	$MOG_{35–55}$ (Encapsulated)	Intranodal	Therapeutic	EAE	PP EAE	[89]
PLGA	200 nm	IL-10 (Encapsulated)	$PLP_{139–151}MOG_{35–55}$ (Encapsulated)	s.c.	Prophylactic & Therapeutic	EAE	RR EAE	[90]
PLGA	1, 30 μm	TGF-β, GM-CSF, VD_3 (Encapsulated)	$MOG_{35–55}$ (Encapsulated)	s.c.	Prophylactic	EAE	PP EAE	[64]

Material		Drug	Antigen	Route	Regimen	Disease	Model	Citation
PLGA	1 or 30 μm	TGF-β, GM-CSF, VD_3 (Encapsulated)	Insulin B_{9-23} Or Denatured Insulin (Encapsulated)	s.c	Preventative & Therapeutic	T1D	NOD	[91, 92]
PLGA	1.5 μm	CpG, GM-CSF (In Hydrogel)	Denatured Insulin (Encapsulated)	s.c.	Preventative	T1D	NOD	[93]
Gold + PEG	60 nm	ITE (Surface)	MOG_{35-55} (Surface)	i.p.	Prophylactic	EAE	PP EAE	[94]
Gold + PEG	60 nm	ITE (Surface)	BDC2.5 (Mim) (Surface)	i.p.	Preventative	T1D	NOD	[95]