Title

An introduction to biomaterial-based strategies for curbing autoimmunity

Journal

Experimental Biology and Medicine, 241(10)

ISSN

1535-3702

Authors

Lewis, Jamal S

Allen, Riley P

Publication Date

2016-05-01

DOI

10.1177/1535370216650294

Peer reviewed

Minireview

An introduction to biomaterial-based strategies for curbing autoimmunity

Jamal S Lewis and Riley P Allen

Department of Biomedical Engineering, University of California, Davis, CA 95616, USA
Corresponding author: Jamal S Lewis. Email: jamlewis@ucdavis.edu

Abstract

Recently, scientists have made significant progress in the development of immunotherapeutics that correct aberrant, autoimmune responses. Yet, concerns about the safety, efficacy, and wide scale applicability continue to hinder use of contemporary, immunology-based strategies. There is a clear need for therapies that finely control molecular and cellular elements of the immune system. Biomaterial engineers have taken up this challenge to develop therapeutics with selective spatial and temporal control of immune cells. In this review, we introduce the immunology of autoimmune disorders, survey the current therapeutic strategies for autoimmune diseases, and highlight the ongoing research efforts to engineer the immune system using biomaterials, for positive therapeutic outcomes in treatment of autoimmune disorders.

Keywords: Immuno-engineering, biomaterials, autoimmune disease, particulate, immunotherapy, tolerogenic

Experimental Biology and Medicine **2016; 241: 1107–1115. DOI: 10.1177/1535370216650294**

Introduction to immune tolerance

The mammalian immune system is composed of a highly coordinated network of organs and cells that serve to rid the host of materials recognized as pathogens.[1,2] Pathogens recognized by the immune system can include bacteria, viruses, and fungal cells. The immune system is divided into two sub-systems: innate immunity and adaptive immunity. The innate immune system encompasses the immediate, non-specific response. Innate immunity often involves physical barriers to pathogens (e.g. skin or mucosal epithelium), the activation of the complement system, local inflammation due to cytokine production, and the uptake of pathogens by antigen presenting cells (APCs) such as macrophages, dendritic cells (DCs), and neutrophils. Adaptive immunity, which is specific to an antigenic epitope, is associated with T and B cell activation, antibody production, and immunological memory. Adaptive immune cells also have the potential to generate an immune response against self-antigens, which can result in a loss of immune tolerance.

Immune tolerance describes *an inability to mount an inflammatory response against a self-antigen.* This inability is vital for maintenance of immune homeostasis and is accomplished by two mechanisms: central tolerance which occurs during the development of adaptive immune cells from stem cells, and peripheral tolerance, a continuous process of immune regulation throughout the host.[3] Central immune tolerance is defined by the negative selection of T and B cells that recognize self-antigens through their membrane bound receptors.[4] For the case of T cells this occurs in the thymus, and for B cells in the bone marrow.[4] Furthermore, peripheral tolerance describes induction of anergy, apoptosis, ignorance, or receptor editing outside the primary lymphoid organs. An anergic cell continually expresses unoccupied antigen receptors, yet is unresponsive to antigen stimulation.[5,6] Whereas, apoptosis is a process in which cells undergo programmed cell death and can be induced through Fas/Fas ligand interactions often found between T cells and thymic epithelial cells.[7] If adaptive lymphocytes are not exposed to their cognate antigen because it is in an immunologically privileged site (e.g. testis), they may become ignorant. Ignorant B cells that are self-reactive to sequestered antigen in inaccessible tissues are never stimulated and can eventually undergo apoptosis. However, their persistence can cause autoimmunity if antigen is released from an immunologically privileged site.[8]

Central tolerance along with peripheral tolerance are essential for the immune system to identify, differentiate, and target self and non-self-antigens.

Autoimmune disease: A breakdown in immune tolerance

When a breakdown in tolerance occurs, autoimmunity ensues. The term ''autoimmune disease'' describes a condition where the immune system responds destructively to

self-antigens, inflicting damage to the host's own tissues.[9] In this scenario, self-reactive lymphocytes avoid central and peripheral tolerance-inducing mechanisms, and mediate an inflammatory response against self-antigens. Self-antigens recognized by autoreactive lymphocytes can range in nature and can be specific to organs or ubiquitous in the body. Examples of organ targets and the various roles of immune cells in autoimmune diseases are shown in Figure 1.

Autoimmune disorders can cause tissue damage, abnormal growth, and changes in organ function. Often, when tissue is destroyed, more self-antigen is released into the periphery leading to exacerbation of the disease. This is especially the case when the antigen is hidden in an immunologically privileged site, such as the eye.[8]

T helper Type 1 (T_{h1}) cells

Autoimmunity can either be T cell or B cell-mediated. The primary orchestrator of T cell-mediated autoimmunity can be T helper Type 1 (T_{h1}), T_{h2}, or Interleukin (IL)-17 secreting T_{h17} cells. The T_{h1} response is dominant in autoimmune diseases such as type I diabetes and rheumatoid arthritis, and leads to the destruction of nerve axons in multiple sclerosis.[10] T_{h1} CD4+ cells secrete pro-inflammatory cytokines such as IL-2, interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and granulocyte-macrophage colony-stimulating factor (GMCSF), efficiently activating macrophage effector functions.[11]

T helper Type 17 (T_{h17}) cells

Another effector cell in autoimmunity, T_{h17} cells were first identified in 2005 and are the cellular source of IL-17.[12,13] T_{h17} cells and tolerance-inducing regulatory T cells (T_{regs}) counteract each other in the development of autoimmune and inflammatory diseases. Accumulation of T_{h17} cells was the first observation to support the notion that IL-17 contributes to the pathogenesis of experimental autoimmune encephalomyelitis (EAE).[14] Additionally, mice deficient in IL-17 are resistant to the onset of collagen-induced arthritis.[15] In humans, T_{h17} cells and their cytokines are also associated with several autoimmune and inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, psoriasis, and inflammatory bowel disease[12,16] (See Figure 1).

T helper Type 2 (T_{h2}) cells/B cells

T helper Type 2/B cell-mediated autoimmune responses are generally characterized by the production of IgG and IgE antibodies against a self-antigen by stimulated B cells.[11,17] Antibodies against self-antigens can create a myriad of systemic problems. Cell receptor autoantibodies can bind to signaling receptors and cause a continual stimulation of the receptor, or block stimulation of the receptor altogether. An example of an autoimmune disease that involves a receptor blocking autoantibody is myasthenia gravis, in which B cells produce autoantibodies against acetylcholine receptors in the synaptic junction, blocking receptor activation by acetylcholine released from the synaptic terminal of

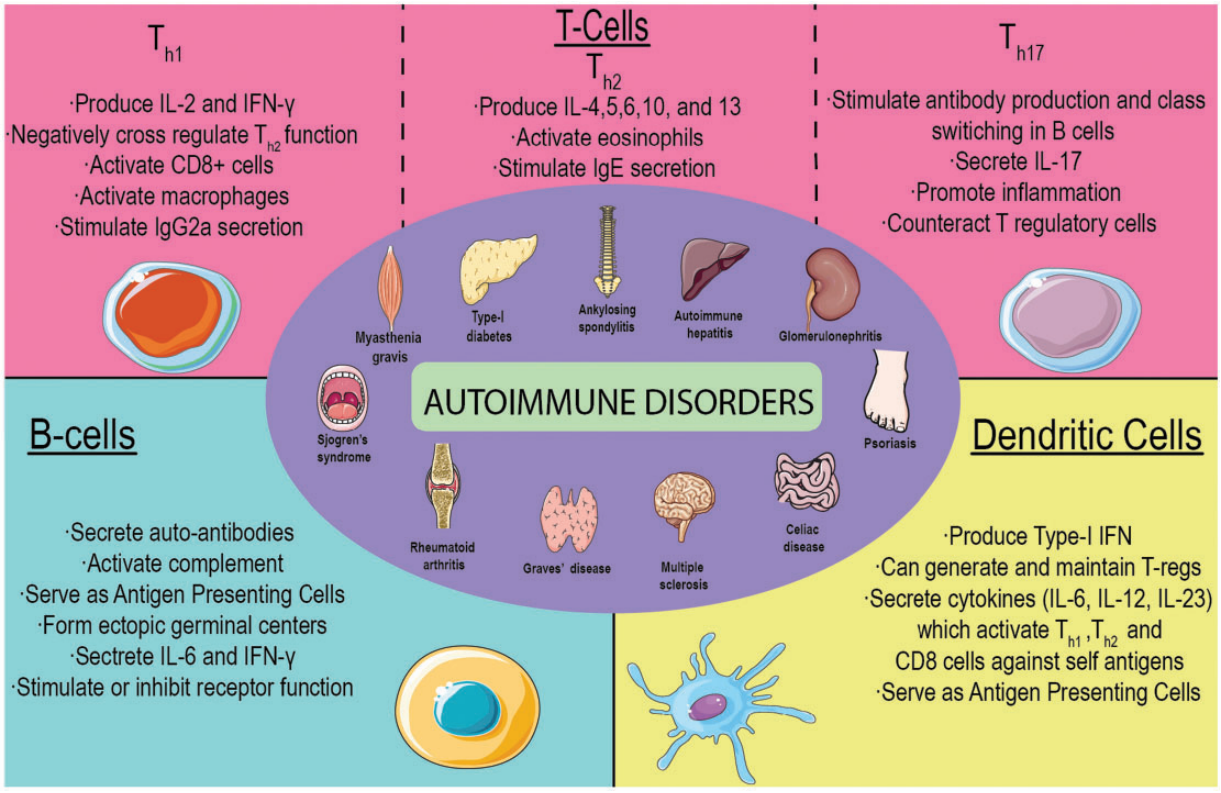

Figure 1 The general contributions of lymphocytes (B cell, T cells and dendritic cells) to the pathogenesis of autoimmune disorders, which affect multiple organs throughout the body.

the neuron.[18] In Grave's disease, the scenario is slightly different, thyroid-stimulating immunoglobulins recognize and bind to the thyrotropin receptor (TSH receptor) which stimulates the secretion of thyroxine and triiodothyronine. The result is high levels of circulating thyroid hormones and hyperthyroidism.[19] It should be noted that T cell subsets typically work in concert with other T cell subsets and immune cell types (e.g. B cells) in the development of autoimmune diseases (See Figure 1).[20] For instance, in type I diabetes, it is evident that T_{h1} and T_{h2} -derived cytokines cooperate to drive β-islet-cell destruction.[20]

At its core, the development of autoimmunity is due to a breakdown in the immune self-recognition. It has been reported that this breakdown is catalyzed by different factors including: genetic abnormalities in the human leukocyte antigen (HLA) regions, newly exposed antigens in the body, infections that overcome tolerance, pathogenic molecular mimicry, development of altered self-antigens due to binding of molecules to cell surfaces, or hormone imbalances.[9,21,22]

Current strategies for immunotherapy of autoimmune diseases

Over the last quarter century, immunotherapy has come to the forefront of basic and clinical research as a leading candidate for the cure of a broad array of immune conditions. In the context of autoimmune diseases, immunotherapy is defined as *a strategy that either interrupts immune dysregulation, or induces specific immune tolerance to self-antigens.* This form of immunologic intervention was first launched in the early 1980s, after clinicians recognized that polyclonal IgG immunoglobulin (IVIG) improved autoimmune thrombocytopenia in patients being treated for immunodeficiency.[23] Today, autoimmune disease treatment accounts for greater than 70% of IVIG clinical use in the US. In addition to IVIG, a host of immuno-modulatory agents and strategies have been developed to curb the increasingly prevalent epidemic of autoimmune and auto-inflammatory disease. Coinciding with breakthroughs in molecular biology, immunologists have created novel immunotherapeutic approaches that can be broadly categorized as non-specific molecular immuno-modulation (e.g. IL-1 agonist), effector cell depletion (e.g. anti-thymocyte globulin [ATG]), and regulatory cell adoptive transfer (e.g. regulatory T cell therapy).

Non-specific molecular immuno-modulation

Non-specific immuno-modulation typically involves the administration of a single agent, or combination of factors with capacity to block auto-inflammatory pathways, induce regulatory cascades, or sequester soluble mediators of autoimmunity. Table 1 describes a number of molecular immuno-modulators that are currently in clinical use. These drugs are typically classified as disease-modifying agents (DMAs), or biological response modifiers (BRMs) based on their ability to modify inflammatory elements of the disease. Moreover, DMAs tend to be non-biologic drugs with wide-ranging effects on immune and other cell types. However, administration of this group of drugs can have adverse side effects, which inspired research to identify and develop immunotherapeutics that more selectively halt the inflammatory mechanisms of autoimmune disease.

Pioneered in the early 1980s, the next generation of immunotherapeutics was predominantly BRMs. These biologics

Table 1 Examples of non-specific molecular immuno-modulators approved for use in autoimmune disease therapy

Name	Type	Target	Mechanism of action	Clinical use
Glucocorticoids (GC)	Non-biologic; steroid	General; GC receptors	NF-κB inhibition; regulatory regions of DNA; suppression of inflammatory cytokine synthesis	RA
Methotrexate	Non-biologic; folic acid analogue	General; folate receptors	Inhibits folate metabolism; increases adenosine (potent immuno-supressor)	RA, JIA, SLE, JD, PS, PA, RA, CrD
Mycophenolate mofetil	Non-biologic; antibiotic from *Penicillium* sp	Guanosine Monophosphate (GMP) synthesis	Inhibits proliferation of T and B lymphocytes; inhibits production of cytokines from T cells, B cell, DCs/ macrophages	SLE, TR, LN
Abatacept	Biologic; CTLA-4 fusion protein	CD80/ CD86	Blocks co-stimulatory molecule binding with CD28 thereby preventing T cell activation.	RA, JIA
Adalimumab	Biologic; human monoclonal antibody	TNF-α	Inhibits inflammatory cytokine – TNF-α	RA, JIA, AS, PsA
Anakinra	Biologic	IL-1	Competitive inhibition of IL-1R	RA
Basiliximab	Biologic; chimeric monoclonal antibody	CD25 (α subunit of IL-2R)	Competitive inhibition of IL-2	T1D, TR

RA: rheumatoid arthritis; JIA: juvenile idiopathic arthritis; SLE: systemic lupus erythematosus; JD: juvenile dermatomyositis; PS: psoriasis; PsA: psoriatic arthritis; CrD: Crohn's disease, TR: transplant rejection; LN: lupus nephritis; T1D: type 1 diabetes.

were bioengineered recombinant proteins and monoclonal antibody products designed to suppress inflammatory responses to self-antigen by targeting critical mediators, such as cytokine receptors (e.g. IL-2 receptor alpha subunit [IL-2Rα; CD25]). IL-2, a key cytokine for the differentiation, survival and functionality of regulatory T cells, was one of the first bioengineered proteins for immunotherapy. Pleiotropic in nature, IL-2 can be both anti-inflammatory and pro-inflammatory, and was first used at high doses to treat cancer.[24] Ironically, research on IL-2 as a cancer therapeutic lead to the discovery that at very low doses, it could bolster the regulatory activity of T_{regs}.[25,26] Clinical trials are currently underway to establish whether low-dose IL-2 could restore the T_{reg}/ effector T cell (T_{eff}) ratio in diseased patients and, thereby be an effective therapeutic for type 1 diabetes and other autoimmune conditions (ClinicalTrials.gov Identifier: NCT02265809). Other examples of BRMs include cytokine inhibitors, such as Adalimumab and Rilonacept, that bind disease-propagating cytokines.[27,28]

Though sales of cytokine inhibitors are now a considerable percentage of the immunotherapeutic market, this class of drugs is not without its share of adverse effects.[29] Reactions at the site of injection are the most common side effects, but can be managed via pretreatment with antihistamines and steroids. However, other more serious reactions, though infrequent, have occurred with the use of cytokine inhibitors including anaphylaxis, thrombocytopenia, neutropenia, heart disease, and induction of autoimmune disorders.[27] Another major side effect is risk of serious infections, particularly bronchial and pulmonary.[30]

Effector cell depletion

It has long been recognized that various subsets of T- and B cells play an important role in the propagation of autoimmune disease and transplant rejection. As such, agents directed at inhibition of these effector cells have been developed. Most notably, antibodies that directly eliminate or suppress the functions of effector immune cells have been used as therapeutic agents for an array of autoimmune disorders. For instance, Alemtuzumab (Lemtrada®), a monoclonal antibody against CD52, is currently used for treatment of multiple sclerosis and organ rejection. CD52 is a ubiquitous receptor found on the surface of lymphocytes and monocytes, but, not on their respective progenitors.[31] Treatment with Alemtuzumab, results in rapid depletion of mature lymphocytes and significant lymphopenia.[32] Unaffected lymphoid progenitor cells then proliferate and replace lymphocytes lost to this treatment. This immune cell reconstitution is dominated by the emergence of regulatory T cells that suppress autoimmune responses.[32] Other monoclonal antibody therapies, such as Teplizumab and Rituximab aim at deleting autoreactive T cells or B cells and increasing the presence of T_{regs}.[33,34]

Predictably, immune cell ablation is often accompanied by severe side effects including risk of infection, induction of carcinogenesis, autoantibody formation, cytopenia, and acute demyelinating complications. Milder effects such as infusion/injection site reactions and abnormalities in lipid profiles are also frequently observed in patients treated with immune cell ablation therapies.[27]

Regulatory cell adoptive augmentation

The development of immunotherapeutics that prompt specific immune responses while minimizing harmful complications is currently a priority for the clinical community. These design criteria, along with a new emphasis on developing patient-centered therapeutic regimens, have led to the development of cell-based therapies for treatment of autoimmune disorders.

There is now an overwhelming body of work that supports the notion that regulatory T cells are critical for controlling autoimmunity and inducing tolerance. Regulatory T cells suppress the activities of self-destructive effector immune cells via mechanisms involving cell–cell contact as well as soluble mediators.[35] Moreover, work by Brusko et al. and others demonstrated that the frequency of functional T_{regs} is significantly reduced in multiple autoimmune disorders.[36,37] Thus, it is not surprising that the researchers have focused on developing T_{regs} as a cell therapy product for the treatment of autoimmune disease. Efforts to translate the success of T_{reg} infusion therapy in animal models of autoimmune disease to humans are being led by the Jeffrey Bluestone group. They first reported that ex vivo-expanded T_{regs} maintained a regulatory profile (high levels of CD25, CD62L, FoxP3 and CTLA-4) and suppressed proliferation of effector T cells in vitro.[38] Further, adoptive transfer of antigen-specific BDC2.5 T_{regs} into new onset, diabetic NOD mice reversed hyperglycemia at a rate of 60%.[38] Clinically, investigators have established that polyclonal T_{reg} therapy is effective for the prevention of graft-versus-host disease (GVHD)[39] and improves β cell function in children with type 1 diabetes.[40] There are currently 12 clinical trials in progress on the safety and efficacy of polyclonal T_{reg} therapy. However, serious concerns on the feasibility of this approach still remain. Especially, given that large-scale production of antigen-specific T_{regs} for autoimmune disease therapy is difficult because of their low bioavailability and lack of stability during expansion.

Another viable cell therapeutic approach that is currently under investigation is dendritic cell-based therapy. Tolerogenic DCs (tDCs) can promote central and peripheral tolerance via a number of mechanisms including T cell anergy, T_{reg} generation, and effector T cell deletion.[41,42] These tDCs are now under intense investigation for use as ''live vaccines'' in antigen-specific immunotherapy and transplantation medicine. In this personalized form of medicine, patient-derived DCs are manipulated ex vivo to a tolerogenic phenotype and then re-infused into the patient as a cellular drug.[42,43] A phase I clinical trial in type 1 diabetics revealed that tDC vaccination is well tolerable and does not induce autoantibody formation. Additionally, beneficial immune responses observed were increased plasma levels of IL-4 and IL-10, and increased frequency of suppressive B220+CD11c-B cells.[44]

Cell-based therapies hold great promise for the generation of antigen-specific tolerance in patients with self-destructive diseases, without the adverse effects that

commonly accompany other immunotherapeutic techniques. However, issues such as consistency and stability of immunophenotype, and exceedingly high manufacturing costs may restrict the broad utilization of this therapeutic approach.[45]

Other immunotherapeutic strategies for autoimmunity that are receiving considerable attention include post-cytokine receptor signaling interference and immune system regeneration using stem cells. These approaches were excellently reviewed by Patterson et al.,[46] and Munir et al.[47] respectively.

Biomaterial-based tolerogenic immunotherapy

Traditionally, the host immune system has been viewed with contempt by biomaterial scientists, due to its reactions, including the foreign body response (FBR), that typically degrade and destroy the functionality of implanted materials and/or devices (e.g. insulin pump). However, there is now recognition that biomaterials can be engineered to manipulate the immune system for therapeutic and diagnostic applications. Biomaterial surface chemistry, surface topography, microscale architecture, and other physiochemical properties have been controlled to either mitigate or propagate immune responses. Herein, we focus on strategies that researchers have employed to dampen self-generated immunity with special emphasis on particulate engineering, tolerogenic drug delivery, and lymph node conditioning.

Particulate engineering for tolerogenic outcomes

With new insights into the immunobiology of APCs, there is now a concentrated effort to develop particulate vehicles for the delivery of antigen and adjuvant. Particulate uptake, antigen processing, antigen presentation, and T cell activation are critical immunological processes that are all innate to APCs, particularly DCs. Additionally, particulate delivery systems protect their cargo from degradation and rapid clearance.[45] Moreover, particle characteristics can influence these processes and therefore direct immunological outcomes.

One particulate parameter that has been manipulated to influence APC functionality and downstream immune responses is size. Particulate vaccines typically vary from 10 nm to 500 μm in diameter, which is similar to the size range of microbial organisms, against which APCs have evolved to purge from the host.[48] Particles below the 500 nm size range can be taken up by endocytosis, a process common to most cells. On the other hand, microparticles (MPs) in the 0.5–7 μm size are readily eaten by APCs through a unique process called phagocytosis.[48] Internalization of particulates by phagocytosis is critical for immune modulation, as following uptake particles enter the phagosome which promotes antigen cross-presentation.[49] Cross-presentation by APCs is the phenomenon where exogenously derived (located outside the cell) antigen is displayed on MHC class I molecules to CD8+ T cells. This process may be important for enlisting all the facets of the adaptive immune system and, mounting effective tolerogenicity.[41] Contrastingly, antigen internalized via endocytosis is poorly cross-presented.[50] Other physical parameters that should be considered in designing particulate vaccines for tolerogenic applications include shape, surface chemistry, and hydrophobicity. These factors have been shown to significantly alter interactions with APCs, as well as, influence their phenotype.[48,51] For example, Champion et al.[52] demonstrated that worm-like particles with very high aspect ratios (>20) exhibits significantly lower phagocytosis by macrophages in comparison to spherical particles of equal volume. With respect to particle surface chemistry, various groups have reported that the surface charge of particles significantly influenced the maturation status and cytokine secretion of engulfing DCs. Jilek et al.[53] showed that exposure of human-derived DCs to anionic poly (lactic-co-glycolic acid) (PLGA) MPs significantly upregulated expression of co-stimulatory markers (CD83 and CD86) and increased secretion of pro-inflammatory cytokines – IL-12 and TNF-α. The hydrophobicity factor, which is interrelated with surface chemistry, is another design consideration for particulate vaccines. Plasma proteins (e.g. immunoglobulin, complement) quickly adsorb onto the surface of hydrophobic particles in the body, thereby earmarking them for clearance by the reticuloendothelial system.[54] Evidently, passive adsorption of proteins onto the surface of particulate vaccines could reduce their in vivo half-life and diminish their therapeutic or diagnostic purposes. Addition of polyethylene glycol (PEG) is a popular approach adopted by researchers to increase the hydrophilicity of particles and thus reduce passive adsorption of plasma proteins.[55,56] PEGylation of particulates has been extensively studied and has been shown to effectively reduce uptake by phagocytic cells in vitro, extend circulation half-life in vivo, and decrease the accumulation of nanoparticles (NPs) in the liver.[57,58] Interestingly, hydrophilicity may be an important factor for pushing suppression of autoimmune reactions. A report by Liu et al.[59] suggested that increased hydrophobicity of PLGA particles promotes particulate uptake, and expression of positively stimulatory surfaces molecules in bone marrow-derived DCs.

The surface chemistry can also be exploited for active targeting of biomaterial-based particulates to specific immune cells. Active targeting of particulates may improve cargo delivery, reduce harmful off-target effects, and initiate immune receptor-signaling pathways.[2] Particulate active targeting can be accomplished by surface absorption or ligation of ligands with high affinity for specific molecules found on the surface of immune cells. In the context of tolerogenic applications, it is vital that engagement of these surface receptors does not stimulate inflammatory pathways. To this end, work done by the Keselowsky[60] group showed that PLGA microspheres with surface-immobilized ligands (DEC205 mAb, CD11c mAb and P-D2 peptide) enhanced particulate uptake by DCs in vitro and in vivo without inducing DC maturation. Santamaria and co-workers[61] further exploited surface modification of biomaterial particulates in designing a novel, artificial

antigen-presenting cell system for remission of auto-immune disorders.

Delivery of tolerance-inducing agents

A prominent, biomaterial-based strategy for manipulation of the immune system is controlled release of immuno-modulatory agents from particulates. Polymeric particles, liposomes, and hydrogels have been used to deliver an array of immuno-modulatory agents, including antigen,[62] nucleic acids,[63] cytokines,[64] and pharmacological drugs,[65] for immunotherapy of auto-inflammatory diseases. As described above, current therapeutic strategies for self-generated inflammation are broadly based on non-specific, molecular or cellular ablation and regulatory immune cell augmentation and are typically accompanied by adverse side effects (e.g. cytokine-release syndrome) or fail to meet required safety standards for biotechnology products. Biomaterial-based particulate systems offer direct delivery of immuno-modulators to specific immune cells for induction of long-term tolerance or immuno-suppression.

Most classically, poly (lactic-co-glycolic acid) (PLGA; or variants thereof) particulates, ranging from nano- to micro-scale in size, have been used as immunotherapeutic, delivery systems. This biopolymer can be tailored by varying lactic to glycolic acid composition and molecular weight. Favorable qualities of PLGA include biocompatibility, biodegradability, and control of physiochemical properties, i.e. size, shape, hydrophobicity, loading, and release kinetics of a wide range of biomolecules. Additionally, reports have indicated that certain formulations of PLGA may inhibit inflammatory immune reactions.[66,67] However, others have contradicted this notion and suggested that PLGA is intrinsically immunogenic.[68] Recently, Phillips and his co-workers. demonstrated that PLGA microspheres loaded with anti-sense oligonucleotides, for co-stimulatory molecules (CD40, CD80, and CD86), passively targeted DCs and manipulated their immuno-regulatory function. This MP system protected NOD mice from T1D by suppressing the expression of these positively stimulatory molecules in APCs that intercepted microspheres. Similarly, Lewis et al.[67] showed that a combinatorial MP system can modify immune cell phenotype and prevent autoimmune diabetes in NOD mice, by delivering a payload of immuno-modulatory agents. They developed a unique system consisting of two classes of PLGA MPs (based on size) – phagocytosable MPs delivering antigen (denatured insulin) and tolerizing drug (vitamin D3) to intracellular receptors, and unphagocytosable MPs to extracellularly deliver DC recruitment and immuno-suppressive biological factors (GM-CSF, TGF-β1). In pre-diabetic NOD mice, this particulate therapy prevented the development of auto-reactive diabetes, though it was unclear what mechanisms are involved in this observed protection.[67] This same group of researchers formulated a combination hydrogel-microparticle vaccine, which included an inflammatory adjuvant, for treatment of type 1 diabetes. They found that a vaccine delivery system, comprising a peptide-based hydrogel loaded with GM-CSF, CpG (unmethylated linear DNA sequence of cytosine nucleotide phosphate-linked to guanine nucleotide), and PLGA MPs encapsulating denatured insulin, significantly prevented autoimmune diabetes onset in pre-diabetic NOD mice. Mechanistically, they postulated that CpG promoted the secretion of the anti-inflammatory cytokine IL-10 from hydrogel-infiltrating DCs, which also captured MP-encapsulated self-antigen.[69] Immuno-engineers have also utilized ''nanogel'' particulates for effective delivery of small molecule drugs to immune cells as a therapeutic measure for systemic lupus erythematosus. Systemic lupus erythematosus is a multi-organ, autoimmune disorder where dysregulated activation of immune cell subsets leads to auto-antibody accumulation in various tissues, which is followed by tissue-destructive inflammation.[70] Following intravenous administration, nano-sized particles amass in several tissues, including those commonly affected in SLE (e.g. lungs, liver). Thus, it is rational to use NPs for co-localization of DMAs with self-reactive, cellular mediators of lupus. Look et al.[71] adopted this approach and established that mycophenolic acid (MPA)-loaded nanogels effectively treated murine lupus.[71]

Existing limitations to tolerogenic MP delivery formulations include the bulk production of particulates, as the large-scale manufacture is often inefficient, difficult, and costly. Additionally, the bioactivity of the encapsulated and surface-immobilized agents can be significantly reduced due to harsh environments these factors may be exposed to during the particle generation process.[45]

Tolerogenic conditioning of lymph nodes

An emerging strategy for autoimmune disease immunotherapy focuses on engineered particulates that release tolerizing drug payloads into secondary lymphoid organs, such as lymph nodes (LNs). The LN is considered the ''command center'' of the immune system, due to the high density of T and B cells housed within these anatomic structures. Moreover, it is where naïve T cells and B cells are primed by APCs such as DCs to initiate adaptive immunity.[72] Additionally, B cells can capture antigen directly in the LN and produce associated antibodies.[73] Typically, vaccines are administered via intramuscular or subcutaneous routes, and are then translocated to the LN via phagocytic cells or lymphatic conduits. Ostensibly, there is significant loss and degradation of antigen and adjuvant during this process, leading to ineffective immunity and other undesirable off-target effects. Precise delivery of vaccine components to the LN promises to boost desired immune responses. To achieve this goal, researchers have applied principles of biomaterial particulate engineering. For example, the research groups of Jeffrey Hubbell and Melody Swartz engineered poly (propylene sulfide) nanoparticles (PPS NPs) for conveying vaccine antigens to LN-resident DCs. Their studies indicated that PPS NPs were passively trafficked to draining lymph nodes via interstitial fluid flow through the lymphatics following subcutaneous injection. Moreover, antigen conjugated to these 20 nm PPS NPs elicited levels of cellular and humoral immunity comparable a co-injection of antigen and lipopolysaccharide (LPS), a powerful immunogenic adjuvant.[74,75] Although these

studies focused on generating immunity, it is reasonable to suggest that this approach with careful selection of adjuvant(s) could induce tolerance directly at the LN. Another direct strategy that may yield positive therapeutic outcomes in an autoimmune setting is intra-lymph nodal (i.LN) injection of controlled release particulates. The Jewell research group showed that i.LN immunization with slow release-formulated adjuvants is a useful approach to prolong and boost prophylactic vaccines while reducing harmful off-target and systemic effects.[76]

Conclusions and outlook

The incidence of autoimmune diseases continues to rise every year, with current remedies either therapeutically inadequate, or accompanied by adverse effects that negate the benefits of their administration. Biomaterial-based immune-engineering holds great potential for rational design of effective and safe immunotherapeutics for autoimmune disorders. Biomaterials can offer a platform for precise spatial and temporal control of immune system-immunotherapeutic interactions. Additionally, biomaterials with inherent immune-modulatory properties are currently under investigation for selective regulation of autoimmune reactions. Although this review focused on the application of biomaterials for immunotherapy of autoimmune disease, biomaterial strategies can also be helpful for building models to better understand the pathogenesis of autoimmune disorders. However, the promise of biomaterial-based modulation of the immune system will never come to fruition if certain challenges are not addressed. Most notably, manufacturability (cost and scale-up of clinical grade materials) of these technologies and elucidation of the intrinsic immunogenicity of biomaterial products in vivo are significant hurdles that must be overcome moving forward to full translation.

Author contributions: JSL and RPA designed and wrote the manuscript. JSL had primary responsibility for its final content.

ACKNOWLEDGEMENTS

JSL gratefully acknowledges the grant support from the National Institutes of Health (award – R43DK100132).

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu Y, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Immunology* 2000;**18**:767–811
2. Lewis JS, Roy K, Keselowsky BG. Materials that harness and modulate the immune system. *MRS Bull* 2014;**39**:25–34
3. von Boehmer H. The developmental biology function and specificity of lymphocyte subsets. *Ann Rev Immunol* 1988;**6**:309–26
4. Nossal GJV. Negative selection of lymphocytes. *Cell* 1994;**76**:229–39
5. Andrews S. The anergic B cell. *Blood* 2010;**115**:4–6
6. Yarkoni Y, Getahun A, Cambier JC. Molecular underpinning of B-cell anergy. *Immunol Rev* 2010;**237**:249–63
7. Castro JE, Listman JA, Jacobson BA, Wang Y, Lopez PA, Ju S, Finn PW, Perkins DL. Fas modulation of apoptosis during negative selection of thymocytes. *Immunity* 1996;**5**:617–27
8. Forrester JV, Xu H, Lambe T, Cornall R. Immune privilege or privileged immunity? *Mucosal Immunol* 2008;**1**:372–81
9. Kamradt T, Mitchison NA. Tolerance and autoimmunity. *N Engl J Med* 2001;**344**:655–64
10. Aarvak T, Chabaud M, Thoen J, Miossec P, Natvig JB. Changes in the Th1 or Th2 cytokine dominance in the synovium of rheumatoid arthritis (RA): a kinetic study of the Th subsets in one unusual RA patient. *Rheumatology (Oxford)* 2000;**39**:513–22
11. Charlton B, Lafferty KJ. The Th1/Th2 balance in autoimmunity. *Curr Opin Immunol* 1995;**7**:793–8
12. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. *Autoimmun Rev* 2014;**13**:668–77
13. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang Y-H, Wang Y, Hood L, Zhu Z, Tian Q, Dong C. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005;**6**:1133–41
14. Kang Z, Wang C, Zepp J, Wu L, Sun K, Zhao J, Chandrasekharan U, DiCorleto P, Trapp B, Ransohoff R, Li X. Act1 mediates IL-17-induced EAE pathogenesis selectively in NG2+ glial cells. *Nature Neuroscience* 2013;**16**:1401–08
15. Nakae S, Nambu A, Sudo K, Iwakura Y. Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J Immunol* 2003;**171**:6173–77
16. Maddur MS, Miossec P, Kaveri SV, Bayry J. Th17 Cells. *Am J Pathol* 2012;**181**:8–18
17. Candon S, McHugh RS, Foucras G, Natarajan K, Shevach EM, Margulies DH. Spontaneous organ-specific Th2-mediated autoimmunity in TCR transgenic mice. *J Immunol* 2004;**172**:2917–24
18. Vrolix K, Fraussen J, Molenaar PC, Losen M, Somers V, Stinissen P, De Baets MH, Martínez-Martínez P. The auto-antigen repertoire in myasthenia gravis. *Autoimmunity* 2010;**43**:380–400
19. Chen CR, Pichurin P, Nagayama Y, Latrofa F, Rapoport B, McLachlan SM. The thyrotropin receptor autoantigen in Graves disease is the culprit as well as the victim. *J Clin Invest* 2003;**111**:1897–1904
20. Azar ST, Tamim H, Beyhum HN, Habbal MZ, Almawi WY. Type I (insulin-dependent) diabetes is a Th1- and Th2-mediated autoimmune disease. *Clin Diagn Lab Immunol* 1999;**6**:306–10
21. Mccarthy DP, Richards MH, Miller SD. Mouse models of multiple sclerosis: experimental autoimmune encephalomyelitis and Theiler's virus-induced demyelinating disease. *Methods Mol Biol* 2012;**900**:1–19
22. Von Boehmer H. Central tolerance: essential for preventing autoimmune disease? *Eur J Immunol* 2009;**39**:2313–6
23. Imbach P, Barandun S, Cottier H, Gugler E, Hassig A, Morell A, Wagner H, Heiniger H. Immunomodulation by intravenous immunoglobulin. *Am J Pediatr Hematol Oncol* 1990;**12**:134–40
24. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev Immunol* 2012;**12**:180–90
25. Rouse M, Nagarkatti M, Nagarkatti PS. The role of IL-2 in the activation and expansion of regulatory T-cells and the development of experimental autoimmune encephalomyelitis. *Immunobiology* 2013;**218**:674–82
26. Nelson BH. IL-2, Regulatory T Cells, and tolerance. *J Immunol* 2004;**172**:3983–8
27. Ostrov BE. Immunotherapeutic biologic agents in autoimmune and autoinflammatory diseases. *Immunol Invest* 2015;**44**:777–802
28. Lapadula G, Marchesoni A, Armuzzi A, Blandizzi C, Caporali R, Chimenti S, Cimaz R, Cimino L, Gionchetti P, Girolomoni G, Lionetti P, Marcellusi A, Mennini FS, Salvarani C. Adalimumab in the treatment of immune-mediated diseases. *Int J Immunopathol Pharmacol* 2014;**27**(1 Suppl): 33–48
29. O'Shea JJ, Kanno Y, Chan AC. In search of magic bullets: the golden age of immunotherapeutics. *Cell* 2014;**157**:227–40
30. Dinarello CA. Anti-cytokine therapeutics and infections. *Vaccine* 2003;**21**(Suppl 2): 24–34

31. Osuji N, Del Giudice I, Matutes E, Morilla a, Owusu-Ankomah K, Morilla R, Dunlop a, Catovksy D. CD52 expression in T-cell large granular lymphocyte leukemia – implications for treatment with alemtuzumab. *Leuk Lymphoma* 2005;**46**:723–7
32. Zhang X, Tao Y, Chopra M, Ahn M, Marcus KL, Choudhary N, Zhu H, Markovic-Plese S. Differential reconstitution of T cell subsets following immunodepleting treatment with alemtuzumab (anti-CD52 monoclonal antibody) in patients with relapsing-remitting multiple sclerosis. *J Immunol* 2013;**191**:5867–74
33. Virgolini L, Marzocchi V. Rituximab in autoimmune diseases. *Biomed Pharmacother* 2004;**58**:299–309
34. Chatenoud L, Bluestone JA. CD3-specific antibodies: a portal to the treatment of autoimmunity. *Nat Rev Immunol* 2007;**7**:622–32
35. Horwitz DA, Zheng SG, Gray JD, Wang JH, Ohtsuka K, Yamagiwa S. Regulatory T cells generated ex vivo as an approach for the therapy of autoimmune disease. *Semin Immunol* 2004;**16**:135–43
36. Longhi MS, Ma Y, Bogdanos DP, Cheeseman P, Mieli-Vergani G, Vergani D. Impairment of CD4+CD25+ regulatory T-cells in autoimmune liver disease. *J Hepatol* 2004;**41**:31–7
37. Brusko TM, Wasserfall CH, Clare-Salzler MJ, Schatz DA, Atkinson MA. Functional defects and the influence of age on the frequency of CD4+CD25+ T-cells in Type 1 diabetes. *Diabetes* 2005;**54**:1407–14
38. Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, Masteller EL, McDevitt H, Bonyhadi M, Bluestone JA. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med* 2004;**199**:1455–65
39. Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, DeFor T, Levine BL, June CH, Rubinstein P, McGlave PB, Blazar BR, Wagner JE. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: Safety profile and detection kinetics. *Blood* 2011;**117**:1061–70
40. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, Grabowska M, Techmanska I, Juscinska J, Wujtewicz MA, Witkowski P, Mlynarski W, Balcerska A, Mysliwska J, Trzonkowski P. Administration of CD4+CD25highCD127-regulatory T cells preserves β-cell function in type 1 diabetes in children. *Diabetes Care* 2012;**35**:1817–20
41. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Ann Rev Immunol* 2003;**21**:685–711
42. Giannoukakis N. Tolerogenic dendritic cells for Type 1 diabetes. *Immunotherapy* 2013;**5**:569–71
43. Suwandi J, Toes R, Nikolic T, Roep BO. Inducing tissue specific tolerance in autoimmune disease with tolerogenic dendritic cells. *Clin Exp Rheumatol* 2015;**33**:S97–103
44. Giannoukakis N, Phillips B, Finegold D, Harnaha J, Trucco M. Phase I (safety) study of autologous tolerogenic dendritic cells in type 1 diabetic patients. *Diabetes Care* 2011;**34**:2026–32
45. Keselowsky BG, Xia CQ, Clare-Salzler M. Multifunctional dendritic cell-targeting polymeric microparticles. *Hum Vaccin* 2011;**7**:37–44
46. Patterson H, Nibbs R, Mcinnes I, Siebert S. Protein kinase inhibitors in the treatment of inflammatory and autoimmune diseases. *Clin Exp Immunol* 2014;**176**:1–10
47. Munir H, McGettrcik H. Mesenchymal stem cell therapy for autoimmune disease: risks and rewards. *Stem Cells Dev* 2015;**24**:2091–100
48. De Temmerman ML, Rejman J, Demeester J, Irvine DJ, Gander B, De Smedt SC. Particulate vaccines: on the quest for optimal delivery and immune response. *Drug Discov Today* 2011;**16**:569–82
49. Yang YW, Hsu PYJ. The effect of poly(d,l-lactide-co-glycolide) microparticles with polyelectrolyte self-assembled multilayer surfaces on the cross-presentation of exogenous antigens. *Biomaterials* 2008;**29**:2516–26
50. Tran KK, Shen H. The role of phagosomal pH on the size-dependent efficiency of cross-presentation by dendritic cells. *Biomaterials* 2009;**30**:1356–62
51. Yoo J-W, Irvine DJ, Discher DE, Mitragotri S. Bio-inspired, bioengineered and biomimetic drug delivery carriers. *Nat Rev Drug Discov* 2011;**10**:521–35
52. Champion JA, Mitragotri S. Shape induced inhibition of phagocytosis of polymer particles. *Pharm Res* 2009;**26**:244–9
53. Jilek S, Ulrich M, Merkle HP, Walter E. Composition and surface charge of DNA-loaded microparticles determine maturation and cytokine secretion in human dendritic cells. *Pharm Res* 2004;**21**:8
54. Neoh KG, Kang ET. Functionalization of inorganic nanoparticles with polymers for stealth biomedical applications. *Polym Chem* 2011;**2**:747
55. Lacasse FX, Filion MC, Phillips NC, Escher E, McMullen JN, Hildgen P. Influence of surface properties at biodegradable microsphere surfaces: effects on plasma protein adsorption and phagocytosis. *Pharm Res* 1998;**15**:312–7
56. Gref R, Lück M, Quellec P, Marchand M, Dellacherie E, Harnisch S, Blunk T, Müller RH. ''Stealth'' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloid Surf B Biointerf* 2000;**18**:301–13
57. Sheng Y, Liu C, Yuan Y, Tao X, Yang F, Shan X, Zhou H, Xu F. Long-circulating polymeric nanoparticles bearing a combinatorial coating of PEG and water-soluble chitosan. *Biomaterials* 2009;**30**:2340–8
58. Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R. Biodegradable long-circulating polymeric nanospheres. *Science* 1994;**263**:1600–3
59. Liu Y, Yin Y, Wang L, Zhang W, Chen X, Yang X, Xu J, Ma G. Surface hydrophobicity of microparticles modulates adjuvanticity. *J Mater Chem B* 2013;**1**:3888
60. Lewis JS, Zaveri TD, Crooks CP, Keselowsky BG. Microparticle surface modifications targeting dendritic cells for non-activating applications. *Biomaterials* 2012;**33**:7221–32
61. Tsai S, Shameli A, Yamanouchi J, Clemente-Casares X, Wang J, Serra P, Yang Y, Medarova Z, Moore A, Santamaria P. Reversal of autoimmunity by boosting memory-like autoregulatory T cells. *Immunity* 2010;**32**:568–80
62. Jiang W, Gupta RK, Deshpande MC, Schwendeman SP. Biodegradable poly(lactic-co-glycolic acid) microparticles for injectable delivery of vaccine antigens. *Adv Drug Deliv Rev* 2005;**57**(3 Spec. Iss.): 391–410
63. Munier S, Messai I, Delair T, Verrier B, Ataman-Önal Y. Cationic PLA nanoparticles for DNA delivery: comparison of three surface polycations for DNA binding, protection and transfection properties. *Colloid Surf B Biointerf* 2005;**43**:163–73
64. Wang NX, Bazdar DA, Sieg SF, von Recum HA. Microparticle delivery of Interleukin-7 to boost T-cell proliferation and survival. *Biotechnol Bioeng* 2012;**109**:1835–43
65. Butoescu N, Seemayer CA, Foti M, Jordan O, Doelker E. Dexamethasone-containing PLGA superparamagnetic microparticles as carriers for the local treatment of arthritis. *Biomaterials* 2009;**30**:1772–80
66. Waeckerle-Men Y, Scandella E, Uetz-Von Allmen E, Ludewig B, Gillessen S, Merkle HP, Gander B, Groettrup M. Phenotype and functional analysis of human monocyte-derived dendritic cells loaded with biodegradable poly(lactide-co-glycolide) microspheres for immunotherapy. *J Immunol Meth* 2004;**287**:109–24
67. Lewis JS, Roche C, Zhang Y, Brusko TM, Wasserfall CH, Atkinson M, Clare-Salzler MJ, Keselowsky BG. Combinatorial delivery of immunosuppressive factors to dendritic cells using dual-sized microspheres. *J Mater Chem B Mater Biol Med* 2014;**2**:2562–74
68. Yoshida M, Babensee JE. Poly(lactic-co-glycolic acid) enhances maturation of human monocyte-derived dendritic cells. *J Biomed Mater Res Part A* 2004;**71**:45–54
69. Yoon YM, Lewis JS, Carstens MR, Campbell-Thompson M, Wasserfall CH, Atkinson MA, Keselowsky BG. A combination hydrogel microparticle-based vaccine prevents type 1 diabetes in non-obese diabetic mice. *Sci Rep* 2015;**5**:13155
70. Rahman A, Isenberg DA. Systemic lupus erythematosus. *N Engl J Med* 2008;**358**:929–39
71. Look M, Stern E, Wang QA, DiPlacido LD, Kashgarian M, Craft J, Fahmy TM. Nanogel-based delivery of mycophenolic acid ameliorates systemic lupus erythematosus in mice. *J Clin Invest* 2013;**123**:1741–9
72. Shu S, Cochran AJ, Huang R-R, Morton DL, Maecker HT. Immune responses in the draining lymph nodes against cancer: implications for immunotherapy. *Cancer Metastasis Rev* 2006;**25**:233–42

73. Suzuki K, Grigorova I, Phan TG, Kelly LM, Cyster JG. Visualizing B cell capture of cognate antigen from follicular dendritic cells. *J Exp Med* 2009;**206**:1485–93
74. Reddy ST, Rehor A, Schmoekel HG, Hubbell JA, Swartz MA. In vivo targeting of dendritic cells in lymph nodes with poly(propylene sulfide) nanoparticles. *J Control Release* 2006;**112**:26–34
75. Kourtis IC, Hirosue S, de Titta A, Kontos S, Stegmann T, Hubbell JA, Swartz MA. Peripherally administered nanoparticles target monocytic myeloid cells, secondary lymphoid organs and tumors in mice. *PLoS One* 2013;**8**:e61646
76. Jewell CM, López SCB, Irvine DJ. In situ engineering of the lymph node microenvironment via intranodal injection of adjuvant-releasing polymer particles. *Proc Natl Acad Sci U S A* 2011;**108**:15745–50