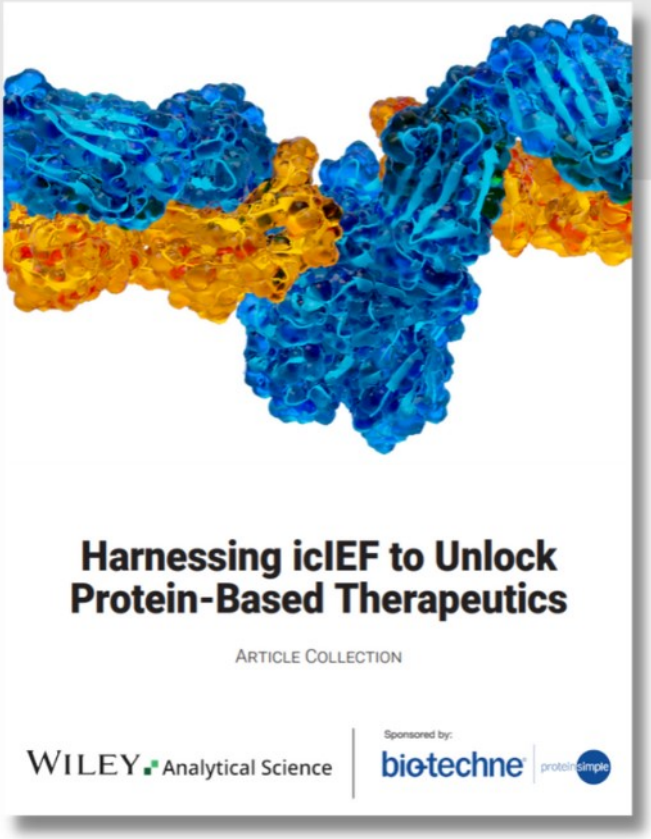
Harnessing icIEF to Unlock Protein-Based Therapeutics



Read the new Article Collection

Keep up to date with the latest developments in biotherapeutics and the range of treatments for various diseases with our latest article collection. Find out how imaged cIEF (icIEF) technique is essential for quality control and analytical development of these drugs, as it accurately determines the surface charge of lipid nanoparticles and the charge heterogeneity of proteins and antibodies.

This article collection aims to provide you with more information on these techniques and technologies, helping you further your research in this field.

bio-techne® | protein simple

WILEY

ADVANCED HEALTHCARE MATERIALS
www.advhealthmat.de

Tissue-Targeted Drug Delivery Strategies to Promote Antigen-Specific Immune Tolerance

*Yuan Rui, Haleigh B. Eppler, Alexis A. Yanes, and Christopher M. Jewell**

During autoimmunity or organ transplant rejection, the immune system attacks host or transplanted tissue, causing debilitating inflammation for millions of patients. There is no cure for most of these diseases. Further, available therapies modulate inflammation through nonspecific pathways, reducing symptoms but also compromising patients' ability to mount healthy immune responses. Recent preclinical advances to regulate immune dysfunction with vaccine-like antigen specificity reveal exciting opportunities to address the root cause of autoimmune diseases and transplant rejection. Several of these therapies are currently undergoing clinical trials, underscoring the promise of antigen-specific tolerance. Achieving antigen-specific tolerance requires precision and often combinatorial delivery of antigen, cytokines, small molecule drugs, and other immunomodulators. This can be facilitated by biomaterial technologies, which can be engineered to orient and display immunological cues, protect against degradation, and selectively deliver signals to specific tissues or cell populations. In this review, some key immune cell populations involved in autoimmunity and healthy immune tolerance are described. Opportunities for drug delivery to immunological organs are discussed, where specialized tissue-resident immune cells can be programmed to respond in unique ways toward antigens. Finally, cell- and biomaterial-based therapies to induce antigen-specific immune tolerance that are currently undergoing clinical trials are highlighted.

1. Introduction

During healthy immunity, the immune system mounts proinflammatory responses to clear pathogenic infections while maintaining tolerance toward host cells and tissues. Disruptions to this delicate balance can lead to autoimmunity, diseases during which self-reactive immune cells drive inflammation and pathology.[1] An estimated 3–5%[2] of the world's population is affected with autoimmune diseases such as multiple sclerosis (MS) and type I diabetes (T1D). Epidemiological data suggest that the prevalence of autoimmune diseases is also rising.[3] There is no cure for most autoimmune diseases, and currently available therapies tackle immunological dysfunction in a nonspecific manner. This can help slow or suppress autoimmune responses, but also healthy immunity, leaving patients susceptible to infections, certain cancers, and other immunotoxic side effects. Even monoclonal antibodies, which are molecularly specific for cell populations in autoimmune pathogenicity, cannot differentiate between healthy and self-reactive immune cells that express these markers, leading to nonspecific immunosuppression.[4] Unwanted immunosuppression is also a problem for cell or organ transplant recipients, who must undergo lifelong immunosuppressant therapy that prevents graft rejection, but also leaves these patients vulnerable to cancer[5] and infectious diseases.[6]

Therapies that promote tolerance that is specific to disease-inducing immune cells while leaving healthy immunity intact

Y. Rui, H. B. Eppler, A. A. Yanes, C. M. Jewell
Fischell Department of Bioengineering
University of Maryland
College Park, MD 20742, USA
E-mail: cmjewell@umd.edu

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adhm.202202238

DOI: 10.1002/adhm.202202238

H. B. Eppler, C. M. Jewell
Biological Sciences Training Program
University of Maryland
College Park, MD 20742, USA

C. M. Jewell
US Department of Veterans Affairs
VA Maryland Health Care System
Baltimore, MD 21201, USA

C. M. Jewell
Robert E. Fischell Institute for Biomedical Devices
College Park, MD 20742, USA

C. M. Jewell
Department of Microbiology and Immunology
University of Maryland Medical School
Baltimore, MD 21201, USA

C. M. Jewell
Marlene and Stewart Greenebaum Cancer Center
Baltimore, MD 21201, USA

could be transformative for patients with autoimmune diseases and transplant recipients. One therapeutic approach to achieve this goal is targeting only immune cells that react against specific identifying molecules – termed "antigens" – on host or transplanted cells. Promoting antigen-specific tolerance requires redirecting the immune responses to these antigens away from inflammation using combinations of immune signals such as antigen fragments, small molecule drugs, and other immunomodulatory molecules. This concept presents a unique opportunity to leverage biomaterial-based drug delivery vehicles, which offer tunable combinatorial immune signal loading and controlled drug release.[7,8]

Another important consideration is design strategies to target cargo to specific immunological tissues or resident immune cell populations. Immune cell programming occurs in specialized lymphoid organs such as the spleen and lymph nodes (LNs), as well as other immunological "hubs" (e.g., lungs, liver, and skin). These organs provide a niche for high concentrations of immune cells to encounter each other, as well as large antigen loads.[9] Biomaterial strategies targeted to these centers for immune reprogramming could promote antigen-specific tolerogenic cell populations that then circulate systemically and selectively suppress autoimmunity or transplant rejection in other parts of the body.

In this review, we first provide an overview of key immune cell subsets involved in the induction of immune tolerance, specifically in the context of autoimmunity and transplant rejection (Section 2). In Section 3, we discuss biomaterial challenges and applications relevant for selective drug delivery to tissue-resident immune cell populations. Finally, in Section 4 we highlight and provide perspective on recent clinical efforts to advance antigen-specific tolerance in patient trials, including recent biomaterials strategies.

2. Key Immune Cells Direct the Nature of Immunity across Autoimmunity and Tolerance

Healthy immunity is orchestrated through a complex interplay between the innate and adaptive immune systems, as well as tissue-specific phenomena (**Figure 1**). When this intricate balance is disrupted, autoimmune diseases can occur, and both innate and adaptive immune cells play important roles in initiating autoimmunity and perpetuating self-reactive inflammation. During healthy immunity, the innate system mounts rapid, but nonspecific, defenses against invading pathogens. Some of these reactions against microbial products have been attributed to the generation of specific autoimmune diseases, which innate immune cells can also exacerbate through localized secretion of inflammatory cytokines.[10] On the other hand, adaptive immunity is highly specific, and the difference between an inflammatory response to clear infections and healthy self-tolerance is defined by the specific antigens as well as the immunological context under which antigens are presented. The majority of our knowledge on autoimmunity centers on the role of the adaptive responses, which include auto-antibody secretion by self-reactive B cells, direct host tissue destruction by CD8+ cytotoxic T cells, and inflammatory cytokine secretion by CD4+ helper T cells. In this section, we describe several immune cell populations involved in adaptive immunity and their roles in orchestrating autoimmunity. While this list is by no means comprehensive, it highlights key populations to which bioengineers are designing targeted drug delivery strategies to facilitate antigen-specific tolerance—the topic of the subsequent sections.

2.1. Dendritic Cells

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that act at the interface between the innate and adaptive immune systems. DCs process and present antigens to lymphocytes – such as T cells and B cells – residing in specialized organs including the spleen that coordinate immune cell interactions (Figure 1a). DCs provide additional signaling to direct T cell function toward immunity or tolerance. Many of these same processes also occur in LNs, such as the B cells processes discuss later below. DCs regulate healthy immunity by recognizing pathogen associated molecular patterns on bacterial and viral pathogens, inducing T cells and natural killer cells to clear infections.[11] Significant evidence demonstrated DCs are also intimately involved in autoimmunity.[12] For example, in MS, DCs contribute to disease induction through self-antigen presentation and inflammatory cytokine secretion, leading to the expansion of pathogenic T cell subsets.[13,14]

DCs have also been shown to be critical in the induction of self-tolerance by supporting the differentiation, proliferation, and persistence of antigen-specific regulatory B cells[15] and T cells.[16,17] Specifically, tolerogenic DCs have been identified as DCs that express surface MHC-I or MHC-II molecules but have low levels of costimulatory molecule expression; this allows them to present antigen without activating inflammatory effector T cell responses, a process that is key to inducing and maintaining self-tolerance.[18] This process is important in preventing inflammation in organs where a large number of antigens are sampled. One example are lung DCs that sample a diverse array of airborne antigen without causing tissue damaging inflammation (Figure 1b). Many biomaterials are currently being developed to recruit and upregulate these tolerogenic DC and APC populations for tolerance induction (see Section 3).

The capacity for tolerogenic DCs to program antigen-specific regulatory T cells to treat autoimmune disease has also been explored in early-phase clinical trials. In a phase 1b clinical trial, Zubizarreta et al. extracted DCs from patient blood and cultured them with the anti-inflammatory drug dexamethasone as well as peptide antigens implicated in MS or autoimmune neuromyelitis optica.[19] Upon infusion back into the patient, these engineered tolerogenic DCs were well-tolerated, and patient peripheral blood mononuclear cells (PBMCs) showed significantly increased anti-inflammatory cytokine IL-10 secretion in response to ex vivo antigen re-stimulation. Additionally, regulatory T cell levels were also found to be increased 12 weeks following treatment. These promising results demonstrated that tolerogenic DCs upregulated several indicators of systemic tolerance in human patients and that these responses were antigen specific. A similar study using tolerogenic DCs loaded with proinsulin peptide antigen demonstrated safety and feasibility in T1D patients (see Section 4 below for more human trial research on antigen-specific tolerance approaches for autoimmunity).[20]

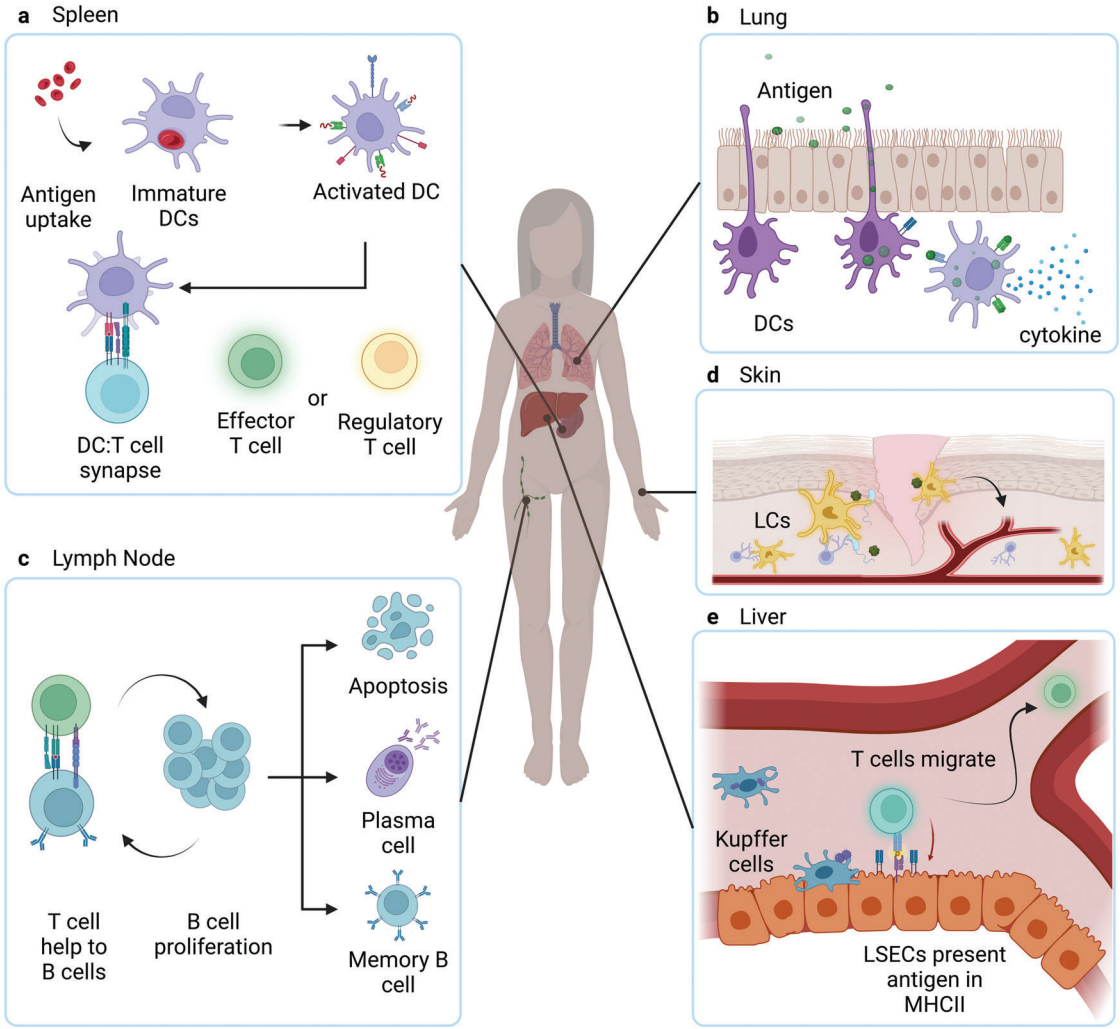


Figure 1. Secondary lymphoid organs coordinate immune cell interactions to support the development of key immune functions. Tissues contain specialized resident immune cells that protect against infection and tissue damage. A) The spleen is the largest secondary lymphoid organ thus a key part of immune development. The spleen filters aged red-blood cells from circulation providing APCs – macrophage and dendritic cells – to diverse blood born antigens. APCs can then present antigen along with costimulation to naïve T and B cells to support tolerance or inflammation. B) APCs in the lung sample inhaled air for disease-causing organisms while preventing inflammation through the secretion of tolerance associated cytokines. This process is important to prevent inflammation-driven damage to sensitive lung tissue. C) LNs are secondary lymphoid organs dispersed throughout the body. In these tissues, APCs engage resident lymphocytes such as T cells and B cells. LNs also provide structured microdomains, such as the important B cell developmental domain – germinal center. There B cells interact with T cells, proliferate, and develop into high affinity antibody producing or memory cells. B cells undergo selection pressure with self-reactive or low affinity cells undergoing apoptosis. D) The skin provides protection by offering a physical barrier rich in tissue-resident APCs. These APCs, called Langerhans Cells (LCs), are important in regulating the balance between tolerance and inflammation. One way that LCs do this is by secreting large amounts of cytokines and rapidly migrate to draining LNs where they support the development of T cells. E) The liver filters blood from the gut. Liver-resident debris clearing cells known as Kupffer cells are important in this process. These cells effectively degrade antigen without stimulating a potent immune response. Simultaneously, liver sinusoidal endothelial cells present antigen without costimulation to promote the development of regulatory T cells. Figure created with Biorender.

2.2. B Cells

Upon exposure to cognate antigen, B cells enter specialized zones in secondary lymphoid organs – including LNs – known as germinal centers to evolve antibodies with higher binding affinity for the antigen.[21] This process is crucial for the generation of robust antibody responses and durable immunological memory against pathogenic infection and during vaccination (Figure 1c).[22,23] During autoimmunity, the same process can be misdirected toward self-antigens to generate auto-antibodies, which numerous studies have shown are critical in the pathogenesis of autoimmune diseases. In MS, for example, cross-reactive B cells may be the pathogenic driver of a 32-fold increase in MS risk observed in people who have previously contracted Epstein-Barr virus (EBV).[24] Lanz et al. demonstrated that antibodies reactive against the central nervous system protein GlialCAM isolated from MS patients were cross-reactive against the EBV transcription factor EBNA1, providing a mechanistic link

between MS and EBV infection.[25] CD19/CD20 antibodies designed to deplete B cells have shown improved outcomes in patients with autoimmune optical neuromyelitis[26] and MS,[27] further demonstrating their pathogenic role in autoimmunity. On the other hand, regulatory B cell subsets have been shown to secrete anti-inflammatory cytokine IL-10[28] and suppress autoimmunity in mouse models of rheumatoid arthritis.[29,30] As further mechanisms of B cell pathogenesis are revealed, biomaterial approaches to promote tolerance through regulation of autoreactive B cell populations are currently under investigation. Furthermore, strategies to induce tolerance through promotion of regulatory B cell subsets are also being evaluated in clinical trials (see Section 4)

2.3. T Cells

There are two main subclasses of T cells: $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells. $CD8^+$ T cells directly kill damaged or infected host cells. $CD4^+$ helper T cells coordinate immunity through inflammatory cytokine secretion and activation of macrophages, B cells, and other T cells. While T cell immunity is critical in protecting against infection, self-reactive T cells drive pathology in many autoimmune diseases. $CD8^+$ T cells destroy insulin-producing β-cells in T1D,[31] while myelin-reactive $CD4^+$[32] and $CD8^+$[33,34] T cells contribute to demyelination of the central nervous system in MS. On the other hand, regulatory T cells (T_{REGS}) are a specialized subset of $CD4^+$ T cells that promote self-tolerance through the secretion of anti-inflammatory cytokines,[35,36] as well as through contact-dependent suppression of self-reactive immune cells.[37,38] Interactions with tolerance promoting antigen presenting cells can cause $CD4^+$ T cells to develop regulatory functions. For example, Langerhan cells (LCs) are key immune cell populations residing in the skin that surveil for pathogens. Simultaneously, these cells protect the skin from damage due to an unrestrained immune response. One-way LCs regulate the balance between immunity and tolerance is through a rapid response to antigen. Upon encountering an antigen, these cells can rapidly migrate to the draining LN and either promote the development of effector T cells or secrete cytokines supporting T_{REG} phenotype (Figure 1d). Loss of T_{REG} immunosuppressive function has been shown to be a major pathogenic mechanism during MS and T1D,[39,40] prompting several early-phase clinical trials to investigate the protective efficacy of patient-derived T_{REG} therapies. In these therapies, the patient's own polyclonal T_{REGS} – T_{REGS} with T cell receptors that recognize a diverse array of antigens – were expanded ex vivo and infused back into the patient (a process known as adoptive transfer therapy). Early studies have shown good safety profiles in transplantation[41,42] and T1D.[43] While the studies above demonstrate that polyclonal T_{REGS} may protect against autoimmune disease, preclinical data suggest that antigen-specific T_{REGS} may be more efficient at controlling pathological immune dysregulation.[44,45] This is likely due to the increased propensity for antigen-specific T_{REGS} to migrate toward and expand in tissues containing high concentrations of cognate antigen, which are usually sites of autoimmune-mediated inflammation. This leads to more potent and persistent control of autoimmune inflammation localized to areas under attack by pathogenic immune cells.[46]

The tolerogenic efficacy of antigen-specific T_{REGS} have recently been tested clinically in allogeneic hematopoietic stem cell transplantation (HSCT), which involves infusing donor blood cells into a genetically dissimilar recipient. Because of the genetically encoded immunological mismatch between donor and host, transplant patients are susceptible to graft-versus-host disease, in which transplanted immune cells recognize host antigens (termed "alloantigens") as foreign and mount a systemic inflammatory response. In a phase I clinical trial, Chen et al. demonstrated that donor-derived T_{REGS} could be engineered to be specific for host alloantigens by ex vivo coculture with tolerogenic DCs extracted from the host.[47] These alloantigen-specific T_{REGS} persisted in the peripheral blood of allogeneic HSCT recipients up to 1 year after transplant, showed upregulation of immune suppressive markers such as CTLA-4 and PD-1, and secreted IL-10, warranting further efficacy trials in suppressing graft-versus-host disease. While T_{REGS}-based cell therapies have shown promising results, many failed trials highlight the challenges that remain in achieving robust efficacy. One such obstacle is the purification and ex vivo expansion of antigen-specific T_{REGS}, which has a very low frequency in the peripheral blood.[48] Strategies to generate and expand antigen-specific T_{REGS} by reprogramming the patient's own immunological organs abrogates this expensive and time-consuming ex vivo cell culture process. Biomaterials that provide precise spatiotemporal control over immunomodulatory signal delivery to polarize patient immune cells toward regulatory phenotypes such as T_{REGS} are actively under investigation in preclinical studies.

3. Biomaterials Have Unique Features to Target Tissue-Specific Immune Cells for Tolerance

In the previous section, we described several important immune cell populations that are involved in orchestrating antigen-specific tolerance. Strategies that leverage the body's inherent ability to program immune cells toward tolerogenic phenotypes are an exciting approach that may overcome shortcomings in existing therapies. Biomaterials facilitate this process by enabling precision delivery of combinatorial immune cues to specific locations in the body. Several preclinical studies have used biomaterial drug delivery approaches to reprogram immunological niches where immune cells naturally encounter antigens and receive signals specifying their responses. Although these targeted delivery areas (e.g., LNs and spleen) are often distinct from the sites where autoreactive cells are actively causing inflammation (e.g., CNS in MS, pancreas in T1D), they serve as immunological hubs where antigen-specific regulatory cells can be mass produced and deployed systemically. In this section we highlight biomaterial design strategies for targeted drug delivery to traditional and emerging immune tissues and other relevant organs to promote antigen-specific tolerance.

3.1. Liver

The liver is the largest internal organ in the body and plays a central role in metabolism and detoxification. Processing 30% of the total blood volume every minute,[49] the liver encounters antigens

deriving both from the gastrointestinal tract as well as arterial blood. This allows the liver to play a key role in immune surveillance as resident immune cells must clear pathogens deriving from the gut as well as systemic circulation. This organ is well-equipped in this capacity, containing the single largest population of macrophages (residential Kupffer cells) and the greatest densities of natural killer cells.[49] On the other hand, the liver is also intimately involved in inducing tolerance to dietary and commensal antigens. Resident APCs such as Kupffer cells and liver sinusoidal endothelia cells (LSECs) are key players in mediating liver tolerance.[50] In particular, Kupffer cells clear cellular debris from the liver without stimulating an inflammatory response. LSECs present antigen to $CD4^+$ T cells via MHC-II but express low levels of costimulatory molecules. This combination of antigen without costimulation makes LSECs a main driver in the development of T_{REGS} (Figure 1e).[51,52]

Most biomaterial drug delivery vehicles designed to target liver immune cells are nanoparticles (NPs) administered intravenously (IV) to take advantage of the high blood flow volume through this organ. NPs can be decorated with ligands that are highly expressed on liver immune cells to enable receptor-mediated active targeting. Liu et al. demonstrated the feasibility of this approach by modifying the surface of polymeric NPs with ligands targeting scavenger and mannose receptors[55] or stabilin receptors,[56] which are highly expressed on LSECs. The authors showed that while NPs in the 200–300 nm range accumulated almost exclusively in the liver, targeting ligands were necessary for antigen colocalization with LSECs. These and other similar studies have motivated the design of NPs decorated with sugar[53] or antibody[57,58] ligands for active targeting of liver-resident immune cells.

Ligand-mediated active targeting of liver immune cells could also be done in a carrier-free manner. This approach has the advantages of delivering a higher density of tolerizing immune signals per mass of administered materials as well as eliminating potentially immunostimulatory effects of the carrier material itself.[59,60] Wilson et al. demonstrated carrier-free liver targeting by modifying protein and peptide antigens with polymeric forms of *N*-acetylgalactosamine (GalNAc) or *N*-acetylglucosamine (GluNAc), which enable them to bind to C-type lectins on Kupffer cells, LSECs, and hepatocytes.[53] The authors found that 3 hours after IV administration, synthetically glycosylated ovalbumin (OVA) accumulated in the liver at a 2-fold higher level compared to un-glycosylated OVA. Two IV administrations of GluNAc-conjugated p31 peptide, which is the disease-relevant antigen in the BDC2.5 T-cell adoptive transfer model of T1D, maintained normal blood glucose for the duration of the experiment, while mice receiving unmodified p31 peptide developed hyperglycemia after just 7 days (**Figure 2**A–C). The authors attributed this therapeutic efficacy to the induction of a significant population of T_{REGS} in the spleen, highlighting the potential of liver-targeted technologies to drive systemic tolerogenic responses.

Alternatively, biomaterial properties can be tuned to enable passive targeting. Saito et al. demonstrated that peptide antigen-carrying polymeric nanoparticles synthesized using poly(D-lactide) (PLA) enabled significant, though incomplete, protection against a mouse experimental autoimmune encephalomyelitis (EAE) model of MS with IV administration of a low particle dose of 1.5 mg per mouse.[54] Curiously, this protection was not observed when particles were formulated with poly(DL-lactide-co-glycolide) (PLG) despite both sets of particles exhibiting similar biophysical characteristics (≈400 nm *z*-average diameter, −40 mV zeta-potential, and 2.5 µg peptide loaded per mg of particle) and similar peptide release kinetics. The authors found that compared to PLG particles, PLA particles persisted longer in the liver and increased liver fractions of inflammatory IFN-γ+ or IL-17+ CD4 T cells (Figure 2D,E). Thus PLA particles are thought to protect against EAE by sequestering pathogenic T cells in the liver and inhibiting their migration to the CNS to induce disease.

Other studies have shown that up to 99% of systemically-administered NPs reach the liver,[61] making hepatically targeted delivery systems highly attractive and relatively feasible. However, in order to remain immunologically active, biomaterial design strategies must avoid clearance by Kupffer cells and reach tolerogenic immune cell populations (i.e., hepatocytes and LSECs) once inside the liver. Future strategies to enable both these goals could benefit from NP surface engineering to manipulate surface charge. In particular, zwitterionic surface coating has been shown to improve NP circulation time[62] as well as selective targeting to the liver,[63] and may be a promising drug delivery approach to liver-resident immune cell populations for tolerance induction.

3.2. Lungs

In their vital function as organs of gas exchange, human lungs inhale almost 11 000 L of air daily containing heavy loads of airborne antigens and pathogens. Lung-resident immune cells are heavily involved in maintaining healthy immunity against pulmonary infections as well as tolerance against harmless inhaled antigens. For example, lung-resident CD11b+ DCs have been shown to play a central role in promoting tolerance to pulmonary antigens in neonatal development in a process partly regulated by the expression of immunomodulatory costimulatory molecule PD-L1.[64] Another group of tolerogenic lung-resident APCs is alveolar macrophages, which have been shown to induce T_{REGS} after exposure to airborne antigens.[65,66] This is due to their high baseline expression of anti-inflammatory cytokine TGF-β and retinal dehydrogenases, which catalyzes the production of retinoic acid to generate inducible T_{REGS}.[67] Lung-resident immune cells may also be involved in orchestrating autoimmunity as myelin-reactive T cells have been reported to undergo genetic reprogramming in the lungs and lung-draining LNs, a process that is required for pathogenic T cells to enter into the CNS and induce disease in a rat EAE model.[68]

The large population of specialized lung-resident immune cells that can be reprogrammed toward potent systemic tolerance is one of several advantages of lung-targeted drug delivery. The physiological structure of the lungs provides a large surface area through which drugs can be readily absorbed and transported directly into the bloodstream. This reduces first-pass metabolism by the liver, which significantly reduces the bioavailability of orally administered drugs.[69] Finally, inhalation delivery is painless and noninvasive, which has the added benefit of enhancing patient compliance.

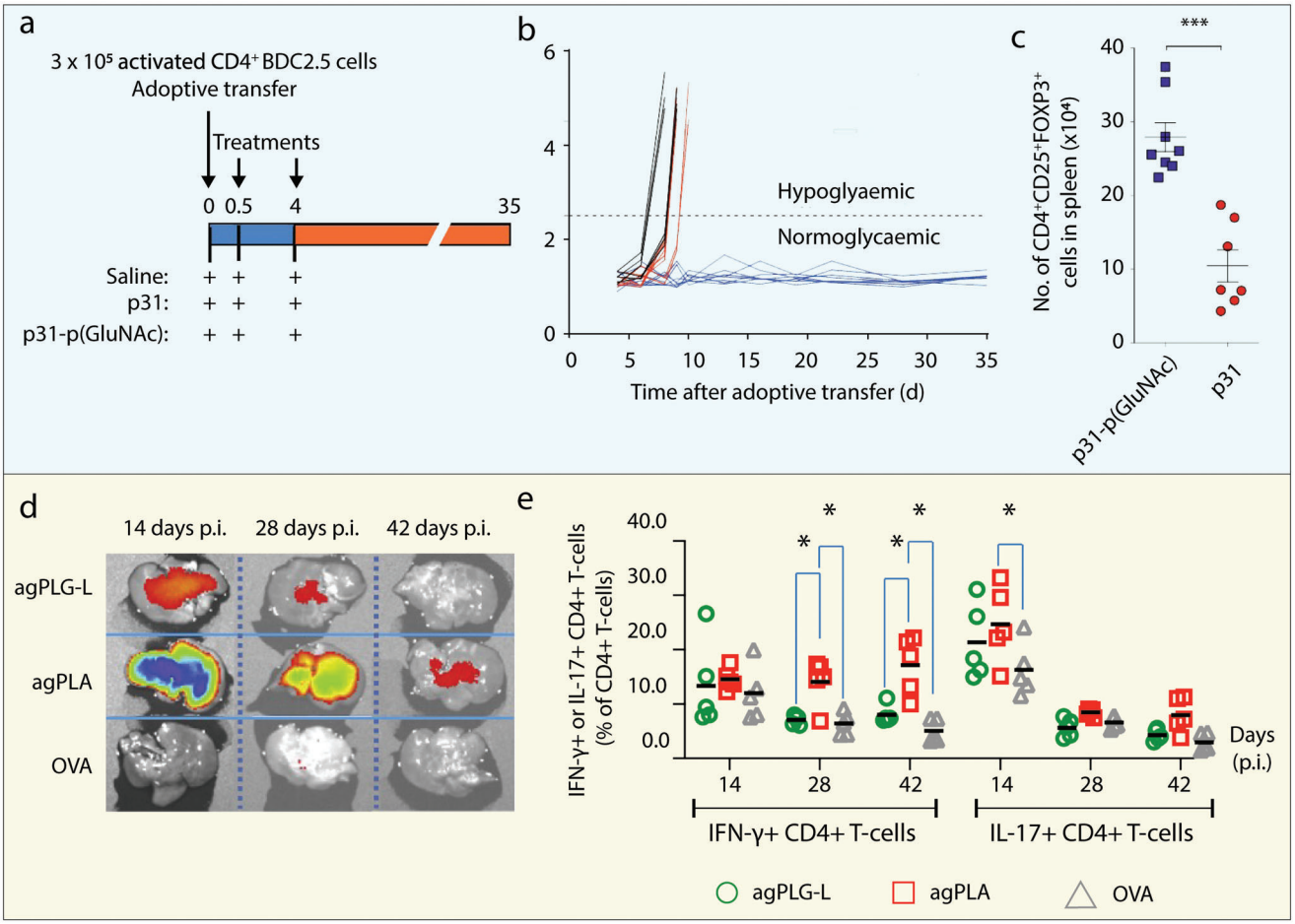


Figure 2. Direct intra-LN injection of PLGA MPs enable sustained release of immune signals and systemic tolerance. A) Whole animal imaging of untreated mouse (control) and mouse with intra-LN injection of fluorescently labeled MPs (i.LN) 24 h post-injection. B) Intra-LN injection of MPs encapsulating fluorescently labeled immune adjuvant polyIC (MP-polyIC) enabled longer signal retention in LNs compared to soluble polyIC or polyIC encapsulated in nanoparticles (NP-polyIC). C) A single intra-LN injection of MPs encapsulating MOG peptide antigen and Rapamycin (MOG/Rapa MP) at peak disease permanently reversed paralysis in mouse EAE. D) Therapeutic efficacy of MOG/Rapa MPs required direct intra-LN injection (*i.LN.*); no significant protection was seen when the same dose of MPs was injected intramuscularly (i.m.). E) Intra-LN injection of MPs restrained inflammatory cell infiltration into the CNS as demonstrated by immunofluorescent analysis of spinal cord sections showing CD3 expression. Scale bar, 10 µm. F) MOG/Rapa MP treatment increased T_{REG} frequencies in nontreated LNs and spleen. (A,B) Adapted with permission.[94] Copyright 2011, PNAS. (C–F) Reproduced with permission.[95] Copyright 2016, Cell Press.

Although pulmonary delivery of soluble immune signals have been used to demonstrate efficacy in mouse EAE models,[71] most preclinical studies have relied on NP encapsulation of immunomodulatory cues. This may be due to the fact that soluble antigen has been shown to clear the lungs rapidly following pulmonary delivery, while antigen encapsulated in particulate form shows nearly 75% retention in the lungs after 1 day.[72] Recent studies have shown that NP retention time in the lungs is predominantly determined by NP size. Haque et al. tracked the fate of poly(lactide-co-glycolide) (PLGA) NPs following intratracheal (IT) administration and found that nearly 40% of 400 nm NPs could still be detected in lung tissue after 7 days while 50 nm NPs were almost completely cleared.[73] To enable sustained pulmonary antigen release, Saito et al. engineered PLGA NPs around 350 nm in size (**Figure 3**A) loaded with a disease-relevant myelin proteolipid protein fragment ($PLP_{139-151}$).[70] Fluorescent signal from Cy5.5-labeled NPs was still detectable in lung tissue 14 days after IT administration (Figure 3B), which is consistent with biodistribution studies reported by other groups using PLGA NPs with similar biophysical properties.[73] IT administration of NPs encapsulating PLP peptide (nano-PLP-IT) delayed EAE disease onset by 1 week compared to IV injection of the same dose of NPs (nano-PLP-IV; Figure 2C). As IV injections led to high NP accumulation in the liver and spleen, the authors attributed this difference in disease kinetics to faster NP internalization and processing by lung-resident APCs compared to APCs in the liver and spleen. Mechanistically, the authors showed that nano-PLP-IT treated animals showed 2-fold higher accumulation of T cells and B cells in the lungs and negligible infiltration of these immune cells into the CNS compared to nanoparticles loaded with irrelevant OVA peptide (Figure 2D). This indicates that therapeutic protection was achieved in part by redirecting pathogenic immune cells away from the CNS to prevent autoimmune pathogenicity. Additionally, Nano-PLP-IT promoted lung

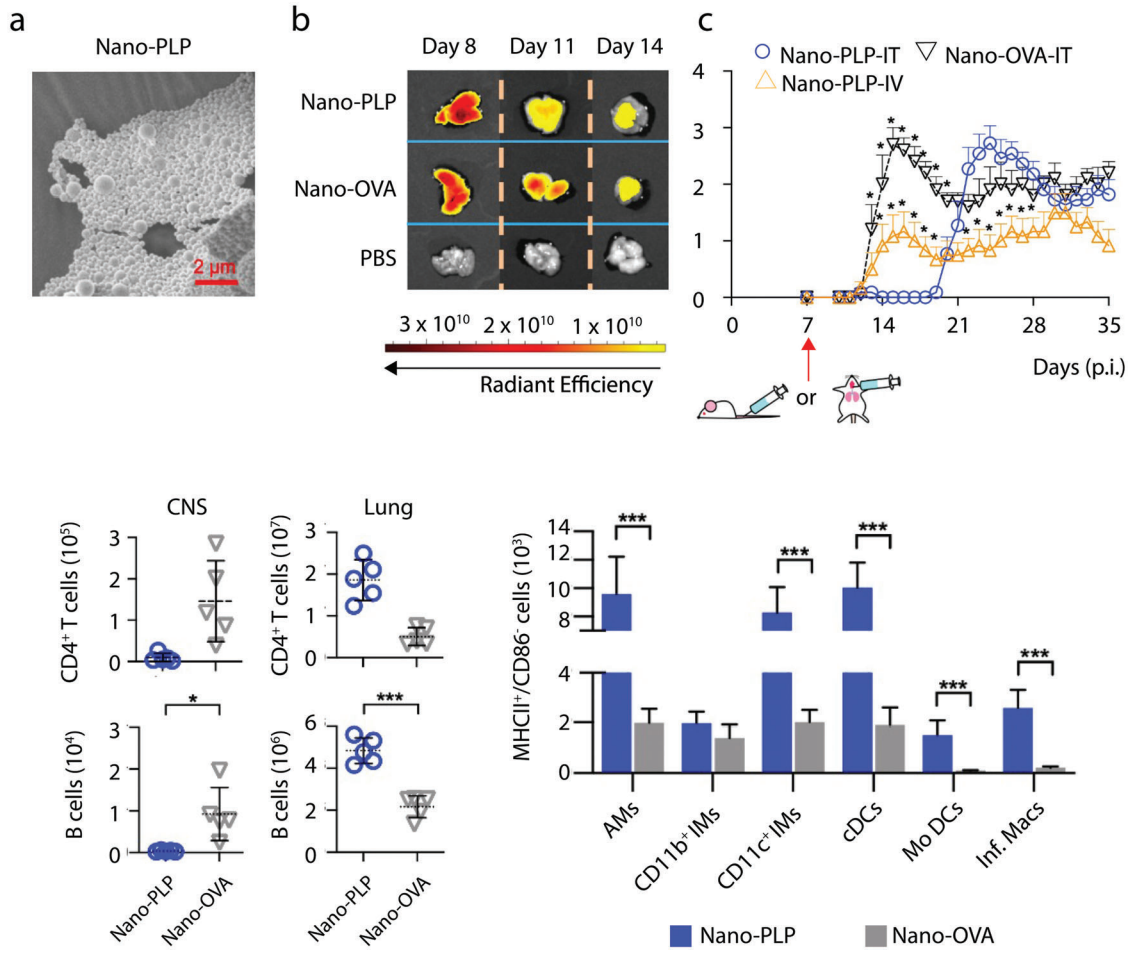


Figure 3. Liver-targeting biomaterials protect from autoimmune disease by pathogenic cell sequestration and T_{REG} upregulation. A) Treatment strategy for liver-targeting synthetically glycosylated p31 antigen peptide in a mouse T1D model. B) Glycosylated antigen (p31-p(GluNAc)) treatment protected mice from T1D-induced hyperglycemia. C) Therapeutic efficacy was partly due to higher fractions of T_{REGS} induced by p31-p(GluNAc) compared to unglycosylated peptide. D) Biomaterial carrier properties can have inherent tolerizing capabilities as PLA nanoparticles carrying antigen (agPLA) maintain longer liver residence time than analogous PLG nanoparticles (agPLG-L). E) agPLA sequestered inflammatory T cells in the liver. (A–C) Adapted with permission.[53] Copyright 2019, Springer Nature. (D,E) Adapted with permission.[54] Copyright 2019, Elsevier.

accumulation of MHC-II$^+$ APCs that do not express the costimulatory molecule CD86 (Figure 3E). This resulted in the induction local T cell anergy, a type of tolerance in which T cells cognate for a specific antigen can no longer mount an immune response.

While the studies described here present compelling evidence that drug delivery to the lungs can induce potent systemic tolerance, IT administration is rarely used in human patients and more clinically relevant pulmonary delivery systems are needed. Recent advances in polymeric and lipid gene delivery materials have demonstrated that nanoparticles encapsulating mRNA can be nebulized and delivered to mice through inhalation, achieving gene expression localized nearly exclusively to the lungs.[74,75] These innovations in noninvasive pulmonary delivery technologies could be exploited for more translational delivery of protein or peptide antigens to enable antigen-specific tolerance in autoimmune diseases. Alternatively, biomaterials could be engineered to accumulate in the lungs following IV administration. Recent studies have shown that surface modification with cationic lipids[63] or ligands that allow NPs to hitchhike on red blood cells[76] enhance pulmonary accumulation of systemically injected biomaterials. Future work could adopt these strategies for lung-targeted drug delivery materials to induce immune tolerance.

3.3. Spleen

Unlike the previous two organs, which contain large numbers of resident immune cells but mainly serve nonimmunological functions, the spleen is a specialized immunological organ. In fact, the spleen is the largest secondary lymphoid organ and serves two main purposes: 1) hematopoiesis (generation of new blood cells) and extraction of aged or dead red blood cells from circulation; 2) facilitating interactions between APCs and lymphocytes.[77] The spleen plays a central role in mounting active immunity against pathogens but has also been implicated in sustaining T_{REG}-mediated tolerance in transplant models.[78] For example, in human multivisceral transplants where the stomach, pancreas,

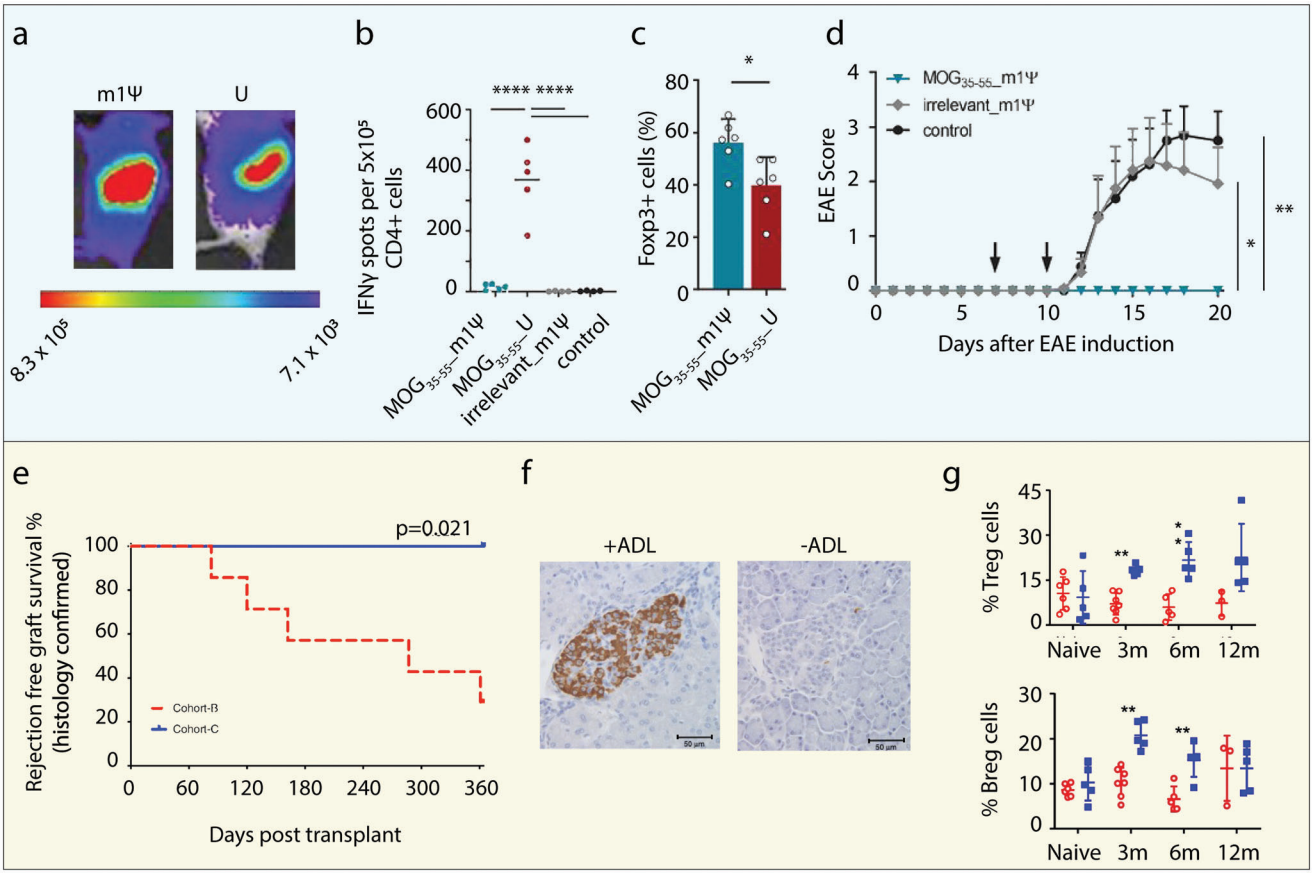


Figure 4. Antigen-loaded NPs provide protection against EAE in mice after pulmonary delivery. A) Representative SEM image of PLGA NPs encapsulating PLP antigen (Nano-PLP). B) Cy5.5-labeled NP signal remained detectable in the lungs at least 14 days after intratracheal administration. C) Intratracheal administration of Nano-PLP (Nano-PLP-IT) delayed disease onset of EAE compared to animals receiving the same dose of Nana-PLP administered IV (Nano-PLP-IV). Therapeutic efficacy of Nano-PLP-IT could be attributed to D) sequestration of T cells and B cells in the lungs and away from the site of pathogenesis in the CNS as well as E) increased anergy (as indicated by $MHCII^{+}CD86^{-}$ expression) among lung-resident APCs. Adapted with permission.[70] Copyright 2020, American Association for the Advancement of Science (AAAS).

intestine, and liver are transplanted en bloc, cotransplantation of donor spleen has been shown to have a moderate protective effect against graft-versus-host disease.[79] Owing to its role in blood filtration and immune surveillance, the spleen is a highly vascularized organ, and the majority of spleen-targeted drug delivery materials are administered IV. In this section, we discuss passive spleen targeting strategies relying on biomaterial physiochemical properties as well as active targeting strategies based on receptor-mediated interactions that have been used preclinically to modulate splenic APCs toward tolerogenic phenotypes.

Passive spleen targeting can be achieved by engineering nanomaterials with anionic surface charge and has been established in lipid-based systems[63] as well as polymeric NPs.[80] Jamison et al. encapsulated insulin peptide antigen in anionic PLGA NPs and demonstrated targeted delivery to spleen APCs. These antigen-bearing NPs were shown to protect insulin-producing pancreatic islets by redirecting pathogenic T cell trafficking toward the spleen in a mouse T1D model.[80] The Kishimoto lab engineered PLGA NPs encapsulating antigen peptide and the small molecule immunomodulator Rapamycin. Upon IV administration, these NPs induced durable, antigen-specific tolerance via splenic APC reprogramming in several mouse models of autoimmune diseases.[81,82] Compared to the studies by Jamison et al., the addition of encapsulated Rapamycin was found to be necessary in promoting antigen-specific T_{REGS}, which provided more durable protection.

Instead of delivering antigen in peptide form, Krienke et al. used slightly anionic lipid NPs to deliver mRNA encoding antigen to splenic CD11c+ APCs (**Figure 4A**). The authors found that modifying the mRNA molecules with 1-methylpseudouridine (m1Ψ mRNA) reduced the inherent inflammatory properties of synthetic single-stranded mRNA. As a result, splenic T cells from m1Ψ mRNA NP-treated animals secreted significantly less inflammatory IFNγ upon restimulation with cognate antigen (Figure 4B). To test the tolerogenic efficacy of this technology, mice induced with EAE were given two IV injections of NP delivering m1Ψ mRNA encoding disease-relevant myelin oligodendrocyte glycoprotein peptide (MOG_{35-55}) before disease onset. Among MOG-specific splenic CD4+ T cells that expanded as a result of treatment, >50% were T_{REGS} (Figure 4C. This population of antigen-specific T_{REGS} provided significant protection against EAE (Figure 4D).[83]

Active targeting strategies have also demonstrated protective efficacy in preclinical animal models. Pishesha et al. covalently

conjugated antigen to single-domain antibody fragments that recognize MHC-II, which is expressed by all professional APCs. A single IV administration of these complexes alleviated disease in mouse models of EAE, T1D, and rheumatoid arthritis in a splenic CD11c+ DC-dependent manner.[85] Rather than choosing a specific targeting ligand, the Miller lab targeted splenic APCs by leveraging the spleen's involvement in clearing apoptotic debris. Apoptotic clearance is a natural process for maintaining healthy self-tolerance that is primarily mediated by specialized splenic marginal zone macrophages.[86,87] To target these cells, the Miller lab engineered apoptotic splenocytes coupled to antigen (Ag-SP). Ag-SP accumulated in the spleen within 30 minutes of IV infusion, where they were taken up by macrophages in the marginal zone. Tolerance was induced by IL-19 production by and PD-L1 expression on macrophages, but long-term maintenance of tolerance required activation of T_{REGS}.[88] The same group recently published follow-up studies in nonhuman primate models of islet transplantation and found that infusion of apoptotic donor leukocytes (ADLs) along with a short-term immunosuppressant therapy protected MHC-I mismatched islet allografts from rejection in 5 of 5 rhesus macaques for greater than 1 year (Figure 4E–G). This efficacy was attributed to ADL inhibition of effector memory T cells as well as nearly 2-fold upregulation of several suppressive T cell subsets.[84]

The efficiency of future spleen-targeted biomaterials administered via the IV route could be enhanced by engineering the interactions between the materials and serum proteins. Recent studies have demonstrated that serum proteins adsorb onto the surface of NPs immediately upon IV injection to form a protein corona, which heavily dictates NP interactions with cells and tissues.[89,90] NP surface modification with the helper lipid 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), for example, has been shown to preferentially target lipid NPs to the spleen due to reduced interactions with apolipoprotein E.[91] Similar strategies for spleen targeting by modulating the protein corona composition on NP surfaces could help reduce the therapeutic dose of immunomodulatory signals and lower the risk of off-target effects.

3.4. Lymph Nodes (LNs)

LNs are secondary lymphoid organs with highly specialized microenvironments that determine effector immune cell responses. The large number of cells and antigens travelling through draining LNs provide a high concentration of antigens and APCs. This facilitates antigen presentation and priming of naïve lymphocytes into effector T cells and B cells. While the antigens presented determine the antigen specificity of mature effector cells, other signals provided in the LN milieu educate lymphocytes toward immunity or tolerance. Simon et al. reported that stromal laminin expression in LNs direct T cell differentiation, with low laminin $\alpha4$:$\alpha5$ ratio driving immunity and high ratios driving tolerance.[92] The dynamics of DC-T cell interactions have also been shown to be important as prolonged interactions between T cells and DCs lead to pro-inflammatory CD8+ T cell activation while brief contact may contribute to the induction of T cell tolerance.[93]

Many LN-targeting biomaterial strategies have relied on size-mediated passive drainage[96,97] or active receptor-mediated targeting of residential APC populations.[98] Galea et al. synthesized ≈100 nm liposomes encapsulating antigen and immunomodulatory small molecule calcitriol to target LNs via passive draining. They found increased PD-L1 and decreased MHC-II expression in DCs in the draining LNs, which effectively suppressed effector T cells while inducing antigen-specific T_{REGS} to produce IL-10.[99] Maulloo et al. reported that antigens directly conjugated to GluNAc sugar molecules improved retention in draining LNs after subcutaneous administration, increased antigen uptake and presentation by LN APCs, and promoted tolerance via CD4+/CD8+ T cell anergy and T_{REGS} induction.[100] Interestingly, the same research group previously reported that IV administration of similar synthetically glycosylated antigens accumulated in the liver to induce tolerance via hepatic APC reprogramming,[53] highlighting the importance of administration route as a part of the design considerations for targeted biomaterial delivery strategies.

In contrast to the above strategies, which aim to target draining LNs after systemic administration of biomaterial vehicles, our lab has taken the approach of directly injecting biomaterial depots into LNs. This strategy physically localizes immunomodulatory cues to treated LNs, which concentrates these signals to the sites of immune cell reprogramming without exposure to other tissues. Intra-LN (i.LN) injections in preclinical mouse models are enabled by subcutaneous injections of a tracer dye, which drains to and allows for visualization of mouse inguinal LNs.[101] Direct i.LN injection of PLGA microparticles (MPs) encapsulating a TLR agonist showed nearly 4-fold higher signal retention at 96 hours postinjection compared to injected soluble TLR agonist (**Figure 5**A,B).[94] MPs encapsulating MOG antigen peptide and Rapamycin inhibited inflammatory T cell infiltration into the spinal cord and promoted systemic antigen-specific T_{REGS}.[95] A single i.LN MP injection at peak disease permanently reversed paralysis in a mouse EAE model (Figure 5C–F). Interestingly, therapeutic protection was completely abrogated by injecting a matching dose of MPs intramuscularly. These studies demonstrated that local LN reprogramming through controlled release of immune signals from biomaterial depots can drive systemic, antigen-specific tolerance. In an open-label clinical trial, 3 i.LN injections of grass pollen allergen over 2 months was shown to be more effective at ameliorating IgE-mediated allergies than conventional treatment of 54 subcutaneous injections over 3 years even though the subcutaneous group received >1000-fold more cumulative allergen dose.[102] This study demonstrates that robust and systemic immunomodulatory responses could be achieved in translatable i.LN injections in humans. Combining this localized injection route with controlled-release biomaterials could enable potent immune regulatory efficacy without harmful systemic exposure to powerful immune cues or drugs. Figure 5

Biomaterials that could be administered systemically, selectively drain to LNs, and persist there for sustained immunomodulation could be an interesting direction for future study. This could be achieved using self-assembly materials, which form intricate structures driven by hydrophobic or electrostatic interactions.[103] Self-assembled materials are particularly relevant for LN targeting because while materials <40 nm in diameter have been shown to enhance drainage into LNs,[96,104] they

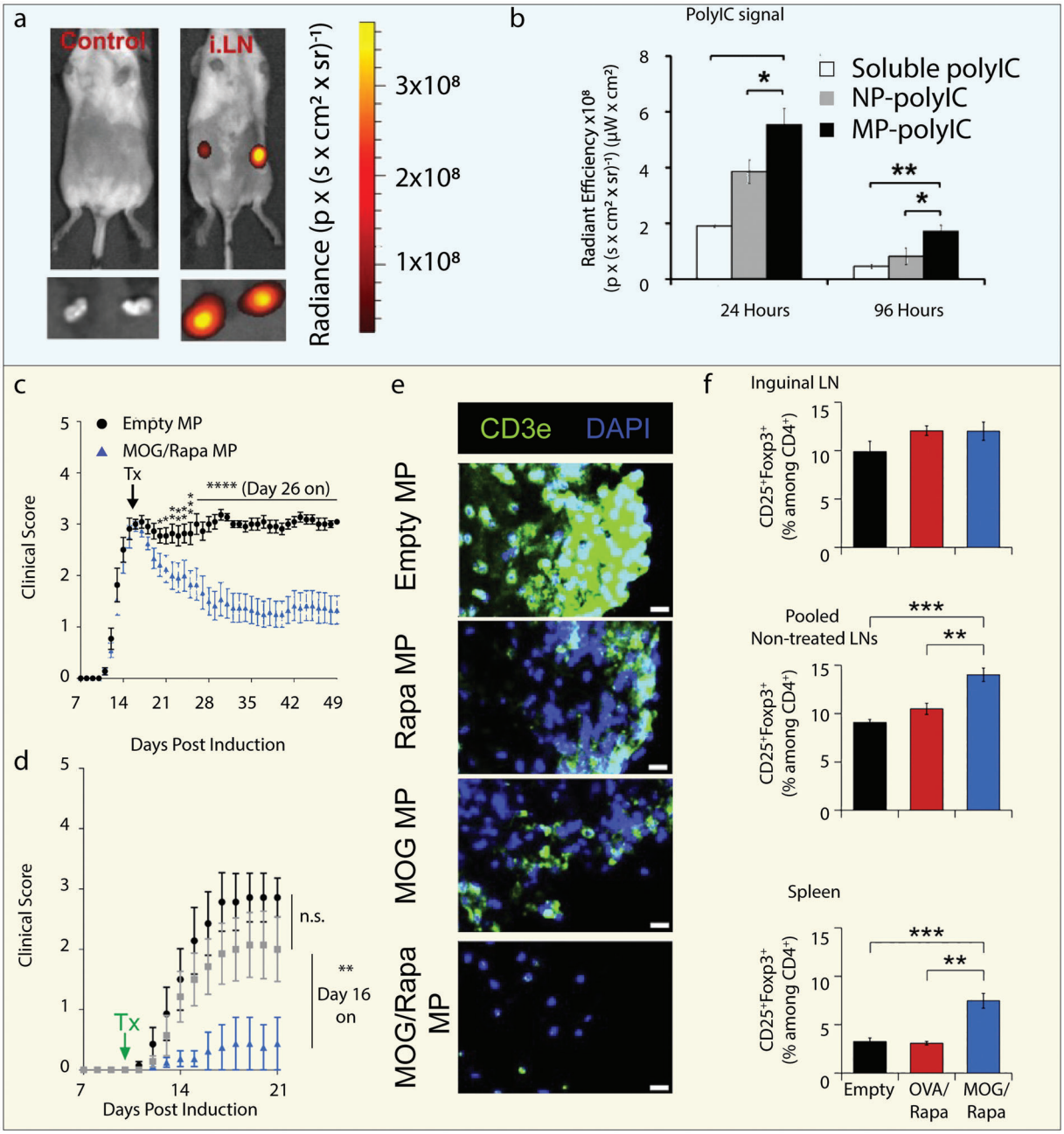


Figure 5. Spleen-targeting biomaterial therapies offer protection in preclinical models of autoimmune disease and transplantation. A) Lipid nanoparticles carrying modified (m1Ψ) or unmodified (U) mRNA enable strong gene expression in the spleen. B) IFNγ secretion was not elevated in CD4$^+$ T cells from animals treated with $MOG_{35\text{-}55}$-m1Ψ after ex vivo restimulation with $MOG_{35\text{-}55}$ peptide. C) Treatment with m1Ψ mRNA encoding the $MOG_{35\text{-}55}$ peptide ($MOG_{35\text{-}55}$-m1Ψ) promoted higher antigen-specific T_{REGS} in C57BL/6 mice as analyzed by $MOG_{35\text{-}55}$-tetramer staining. D) Two treatments (black arrows) of 20 µg $MOG_{35\text{-}55}$-m1Ψ prior to onset of disease prevented EAE-induced paralysis in mice. E) Rhesus macaques receiving apoptotic donor leukocyte ADL) infusions maintained stable tolerance of MHCI-mismatched pancreatic islet allografts for at least 1 year after engraftment. F) Intact, insulin-stained donor islets were found only in recipients receiving ADL treatment 1 year post-transplant. G) Graft tolerance was attributed to elevated levels of circulating regulatory immune cell network. (A–D) Adapted with permission.[83] Copyright 2021, AAAS. (E–G) Adapted with permission.[84] Copyright 2019, Springer Nature.

must assemble into much larger structures to enhance retention within LNs and prolonged immune signaling. Environmentally responsive materials that self-assemble into macrostructures in response to pH,[105] temperature, or light stimuli, could be engineered to enhance LN retention of injected materials.

3.5. Skin

The skin is our first line of defense that not only acts as a physical barrier but can also serve as a potent immunologic organ. Skin is composed of two main layers, the epidermis and dermis. The

epidermal layer contains APCs such as epidermal dendritic cells and Langerhans cells (LCs). LCs are skin resident macrophages involved in in situ antigen presentation but also have the unique capability to traffic to draining LNs.[106] Evidence suggests that LCs are implicated in skin-mediated tolerance through IL-10 production and T_{REG} induction, making them an attractive target for biomaterial antigen delivery.[107,108] Beneath the epidermis lies the dermal layer, where blood vessels, neurons, and lymphatic vessels run throughout to facilitate local inflammation during infection and skin repair.[106] The high concentration of APCs in the skin makes this organ an attractive target for induction of antigen-specific tolerance. Using a cynomolgus-macaque model of EAE, Fovet et al. demonstrated that intradermal injection of MOG peptide fused to antibodies directed against dendritic cell receptors protected against disease.[109] This efficacy was attributed to reduced activation of inflammatory effector CD4+ T cells as well as induction of MOG-specific T_{REGS}, highlighting that potent, systemic tolerance can be achieved by modulating skin-resident immune cells. To access these skin-resident immune cells in a more clinically translational manner, researchers have dived into the use of microneedle (MN) patches, which are an attractive alternative to hypodermic needles as they can reduce trauma at site of treatment, decrease pain, and improve antigen delivery and retention in the skin.[110] Stainless steel, silicone, and polymer MN formulations have been used to fabricate coated, solid, and dissolvable microneedles loaded with antigen or peptide of interest.[107,108,111]

Taking advantage of the abundant population of APCs in the skin, the Hanna lab applied peptide coated MNs to the skin of mice to deliver endogenous antigen to induce tolerance in a T1D model.[110] The authors fabricated arrays of stainless-steel needles to deliver peptides of varying hydrophobicity and demonstrated that the more hydrophobic BDC2.5 mimotope showed longer retention in the skin than the WE14 peptide which had increased water solubility. To test the hypothesis that sustained antigen presentation can promote tolerance induction, islet-specific BDC2.5 T cells were transferred into NOD mice treated with 2 μg peptide delivered via MN or injected intradermally. After 72 h, MN treatment elicited a more consistent level of proliferation in skin draining-LNs compared to intradermal (ID) injections using the same dose of peptide.[110] These results illustrate that the larger surface area covered by MN can enable more efficient antigen delivery to skin-resident LCs and amplify immune responses.

Sustained release of antigen peptides could be further improved by encapsulating peptides in polymeric nanoparticles before coating them onto MN's.[111] MN strategies to enable direct, sustained antigen delivery to skin APCs were further demonstrated by the Kim lab. They manufactured microneedle patches loaded with house dust mite allergen (*D. Farinae extract*; *DfE*) to evaluate its efficacy as a novel allergen specific immunotherapy to treat atopic dermatitis (AD). 10 μg DfE delivered via MNs was able to suppress pro-allergic Th2 cytokines IL-4 and IL-13 while increasing IL-10 production and T_{REGS}.[107] Interestingly, 100 μg DfE injected subcutaneously was required to achieve similar efficacy to the 10 μg DfE MN patch, with 10 μg DfE subcutaneous injection having no effect. These studies highlight that biomaterial-based MNs can be used to engineer the large number of skin-resident immune cells during allergen therapy.

3.6. In Situ Immune Cell Reprogramming

So far, we have discussed design strategies that target biomaterials to tissue-resident immune cells in several organs. An alternative strategy is to use biomaterials to recruit immune cells to the site of injection and engineer immune responses in situ. The Keselowsky lab engineered such an "artificial immune organ" by using a dual-sized microparticle system to deliver antigen and cytokines. In these studies, PLGA MPs ≈30 μm in diameter were designed to be nonphagocytosable and release GM-CSF for DC recruitment as well as TGF-β for tolerogenic reprogramming. Simultaneously, phagocytosable MPs ≈1 μm were used for intracellular delivery of self-antigen and vitamin-D3, which induces tolerance by binding intracellular transcription factors. Upon sub-cutaneous delivery, the larger, un-phagocytosable MP populations recruited DCs, which phagocytosed and trafficked smaller MPs to draining LNs. There, these tolerogenic DCs promoted antigen-specific T_{REGS} to impart protective efficacy in mouse T1D[112,113] and EAE[114] models. Chen et al. engineered immune-homeostatic microparticles (IHMs) that induced tolerance through immune cell recruitment in circulation. These IHMs were designed to release monocyte chemotactic protein-1 to recruit activated T cells, which underwent apoptosis after binding to Fas ligand decorating the particles. As apoptosis is a naturally tolerizing process that produces anti-inflammatory cytokines, IHMs engineered to codeliver self-antigens simultaneously depleted pathogenic T cells while expanding antigen-specific T_{REGS} to reduce disease burden in mouse models of colitis, T1D, and EAE.[115]

4. Clinical Perspectives

There are currently no cures for autoimmune disease such as MS and T1D, and most available treatments are disease modifying therapies aimed at controlling symptoms or slowing disease progression. In the case of MS, for example, FDA-approved disease modifying therapies include: generally immunosuppressive anti-inflammatory cytokines (e.g., beta interferons[116]); DNA synthesis disruptors to inhibit rapidly dividing pathogenic T cells (e.g., teriflunomide[117]); and monoclonal antibodies for lymphocyte depletion (e.g., ocrelizumab,[27] natalizumab[118]). Although these therapies provide symptom relief to patients, most of them act through suppressing activated immune cells, which include pathogenic autoreactive populations but also immune cells involved in fighting cancer or infections. The resulting risks of immunocompromise seriously hamper the quality of life of autoimmunity or organ transplant patients, who need to take these treatments for the rest of their lives.

In response to the challenges discussed above, a steady stream of therapies that promote antigen-specific tolerance are currently undergoing clinical testing. Among these, a majority involve ex vivo engineering of tolerogenic immune cells, which are then adoptively transferred back into the patient (**Table 1**). Although these treatments still need to overcome major hurdles before reaching the clinic, adoptive cell transfer immunotherapies have previously demonstrated efficacy in cancer treatment. One such treatment, the prostate cancer therapy Sipuleucel-T,[119–121] which consists of autologous DCs stimulated ex vivo to react against prostate cancer antigen, highlights the therapeutic potential of

Table 1. Recent clinical trials promoting antigen-specific tolerance.

	Design	Route	Disease	Outcome	Trial ID	Ref.
DC-based therapy	Autologous DCs tolerized with dexamethasone and loaded with mixture of myeline peptides	IV	MS	Safety and tolerability; increased IL-10 production after ex vivo antigen restimulation; increased T_{REG} frequency	NCT 0228367	[19]
DC-based therapy	Autologous DCs tolerized with vitamin-D3 and loaded with mixture of myeline peptides	IV or i.LN	MS	Ongoing	NCT 02618902 NCT 02903537	[122]
Apoptotic cell-based therapy	Autologous PBMCs chemically coupled to mixture of myelin peptides	IV	MS	Safety and tolerability; decrease in antigen-specific T cell responses	EudraCT #2008-004408-29	[123]
Apoptotic cell-based therapy	Donor PBMCs treated with mitomycin C to induce apoptosis	IV	Living donor kidney transplant	Safety and tolerability; increase in regulatory B cell frequency	NCT 02560220	[124]
Biomaterial-based therapy	PLGA NPs encapsulating gluten protein antigen (gliadin)	IV	Celiac disease	Reduction in interferon-γ; reduction in circulating effector T cells following 14-day gluten challenge	NCT 03738475	[125]

antigen-specific cell therapies. Indeed, several recent clinical trials have engineered tolerogenic autologous DCs using combinations of antigen peptides and tolerizing steroids[19] or small molecules.[122] Other groups have focused on inducing tolerance through apoptotic cell-based therapies, where PBMCs bearing relevant antigens are induced to display tolerizing apoptotic markers before IV infusion into patients. Lutterotti et al.[123] used this approach in a first-in-human trial in MS patients by chemically coupling autologous PBMCs to a cocktail of seven myelin peptides using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide chemistry, which induces cellular apoptosis. In addition to good safety and tolerability, this phase I trial showed that patients receiving a dose of $>1 \times 10^9$ cells saw a decrease in T cell proliferation in response to ex vivo peptide restimulation, suggesting that an antigen-specific tolerizing effect was achieved. Apoptotic PBMCs also demonstrated promising results in a phase I trial involving patients receiving living donor kidney transplants. In this study, donor PBMCs (which already expressed relevant donor antigens) were modified ex vivo to undergo apoptosis using mitomycin C, an alkylating agent that causes cell death via DNA transcription inhibition.[124] Patients receiving up to 1.5×10^8 cells per kg of body weight prior to kidney transplant in addition to post-transplantation immunosuppression showed no donor-specific antibodies or rejection episodes, suggesting good tolerability. Compared to control transplantation cohorts, patients that received apoptotic cell therapy demonstrated up to 68-fold increases in $CD19^+CD24^{hi}CD38^{hi}$ regulatory B cells, the majority of which secreted IL-10. Importantly, patient cells showed no reactivity upon ex vivo restimulation with donor blood cells at 360 days post-transplantation but preserved reactivity against third-party cells, indicating that tolerance was antigen-specific.

Results from some of the first biomaterial-based therapies have also demonstrated antigen-specific tolerance in early-phase trials in patients with celiac disease, in which gluten protein induces unwanted immune activation. In this study, negatively-charged PLGA NPs encapsulating the gluten protein gliadin were infused into celiac disease patients, who then completed a 14-day gluten challenge.[125] Patients who received NP treatment showed an 88% reduction in interferon-γ secretion compared to placebo, and circulating levels of $CD4^+$, $CD8^+$, and $\gamma\delta$ effector T cells were also reduced. These promising results suggest that biomaterial-based therapies could be safe and effective at promoting antigen-specific tolerance in humans. Similar therapies could be produced by substituting in different antigens to produce highly translatable, off-the-shelf therapies for other autoimmune diseases.

5. Conclusions

Therapeutic strategies enabling antigen-specific immune tolerance could be transformative for patients with autoimmune diseases. In the past decade, a small number of exciting cell therapies have demonstrated good tolerability and evidence of antigen-specific tolerance in human patients, and may be on the horizon for autoimmune disease treatment. Cell therapies are inherently complex and expensive to manufacture, however, and the ultimate goal for autoimmune therapies should be off-the-shelf therapeutics that direct the body's own immune cell programming and expansion machinery to promote robust, antigen-specific tolerance. Toward this end, many exciting biomaterial approaches are currently in development to target immune organs and educate tissue-resident immune cells toward tolerance. Preclinical studies have demonstrated that these approaches induce tolerance through diverse mechanisms including sequestering self-reactive pathogenic cells away from locations where they induce disease, promoting tolerogenic DCs and T_{REGS} responses, and leveraging the body's naturally tolerizing process for clearing apoptotic debris to induce an anti-inflammatory response. Some of these technologies are already in early stages of clinical translation. Looking ahead, strategies that combine advances in biomaterials with new findings in autoimmune pathogenesis[24,25] or self-antigens common in most patients[126] would greatly facilitate the translation of these technologies to the clinic.

Acknowledgements

Y.R. and H.B.E. contributed equally to this work. This work was supported by United States National Institutes of Health award # R01AI169686, R01EB026896, R01AI144667, R01EB027143 and United States Department of Veterans Affairs Award No. I01BX003690 to C.M.J.

Conflict of Interest

C.M.J. is an employee of the VA Maryland Health Care System. The views reported here do not reflect the views of the VA or United States Government. C.M.J. has an equity position with Vaccitech plc. Beyond these declarations, there are no other Conflict of Interest to report.

Keywords

autoimmunity, biomaterials, drug delivery, immune tolerance, immunotherapy, nanoparticles and microparticles

Received: September 1, 2022
Revised: November 15, 2022
Published online: December 15, 2022

[1] M. Wahren-Herlenius, T. Dörner, *Lancet* **2013**, *382*, 819.
[2] L. Wang, F.-S. Wang, M. E. Gershwin, *J. Intern. Med.* **2015**, *278*, 369.
[3] A. Lerner, P. Jeremias, T. Matthias, *Int. J. Celiac Dis.* **2015**, *3*, 151.
[4] D. B. Clifford, A. Deluca, D. M. Simpson, G. Arendt, G. Giovannoni, A. Nath, *Lancet Neurol.* **2010**, *9*, 438.
[5] S. A. Acuna, *Transplant. Rev.* **2018**, *32*, 218.
[6] M. B. Roberts, S. Izzy, Z. Tahir, A. Al Jarrah, J. A. Fishman, J. El Khoury, *Transplant Infect. Dis.* **2020**, *22*, e13407.
[7] R. S. Oakes, E. Froimchuk, C. M. Jewell, *Adv. Ther.* **2019**, *2*, 1800157.
[8] J. M. Gammon, C. M. Jewell, *Adv. Healthcare Mater.* **2019**, *8*, 1801419.
[9] A. N. Theofilopoulos, D. H. Kono, R. Baccala, *Nat. Immunol.* **2017**, *18*, 716.
[10] V. Saferding, S. Blüml, *J. Autoimmun.* **2020**, *110*, 102382.
[11] E. Martin-Gayo, X. G. Yu, *Front. Immunol.* **2019**, *10*, https://doi.org/10.3389/fimmu.2019.01306.
[12] D. Ganguly, S. Haak, V. Sisirak, B. Reizis, *Nat. Rev. Immunol.* **2013**, *13*, 566.
[13] M. Greter, F. L. Heppner, M. P. Lemos, B. M. Odermatt, N. Goebels, T. Laufer, R. J. Noelle, B. Becher, *Nat. Med.* **2005**, *11*, 328.
[14] G. Huang, Y. Wang, P. Vogel, T.-D. Kanneganti, K. Otsu, H. Chi, *Nat. Immunol.* **2012**, *13*, 152.
[15] J. Boldison, L. C. Da Rosa, J. Davies, L. Wen, F. S. Wong, *Cell Mol. Immunol.* **2020**, *17*, 843.
[16] J. Suffner, K. Hochweller, M.-C. Kühnle, X. Li, R. A. Kroczek, N. Garbi, G. J. Hämmerling, *J. Immunol.* **2010**, *184*, 1810.
[17] U. Sela, P. Olds, A. Park, S. J. Schlesinger, R. M. Steinman, *J. Exp. Med.* **2011**, *208*, 2489.
[18] O. Morante-Palacios, F. Fondelli, E. Ballestar, E. M. Martínez-Cáceres, *Trends Immunol.* **2021**, *42*, 59.
[19] I. Zubizarreta, G. Flórez-Grau, G. Vila, R. Cabezón, C. EspañA, M. Andorra, A. Saiz, S. Llufriu, M. Sepulveda, N. Sola-Valls, E. H. Martinez-Lapiscina, I. Pulido-Valdeolivas, B. Casanova, M. Martinez Gines, N. Tellez, C. Oreja-Guevara, M. Español, E. Trias, J. Cid, M. Juan, M. Lozano, Y. Blanco, L. Steinman, D. Benitez-Ribas, P. Villoslada, *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 8463.
[20] T. Nikolic, J. J. Zwaginga, B. S. Uitbeijerse, N. J. Woittiez, E. J. de Koning, H.-J. Aanstoot, B. O. Roep, *Lancet Diab. Endocrinol.* **2020**, *8*, 470.
[21] R. A. Elsner, M. J. Shlomchik, *Immunity* **2020**, *53*, 1136.
[22] N. Pardi, M. J. Hogan, M. S. Naradikian, K. Parkhouse, D. W. Cain, L. Jones, M. A. Moody, H. P. Verkerke, A. Myles, E. Willis, C. C. Labranche, D. C. Montefiori, J. L. Lobby, K. O. Saunders, H.-X. Liao, B. T. Korber, L. L. Sutherland, R. M. Scearce, P. T. Hraber, I. N. Tombácz, H. Muramatsu, H. Ni, D. A. Balikov, C. Li, B. L. Mui, Y. K. Tam, F. Krammer, K. Karikó, P. Polacino, L. C. Eisenlohr, et al, *J. Exp. Med.* **2018**, *215*, 1571.
[23] U. Sahin, A. Muik, E. Derhovanessian, I. Vogler, L. M. Kranz, M. Vormehr, A. Baum, K. Pascal, J. Quandt, D. Maurus, S. Brachtendorf, V. Lörks, J. Sikorski, R. Hilker, D. Becker, A.-K. Eller, J. Grützner, C. Boesler, C. Rosenbaum, M.-C. Kühnle, U. Luxemburger, A. Kemmer-Brück, D. Langer, M. Bexon, S. Bolte, K. Karikó, T. Palanche, B. Fischer, A. Schultz, P.-Y. Shi, et al, *Nature* **2020**, *586*, 594.
[24] K. Bjornevik, M. Cortese, B. C. Healy, J. Kuhle, M. J. Mina, Y. Leng, S. J. Elledge, D. W. Niebuhr, A. I. Scher, K. L. Munger, A. Ascherio, *Science* **2022**, *375*, 296.
[25] T. V. Lanz, R. C. Brewer, P. P. Ho, J.-S. Moon, K. M. Jude, D. Fernandez, R. A. Fernandes, A. M. Gomez, G.-S. Nadj, C. M. Bartley, R. D. Schubert, I. A. Hawes, S. E. Vazquez, M. Iyer, J. B. Zuchero, B. Teegen, J. E. Dunn, C. B. Lock, L. B. Kipp, V. C. Cotham, B. M. Ueberheide, B. T. Aftab, M. S. Anderson, J. L. Derisi, M. R. Wilson, R. J. M. Bashford-Rogers, M. Platten, K. C. Garcia, L. Steinman, W. H. Robinson, *Nature* **2022**, *603*, 321.
[26] B. A. C. Cree, J. L. Bennett, H.o J. Kim, B. G. Weinshenker, S. J. Pittock, D. M. Wingerchuk, K. Fujihara, F. Paul, G. R. Cutter, R. Marignier, A. J. Green, O. Aktas, H.-P. Hartung, F. D. Lublin, J. Drappa, G. Barron, S. Madani, J. N. Ratchford, D. She, D. Cimbora, E. Katz, *Lancet* **2019**, *394*, 1352.
[27] X. Montalban, S. L. Hauser, L. Kappos, D. L. Arnold, A. Bar-Or, G. Comi, J. De Seze, G. Giovannoni, H.-P. Hartung, B. Hemmer, F. Lublin, K. W. Rammohan, K. Selmaj, A. Traboulsee, A. Sauter, D. Masterman, P. Fontoura, S. Belachew, H. Garren, N. Mairon, P. Chin, J. S. Wolinsky, *N. Engl. J. Med.* **2017**, *376*, 209.
[28] L. Radomir, M. P. Kramer, M. Perpinial, N. Schottlender, S. Rabani, K. David, A. Wiener, H. Lewinsky, S. Becker-Herman, R. Aharoni, R. Milo, C. Mauri, I. Shachar, *Nat. Commun.* **2021**, *12*, 1893.
[29] C. J. M. Piper, E. C. Rosser, K. Oleinika, K. Nistala, T. Krausgruber, A. F. Rendeiro, A. Banos, I. Drozdov, M. Villa, S. Thomson, G. Xanthou, C. Bock, B. Stockinger, C. Mauri, *Cell Rep.* **2019**, *29*, 1878.
[30] E. C. Rosser, C. J. M. Piper, D. E. Matei, P. A. Blair, A.© F. Rendeiro, M. Orford, D. G. Alber, T. Krausgruber, D. Catalan, N. Klein, J. J. Manson, I. Drozdov, C. Bock, L. R. Wedderburn, S. Eaton, C. Mauri, *Cell Metab.* **2020**, *31*, 837.
[31] S. V. Gearty, F. Dündar, P. Zumbo, G. Espinosa-Carrasco, M. Shakiba, F. J. Sanchez-Rivera, N. D. Socci, P. Trivedi, S. W. Lowe, P. Lauer, N. Mohibullah, A. Viale, T. P. Dilorenzo, D. Betel, A. Schietinger, *Nature* **2022**, *602*, 156.
[32] C. A. Dendrou, L. Fugger, M. A. Friese, *Nat. Rev. Immunol.* **2015**, *15*, 545.
[33] C. Skulina, S. Schmidt, K. Dornmair, H. Babbe, A. Roers, K. Rajewsky, H. Wekerle, R. Hohlfeld, N. Goebels, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 2428.
[34] J. J. Sabatino, M. R. Wilson, P. A. Calabresi, S. L. Hauser, J. P. Schneck, S. S. Zamvil, *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 25800.
[35] K. Wing, S. Sakaguchi, *Nat. Immunol.* **2010**, *11*, 7.
[36] M. Dominguez-Villar, D. A. Hafler, *Nat. Immunol.* **2018**, *19*, 665.
[37] K. Wing, Y. Onishi, P. Prieto-Martin, T. Yamaguchi, M. Miyara, Z. Fehervari, T. Nomura, S. Sakaguchi, *Science* **2008**, *322*, 271.
[38] B. Akkaya, Y. Oya, M. Akkaya, J. Al Souz, A. H. Holstein, O. Kamenyeva, J. Kabat, R. Matsumura, D. W. Dorward, D. D. Glass, E. M. Shevach, *Nat. Immunol.* **2019**, *20*, 218.

[39] V. Viglietta, C. Baecher-Allan, H. L. Weiner, D. A. Hafler, *J. Exp. Med.* **2004**, *199*, 971.
[40] S. Lindley, C. M. Dayan, A. Bishop, B. O. Roep, M. Peakman, T. I. M. Tree, *Diabetes* **2005**, *54*, 92.
[41] J. M. Mathew, J. H-Voss, A. Lefever, I. Konieczna, C. Stratton, J. He, X. Huang, L. Gallon, A. Skaro, M. J. Ansari, J. R. Leventhal, *Sci. Rep.* **2018**, *8*, 7428.
[42] A. Roemhild, N. M. Otto, G. Moll, M. Abou-El-Enein, D. Kaiser, G. Bold, T. Schachtner, M. Choi, R. Oellinger, *BMJ* **2020**, *371*, m3734
[43] A. Bluestone Jeffrey, H. Buckner Jane, M. Fitch, E. Gitelman Stephen, S. Gupta, K. Hellerstein Marc, C. Herold Kevan, A. Lares, R. Lee Michael, K. Li, W. Liu, S. A. Long, L. M. Masiello, V. Nguyen, A. L. Putnam, M. Rieck, P. H. Sayre, Q. Tang, *Sci. Transl. Med.* **2015**, *7*, 315ra189.
[44] F. P. Legoux, J.-B. Lim, A. W. Cauley, S. Dikiy, J. Ertelt, T. J. Mariani, T. Sparwasser, S. S. Way, J. J. Moon, *Immunity* **2015**, *43*, 896.
[45] J. D. Ooi, J. Petersen, Y. H. Tan, M. Huynh, Z. J. Willett, S. H. Ramarathinam, P. J. Eggenhuizen, K. L. Loh, K. A. Watson, P. Y. Gan, M. A. Alikhan, N. L. Dudek, A. Handel, B. G. Hudson, L. Fugger, D. A. Power, S. G. Holt, P. T. Coates, J. W. Gregersen, A. W. Purcell, S. R. Holdsworth, N. L. La Gruta, H. H. Reid, J. Rossjohn, A. R. Kitching, *Nature* **2017**, *545*, 243.
[46] P. Sagoo, N. Ali, G. Garg, F. O. Nestle, R. I. Lechler, G. Lombardi, *Sci. Transl. Med.* **2011**, *3*, 83ra42.
[47] P. P. Chen, A.-M. Cepika, R. Agarwal-Hashmi, G. Saini, M. J. Uyeda, D. M. Louis, B. Cieniewicz, M. Narula, L. C. Amaya Hernandez, N. Harre, L. Xu, B. C. Thomas, X. Ji, P. Shiraz, K. M. Tate, D. Margittai, N. Bhatia, E. Meyer, A. Bertaina, M. M. Davis, R. Bacchetta, M. G. Roncarolo, *Sci. Transl. Med.* **2021**, *13*, eabf5264.
[48] E. L. Masteller, Q. Tang, J. A. Bluestone, *Antigen-Specific Regulatory T Cells—Ex Vivo Expansion and Therapeutic Potential*, Vol. *18*, Elsevier, Amsterdam **2006**, pp. 103–110.
[49] C. N. Jenne, P. Kubes, *Nat. Immunol.* **2013**, *14*, 996.
[50] D. G. Doherty, *J. Autoimmun.* **2016**, *66*, 60.
[51] A. Carambia, B. Freund, D. Schwinge, M. Heine, A. Laschtowitz, S. Huber, D. C. Wraith, T. Korn, C. Schramm, A. W. Lohse, J. Heeren, J. Herkel, *J. Hepatol.* **2014**, *61*, 594.
[52] S. Shetty, P. F. Lalor, D. H. Adams, *Nat. Rev. Gastroenterol. Hepatol.* **2018**, *15*, 555.
[53] D. S. Wilson, M. Damo, S. Hirosue, M. M. Raczy, K. Brünggel, G. Diaceri, X. Quaglia-Thermes, J. A. Hubbell, *Nat. Biomed. Eng.* **2019**, *3*, 817.
[54] E. Saito, R. Kuo, K. R. Kramer, N. Gohel, D. A. Giles, B. B. Moore, S. D. Miller, L. D. Shea, *Biomaterials* **2019**, *222*, 119432.
[55] Q.i Liu, X. Wang, X. Liu, S. Kumar, G. Gochman, Y. Ji, Y.u-P. Liao, C. H. Chang, W. Situ, J. Lu, J. Jiang, K.-C. Mei, H. Meng, T. Xia, A. E. Nel, *ACS Nano* **2019**, *13*, 4778.
[56] Q. Liu, X. Wang, X. Liu, Y.-P. Liao, C. H. Chang, K.-C. Mei, J. Jiang, S. Tseng, G. Gochman, M. Huang, Z. Thatcher, J. Li, S. D. Allen, L. Lucido, T. Xia, A. E. Nel, *ACS Nano* **2021**, *15*, 1608.
[57] C. Kappel, C. Seidl, C. Medina-Montano, M. Schinnerer, I. Alberg, C. Leps, J. Sohl, A.-K. Hartmann, M. Fichter, M. Kuske, J. Schunke, G. Kuhn, I. Tubbe, D. Paßlick, D. Hobernik, R. Bent, K. Haas, E. Montermann, K. Walzer, M. Diken, M. Schmidt, R. Zentel, L. Nuhn, H. R.g Schild, S. Tenzer, V. Mailänder, M. Barz, M. Bros, S. Grabbe, *ACS Nano* **2021**, *15*, 15191.
[58] I. N. TombáCz, D. Laczkó, H. Shahnawaz, H. Muramatsu, A. Natesan, A. Yadegari, T. E. Papp, M.-G. Alameh, V. Shuvaev, B. L. Mui, Y. K. Tam, V. Muzykantov, N. Pardi, D. Weissman, H. Parhiz, *Mol. Ther.* **2021**, *29*, 3293.
[59] J. I. Andorko, K. G. Pineault, C. M. Jewell, *J. Biomed. Mater. Res., Part A* **2017**, *105*, 1219.
[60] S. A. Shah, R. S. Oakes, S. M. Kapnick, C. M. Jewell, *Front. Immunol.* **2022**, *13*, 843355.
[61] F. Campbell, F. L. Bos, S. Sieber, G. Arias-Alpizar, B. ¸R.n E. Koch, J. Huwyler, A. Kros, J. Bussmann, *ACS Nano* **2018**, *12*, 2138.
[62] M. A. Jackson, T. A. Werfel, E. J. Curvino, F. Yu, T. E. Kavanaugh, S. M. Sarett, M. D. Dockery, K. V. Kilchrist, A. N. Jackson, T. D. Giorgio, C. L. Duvall, *ACS Nano* **2017**, *11*, 5680.
[63] Q. Cheng, T. Wei, L. Farbiak, L. T. Johnson, S. A. Dilliard, D. J. Siegwart, *Nat. Nanotechnol.* **2020**, *15*, 313.
[64] E. S. Gollwitzer, S. Saglani, A. Trompette, K. Yadava, R. Sherburn, K. D. Mccoy, L. P. Nicod, C. M. Lloyd, B. J. Marsland, *Nat. Med.* **2014**, *20*, 642.
[65] M. Guilliams, I. De Kleer, S. Henri, S. Post, L. Vanhoutte, S. De Prijck, K. Deswarte, B. Malissen, H. Hammad, B. N. Lambrecht, *J. Exp. Med.* **2013**, *210*, 1977.
[66] P. Soroosh, T. A. Doherty, W. Duan, A. K. Mehta, H. Choi, Y. F. Adams, Z. Mikulski, N. Khorram, P. Rosenthal, D. H. Broide, M. Croft, *J. Exp. Med.* **2013**, *210*, 775.
[67] J. A. Hall, J. R. Grainger, S. P. Spencer, Y. Belkaid, *Immunity* **2011**, *35*, 13.
[68] F. Odoardi, C. Sie, K. Streyl, V. K. Ulaganathan, C. Schläger, D. Lodygin, K. Heckelsmiller, W. Nietfeld, J. Ellwart, W. E. F. Klinkert, C. Lottaz, M. Nosov, V. Brinkmann, R. Spang, H. Lehrach, M. Vingron, H. Wekerle, C. Flügel-Koch, A. Flügel, *Nature* **2012**, *488*, 675.
[69] J. S. Patton, P. R. Byron, *Nat. Rev. Drug Discovery* **2007**, *6*, 67.
[70] E. Saito, S. J. Gurczynski, K. R. Kramer, C. A. Wilke, S. D. Miller, B. B. Moore, L. D. Shea, *Sci. Adv.* **2020**, *6*, eabc9317.
[71] C. Kuehl, S. Thati, B. Sullivan, J. Sestak, M. Thompson, T. Siahaan, C. Berkland, *J. Pharm. Sci.* **2017**, *106*, 3293.
[72] A. V. Li, J. J. Moon, W. Abraham, H. Suh, J. Elkhader, M. A. Seidman, M. Yen, E.-J. Im, M. H. Foley, D. H. Barouch, D. J. Irvine, *Sci. Transl. Med.* **2013**, *5*, 204ra130.
[73] S. Haque, C. W. Pouton, M. P. Mcintosh, D. B. Ascher, D. W. Keizer, M. R. Whittaker, L. M. Kaminskas, *Nanomed.: Nanotechnol., Biol., Med.* **2020**, *30*, 102291.
[74] M. P. Lokugamage, D. Vanover, J. Beyersdorf, M. Z. C. Hatit, L. Rotolo, E. S. Echeverri, H. E. Peck, H. Ni, J.-K. Yoon, Y. Kim, P. J. Santangelo, J. E. Dahlman, *Nat. Biomed. Eng.* **2021**, *5*, 1059.
[75] A. K. Patel, J. C. Kaczmarek, S. Bose, K. J. Kauffman, F. Mir, M. W. Heartlein, F. Derosa, R. Langer, D. G. Anderson, *Adv. Mater.* **2019**, *31*, 1805116.
[76] L. T. Ferguson, E. D. Hood, T. Shuvaeva, V. V. Shuvaev, M. C. Basil, Z. Wang, J. Nong, X. Ma, J. Wu, J. W. Myerson, O. A. Marcos-Contreras, J. Katzen, J. M. Carl, E. E. Morrisey, E. Cantu, C. H. Villa, S. Mitragotri, V. R. Muzykantov, J. S. Brenner, *ACS Nano* **2022**, *16*, 4666.
[77] S. M. Lewis, A. Williams, S. C. Eisenbarth, *Sci. Immunol.* **2019**, *4*, eaau6085.
[78] N. Gagliani, T. Jofra, A. Valle, A. Stabilini, C. Morsiani, S. Gregori, S. Deng, D. M. Rothstein, M. Atkinson, M. Kamanaka, R. A. Flavell, M. G. Roncarolo, M. Battaglia, *Am. J. Transplant.* **2013**, *13*, 1963.
[79] T. Kato, A. G. Tzakis, G. Selvaggi, J. J. Gaynor, H. Takahashi, J. Mathew, R. Garcia-Morales, E. Hernandez, A. David, S. Nishida, D. Levi, J. Moon, E. Island, G. Kleiner, P. Ruiz, *Ann. Surg.* **2007**, *246*, 436.
[80] B. L. Jamison, T. Neef, A. Goodspeed, B. Bradley, R. L. Baker, S. D. Miller, K. Haskins, *J. Immunol.* **2019**, *203*, 48.
[81] R. A. Lamothe, P. N. Kolte, T. Vo, J. D. Ferrari, T. C. Gelsinger, J. Wong, V. T. Chan, S. Ahmed, A. Srinivasan, P. Deitemeyer, R. A. Maldonado, T. K. Kishimoto, *Front. Immunol.* **2018**, *9*, https://doi.org/10.3389/fimmu.2018.00281.
[82] R. A. Maldonado, R. A. Lamothe, J. D. Ferrari, A.-H. Zhang, R. J. Rossi, P. N. Kolte, A. P. Griset, C. O'Neil, D. H. Altreuter, E. Browning, L. Johnston, O. C. Farokhzad, R. Langer, D. W. Scott, U. H. Von Andrian, T. K. Kishimoto, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E156.
[83] C. Krienke, L. Kolb, E. Diken, M. Streuber, S. Kirchhoff, T. Bukur, Ö. Akilli-Öztürk, L. M. Kranz, H. Berger, J. Petschenka, M. Diken,

S. Kreiter, N. Yogev, A. Waisman, K. Karikó, Ö. TüReci, U. Sahin, *Science* **2021**, *371*, 145.
[84] A. Singh, S. Ramachandran, M. L. Graham, S. Daneshmandi, D. Heller, W. L. Suarez-Pinzon, A. N. Balamurugan, J. D. Ansite, J. J. Wilhelm, A. Yang, Y. Zhang, N. P. Palani, J. E. Abrahante, C. Burlak, S. D. Miller, X. Luo, B. J. Hering, *Nat. Commun.* **2019**, *10*, 3495.
[85] N. Pishesha, T. Harmand, L. Y. Smeding, W. Ma, L. S. Ludwig, R. Janssen, A. Islam, Y. J. Xie, T. Fang, N. Mccaul, W. Pinney, H. R. Sugito, M. A. Rossotti, G. Gonzalez-Sapienza, H. L. Ploegh, *Nat. Biomed. Eng.* **2021**, *5*, 1389.
[86] T. L. Mcgaha, M. C. I. Karlsson, *Immunol. Rev.* **2016**, *269*, 26.
[87] T. L. Mcgaha, Y. Chen, B. Ravishankar, N. Van Rooijen, M. C. I. Karlsson, *Blood* **2011**, *117*, 5403.
[88] D. R. Getts, D. M. Turley, C. E. Smith, C. T. Harp, D. Mccarthy, E. M. Feeney, M. T. Getts, A. J. Martin, X. Luo, R. L. Terry, N. J. C. King, S. D. Miller, *J. Immunol.* **2011**, *187*, 2405.
[89] D. Glancy, Y. Zhang, J. L. Y. Wu, B. Ouyang, S. Ohta, W. C. W. Chan, *J. Controlled Release* **2019**, *304*, 102.
[90] Y. Zhang, J. L. Y. Wu, J. Lazarovits, W. C. W. Chan, *J. Am. Chem. Soc.* **2020**, *142*, 8827.
[91] R. Zhang, R. El-Mayta, T. J. Murdoch, C. C. Warzecha, M. M. Billingsley, S. J. Shepherd, N. Gong, L. Wang, J. M. Wilson, D. Lee, M. J. Mitchell, *Biomater. Sci.* **2021**, *9*, 1449.
[92] T. Simon, L. Li, C. Wagner, T. Zhang, V. Saxena, C. C. Brinkman, L. H. Tostanoski, S. Ostrand-Rosenberg, C. Jewell, T. Shea-Donohue, K. Hippen, B. Blazar, R. Abdi, J. S. Bromberg, *Transplantation* **2019**, *103*, 2075.
[93] S. P. Hugues, L. Fetler, L. Bonifaz, J. Helft, F. Amblard, S. Amigorena, *Nat. Immunol.* **2004**, *5*, 1235.
[94] C. M. Jewell, S. C. Bustamante LóPez, D. J. Irvine, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 15745.
[95] L. H. Tostanoski, Y.-C. Chiu, J. M. Gammon, T. Simon, J. I. Andorko, J. S. Bromberg, C. M. Jewell, *Cell Rep.* **2016**, *16*, 2940.
[96] G. P. Howard, G. Verma, X. Ke, W. M. Thayer, T. Hamerly, V. K. Baxter, J. E. Lee, R. R. Dinglasan, H.-Q. Mao, *Nano Res.* **2019**, *12*, 837.
[97] S. Kang, S. Ahn, J. Lee, J. Y. Kim, M. Choi, V. Gujrati, H. Kim, J. Kim, E.-C. Shin, S. Jon, *J. Controlled Release* **2017**, *256*, 56.
[98] A. Schudel, D. M. Francis, S. N. Thomas, *Nat. Rev. Mater.* **2019**, *4*, 415.
[99] R. Galea, H. J. Nel, M. Talekar, X. Liu, J. D. Ooi, M. Huynh, S. Hadjigol, K. J. Robson, Y. T. Ting, S. Cole, K. Cochlin, S. Hitchcock, B. Zeng, S. Yekollu, M. Boks, N. Goh, H. Roberts, J. Rossjohn, H. H. Reid, B. J. Boyd, R. Malaviya, D. J. Shealy, D. G. Baker, L. Madakamutil, A. R. Kitching, B. J. O'sullivan, R. Thomas, *JCI Insight* **2019**, *4*, e126025.
[100] C. D. Maulloo, S. Cao, E. A. Watkins, M. M. Raczy, A. S. Solanki, M. Nguyen, J. W. Reda, H.-N. Shim, D. S. Wilson, M. A. Swartz, J. A. Hubbell, *Front. Immunol.* **2021**, *12*, https://doi.org/10.3389/fimmu.2021.714842.
[101] J. I. Andorko, L. H. Tostanoski, E. Solano, M. Mukhamedova, C. M. Jewell, *J. Visualized Exp.* **2014**, *83*, e50984.
[102] G. Senti, B. M. Prinz Vavricka, I. Erdmann, M. I. Diaz, R. Markus, S. J. Mccormack, J. J. Simard, B. WüThrich, R. Crameri, N. Graf, P. Johansen, T. M. KüNdig, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17908.
[103] E. Froimchuk, S. T. Carey, C. Edwards, C. M. Jewell, *Acc. Chem. Res.* **2020**, *53*, 2534.
[104] S. Zinkhan, A. Ogrina, I. Balke, G. Reseviča, A. Zeltins, S. De Brot, C. Lipp, X. Chang, L. Zha, M. Vogel, M. F. Bachmann, M. O. Mohsen, *J. Controlled Release* **2021**, *331*, 296.
[105] H. Wu, V. Estrella, M. Beatty, D. Abrahams, A. El-Kenawi, S. Russell, A. Ibrahim-Hashim, D. L. Longo, Y. K. Reshetnyak, A. Moshnikova, O. A. Andreev, K. Luddy, M. Damaghi, K. Kodumudi, S. R. Pillai, P. Enriquez-Navas, S. Pilon-Thomas, P. Swietach, R. J. Gillies, *Nat. Commun.* **2020**, *11*, 4113.
[106] H. C. West, C. L. Bennett, *Front. Immunol.* **2018**, *8*, https://doi.org/10.3389/fimmu.2017.01941.
[107] J. H. Kim, J. U. Shin, S. H. Kim, J. Y. Noh, H. R. Kim, J. Lee, H. Chu, K. Y. Jeong, K. H. Park, J. D. Kim, D. H. Jeong, T.-S. Yong, J.-W. Park, K. H. Lee, *Biomaterials* **2018**, *150*, 38.
[108] E. Shklovskaya, B. J. O'sullivan, L. G. Ng, B. Roediger, R. Thomas, W. Weninger, B. Fazekas De St Groth, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 18049.
[109] C.-M. Fovet, L. Stimmer, V. Contreras, P. Horellou, A. Hubert, N. Seddiki, C. Chapon, S. Tricot, C. Leroy, J. Flament, J. Massonneau, N. Tchitchek, B. A. 'T Hart, S. Zurawski, P. Klucar, P. Hantraye, K. Deiva, G. Zurawski, S. Oh, R. Le Grand, C. Serguera, *EBioMedicine* **2019**, *47*, 492.
[110] X. Zhao, J. C. Birchall, S. A. Coulman, D. Tatovic, R. K. Singh, L.i Wen, F. S. Wong, C. M. Dayan, S. J. Hanna, *J. Controlled Release* **2016**, *223*, 178.
[111] A. F. Lima, I. R. Amado, L. R. Pires, *Polymers* **2020**, *12*, 3063.
[112] J. S. Lewis, N. V. Dolgova, Y. Zhang, C. Q. Xia, C. H. Wasserfall, M. A. Atkinson, M. J. Clare-Salzler, B. G. Keselowsky, *Clin. Immunol.* **2015**, *160*, 90.
[113] J. S. Lewis, J. M. Stewart, G. P. Marshall, M. R. Carstens, Y. Zhang, N. V. Dolgova, C. Xia, T. M. Brusko, C. H. Wasserfall, M. J. Clare-Salzler, M. A. Atkinson, B. G. Keselowsky, *ACS Biomater. Sci. Eng.* **2019**, *5*, 2631.
[114] J. J. Cho, J. M. Stewart, T. T. Drashansky, M. A. Brusko, A. N. Zuniga, K. J. Lorentsen, B. G. Keselowsky, D. Avram, *Biomaterials* **2017**, *143*, 79.
[115] X. Chen, X. Yang, P. Yuan, R. Jin, L. Bao, X. Qiu, S. Liu, T. Liu, J. J. Gooding, W. Chen, G. Liu, Y. Bai, S. Liu, Y. Jin, *Sci. Transl. Med.* **2021**, *13*, eaaw9668.
[116] E. Kingwell, E. Leray, F. Zhu, J. Petkau, G. Edan, J. Oger, H. Tremlett, *Brain* **2019**, *142*, 1324.
[117] P. O'connor, J. S. Wolinsky, C. Confavreux, G. Comi, L. Kappos, T. P. Olsson, H. Benzerdjeb, P. Truffinet, L. Wang, A. Miller, M. S. Freedman, *N. Engl. J. Med.* **2011**, *365*, 1293.
[118] L. Steinman, *Nat. Rev. Drug Discovery* **2005**, *4*, 510.
[119] M. A. Cheever, C. S. Higano, *Clin. Cancer Res.* **2011**, *17*, 3520.
[120] C. S. Higano, A. J. Armstrong, A. O. Sartor, N. J. Vogelzang, P. W. Kantoff, D. G. Mcleod, C. M. Pieczonka, D. F. Penson, N. D. Shore, J. Vacirca, R. S. Concepcion, R. F. Tutrone, L. T. Nordquist, D. I. Quinn, V. Kassabian, M. C. Scholz, M. Harmon, R. C. Tyler, N. N. Chang, H. Tang, M. R. Cooperberg, *Cancer* **2019**, *125*, 4172.
[121] R. R. Mckay, J. M. Hafron, C. Ferro, H. M. Wilfehrt, K. Fitch, S. C. Flanders, M. D. Fabrizio, M. T. Schweizer, *Adv. Ther.* **2020**, *37*, 4910.
[122] B. Willekens, S. Presas-Rodríguez, M. Mansilla, J. Derdelinckx, W.-P. Lee, G. Nijs, M. De Laere, I. Wens, P. Cras, P. Parizel, W. Van Hecke, A. Ribbens, T. Billiet, G. Adams, M.-M. Couttenye, J. Navarro-Barriuso, A. Teniente-Serra, B. Quirant-Sánchez, A. Lopez-Diaz De Cerio, S. Inogés, F. Prosper, A. Kip, H. Verheij, C. C. Gross, H. Wiendl, M. Van Ham, A. Ten Brinke, A. M. Barriocanal, A. Massuet-Vilamajó, N. Hens, et al, *BMJ Open* **2019**, *9*, e030309.
[123] A. Lutterotti, S. Yousef, A. Sputtek, K. H. StüRner, J.-P. Stellmann, P. Breiden, S. Reinhardt, C. Schulze, M. Bester, C. Heesen, S. Schippling, S. D. Miller, M. Sospedra, R. Martin, *Sci. Transl. Med.* **2013**, *5*, 188ra175.
[124] C. Morath, A. Schmitt, C. Kleist, V. Daniel, G. Opelz, C. Süsal, E. Ibrahim, F. Kälble, C. Speer, C. Nusshag, L. Pego Da Silva, C. Sommerer, L. Wang, M. Ni, A. HüCkelhoven-Krauss, D. Czock, U. Merle, A. Mehrabi, A. Sander, M. Hackbusch, C. Eckert, R. Waldherr, P. Schnitzler, C. Müller-Tidow, J. D. Hoheisel, S. A. Mustafa, M. S. S. Alhamdani, A. S. Bauer, J. Reiser, M. Zeier, et al, *J. Clin. Invest.* **2020**, *130*, 2364.
[125] C. Kelly, J. A. Murray, D. A. Leffler, D. R. Getts, A. C. Bledsoe, G. Smithson, M. R. First, A. Morris, M. Boyne, A. Elhofy, T.-T. Wu, J. R.

Podojil, S. D. Miller, R. Fogel, T. L. Freitag, M. Gerber, P. K. Haynes, M. Koren, M. Matson, S. Meri, T. H. Oliphant, B. E. Rizzardi, J. Silvester, M. Turner, *Gastroenterology* **2021**, *161*, 66.

[126] R. Planas, R. Santos, P. Tomas-Ojer, C. Cruciani, A. Lutterotti, W. Faigle, N. Schaeren-Wiemers, C. Espejo, H. Eixarch, C. Pinilla, R. Martin, M. Sospedra, *Sci. Transl. Med.* **2018**, *10*, eaat4301.

Yuan Rui is a post-doctoral associate in the Fischell Department of Bioengineering at the University of Maryland. Her research focuses on designing nanoparticle and microparticle systems for safe and efficient delivery of bioactive molecules to modulate immune function. Specifically, she is interested in developing immunotherapies for autoimmune disease. She earned her Ph.D. in biomedical engineering from the Johns Hopkins School of Medicine in 2021.

Haleigh B. Eppler is a biological science Ph.D. candidate working in the Fischell Department of Bioengineering at the University of Maryland College Park. Her research uses biomaterials to generate immunological understanding. In particular, she is interested in using biomaterials to probe cellular processes occurring during autoimmune disease.

Alexis A. Yanes is a research technician and faculty specialist in the Fischell Department of Bioengineering at the University of Maryland, College Park. He received his B.S. in animal science from the University of Maryland where he currently supports lab operations at the interface of biomaterials and immunotherapy.

Christopher M. Jewell is the MPower Professor and the Minta Martin Professor of Engineering at the University of Maryland in the Fischell Department of Bioengineering. He is also a Research Health Scientist with the United States Department of Veterans Affairs. Dr. Jewell's research centers on using biomaterials to study and direct immune process. These efforts have been supported by $25M in funding, resulting in over 120 manuscripts. Dr. Jewell is a fellow of the Biomedical Engineering Society (BMES), the American Institute for Medical and Biological Engineering (AIMBE), the Controlled Release Society (CRS), and the Alliance for Cancer Gene Therapy (ACGT).