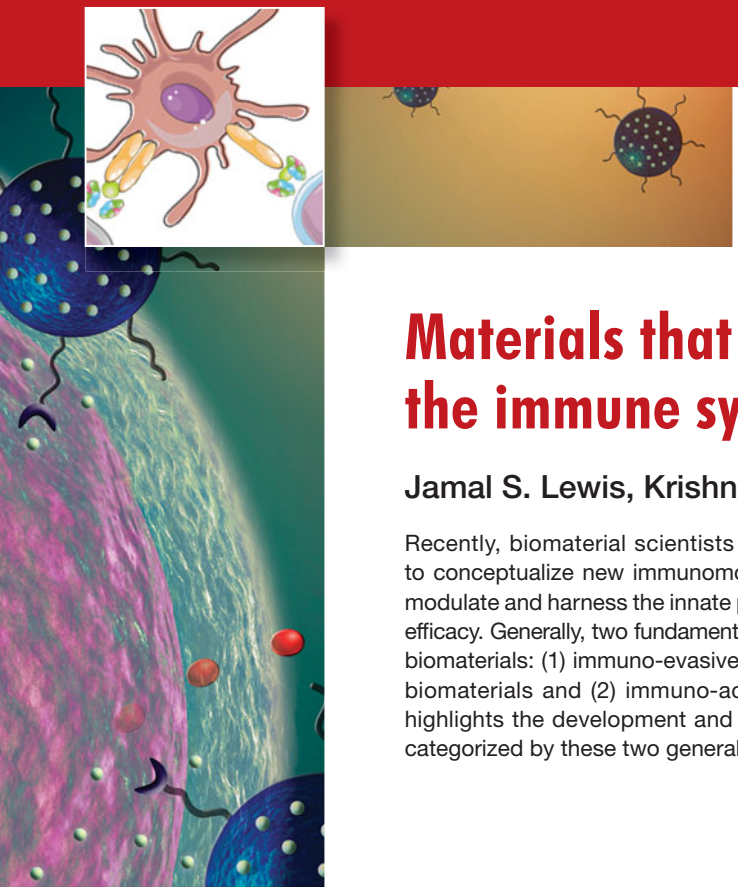


Materials that harness and modulate the immune system

Jamal S. Lewis, Krishnendu Roy, and Benjamin G. Keselowsky

Recently, biomaterial scientists have married materials engineering and immunobiology to conceptualize new immunomodulatory materials. This special class of biomaterials can modulate and harness the innate properties of immune functionality for enhanced therapeutic efficacy. Generally, two fundamental strategies are followed in the design of immunomodulatory biomaterials: (1) immuno-evasive (immuno-mimetic, immuno-suppressing, or immuno-inert) biomaterials and (2) immuno-activating or immuno-enhancing biomaterials. This article highlights the development and application of a number of immunomodulatory materials, categorized by these two general approaches.

Immune system introduction

The mammalian immune system is a highly complex, yet organized, network of organs, cells, and biomolecules whose primary function is to defend the body from any recognized foreign entity, including viruses and bacteria.[1] This defense system deploys two concerted sets of mechanisms to shield the body from would-be invaders: (1) the antigen-independent, nonspecific innate immune response that is activated immediately upon pathogen invasion, and (2) the temporally delayed, antigen-specific, adaptive immune response. Innate immunity employs a set of instant, protective measures that are specific to broad classes of pathogenic molecules. For instance, the skin and mucosal linings are central to the innate immune system forming a protective barrier from numerous pathogenic agents.[1,2] Additionally, innate immune responses include precise elements that allow for efficient molecular recognition and removal of non-self-entities (identified as not belonging to the host) and, further, engagement of the adaptive arm of the immune system via co-stimulatory signaling.[1,2] Essential to these responses are molecules of the complement system, chemokines and cytokines such as interferons, as well as cells such as neutrophils, macrophages, natural killer cells, and of particular note, dendritic cells (DCs) (see review on innate immunity in References 1 and 2).

The adaptive immune system, found only in vertebrates, is thought to be born out of evolutionary necessity and is involved in the development of long-term memory for newly encountered antigens.[3] Adaptive immunity is characterized by (1) an extraordinary repertoire of receptor molecules that result from somatic recombination (a mechanism of genetic recombination in the early stages of immunoglobulin [Ig] and T-cell receptors [TCRs] production of the immune system) and (2) immunological memory. With a diverse repertoire of molecules, which are typically on the cell surface and/or secreted by T cells (lymphocytes with multiple subsets, including those that attack infected or cancerous cells, direct the immune responses, and curb excessive immune reactions) and B cells (lymphocytes that primarily differentiate into plasma cells that secrete antibodies to attack foreign antigens), the adaptive arm of the immune system is highly effective in specifically tailoring immune reactions toward a newly defined pathogenic threat, while reducing any potential collateral damage to host tissue. Moreover, clonal expression and selection generate mechanisms by which long-term memory of the immune system is developed and preserved, which provides protective immunity from subsequent challenge by the same pathogen.[4] Together with T cells and B cells, the central effectors for adaptive immune responses include immunoglobulins and a wide array of cytokines. **Figure 1** illustrates a simplified

Jamal S. Lewis, University of Florida, Gainesville; jamalslewis@ufl.edu
Krishnendu Roy, University of Texas at Austin; kroy@mail.utexas.edu
Benjamin G. Keselowsky, University of Florida; bkeselowsky@bme.ufl.edu
DOI: 10.1557/mrs.2013.310

© 2014 Materials Research Society

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

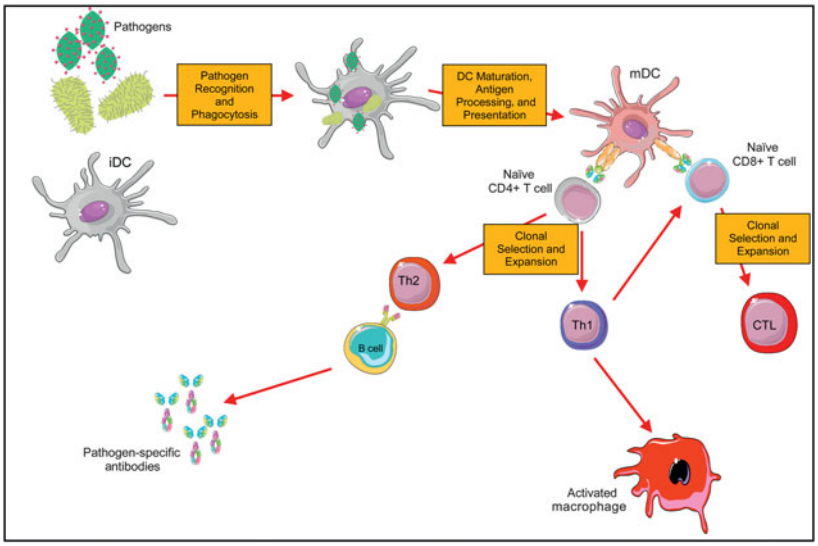


Figure 1. Simplified cascade of immune reactions to microbial agents. Naïve CD4+ T cells differentiate into helper T cells, Th1, and Th2; naïve CD8+ T cells differentiate into CTLs (and other cell types not shown). Professional antigen-presenting cells, including iDCs, recognize and engulf pathogens. They process the pathogenic material to peptides, which are then displayed to either CD4+ naïve T cells via MHC-II or CD8+ naïve T cells through MHC-I. These selected naïve T cells undergo clonal expansion and activation and mediate the adaptive immune response to the pathogen either through antibodies (humoral) or cellular elements (e.g., CTL). Note: iDC, immature dendritic cell; mDC, mature dendritic cell; Th, helper T cell; CTL, cytotoxic T lymphocyte; MHC-I, major histocompatibility complex-I; MHC-II, major histocompatibility complex-II.

cascade of immune reactions to microbial agents. Clearly, the mammalian immune system has evolved diverse and dynamic strategies to recognize, nullify, and eliminate pathogenic infections with efficacy and minimal collateral damage to host tissue. However, immune reactions are not reserved for invading pathogens only. Biomaterials, now frequently implanted into humans, can also elicit responses from the immune system.

Immuno-evasive biomaterials

It has long been recognized that implantation of biomaterials in bulk form triggers a significant response from the host immune system, collectively referred to as the foreign body reaction (reviewed by Anderson et al.).[5]

Recently, biomedical engineers have recognized that biomaterials can be designed to modulate host immune responses, including the foreign body reaction, allowing for improved therapeutic and diagnostic applications. Biomaterial surface chemistry, surface topography, and microscale architecture are all general approaches that have been applied to reduce immune reactions to implanted materials by limiting protein deposition, which controls immune cell interaction and activation.[6] Examples of these approaches are summarized in **Table I**. However, where physicochemical methods have reached their limit of benefit, research efforts to design biomaterials that modulate pro-inflammatory immune responses associated with implantation of biomaterials have intensified. One track of biomaterial design considered to have tremendous potential for attenuation of inflammatory immune reactions to biomaterials is "immuno-evasive biomaterials." Often referred to as "immuno-mimetic materials," these are biomaterials that borrow concepts from nature to direct specific immune responses at the molecular and cellular levels.

Evasive strategies of infectious agents

Pathogens of the viral and bacterial classes, because of their evolution to avoid immune system detection, interception, and clearance, are regarded as the inspiration behind the development of this genre of materials. The general strategies used by these microbial agents include (1) surface camouflage/obstruction, (2) phagocytic inhibition, (3) secretion of anti-inflammatory cytokines/homologues, and (4) negation/degradation of inflammatory mediators. Novak et al. compiled an excellent review of microbial immune evasive strategies and their adoption by biomaterials.[13] These pathogenic agents have evolved to survive, and even thrive, in mammalian hosts, withstanding constant assault by immune defense mechanisms. They could therefore inform biomaterial engineers on the design of materials with the ability to escape immune reactions.

Anti-inflammatory mediators for surface immobilization

Historically, to modulate inflammatory responses to biomaterial implants, material scientists have manipulated material type, surface chemistry, and topography (Table I). These properties can influence protein deposition, provisional matrix formation (temporary extracellular matrix that is remodeled over time), immune cell attachment, and ultimately host immune responses.[6] Studies such as those by Acharya et al. suggest that surface immobilization of naturally derived bio-ligands is an approach with potential to modulate inflammatory responses to biomaterial implants.[14] Hume et al. adopted an approach of this category, functionalizing polyethylene glycol (PEG) hydrogels with covalently immobilized immunosuppressive biological agents—transforming growth factor 1 (TGF-β1) and interleukin-10 (IL-10)—to reduce inflammatory responses to cell-laden material carriers. They immobilized TGF-β1 and IL-10 into PEG hydrogel networks via thiol-acrylate polymerization and showed that the presence of these two anti-inflammatory cytokines significantly down-regulates dendritic cell maturation markers, including IL-12 and major histocompatibility complex (MHC)-II *in vitro*.[15] Kim et al. also showed that surface immobilization of anti-inflammatory cytokines is a viable method to attenuate host immune responses *in vitro*. In this work, a fusion protein of recombinant human IL-1 receptor antagonist (IL-1ra) and elastin-like peptide was immobilized on self-assembling monolayers of carboxyl-terminated alkanethiol.

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

Table I. Classical approaches to prevent immune responses to implanted biomaterials.

General Approach	Specific Strategy	Example	Factor	Ref.
Biomaterial/Surface Chemistry	Hydrophilic material	Jones et al. reported that hydrophilic or neutral material implantation generally limited monocyte/macrophage adhesion and reduced foreign-body giant cell formation.	Polyacrylamide, sodium salt of poly(acrylic acid)	12
	Surface coating material with non-fouling synthetic materials	Bayramoglu et al. demonstrated that PHEMA hydrogels with conjugated heparin significantly reduced fibrinogen adsorption and platelet adhesion.	PHEMA hydrogel	11
	Surface-immobilized non-fouling moieties	Titanium surfaces made resistant to fibroblast adhesion by surface immobilization of non-fouling PEG moieties tethered via peptide mimics of mussel adhesive proteins.	PEG-DOPA	8
	Surface coating material with naturally derived materials	Marchant et al. showed that a polyethylene substrate could be rendered resistant to protein adsorption by coating with a modified dextran surfactant.	Dextran	7
Surface Physicality	Surface topography	Work done by Chen and collegues suggests that topographical cues on biomaterial surfaces can influence macrophage cell morphology and cytokine secretion *in vitro* and adhesion *in vivo*.	Micron scale patterns	9
	Surface roughness	A study by Deligianni et al. reported that cellular attachment of human bone marrow cells on Ti6Al4V surfaces was a function of increasing micro/sub-micron scale roughness.	Micro/sub-micron scale roughness	10

Note: PHEMA, poly 2-hydroxyethylmethacrylate; PEG, poly(ethylene glycol); DOPA, dihydroxyphenylalanine.

When lipopolysaccharide (LPS)-matured, human monocytes were cultured on this surface, there was a significant reduction in inflammatory cytokine production compared to control surfaces.[16]

Additionally, a number of studies have demonstrated that implanted materials can evade host immune responses through surface immobilization of pro-wound healing factors. These pro-wound healing factors do not directly influence immune responses but prevent material degradation by accelerating tissue regeneration around the implanted material. For instance, Aizawa et al. prepared an agarose hydrogel functionalized with an adhesive motif—glycine-arginine-glycine-glutamic acid-serine—and a neural stem cell differentiation factor—platelet-derived growth factor via photochemistry. This composite biomaterial supported neural stem/progenitor cell attachment and further preferential differentiation to oligodendrocytes—a neural cell type responsible for production of the myelin sheet that insulates axons.[17] Moreover, this study elucidated the potential of biomaterials to control tissue regeneration in a spatiotemporal manner, which could also influence host immune response and reduce material disintegration.

Release of anti-inflammatory and wound healing agents

Another major strategy used to design immuno-evasive biomaterials is the controlled release of tolerance-inducing and/or pro-wound healing agents from pre-fabricated depots within the biomaterial. The rationale of this approach is similar to that described previously for surface-immobilized mediators (i.e., factors are either anti-inflammatory—acting to directly mitigate immune responses—or pro-regenerative—expediting cellular coverage of the biomaterial and thereby protecting it from immune attack). However, the method of factor delivery differs—immobilized versus soluble. Because of the unrestricted mobility, this approach may offer a broader range of mechanisms to execute immune evasion, including inhibition of formation and secretion of pro-inflammatory factors (e.g., prostaglandins and leukotrienes), diminution of inflammatory cell recruitment pathways, and suppression of pro-inflammatory molecule production (e.g., IL-12). For instance, Hahn et al. demonstrated that hyaluronic acid (HA) hydrogels loaded with the anti-inflammatory agent vitamin E succinate (VES) produce an anti-inflammatory, non-fouling biomaterial.[18] It has been long recognized that hydrogels have major potential for immuno-isolation in a mode similar to that of bacterial biofilms.[19] Hahn and colleagues demonstrated that this physical barrier to immune intervention could be further functionalized with anti-inflammatory drugs. The HA-VES composite under *in vitro* conditions elicited poor monocyte adhesion and significantly reduced secretion of the pro-inflammatory cytokine tumor necrosis factor α (TNF-α) by LPS-activated macrophages, a result directly attributed to the release of VES from the hydrogel matrix.[18] Others have adopted similar hydrogel delivery approaches delivering anti-inflammatory cytokines, blocking proteins, pro-wound healing factors, or genetic transcripts for pro-regenerative/pro-inflammatory agents.[20–22]

Other immuno-evasive biomaterial strategies

Lin et al. demonstrated that hydrogel film use could be expanded to approaches for neutralization of inflammatory molecules.

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

This group developed a peptide-functionalized, PEG-based hydrogel capable of sequestering the pro-inflammatory cytokine, TNF-α. Although hydrogel coatings can act as a physical barrier to immune cells, soluble mediators such as TNF-α and reactive oxidative species can still penetrate the semi-permeable hydrogel and mediate implant failure. The TNF-α antagonizing peptide WP9QY was incorporated into a PEG hydrogel, and the composite demonstrated efficacy in prolonging survival time and functionality of encapsulated PC12 cells and mouse islets through sequestration of TNF-α.[22]

Another interesting approach to immuno-isolation of implants is cell-based surface modification. Various studies have determined that implantation of materials into either subcutaneous (beneath the skin) lean tissues or epididymal (occurring in or around the epididymis) fat tissues have very different healing and host immune responses.[23] Implants in subcutaneous lean tissue result in extensive fibrous capsule formation and reduced vascularization in implant-adjacent tissues, which could be detrimental to the function of the implanted device. In contrast, implants in the epididymal fat pads of rats exhibit less evidence of fibrous capsule formation and extensive neovascularization in implant-adjacent tissues.[24] The mechanisms by which this reduction in foreign body response severity occurs are yet to be clarified. However, groups have shown that adipose-derived stromal cells (ASCs; pluripotent progenitor cells found in fatty tissues) have exceptional regenerative qualities. Additionally, ASCs secrete a number of anti-inflammatory and pro-wound healing cytokines.[24] This has led to the development of ASC biomaterial coatings as a means of immuno-isolation. Prichard et al. confirmed that ASC attachment to different biomaterials is feasible using various immobilization schemes.[24]

Immuno-activating biomaterials

The previous discussion centered on the design of biomaterials for evasion or minimization of host immune responses against implanted materials. However, biomaterials can also be designed to harness the power of the host's immune responses and provide therapeutic effects. Perhaps the first applications of this concept are vaccines utilizing non-biological adjuvant materials (e.g., alum). An adjuvant is a substance that can amplify immune responses to an accompanying antigen but alone does not evoke adaptive immune responses.[25] Vaccines, first developed by Edward Jenner in 1796 have been hailed as the greatest success of modern medicine. Since then, vaccines have been advanced to control and, in some cases, eliminate lethal infectious diseases such as smallpox, polio, measles, and malaria. In spite of the many successes, barriers to expanding vaccine utility exist, particularly with regard to their safety, efficacy, and scalability. Current vaccine preparations typically include an antigen or live attenuated microorganism, an adjuvant to boost immune responses, and a delivery system for targeted delivery.[26] Live attenuated microorganisms are altered to reduce virulence but still carry the risk of reversion to an infectious state. Substitutes for this vaccine constituent include inactivated microbes, protein subunits, DNA, and recombinant vectors. These options are only marginally safer than live attenuated vaccines but only stimulate weak immune responses, necessitating use of adjuvants.[26]

Clinically, the list of adjuvants approved for use in humans is very short: in the United States, aluminum hydroxides or phosphates (e.g., alum) are the only adjuvants approved by the Food and Drug Administration (FDA).[27] This lack of viable options, in addition to an increased understanding of innate immunity, has spurred an upturn in adjuvant research. New insights into the ability of the immune system to control both the magnitude and direction of immune responses have stimulated novel vaccine design. Moreover, it is now clear that immunity is intimately involved in a plethora of medical conditions, including cancer, allergy, and autoimmune diseases (e.g., Type I diabetes).[28] Vaccination strategies against these 21st century diseases have propelled a deeper investigation of adjuvants that could direct specific immune responses. The use of biomaterials serving as an integral adjuvant component holds much promise.

Vaccine design

As mentioned previously, the immune system uses two distinct yet synergistic mechanisms to respond to pathogenic threats, generally referred to as the innate and adaptive immune responses. The overall physiological response, depicted in Figure 1, begins with the antigen presenting cells (APCs) of the innate immune system, which recognize and intercept pathogens. The most efficient APC is the dendritic cell (DC). Following phagocytosis (process of engulfing) of debris in peripheral tissue, DCs migrate to lymph nodes, where they present processed, pathogenic peptide to T cells and B cells. Degraded antigen fragments are presented on the surface of DCs via two types of major histocompatible complex molecules that provide signals via the T cell receptor complex to instigate T cell selection, expansion, and activation. The antigen presenting pathway is critical to the nature of the adaptive immune response. Endogenously derived (located within the cell cytosol) peptides are displayed primarily via Type I MHCs (expressed on all multi-nucleated cells). Peptide-MHC-I complexes interact with CD8+ T cells. On the other hand, MHC-II complexes (expressed on APCs) are loaded primarily with epitopes from exogenous antigenic sources and interact with CD4+ T cells. While these are considered the primary responses, recently, it has been shown that subsets of DCs are capable of efficient presentation of exogenously derived (located outside the cell) antigen fragments on MHC-I to CD8+ T cells; this phenomenon is referred to as cross-presentation.[29] Conversely, DCs can also undergo autophagy in response to certain pathogens, resulting in presentation of endogenous antigen on MHC-II complexes.[30]

Antigen presentation by DCs to CD8+ T cells can stimulate cytotoxic T lymphocytes, which can directly kill pathogen-infected cells by a number of mechanisms, including apoptosis (programmed cell death) and cell lysis (breakdown of the

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

integrity of the cell).[29] Dendritic cells also induce naïve CD4+ T cells to differentiate into helper T cells, Th1, and Th2, which facilitate cell-mediated and humoral immunity, respectively. These cells assist immune reactions via secretion of specific cytokines. It should also be noted that CD4+ T cell subsets have also been linked to immune suppression and may be important in the control of autoimmune dysfunction.[31,32] Finally, B cells also have antigen processing and presentation ability, but it is through interacting with APCs and helper T cells that B cells are fully activated and induced to differentiate into plasma cells that secrete high levels of antigen-specific antibodies.[2] For an effective vaccine, it is imperative that the relevant immune pathway is induced.[25] Therefore, these different modes of immunity must be considered in adjuvant selection and design.

Adjuvants used in current vaccines are biased toward initiation of humoral immunity (branch of immunity mediated by antibodies) and are often marked by significant increases in antibody titer (measurement of antibody production) and circulation time. However, with higher safety standards, the rapid emergence of resistance, and the broader spectrum of diseases requiring intervention, there has been a shift in focus to promote cell-mediated immunity.[33] It is thought that guiding dendritic cell response is central to controlling the direction (i.e., cell-mediated, humoral, or regulatory) and magnitude of immunity. Another important factor in vaccinology being reevaluated is the route of administration. The site of vaccine delivery is also the location of immune responses with maximum intensity, specifically comparing mucosal versus parenteral immunity.[34] Further, the vaccine administration site has been shown to also influence antibody isotype production. Pathogen infiltration typically occurs through mucosal regions (i.e., oral, nasal, genital membranes) and stimulates production of a breadth of antibody isotypes by plasma cells. In contrast, parenterally delivered vaccines primarily elicit immunoglobulin G antibodies,[33] and most vaccines are administered parenterally via intramuscular injection.[35] Vaccine delivery to one mucosal surface can prime other mucosal regions because of a shared immune mucosal system.[36] However, mucosal surfaces present several difficulties for vaccine delivery due to physiological barriers, including pH, enzyme activity, and membrane clearance mechanisms. For effective vaccination, large and repeated doses of antigen and adjuvant are required; otherwise immune tolerance to the delivered antigen may be induced.[36]

There is clear need for improvement in vaccine formulation and delivery strategies to meet revised vaccine safety and efficacy standards. These goals can be achieved by focusing on (1) the development of vaccine delivery modes, and (2) targeting of DCs for activation and control of cell-mediated immunity.[37] Both of these areas can be addressed by engineering adjuvants, and biomaterials can play a large role in this regard.

Adjuvant considerations

Early on in vaccine development, it was recognized that soluble protein antigens were far less immunogenic than protein antigens presented in particulate formulations.[26] Calcium phosphates and later aluminum hydroxide or aluminum phosphates in gel form (alum) were recruited to act as adjuvants (i.e., enhance immune responses to vaccine antigens). Today, alum remains the only FDA approved adjuvant for use in humans in the United States.[38] Likewise, in Europe, the only approved adjuvant is MF59 (a squalene-based proprietary mixture), a potent inducer of both cellular and humoral immune responses. However, the precise mode of action and the potential to induce autoimmunity remain significant drawbacks for use of MF59.[39] Since the 1980s, there has been extensive research conducted to increase vaccine adjuvant options. Experimental adjuvants currently under investigation to improve cell-mediated immunity include monophosphoryl lipid A (MPLA),[40] cytosine-phosphate-guanine (CpG) DNA motifs,[41] and modified cholera enterotoxin.[42] However, the toxic nature of these microbial-derived potentiators continues to be a major safety concern for use in humans.[26]

The primary considerations of new adjuvant design include (1) delivery of antigen to APCs, (2) protection of antigen integrity, and (3) activation of innate immunity.[38] DCs are the most effective antigen-presenting cells and the most effective at initiating cell-mediated immunity,[29] therefore, it is pertinent to convey antigen efficiently to DCs. Dendritic cell-based immunotherapy is a rapidly growing strategy to accomplish direct delivery of antigen to DCs.[43] In this *ex vivo* approach, monocytes are isolated from the patient's blood and matured into DCs *in vitro* using soluble differentiation cues. These *in vitro*-derived DCs are then pulsed with relevant antigen and subsequently reintroduced to the patient's body.[44] Although an exogenously loaded DC vaccine is now in clinical trial for prevention of Type I diabetes,[45] this approach is largely seen as a proof-of-concept exercise due to limitations that restrict its widespread application. DC-based immunotherapy limitations include plasticity and complexity of DC maturation, DC viability and trafficking, *ex vivo* cell stability, shelf-life, and high manufacturing costs.[46,47] Nevertheless, Dendreon Corp. has commercialized ProVenge—an active immunotherapy with autologous DCs that were loaded *ex vivo* with a fusion protein containing prostatic acid phosphatase for prostate cancer treatment.[48] For most patients, this immunotherapy is not more efficacious than chemotherapy. However, it may be beneficial to patients who are refractory to standard chemotherapeutic regimes.[49] A more practical and sustainable strategy would be *in vivo* delivery of antigen and modulating factors to DCs. To this end, researchers have co-administered antigen with DC-recruiting cytokines to attract DCs to the injection site.[50] Others have encapsulated antigen in particle-based vehicles for protection and targeted delivery to these specialized phagocytic cells.[51]

Vaccine efficacy can be dramatically reduced due to degradation of antigen by various physiological mechanisms. Stabilization of the antigen via adjuvant formulation is therefore a critical feature of vaccine design. For effective vaccination, the antigen must remain intact until uptake by APCs.

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

Antigen adsorption onto alum is the only approach approved in the United States, which circumvents the limited half-life of macromolecular antigens and the host's degradative mechanisms.[38] Alternatively, DC-based immunotherapy has also been investigated with DCs transfected with antigen-coding genetic material such that intact antigen is produced endogenously (derived from the cell's own machinery) and loaded into MHC-I by the cell's own machinery.[52]

Manipulation of innate immune elements, particularly DCs, is considered to be the most promising strategy to boost adjuvant activity and vaccine efficacy.[26] To this extent, research is being conducted to develop adjuvants that will provide for DC activation and initiation of adaptive immunity. One such immune stimulant that has been investigated for this purpose is Freund's complete adjuvant (FCA), which is composed of mineral oil and inactivated mycobacteria.[53] Despite being an effective adjuvant, FCA causes severe inflammation, and therefore is currently only approved for use in restricted animal studies.[53]

Another immune modulator that has been extensively researched for enhancement of adjuvant activity is LPS—an endotoxin and ligand for Toll-like receptors (TLR, a major receptor class involved in innate immunity). Unfortunately, LPS also stimulates a too vigorous immune reaction, potentially resulting in sepsis.[54] Some adjuvant researchers aim to modify TLR agonists (molecules with high binding affinity for TLR) to elicit cellular immune responses without the unwanted collateral damage accompanying sepsis. TLRs, discussed in-depth in a review by Akira et al.,[55] are transmembrane proteins found in either the cell surface or intracellular endosomal (pertaining to the endosome, an intracellular compartment involved in intracellular transport) membranes. Their location, dictated by the type of immune response induced, gives these pattern recognition receptors (PRRs) direct access to pathogen associated molecular patterns found in bacteria and/or viruses. Pathogen associated molecular patterns are structures that are not native to mammalian cells and include double-stranded RNA (e.g., Poly (I:C)), single-stranded RNA, and unmethylated CpG DNA motifs.[55] The association of this class of molecules with TLRs generally leads to downstream activation of inflammation-linked transcription factors, most notably the nuclear factor kappa B (NF-κB), the quintessential inflammation signaling pathway.[33]

Other PRRs that have also been identified for potential anti-pathogenic vaccine applications include retinoic acid-inducible gene 1(RIG-1)-like receptors, nucleotide-binding domain-like receptors, and C-type lectin receptors.[2] These receptors, localized in the cytoplasm, detect different components of bacteria and viral vectors, and generally trigger an inflammatory response via NF-κB activation.[2] The use of PRR-binding ligands to enhance immune responses to "weak" antigens offers a potential solution to improving vaccine efficacy by induction of cell-mediated immunity. However, there may still be cytotoxic side effects to contend with due to the potent inflammation induced, and this could restrict broad-scale application of this approach. To mitigate this, utilization of controlled release particulate biomaterial delivery systems may be able to minimize diffusion from the injection site and localize immunomodulatory molecules with PRRs in APCs.[37]

Polymeric particulates as vaccine adjuvants

Biomaterial particulate systems have gained widespread attention as a viable option for delivery of vaccines in the last quarter century.[56] Polymeric particles at the micron and submicron sizes are contemporary immunotherapy tools being used to deliver antigen,[57] adjuvant,[58] nucleic acids,[59] and pharmacological drugs.[60] Perhaps the most commonly employed biomaterial for delivery systems is poly(lactic-*co*-glycolic acid) (PLGA) or variants thereof. This biopolymer can be tailored by varying lactic to glycolic acid composition, has been extensively characterized, and has demonstrated qualities such as biocompatibility and biodegradability.[56] Additionally, PLGA particulate systems offer control over size and shape of the delivery system, hydrophobicity, loading, and release kinetics of a wide range of biomolecules. These physicochemical properties work in concert to influence particle uptake, modulation of immunogenicity, and antigen processing and presentation. Furthermore, PLGA particulates provide capability for surface functionalization.[61] These qualities combined make PLGA particulate systems well suited for vaccine delivery to APCs such as DCs.

There are a number of other biomaterial delivery systems currently under investigation for vaccine adjuvant purposes. For instance, poly(ε-caprolactone) has been identified as a suitable material for immunotherapeutic applications due to its hydrophobicity, biodegradability, ease of vaccine component incorporation, tunable release kinetics, and FDA approval in devices.[62]

Liposomes and micelles, micro- and nanoscale aggregates of phospholipid molecules, have also found a niche in immunotherapeutic delivery, although their stability may limit clinical application.[63] Interestingly, liposomes have inspired the development of a new class of delivery system called polymersomes, which are effective carriers of vaccine components and have a longer half-life than liposomes. These nano-sized structures are built from copolymers and graft copolymers that form a shell, which can encapsulate hydrophilic elements, while entrapping hydrophobic components within the shell itself.[64]

Naturally derived biomaterials have also demonstrated suitable adjuvant qualities. Because of their superior antibacterial properties, biocompatibility, and low toxicity compared to synthetic modules, natural polymers are now being investigated as vaccine carriers (e.g., dextran).[65] However, the limited availability of such naturally derived polymers may prevent their widespread use. Some researchers have combined both synthetic and naturally derived biopolymers to produce combined immunomodulatory delivery systems with properties customized to the vaccine application. Others have recognized that the dynamic conditions *in vivo* can influence

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

vaccine efficacy and addressed this potential hurdle by developing physiologically responsive (e.g., pH) particulate delivery systems.[66]

Biomaterial particulate adjuvant applications

Novel biomaterial particle-based delivery systems are being investigated as adjuvants that can tailor immune responses to the specific condition affecting the host. This section highlights a number of biomaterial particulate delivery approaches used in an adjuvant capacity, along the lines of augmenting either immunogenic or tolerogenic (tolerance-inducing) responses.

Immunogenic vaccine applications

Recently, injectable, particle-based material carriers have received much attention for their ability to efficiently deliver and improve immunotherapeutics, particularly for immunogenic applications (**Figure 2**). Biomaterial particulates can deliver antigen and immunostimulatory molecules to DCs, promote antigen-specific immunity, and have tunable properties that make them attractive for vaccine adjuvants. The most popular biomaterial employed for this purpose is PLGA. For instance, Elamanchili et al. demonstrated a PLGA nanoparticle-based delivery system that is a promising vaccine carrier approach for DC-mediated immune therapy.[67] An *in vitro* study by this group illustrated that PLGA nanoparticles, encapsulating the TLR4 agonist, MPLA, influenced upregulation of the positively stimulatory molecules (MHC-II and CD86) in murine-derived DCs. Inclusion of an antigen (MUC1 peptide) in the particulate formulation resulted in DCs with the ability to prime naïve T cells from unimmunized normal and MUC1 transgenic mice. Kasturi et al. conducted similar work, demonstrating that activation of innate immunity via TLRs significantly boosts vaccine efficacy. This group used a PLGA nanoparticulate system to deliver a combination of TLR 4 and TLR 7 agonists along with antigen to boost antigen-specific and T cell responses in mice. Further, the same nanoparticle-based delivery system induced robust immunity against pandemic H1N1 influenza in rhesus macaques.[68] Interestingly, it has been shown that nanoparticle-based systems are passively trafficked to draining lymph nodes via interstitial fluid flow to lymphatics following subcutaneous injection, improving vaccine efficacy via direct delivery to antigen-presenting cells and a controlled release of antigen depot.[69]

Another class of synthetic materials that is being explored for adjuvant qualities are polyanhydrides. Torres et al.[70] reported on the adjuvant activity of polyanhydride microparticles without additional immunomodulatory agents. This study demonstrated that microparticles composed of different polyanhydride chemistries (1,6-bis(*p*-carboxyphenoxy) hexane [CPH] and sebacic acid or 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane and CPH) can shape the phenotype and inflammatory secretion profile of *in vitro* murine-derived DCs in a chemistry-dependent fashion.[70] This relationship also influenced downstream antigen-specific proliferation of CD4+ and CD8+ T cells *in vitro*, suggesting that polyanhydride particulate systems can be tailored to control the direction of immune response, with potential as an immunogenic vaccine adjuvant against a wide range of pathogens.

Particulate systems can provide delivery of genetic material in a defined spatial and temporal manner for immunotherapeutic applications. Singh et al. used an injectable, polymer gel-particulate composite system for tuned simultaneous delivery of a chemoattractant (MIP3α; to induce DC migration to the biomaterial composite), interleukin-10 (IL-10) siRNA, and antigen plasmid DNA in order to modulate infiltrating APC phenotypes for *in vivo* cancer immunotherapy. They demonstrated that the hydrogel matrix had suitable degradability with controlled release of chemokine to attract significant numbers of DCs over a sustained period *in vitro*. In sum, the composite gel-particulate system attracts DCs, provides particulate antigens, and directs them toward an inflammatory phenotype.[71] This study demonstrated the versatility of this modular approach as well as promise for *in vivo*-specific immunotherapies for immune-related disease.

In an effort to further synchronize site-specific targeting of drug delivery with therapeutic or

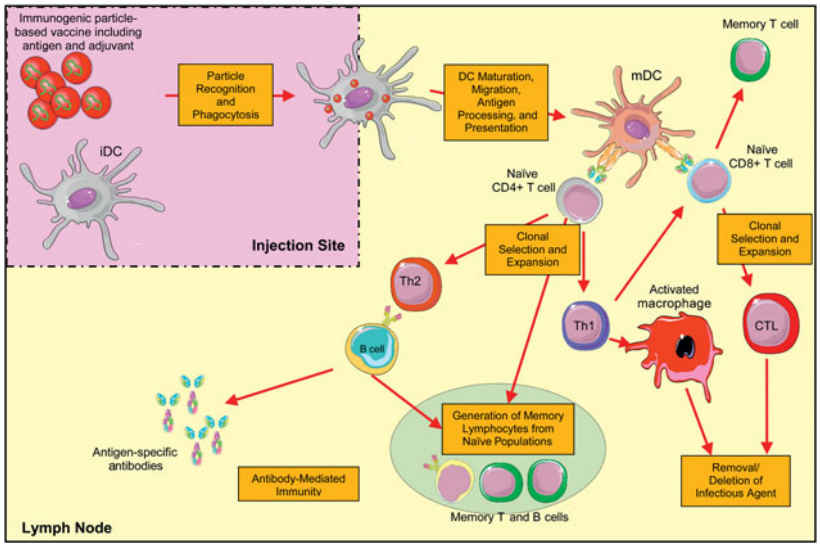


Figure 2. Possible immune mechanisms of immunogenic vaccines. Immature dendritic cells (DCs) recognize and engulf vaccine-loaded microparticles. After particle degradation and processing of the pathogenic antigen contained in the microparticles to peptides, the DCs mature and display antigenic peptide to either CD4+ naïve T cells via MHC-II or CD8+ naïve T cells through MHC-I. These selected naïve T cells undergo clonal expansion and activation, and mediate the adaptive immune response to the antigen either through antibodies (humoral) or cellular elements (e.g., CTL). Note: iDC, immature dendritic cell; mDC, mature dendritic cell; Th, helper T cell; CTL, cytotoxic T lymphocyte; MHC-I, major histocompatibility complex-I; MHC-II, major histocompatibility complex-II; TGF-β1, transforming growth factor beta 1.

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

diagnostic need, polymeric materials have been designed that respond to local physical, chemical, and biological cues, including pH, temperature, monosaccharides, and light.[72] Most of this stimuli-responsive work has focused on using polymeric particulate systems, which can be tailored to release therapeutics at the subcellular, cellular, tissue, or organ levels,[73] and which may prove impactful for immunotherapy. Free, soluble proteins are normally endocytosed into vesicles that subsequently become acidified and can contain degradative enzymes. To develop more effective vaccines against infectious agents and cancerous growths, it is important to promote delivery of protein antigens to the cytosol where they can access the MHC-I presentation pathway of antigen presenting cells. Endosome-disrupting particulate systems are a promising approach to achieve cytosolic delivery of protein therapeutics, thereby escaping antigen exposure to the acidic and degradative endosomal/lysosomal compartment.[74] Foster et al. illustrated that pH-responsive poly(propylacrylic acid) particulate systems with incorporated protein antigen could enhance CD8+ cytotoxic T cell generation as well as humoral responses to antigen challenge in thymus tumor-bearing wild type mice.[75]

Finally, self-assembling peptides have also been explored as promising immune adjuvants that physically present immuno-relevant T cell and B cell antigens. Notably, Rudra et al. illustrated the broad utility of self-assembling peptides as chemically defined adjuvants. Their investigation demonstrated that a short fibrilizing peptide (Q11; Ac-QQKFQFQFEQQ-Am) with covalently bound OVA323–339 epitope (ISQAVHAAHAEINEAGR) antigen is capable of raising high antibody titers against the antigen in the absence of adjuvant in a mouse model.[76] This research is promising for the development of self-adjuvanting vaccine systems.

Tolerance-inducing vaccine applications

Scientists have also utilized the carrier capacity of biomaterial particle-based systems for *in vivo* delivery of vaccine components, including antigen and immunomodulatory agents, to DCs for treatment of autoimmune disease and transplant rejection (**Figure 3**). For instance, a study by Phillips et al. demonstrated PLGA microspheres loaded with anti-sense oligonucleotides specific for co-stimulatory molecules that are involved in DC T-cell stimulation, passively targeted DCs, and manipulated their immunoregulatory function by suppressing the expression of these molecules integral for T-cell activation by DCs. The same group then successfully protected from Type I diabetes in non-obese diabetic (NOD) mice through *in vivo* targeting with microparticles that suppressed APC activation upon interception.[77] Alternately, microparticulate systems can potentially modify *in vivo* DC phenotypes by delivering a payload of immunosuppressive agent(s). For example, DCs that phagocytose rapamycin-loaded PLGA MPs have a reduced ability to activate T cells. Critically, this intracellularly targeted delivery of rapamycin to DCs provided more effective suppression than extracellular rapamycin delivery.[78]

Active targeting of MPs may prove important for augmenting delivery of adjuvants and antigen to DCs. This can be accomplished by surface ligation of ligands that specifically bind molecules on the surface of DCs. However, for tolerance-inducing vaccine applications, it is pertinent that targeting of these DC receptors do not stimulate inflammatory pathways. To this extent, Lewis et al. reported that poly(d, l lactic-*co*-glycolic acid) microspheres with surface immobilized ligands (DEC205 and CD11c antibodies and P-D2 peptide) are capable of enhanced DC targeting *in vitro* and *in vivo* without stimulating DC activation (**Figure 4**).[79] Similarly, Bandyopadhyay et al. demonstrated that DEC205 antibody-tethered PLGA nanoparticles not only efficiently target DCs for uptake but also increase production of IL-10 in DCs.[80] These findings could also be instructive for the use of artificial APCs to prevent and reverse autoimmune diseases. Along this line, researchers were able to prevent and reverse diabetes in NOD mice using peptide-MHC complex coated iron oxide (FeO) nanoparticles. Santamaria and co-workers demonstrated that nanoparticles coated with disease-relevant peptide-major histocompatibility Type I complexes (pMHC-I-nanoparticles) expanded cognate (with complementary TCRs)

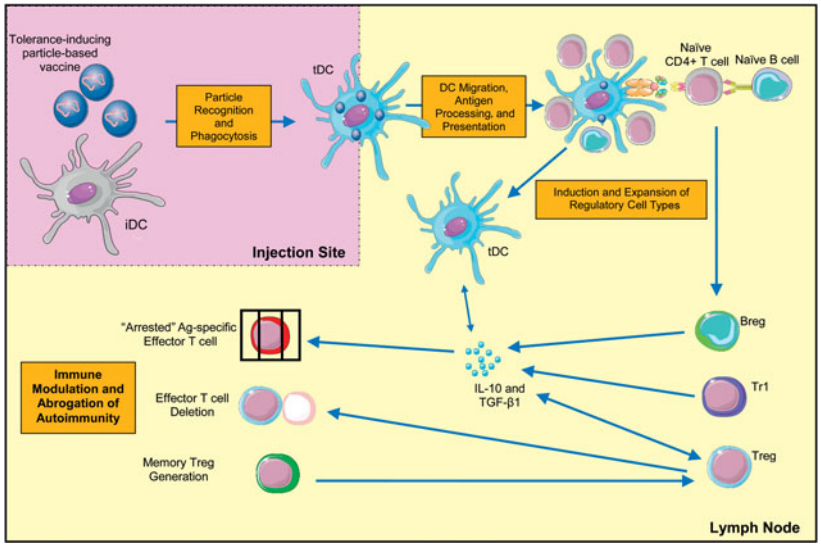


Figure 3. Possible immune mechanisms of tolerance-inducing vaccines. Immature dendritic cells (DCs) recognize and engulf particle-based vaccine. After particle degradation and antigen processing, the tDCs display peptides to selected CD4+ naïve T cells via MHCII in a tolerance-inducing manner. This tDC-naïve CD4+ T-cell interaction produces a number of antigen-specific regulatory T-cell types that halt autoimmune reactions. Note: iDC, immature dendritic cell; tDC, tolerogenic dendritic cell; Breg, regulatory B cell; Treg, FoxP3+ regulatory T cell; Tr1, Type I regulatory T cell; IL-10, interleukin-10; TGF-β1, transforming growth factor beta 1; MHC-II, major histocompatibility complex-II; CTL, cytotoxic T lymphocyte.

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

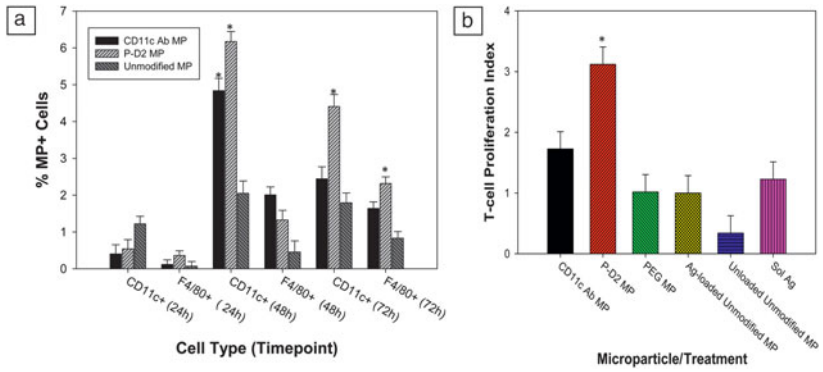


Figure 4. (a) Phagocytosis and trafficking of microparticles (MPs) by dendritic cells (DCs) and macrophages (MΦs) are enhanced through MP surface modification. Mice were given footpad injections containing ligand-conjugated, fluorescent dye-loaded MPs along with control MPs. Draining lymph nodes were recovered, processed, and stained for CD11c (DC) and F4/80 (MΦ) surface markers followed by flow cytometry analysis. Mean and standard deviations of the percentage of DCs and MΦs associated with MPs (MP+) are plotted, and pair-wise statistical significance is shown by *. (b) Surface modification of MPs improved prolonged antigen presentation by DCs. DCs were cultured with either MPs loaded with peptide (antigen) or soluble peptide (as a control, at an equal mass to that loaded in the MPs), followed by washing to remove unbound MPs. Subsequently, freshly isolated CD4+ T cells were added to culture wells after four days (to determine prolonged antigen presentation) and co-cultured in a three-day mixed lymphocyte reaction. T cell proliferation was then measured as a measure of functional antigen presentation. Note: PEG, poly(ethylene glycol); Sol, soluble. Adapted from Reference 79.

auto-regulatory CD8+ T cells in animals, suppressed the recruitment of non-cognate specificities, prevented disease in pre-diabetic mice, and restored normoglycemia (normal concentration of sugar in the blood) in diabetic animals.[81]

Concluding remarks

Recent recognition that the fields of immunity and biomaterials have begun to intertwine has spurred the intentional design of immunomodulatory biomaterials. These materials aim to harness the power of the immune system and can be generally classified into two categories: (1) immuno-evasive biomaterials and (2) immuno-activating biomaterials.

Immuno-evasive materials are being conceived out of a need to attenuate inflammatory reactions to biomaterial implantation. Initially, efforts to mitigate immune responses to implanted biomaterials involved manipulation of biomaterial surface chemistry, surface topography, and microscale architecture to limit protein deposition, and thereby limit immune cell adhesion. However, generally these approaches have only been moderately successful, prompting research into newer methods to neutralize immunity. To this extent, scientists are borrowing features of pathogenic immune evasion and applying them to implantable devices. Functionalization of biomaterials with extracellular matrix ligands, anti-inflammatory mediators, wound-healing agents, immuno-depleting hydrogels, and other bioinspired techniques are promising strategies for biomaterial immune evasion.

New insights into the inflammatory interactions of materials with immune cells, along with new developments in adjuvant immunology, have inspired the design of materials that harness the host's pro-immune responses—immuno-activating biomaterials. In retrospect, one of the first immuno-active biomaterials was alum, which acts as a non-biological adjuvant in vaccine formulations against microbial infections. Today, because of improved safety standards and a growing appreciation for the overlap of immunology and biomaterials, material-based adjuvant research has intensified. Particle-based systems that can deliver antigen and immuno-modulators have great potential for immunotherapeutic applications. Advantages of the use of particulate vaccine carriers include: (1) protection and stability of antigen, (2) multi-modal delivery of antigen and immunomodulator(s), (3) efficient delivery of vaccine components to dendritic cells, and therefore modulation of cell-mediated immunity, (4) delivery through multiple routes, and (5) applicability to a wide range of immune-related conditions. Further advances in immuno-biology and biomaterials engineering are expected to see an increasing role for particle-based systems in the next generation of vaccines.

Acknowledgments

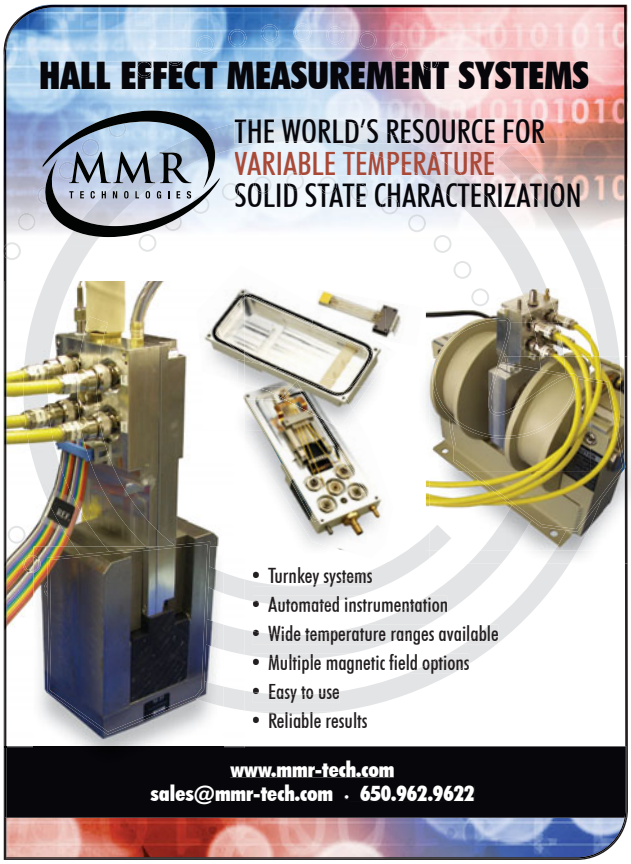
This work was supported in part by grants from the National Institutes of Health, R01DK091658 and R21A1094360.

References

1. J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.T. Liu, B. Pulendran, K. Palucka, *Annu. Rev. Immunol.* **18**, 767 (2000).
2. S. Akira, S. Uematsu, O. Takeuchi, *Cell* **124**, 783 (2006).
3. A. Iwasaki, R. Medzhitov, *Science* **327**, 291 (2010).
4. R. Medzhitov, C.A. Janeway, *Curr. Opin. Immunol.* **9**, 4 (1997).
5. J.M. Anderson, *Annu. Rev. Mater. Res.* **31**, 81 (2001).
6. R.M. Boehler, J.G. Graham, L.D. Shea, *Biotechniques* **51**, 239 (2011).
7. R.E. Marchant, S. Yuan, G. Szakalasgratzl, *J. Biomater. Sci., Polym. Ed.* **6**, 549 (1994).
8. J.L. Dalsin, L. Lin, S. Tosatti, J. Vörös, M. Textor, P.B. Messersmith, *Langmuir* **21**, 640 (2005).
9. S. Chen, A. Jones, Y. Xu, H.Y. Low, J.M. Anderson, K.W. Leong, *Biomaterials* **31**, 3479 (2010).
10. D.D. Deligianni, N. Katsala, S. Ladas, D. Sotiropoulou, J. Amedee, Y.F. Missirlis, *Biomaterials* **22**, 1241 (2001).
11. G. Bayramoglu, M. Yilmaz, E. Batislam, M. Arica, *J. Appl. Polym. Sci.* **109**, 749 (2008).
12. J.A. Jones, D.T. Chang, H. Meyerson, E. Colton, I.K. Kwon, T. Matsuda, J.M. Anderson, *J. Biomed. Mater. Res. A* **83A**, 585 (2007).
13. M.T. Novak, J.D. Bryers, W.M. Reichert, *Biomaterials* **30**, 1989 (2009).
14. A.P. Acharya, N.V. Dolgova, M.J. Clare-Salzler, B.G. Keselowsky, *Biomaterials* **29**, 4736 (2008).
15. P.S. Hume, J. He, K. Haskins, K.S. Anseth, *Biomaterials* **33**, 3615 (2012).
16. D.H. Kim, J.T. Smith, A. Chilkoti, W.M. Reichert, *Biomaterials* **28**, 3369 (2007).
17. Y. Aizawa, N. Leipzig, T. Zahir, M. Shoichet, *Biomaterials* **29**, 4676 (2008).
18. S.K. Hahn, S. Jelacic, R.V. Maier, P.S. Stayton, A.S. Hoffman, *J. Biomater. Sci., Polym. Ed.* **15**, 1111 (2004).
19. N.Y. Kostina, C. Rodriguez-Emmenegger, M. Houska, E. Brynda, J. Michalek, *Biomacromolecules* **13**, 4164 (2012).
20. C.C. Lin, P.D. Boyer, A.A. Aimetti, K.S. Anseth, *J. Control. Release* **142**, 384 (2010).
21. P.Y. Lee, Z.H. Li, L. Huang, *Pharm. Res.* **20**, 1995 (2003).
22. C.C. Lin, A.T. Metters, K.S. Anseth, *Biomaterials* **30**, 4907 (2009).

https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press

23. H.L. Prichard, W.M. Reichert, B. Klitzman, *Biomaterials* **28**, 936 (2007).
24. H.L. Prichard, W. Reichert, B. Klitzman, *Stem Cells* **26**, 2691 (2008).
26. P.H. Makela, *FEMS Microbiol. Rev.* **24**, 9 (2000).
25. R. Edelman, *Rev. Infect. Dis.* **2**, 370 (1980).
27. R.K. Gupta, *Adv. Drug Delivery Rev.* **32**, 155 (1998).
28. M.P. Girard, *Ann. Pharm. Fr.* **67**, 203 (2009).
29. J. Banchereau, R.M. Steinman, *Nature* **392**, 245 (1998).
30. S. Morris, M.S. Swanson, A. Lieberman, M. Reed, Z. Yue, D.M. Lindell, N.W. Lukacs, *J. Immunol.* **187**, 3953 (2011).
31. S. Sakaguchi, T. Yamaguchi, T. Nomura, M. Ono, *Cell* **133**, 775 (2008).
32. S. Sakaguchi, M. Ono, R. Setoguchi, H. Yagi, S. Hori, Z. Fehervari, J. Shimizu, T. Takahashi, T. Nomura, *Immunol. Rev.* **212**, 8 (2006).
33. J. Leleux, K. Roy, *Adv. Healthcare Mater.* **2**, 72 (2013).
34. J. Holmgren, C. Czerkinsky, *Nat. Med.* **11**, S45 (2005).
35. D. Kaul, P.L. Ogra, *Dev. Biol. Stand.* **95**, 141 (1998).
36. E.J. Ryan, L.M. Daly, K.H.G. Mills, *Trends Biotechnol.* **19**, 293 (2001).
37. S.K. Mallapragada, B. Narasimhan, *Int. J. Pharm.* **364**, 265 (2008).
38. K.S. Jones, *Biotechnol. Progr.* **24**, 807 (2008).
39. G. Ott, G.L. Barchfeld, D. Chernoff, R. Radhakrishnan, P. van Hoogevest, G. Van Nest, *Pharm. Biotechnol.* **6**, 277 (1995).
40. W. Quintilio, F.S. Kubrusly, D. Iourtov, C. Miyaki, M.A. Sakauchi, F. Lucio, S.d.C. Dias, C.S. Takata, E.N. Miyaji, H.G. Higashi, L.C. Leite, I. Raw, *Vaccine* **27**, 4219 (2009).
41. X.P. Ioannou, S.M. Gomis, R. Hecker, L.A. Babiuk, S. Littel-van den Hurk, *Vaccine* **21**, 4368 (2003).
42. C. Ruedl, C. Rieser, N. Kofler, G. Wick, H. Wolf, *Vaccine* **14**, 792 (1996).
43. H. Matsue, A. Morita, K. Matsue, A. Takashima, *J. Dermatol. (Tokyo)* **26**, 757 (1999).
44. S.A.W. Fadilah, S.K. Cheong, *Malaysian J. Pathol.* **29**, 1 (2007).
45. www.clinicaltrials.gov.
46. D. Yi, S. Appel, *Scand. J. Immunol.* **78**, 167 (2013).
47. B.G. Keselowsky, C.Q. Xia, M. Clare-Salzler, *Hum. Vaccines* **7**, 37 (2011).
48. J.A. McIntyre, D. Fernandez, *Drugs Future* **30**, 892 (2005).
49. C. Doehn, T. Boehmer, I. Kausch, M. Sommeraur, D. Jocham, *Biodrugs* **22**, 71 (2008).
50. O.A. Ali, G. Dranoff, D.J. Mooney, *Proc. Annu. Meet. Am. Assoc. Cancer Res.* **48**, 652 (2007).
51. D. Lemoine, F. Wauters, S. Bouchend'homme, V. Preat, *Int. J. Pharm.* **176**, 9 (1998).
52. Y.Z. Chen, X.L. Yao, Y. Tabata, S. Nakagawa, J.Q. Gao, *Clin. Dev. Immunol.* **2010**, 565643 (2010).
53. E. Claassen, W. Deleeuw, P. Degreeve, C. Hendriksen, W. Boersma, *Res. Immunol.* **143**, 478 (1992).
54. A. Ianaro, M. Tersigni, F. D'Acquisto, *Mini Rev. Med. Chem.* **9**, 306 (2009).
55. S. Akira, K. Takeda, T. Kaisho, *Nat. Immunol.* **2**, 675 (2001).
56. W. Jiang, R.K. Gupta, M.C. Deshpande, S.P. Schwendeman, *Adv. Drug Delivery Rev.* **57**, 391 (2005).
57. D.T. Ohagan, D. Rahman, J.P. Mcgee, H. Jeffery, M.C. Davies, P. Williams, S.S. Davis, S.J. Challacombe, *Immunology* **73**, 239 (1991).
58. T.E. Rajapaksa, D.D. Lo, *Curr. Immunol. Rev.* **6**, 29 (2010).
59. P.M. Mountziaris, D.C. Sing, S.A. Chew, S.N. Tzouanas, E.D. Lehman, F. Kasper, A.G. Mikos, *Pharm. Res.* **28**, 1370 (2011).
60. G. Mohammadi, H. Valizadeh, M. Barzegar-Jalali, F. Lotfipour, K. Adibkia, M. Milani, M. Azhdarzadeh, F. Kiafar, A. Nokhodchi, *Colloids Surf., B* **80**, 34 (2010).
61. Y. Waeckerle-Men, M. Groettrup, *Adv. Drug Delivery Rev.* **57**, 475 (2005).
62. M.A. Benoit, B. Baras, J. Gillard, *Int. J. Pharm.* **184**, 73 (1999).
63. G. Gregoriadis, *Trends Biotechnol.* **13**, 527 (1995).
64. D.M. Webster, P. Sundaram, M.E. Byrne, *Eur. J. Pharm. Biopharm.* **84**, 1 (2013).
65. X. Li, Kong, S. Shi, X. Zheng, G. Guo, Y. Wei, Z. Qian, *BMC Biotech.* **8**, 89 (2008).
66. W.N. Haining, D.G. Anderson, S.R. Little, M.S. von Berwelt-Baildon, A.A. Cardoso, P. Alves, K. Kosmatopoulos, L.M. Nadler, R. Langer, D.S. Kohane, *J. Immunol.* **173**, 2578 (2004).
67. P. Elamanchili, M. Diwan, M. Cao, J. Samuel, *Vaccine* **22**, 2406 (2004).
68. S.P. Kasturi, I. Skountzou, R.A. Albrecht, D. Koutsonanos, T. Hua, H.I. Nakaya, R. Ravindran, S. Stewart, M. Alam, M. Kwissa, F. Villinger, N. Murthy, J. Steel, J. Jacob, R.J. Hogan, A. Garcia-Sastre, R. Compans, B. Pulendran, *Nature* **470**, 543 (2011).
69. S. Hirosue, I.C. Kourtis, A.J. van der Vlies, J.A. Hubbell, M.A. Swartz, *Vaccine* **28**, 7897 (2010).
70. M.P. Torres, J.H. Wilson-Welder, S.K. Lopac, Y. Phanse, B. Carrillo-Conde, A.E. Ramer-Tait, B.H. Bellaire, M.J. Wannemuehler, B. Narasimhan, *Acta Biomater.* **7**, 2857 (2011).
71. A. Singh, S. Suri, K. Roy, *Biomaterials* **30**, 5187 (2009).
72. D. Roy, J.N. Cambre, B.S. Sumerlin, *Prog. Polym. Sci.* **35**, 278 (2010).
73. M. Motornov, Y. Roiter, I. Tokarev, S. Minko, *Prog. Polym. Sci.* **35**, 174 (2010).
74. S. Ganta, H. Devalapally, A. Shahiwala, M. Amiji, *J. Control. Release* **126**, 187 (2008).
75. S. Foster, C.L. Duvall, E.F. Crownover, A.S. Hoffman, P.S. Stayton, *Bioconjugate Chem.* **21**, 2205 (2010).
76. J.S. Rudra, S. Mishra, A.S. Chong, R.A. Mitchell, E.H. Nardin, V. Nussenzweig, J.H. Collier, *Biomaterials* **33**, 6476 (2012).
77. B. Phillips, K. Nylander, J. Harnaha, J. Machen, R. Lakomy, A. Styche, K. Gillis, L. Brown, D. Lafreniere, M. Gallo, J. Knox, K. Hogeland, M. Trucco, N. Giannoukakis, *Diabetes* **57**, 1544 (2008).
78. S. Jhunjhunwala, G. Raimondi, A. Thomson, S. Little, *J. Control. Release* **133**, 191 (2009).
79. J.S. Lewis, T.D. Zaveri, C.P. Crooks, B.G. Keselowsky, *Biomaterials* **33**, 7221 (2012).
80. A. Bandyopadhyay, R.L. Fine, S. Demento, L.K. Bockenstedt, T.M. Fahmy, *Biomaterials* **32**, 3094 (2011).
81. S. Tsai, A. Shameli, J. Yamanouchi, X. Clemente-Casares, J. Wang, P. Serra, Y. Yang, Z. Medarova, A. Moore, P. Santamaria, *Immunity* **32**, 568 (2010). □





https://doi.org/10.1557/mrs.2013.310 Published online by Cambridge University Press