Published in final edited form as:

J Mater Chem B Mater Biol Med. 2014 May 7; 2(17): 2409–2421. doi:10.1039/C3TB21549K.

Engaging adaptive immunity with biomaterials

Carolina Mora-Solano and Joel H. Collier*

University of Chicago, Department of Surgery

Abstract

Adaptive immune responses, characterized by T cells and B cells engaging and responding to specific antigens, can be raised by biomaterials containing proteins, peptides, and other biomolecules. How does one avoid, control, or exploit such responses? This review will discuss major properties and processes that influence biomaterials-directed adaptive immunity, including the physical dimensions of a material, its epitope content, and its multivalency. Selected strategies involving novel biomaterials designs will be discussed to illustrate these points of control. Specific immunological processes that biomaterials are being developed to direct will be highlighted, including minimally inflammatory scaffolds for tissue repair and immunotherapies eliciting desired B cell (antibody) responses, T cell responses, or tolerance. The continuing development of a knowledge base for specifying the strength and phenotype of biomaterials-mediated adaptive immune responses is important, not only for the engineering of better vaccines and immunotherapies, but also for managing immune responses against newer generations of increasingly biological and biomolecular materials in contexts such as tissue repair, tissue engineering, or cell delivery.

1. Introduction

In the past few decades, researchers working in the field of Biomaterials have developed an expansive set of exciting new hybrid biological/synthetic materials and are applying them towards diverse clinical objectives, including tissue engineering, wound healing, cell-based therapies, immunotherapies, and the delivery of genes, drugs, proteins, and other therapeutics for a broad spectrum of diseases. Arguably the dominant theme in contemporary biomaterials research is an ever-increasing reliance on incorporated biomolecules to confer specific biological activity.¹ Reasons for including biomolecules are extremely varied and include targeting (via antibodies or other affinity tags), cell adhesion, specific proteolysis, selective cell ablation, specific enzyme activity, homing factors for various cell populations, and modulators of inflammation, to name a few. However, along with the now commonplace integration of biological macromolecules into biomaterials come new considerations in biomaterials design, not the least of which are adaptive immune responses. Previous materials generally lacking in potentially immunogenic components such as the metals, polymers, and ceramics in current widespread clinical use have largely been able to avoid adaptive immunity, but next-generation biomaterials will not necessarily have that luxury. Fortunately, the very explosion of available chemistries and strategies seen in biomaterials recently is enabling not just the avoidance of adaptive immunity, but a deeper understanding and engagement of immunological processes as well, as evidenced by the increasing introduction of new vaccine platforms and other immunomodulating biomaterials. This article is focused on the emerging concepts, strategies, and underlying basic knowledge that is enabling us to avoid, control, or exploit biomaterials-directed

*corresponding author: Joel H. Collier, Associate Professor, Department of Surgery, Committee on Immunology, Committee on Molecular Medicine, University of Chicago, 5841 S. Maryland Ave ML 5032, Chicago, IL 60637, Tel: 773-834-4161, Fax: 773-834-4546, collier@uchicago.edu.

adaptive immune responses. The research area in this field is growing rapidly, so our goal is not to comprehensively cover all the work related to it, but rather to highlight studies and findings that illustrate the field's current state and some of its emerging directions. Other recent reviews emphasize the perspective of inflammation and innate responses,²⁻⁵ the immunology of nanotechnology and nanoparticles,²⁻⁹ immunoengineering,^{6,7,10,11} and peptide vaccines.^{12,13}

2. Basic Biomaterials Properties for Exploiting Adaptive Immunity

Adaptive immunity consists of T cell and B cell responses to specific antigens, and it is initiated by the interaction of these cells with antigen-presenting cells (APCs) in the lymph node (Figure 1). B cells become activated after cross-linking of their B cell receptors (BCRs), but require activated antigen-specific T follicular helper cells (T_{FH}) for maturation into long-lived antibody-producing memory B cells or plasma cells.^{9,14,15} Meanwhile, naive T cells require appropriately presented antigen (signal 1) and co-stimulation (signal 2).^{13,16} Both halves of this two-signal mechanism represent fundamental considerations for biomaterials design, for maximizing and for avoiding strong adaptive immune responses.^{13,16} Antigens are taken up by professional antigen-presenting cells (APCs, i.e. dendritic cells, B cells, and macrophages) and processed, and the resulting epitopes are displayed on the cell surface within the major histocompatibility complex (MHC). This epitope-MHC complex binds to the T cell receptor (TCR) to provide signal 1. Signal 2 is provided when the APC becomes activated and displays costimulatory receptors (e.g. CD80, CD86, CD40). Design strategies to activate co-stimulation, e.g. inclusion of danger-associated or pathogen-associated immunostimulatory ligands for pattern recognition receptors (PRRs), have been reviewed previously.^{6,7,10,11,17} Traditional vaccines prepared from live, attenuated, or inactivated whole pathogens contain a diverse mixture of antigens as well as molecules that can activate APCs and promote secretion of inflammatory cytokines, generally leading to strong immune responses, but chemically defined materials are providing new levels of control for specifying and shaping such responses.

In this section, we will highlight three of the most important compositional characteristics of biomaterials that influence adaptive immune responses: their epitope content, size, and multivalency. Together, these three factors strongly influence the pathways by which antigen-containing biomaterials are processed and responded to by the immune system.

2.1. Epitope content

Adaptive immune responses cannot proceed without competent epitopes, so efforts to specify the epitope content of a given biomaterial are an appropriate first-order point of control. Vaccines typically require the presence of a T cell helper epitope to activate CD4+ T cells, and either a B cell specific or CD8+ specific epitope, to induce neutralizing antibodies or cytotoxic responses, respectively.^{10,13,18} In the fields of bioengineering and biomaterials, the word “epitope” has come to be used at times to indicate a biofunctional or biomolecular component, or a ligand in general, but here we are employing the classical definition: the sequence on a molecule that can be recognized by antibodies, by BCRs, or TCRs. Epitopes are usually peptide sequences, and they can be incorporated into biomaterials directly or can arise *in situ* as APCs process larger antigens, proteins, cells, or debris associated with the biomaterial.

Two pathways exist for antigen processing and epitope presentation.^{10,18} In the classical MHC-II pathway, extracellular antigens are taken up via phagocytosis, endocytosis, or macropinocytosis, digested in endosomal/lysosomal compartments, loaded into MHC-II molecules, and presented on the APC surface for CD4+ helper T cells to recognize.^{10,18} Intracellular antigens (after infection with intracellular pathogens), or extracellular ones that

escape to the cytosol through cross-presentation pathways, are digested via the proteasome into short peptide fragments, transported into the endoplasmic reticulum, loaded into MHC-I, and presented on the APC surface for cytotoxic, CD8⁺ T cell recognition.^{10,18} Thus, a competent T cell epitope must successfully be processed through one of these entire pathways. A B cell epitope, on the other hand, is simply a sequence (linear or conformational) on a peptide or protein that can bind antibodies or BCRs directly; processing is not necessarily required.¹⁹

Currently, predictive algorithms are increasingly employed to determine the likelihood for a given amino acid sequence to act as a functional epitope.²⁰ In general, predictive power is greatest for MHC-I binding peptides,^{20,21} but MHC-II^{22,23} and B cell epitope prediction¹⁹ is rapidly improving. MHC-I epitope prediction can be based on multiple points along the antigen processing pathway, including proteasomal cleavage, MHC binding, engagement of TCRs, the 3D structure of the epitope, or a consensus combination of more than one of these considerations.²¹ MHC-I molecules are known to bind peptides 8-10 amino acids (aa) long whereas MHC-II molecules can bind peptides 9-40 aa in length, even though the core binding motif is only 9aa long.²³ These length differences mean that an MHC-II-binding epitope can extend out of the open ends of the MHC surface and bind with different registers or aa contacts, making prediction more complicated than for MHC-I-binding epitopes. B cell epitope prediction is also challenging because discontinuous stretches of amino acids often form conformational epitopes, requiring knowledge or calculation of the 3D structure of the protein.¹⁹ Although prediction tools continue to be refined and are not yet 100% reliable, an online resource such as the Immune Epitope Database and Analysis Resource (IEDB, www.iedb.org) is a helpful entry point for biomaterials researchers seeking to predict the epitope content of their constructs.^{24,25}

Consideration of epitope content is perhaps the most critical parameter for biomaterials-driven adaptive immune responses, since the presence or absence of T and B cell epitopes on a particle, fiber, scaffold, or other biomaterial can mean the difference between no detectable immune response and a vigorous one. For example, peptides that self-assemble into nanofibers do not raise detectable antibody responses unless competent B and T cell epitopes are present in the peptide sequence, in which case the antibody responses are strong and durable.²⁶⁻²⁹

2.2. Size

The physical dimensions of a biomaterial profoundly influence adaptive immune responses.^{9,30} For particulate vaccines and delivery systems, size modulates biodistribution, active and passive targeting, cellular uptake mechanisms, and the intracellular fate of antigens,³¹⁻³⁶ which cumulatively direct the magnitude and quality of the adaptive immune response elicited.^{29,34,37-47} APCs such as dendritic cells and macrophages are capable of recognizing and responding to particles ranging from the size of viruses (10-200 nm), to bacteria (1-8 μ m), and cells (>1 μ m),^{7,9,30} and immunomodulatory biomaterials have been designed in each of these ranges.

As most immunotherapeutic platforms are delivered to peripheral tissues, size regulates whether or not biomaterials can access APC-enriched anatomical locations, such as the lymph nodes. It is critical that the biomaterial or its derivatives traffic correctly to and within the lymph node, so that it can interact with the necessary cell types to elicit a response. The lymphatic system mediates and regulates the transport of molecules and cells between tissue compartments and the blood.^{48,49} Entry into lymphatic vessels is possible owing to the presence of gaps and permeable cell-cell junctions between endothelial cells.^{11,33,48,49} The anatomy of lymph nodes is composed roughly of a cortex, paracortex, and medulla circumscribed by a sinus. B cell follicles are located in the cortical region, while T cells

localize to the paracortical region. Lymphocytes enter the lymph nodes at specialized endothelia called high endothelial venules (HEVs) and migrate within the nodes following specific chemokine gradients.^{48,49} DCs, on the other hand, can be found throughout the node but are present in higher numbers near the T cell zone and the medulla.^{48,49} Incoming fluid from lymph vessels is sampled first at the sinus, which is lined with mostly macrophages but also DCs.^{33,48-50} In this region, molecules larger than 70kDa or 7nm in diameter drain along the sinus and can be internalized by APCs.^{11,33,34,49,50} Lower molecular weight materials, in contrast, can reach other compartments of the lymph nodes directly, without being carried there by APCs, as they can be transported inside small channels in the lymph node known as fibroblastic reticular conduits (FRCs).^{49,51,52} B cells and immature resident DCs, but not mature immigrating DCs, are able to probe into these conduits to sample antigens.^{50,53-55} Materials >100nm are less successful at passive transport through the lymphatic system by interstitial flow, but can be carried to nodes following uptake and processing by migrating DCs.^{33,35,40} Alternatively they can be specifically delivered to nodes via intranodal injections.⁵⁶

Owing to the strict size exclusion properties and permeability of the lymphatic system, small soluble antigens (<70kDa) arrive from a site of delivery or infection within minutes and drain through the FRCs.⁵²⁻⁵⁵ Larger particles take longer to reach lymph nodes but are detectable, although at much lower intensity, anywhere between hours (<50nm) to days (>50nm) after peripheral injection, suggesting uptake by APCs at the lymph nodes or in tissues, respectively.^{29,33-35,37,39,40,44} The cutoff size for effective drainage of particles to lymph nodes was found to be 45nm,³³ which is larger than the size limit for rapid elimination via the kidneys at 5.5nm³² but much lower than the size limit (>1 μ m) for rapid clearance by the reticuloendothelial system (liver, spleen, lymph nodes and bone marrow).³⁰ While specifically investigating lymph node targeting, 30nm-size fluorescent virus-like particles (Figure 2a) and 20nm nanoparticles could be detected in lymph nodes within 2h hours.^{33,35} In contrast, larger (100nm, 500nm or 1 μ m) particles are barely detected in the lymph nodes by 24h and largely remain at the injection site.^{33,35,44} Interestingly, particles <50nm delivered via the skin (subcutaneously or intradermally) can reliably target an impressive ~50% of DCs in the lymph nodes, of which CD8a+ DCs constitute a population important for cross-presentation of antigen to CD8+ T cells.^{34,35} For example, 25nm particles modified with polyhydroxylated surfaces (to mediate complement binding) induced CD8 T cell responses at levels comparable to co-injection of soluble LPS and antigen.³⁴

Larger (>50nm) biomaterials also can elicit strong responses in the lymph nodes. Some of these materials have been carefully designed to degrade under certain environmental or cellular conditions, while others may function due to heterogeneity in the size of the particles or due to partial degradation, two alternatives that need to be investigated further. For example, ~200nm ICMVs were specifically designed for rapid degradation by phagosomal phospholipases following uptake by DCs to promote cross-presentation and CD8 T cell responses (Figure 2b).³⁹ When these particles were synthesized with a malaria antigen that was incorporated to the surface in a multivalent display format, these particles elicited long-lasting (>1year) antibody responses following a prime/boost regimen, and the strong antibody responses correlated with sustained presence of ICMVs in the lymph nodes, formation of germinal centers, and development of T follicular helper (T_{FH}) cells.⁴⁰ Similarly, ~1 μ m-long peptide nanofibers (Figure 2c) composed of the self-assembling peptide Q11 (QQKFQFQFEQQ) synthesized in tandem with the OVA₃₂₃₋₃₃₉ peptide (which contains both B cell and T cell epitopes) were found to elicit high IgG titer antibodies and a 100-fold higher frequency of germinal center B cells compared to alum-adjuvanted OVA₃₂₃₋₃₃₉.^{26,27,29} Finally, peptide amphiphiles conjugated to OVA-specific MHC class I peptide SIINFELK peptide (DiC16-OVA) that form cylindrical micelles 50-300nm in length and less than 10nm in diameter were shown to significantly delay the growth of OVA-

expressing tumor cells and extend the survival of tumor-bearing mice compared to immunization with soluble OVA administered in incomplete Freund's adjuvant.⁴⁵

Thus, in designing immunostimulatory biomaterials where specific targeting of lymph node-resident DC populations is warranted, small particles appear to be the most suitable candidates. In particular, small particles with the proper immunostimulatory surface characteristics appear to be effective at targeting and activating a significant number of lymph-borne DCs, making them highly desirable for cross-presentation and CD8⁺ T cell activation responses. On the other hand, unless care is taken to achieve very small particles, slower trafficking by DCs and a bias toward CD4⁺ T cell and antibody responses seem to be the most likely route for most biomaterial particulates.^{26-29,37,40,44,46,47,53,57} Yet, given that larger particles can still elicit CD4⁺ and CD8⁺ T cells,^{29,37,39,44-46,57} it is plausible that fragments from larger particles are capable of targeting lymph nodes directly, or that targeting APCs in peripheral tissues is sufficient to induce those responses. Although size is now a recognized parameter of major importance for designing immunostimulatory biomaterials, the ultimate fate of different nanoparticle and microparticle systems and how they and their byproducts contribute to adaptive immune responses continues to be under considerable investigation.

2.3. Multivalency

In addition to the identity of the epitopes, their multivalency is also critical to the adaptive immune response, particularly for B cell activation.^{9,58,59} Multivalency in this context refers to the repetitive display of an antigen at high local density on the material. Synthetic multivalent materials cause crosslinking of BCRs that are specific for that antigen, thus lowering the threshold for B cell activation.^{9,58,59} Highly multivalent antigen presentation has been achieved for biomaterials by using synthetic scaffolds or recombinant proteins, and these can elicit strong antibody responses in both T-cell independent and T cell-dependent systems; here we focus primarily on the latter. The first multi-antigenic peptide (MAP) vaccine was designed over 25 years ago and consisted of a fully synthetic branching oligolysine macromolecule that incorporated multiple copies of peptide epitopes.^{60,61} In most cases, the antibodies produced reacted to the native protein as well as the peptide.⁶⁰ Further, incorporation of malaria antigen-specific B and T cell epitopes was found to protect mice after a malaria parasite challenge and protection required the presence of both the B cell and the T cell epitopes in the scaffold, suggesting a T cell dependence for antibody production.⁶¹

The requirement for repetitiveness and organized multivalent display to elicit antibodies was later corroborated using viruses,⁶² recombinant proteins bearing multiple copies of the same epitope,⁶³ and synthetic or derived particles mimicking viral structures known as virus-like particles (VLPs, Figure 2a).^{38,41,42,64-66} The organized MAP format promotes BCR activation and can reverse B cell tolerance to soluble antigens.⁶⁷ Some of these synthetic and bio-inspired multivalent vaccine constructs can promote T cell-dependent antibody responses in the presence of diminishing doses of adjuvant (also called dose sparing) or in the absence of exogenous danger signals.^{38-41,43,68} For example, Moon, Irvine, and coworkers developed lipid nanoparticles composed of layers of stapled lipid vesicles entrapping recombinant protein antigens from a malaria parasite, and additionally coated the particles' surfaces with antigen for multivalent display.⁴⁰ The multivalent presentation of the antigen increased by almost a full order of magnitude the antibody titers compared to nanoparticles that only encapsulated the antigen for controlled release. When delivered to mice in the presence of monophosphoryl lipid A (MPLA), these particles required a much lower dose of both antigen and adjuvant to elicit broad and durable humoral responses (Figure 2b). Further, the responses included germinal center formation and differentiation of CD4 T cells to become T Follicular Helper (TFH) cells. Also, multivalent antigen effects on

B cells are also likely to be an important component of the responses seen with self-assembled peptides (Figure 2c).²⁶⁻²⁸

3. Immunological Outcomes Achievable with Biomaterials

3.1. Antibody responses without inflammation or rejection

Much of the interest in immunomodulating biomaterials is being driven by the need for new vaccines and immunotherapies, but other biomedical research areas such as tissue engineering and surgical repair are also benefitting from increased immunological understanding and control. For example, in the development of new biologically active materials for tissue repair, any inflammatory adaptive immune responses to epitopes engineered into the material could lead to the undesirable outcome of rejection. One longstanding strategy for rendering allograft or xenograft tissues minimally immunogenic is the removal of cells and cellular debris via chemical treatments or detergents. This strategy significantly depletes immunogenic cellular components such as the oligosaccharide α -Gal membrane antigen, HLA/MHC molecules, and genetic materials.^{3,5,69} Even whole organs can be sufficiently decellularized to the point that there is little evidence of rejection.⁷⁰ Decellularized matrix products in current clinical usage are manufactured from a range of sources, including human, porcine or bovine dermis, porcine small intestinal submucosa (SIS), pericardium from a variety of species, and porcine heart valves.⁶⁹ Even though decellularized matrices assuredly still contain non-self antigens, they are well tolerated *in vivo* and exhibit constructive remodeling. Although the widespread clinical use of such scaffolds may appear to suggest that these materials are effectively “inert” to adaptive immunity, studies conducted over the past decade have shown that adaptive immunity is, on the contrary, substantially engaged. These biologically sourced materials are molecularly complex, but they have led to interesting observations with respect to immune responses that are likely to have significant relevance to engineered biomolecular biomaterials in general. Here we discuss the immune response to SIS as a model for non-inflammatory, pro-healing responses, followed by emerging evidence that engineered materials can elicit these responses as well.

3.1.1. Small intestinal submucosa—In 2001, Metzger and colleagues found that SIS raised a vigorous antibody response in mice, even though it demonstrated excellent tissue compatibility and did not elicit any signs of rejection or inflammation in the tissue (Figure 3a-c).⁷¹ The antibody response to this material was polarized towards the IgG1 subclass, and the strength of the IgG1 response was as high as with extracts of SIS injected with complete Freund's adjuvant (CFA, a very strong adjuvant). Despite this vigorous antibody response, the implants performed well, exhibiting productive remodeling, resolved inflammation, and overall acceptance of the material by 28 days. The antibody response was dependent on T cells, as no anti-SIS antibodies were detected in T cell knockout mice.⁷¹ Thus, the CD4⁺ T helper (Th) cell phenotype may provide a hint about the mechanism of this immunogenic but non-inflammatory clinical profile. Th phenotypes can take many forms, the best known of which are Th1 and Th2. Th1 responses are characterized by the production of pro-inflammatory cytokines such as interferon gamma (IFN- γ), and interleukin 2 (IL-2), which can induce classically activated macrophages, stimulate the production of complement-fixing antibody isotypes such as IgG2a and IgG2b, the differentiation of CD8⁺ cells to a cytotoxic phenotype, and inflammation. In contrast, Th2 responses are characterized by the production of IL-4 and IL-10 by T cells, which do not cause macrophages to become classically activated, and which lead to the production of non-complement-fixing antibody isotypes such as IgG1.^{4,72} Thus, the IgG1 dominance in the response to SIS is consistent with Th2 polarization. Cytokine analysis of the implants further supported the conclusion that Th2 responses were favored by the SIS, as IL-4 was upregulated and IFN- γ was

downregulated relative to xenogeneic tissue positive controls.⁷¹ While these experiments were conducted in mice, similar results have been observed in humans and non-human primates. In humans, the SIS used in hernia repair procedures raises significant antibody responses, with titers peaking from 2-6 weeks and diminishing thereafter,⁷³ yet it is not associated with signs of clinical rejection or other complications.⁷⁴ When human decellularized dermal tissue was implanted into monkeys, it also elicited a similar, early, and transient antibody response.⁷⁵

The simultaneous production of antibodies and the absence of tissue inflammation in response to decellularized matrices raise the question of whether the immune and inflammatory processes influence each other in this context, and if so, by what mechanisms? Decellularized matrices contain a number of candidate molecules that could exert significant immunomodulatory effects. Seventer and colleagues found that *in vitro*, TGF- β released from SIS could inhibit Th1 cell expansion and cytokine secretion.⁷⁶ Decellularized matrices, when applied to a polypropylene mesh, can also attenuate the fibrotic response that polypropylene otherwise elicits, further suggesting an immunomodulatory role.⁷⁷ Interestingly, decellularized matrices have received interest recently as adjuvants for vaccines. For example, when SIS was formulated into particles and combined with non-self antigens or hapten-protein conjugates, it increased the magnitude of the antibody response (primarily IgG1) against the antigens or haptens, again without promoting significant inflammation.⁷⁸ Little is known about which proteins are immunodominant, what the affinity of the antibody responses are, or how diverse of an antibody and T cell repertoire is generated, as titers have been measured against multi-component extracts of the implanted material. Conclusively identifying the epitopes and/or the main immunomodulating components that positively and negatively influence these processes is challenging. One way to address this gap in knowledge is to use engineered materials with defined epitopes and immunomodulatory components.

3.1.2. Synthetic peptide-based materials—In part to provide chemically defined scaffolds with which to mechanistically study biomaterial/immune interactions, and in part to efficiently engineer multicomponent materials for tissue engineering and vaccines, in our group we have designed biomaterials using molecular self-assembly. Using gels and nanofibers composed of self-assembling peptides, proteins, and peptide/polymer conjugates, we have recently observed that scaffolds with far less molecular complexity than decellularized matrices nevertheless raise immune responses that share many of the same characteristics (Figure 3d-g), namely the provocation of a vigorous antibody response but almost no signs of inflammation.²⁹ For example, the fibrillizing peptide Q11 assembles into supramolecular nanofibers and hydrogels, and it raises strong and durable antibody responses, of a Th1/Th2 balanced phenotype, when attached to peptides or proteins containing a competent B cell or T cell epitope.²⁶ Other self-assembling peptides elicit similar responses.²⁷ When OVA-Q11 peptide nanofibers were delivered intraperitoneally, again there was no detectable infiltration of inflammatory cell types or production of inflammatory cytokines.²⁹ Analyzing the behavior of T cells responding to these materials, we found that the materials induced differentiation into T follicular helper cells, but T cells were only moderately responsive in terms of Th1/Th2 differentiation, producing modest amounts of both IFN- γ and IL-4 compared with alum-adjuvanted positive controls.²⁹ Q11 nanofibers conjugated to protein antigens raised antibodies primarily of the IgG1 isotype, suggesting a Th2 polarization for these materials.⁷⁹

Tests of the self-assembled peptide materials presenting the OVA peptide epitope in T cell-knockout mice showed that T cells were required for the strong antibody responses these materials elicit.²⁷ This does not rule out, however, that T-independent and T-dependent processes may act in parallel to influence different aspects of the total response, a possibility

that may provide interesting future study. Although we have endeavored to determine the signaling mechanism by which self-assembled peptide nanofibers lead to vigorous antibody responses, to date only myeloid differentiation factor 88 (MyD88) has been identified as a necessary signaling component,²⁸ a shared adapter protein downstream of several TLRs. Further, we have ruled out the necessity of TLR-4,⁷⁹ TLR-2,²⁸ TLR-5,²⁸ and the NOD-like receptor, NALP3,²⁸ which is involved in inflammasome signaling and secretion of mature inflammatory cytokines such as IL-1 β .⁸⁰ Collectively, studies with decellularized xenogeneic tissues and self-assembled peptide nanofibers and matrices illustrate that strong antibody responses can occur without inflammation or rejection of the material, opening up possibilities for active immunomodulation using biomaterials, both in the context of tissue repair and in rational vaccine development.

3.2. T cell activation

3.2.1. Activating T cells through DCs—Because antigen-presentation and activation by APCs is the first step in T cell activation, many novel biomaterials platforms seek to specify T cell phenotypes by first specifying the phenotypes of APCs. From the outset, targeting nanoparticles to DCs instead of macrophages is important because DCs are much more efficient at antigen presentation and T cell stimulation.⁸¹ Dendritic cells' capacity for antigen processing and presentation in MHC complexes is also less dependent on the cellular uptake mechanism or activation status.⁸² Furthermore, DCs are enriched in the areas of the lymph node where naive T cells are activated, and where B cells and macrophages are for the most part excluded. A critical point of control for specifying the activation of CD4+ or CD8+ T cells by DCs is whether the processed antigens are ultimately presented in MHC-II or MHC-I molecules, respectively.^{10,18} Thus, biomaterials strategies to elicit responses may focus on i) delivery to appropriate DC subtypes, ii) activation of DCs, or iii) cross-presentation of the antigen. Here we focus on strategies to activate CD8+ T cells via DCs, as this has been the main area of progress so far.

One strategy is to target DC-rich areas in lymphoid tissues, thus maximizing the chances for encounter with DC subtypes specialized for cross-presentation of exogenous antigens. For example, the use of particle size to target lymph node resident DCs was discussed above (section 2.2). It is generally accepted that conventional (c)DCs, which express CD8 on their surfaces if they reside in lymphoid tissues or CD103 in peripheral tissues, are the most efficient at cross-presentation.⁸³ These cells can secrete particularly high levels of IL-12, which results in CD4+ Th1 polarization and CD8+ T cell activation. Importantly, not all lymphoid-derived CD8-expressing DCs are fully licensed for cross-presentation; thus, a second strategy is to include appropriate immunostimulators to activate these and other DCs. In the spleen, CD8+ DCs normally present antigen on MHC-II, but can also be induced to cross-present after full maturation during inflammation or infection, due to exposure to “licensing” factors such as the cytokine granulocyte-macrophage colony stimulating factor (GM-CSF) and TLR ligands like CpG.⁸⁴ Other DC subtypes have also been shown to cross-present when stimulated by biomaterials containing these factors. In the skin, plasmacytoid DCs (CD11c+PDCA-1+), myeloid DCs (CD11c+CD11b+), and cDCs (CD11c+CD8+) were successfully recruited to a PLGA scaffold incorporating tumor lysate as the antigen, together with CpG and GM-CSF as infection-mimicking agents.^{85,86} This scaffold design elicited vigorous CD8+ T cell responses and Th1 cytokine production (IFN γ and IL-2) *in situ*, resulting in the extended survival of mice after a challenge with a highly metastatic and aggressive melanoma tumor cell line.⁸⁵ In further work, it was also found that this scaffold attenuated Tregs by decreasing the Treg-polarizing immunosuppressive cytokines TGF- β and IL-10. Two immunizations with this polymer vaccine significantly delayed the progression of tumors and attained complete regression of tumors in up to 50% of the animals.⁸⁶

Another strategy for enhancing CD8⁺ T cell responses is to promote the rapid escape of antigen from endosomal compartments into the cytosol, which enhances the opportunity for MHC-I antigen processing and presentation (see section 2.1). One critical parameter in this regard is the biomaterial's chemical stability under different environments and intracellular compartments. For example, ICMVs are subject to enzymatic degradation following endocytosis of the particles by DCs, and this mechanism is thought to maximize the chance of endosomal escape into the cytosol required for antigen cross-presentation.³⁹ Indeed, immunization using these particles, which incorporated OVA protein in the aqueous core and MPLA as adjuvant within the lipid layers, induced a 14-fold higher frequency of CD8⁺OVA-tetramer⁺ T cells compared to soluble OVA and MPLA adjuvant.³⁹ Another platform designed for cross-presentation is oxidation-sensitive polymersomes composed of self-assembled block co-polymers of hydrophilic PEG and hydrophobic poly(propylene) sulfide (PPS).⁸⁷ These vesicles are designed to encapsulate antigen and adjuvant in the aqueous core or in the vesicle membrane, respectively, based on their relative hydrophilicity. Oxidation of a small amount of PPS to sulfoxide leads to the restructuring and release of any loaded components. Owing to this oxidation sensitivity, PEG-bl-PPS polymersomes were found to localize in both endosomes and the cytosol.⁸⁸ When polymersomes were loaded the TLR7 agonist gardiquimod or the TLR7/8 agonist R848, they were found to induce DC maturation; when DCs were primed with polymersomes loaded with OVA protein they were able to stimulate proliferation of OTII transgenic CD8⁺ T cells *in vitro*.⁸⁸ A recent study also compared the effect of restructuring the components of PPS polymersomes, testing the combined effects of size and mode of antigen release on CD4⁺ and CD8⁺ T cell responses.⁴⁷ 30 nm particles consisting of a solid core of PPS and a hydrophilic PEG corona were compared to 125 nm PEG-bl-PPS polymersomes. In the former case, OVA protein was anchored to the surface via a reduction-sensitive bond, whereas in the latter case the antigen was encapsulated and released upon oxidation of PPS.⁴⁷ In agreement with previous studies investigating the effect of size and reduction-sensitive release from endosomes for the induction of cross-presentation by nanoparticles, the smaller nanoparticles were more effective at inducing CD8⁺ T cell responses.

3.2.2. Direct T cell targeting strategies—Precise targeting of particles to DCs can be a challenging task. First, detailed knowledge of the phenotypic and functional heterogeneity of DCs, as well as their origin and development, is incomplete and evolving,⁸³ and this gap in understanding hinders the development of chemically-targeted adjuvants and immunotherapies. Second, there are major differences between the DC phenotypes of mice and humans, so even targeting agents that succeed in experimental models may not translate immediately to human use.^{4,89-91} In light of these challenges, several materials are in development that can directly activate T cells, bypassing the need for professional APCs (Figure 4). These APC-mimicking particles are called artificial APCs (APCs). Initially used as tools to understand the basic requirements for T cell activation,⁹² some of the more recent designs have been used not only for T cell stimulation *ex-vivo*, e.g. for adoptive cell therapy (ACT), but also for *in vivo* stimulation in mouse models.

The most commonly used material designs incorporate high-density presentation of signal 1 (peptide/MHC complex) and signal 2 (costimulation, anti-CD28) on the particle surface, but there also examples of others incorporating cytokines such as IL-2 (signal 3). For non-antigen-specific T cell stimulation, a TCR-activating antibody against CD3 (anti-CD3) can be used. Even for this relatively straightforward composition, biomaterial properties such as particle shape and surface area can have a significant effect on cell stimulation, as has been observed for anti-CD3 adsorbed on single walled carbon nanotube (SWNT) bundles.⁹³ In multi-component designs, both physical and chemical properties must be considered. For example, spherical aAPCs have been fabricated from biodegradable PLGA microparticles encapsulating the T cell stimulating cytokine interleukin 2 (IL-2) (Figure 4a).^{94,95} An

avidin-palmitic acid conjugate was attached to the particle surface during synthesis, allowing a high density of biotinylated anti-CD3, anti-CD28 or peptide/MHC complexes to be displayed on the surface. Interestingly, micron-sized particles but not nanoscale particles were optimal for *in vitro* CD8+ T cell stimulation,⁹⁴ perhaps mimicking more closely the surface area presented by the APC. Also, a combination of all three signals on the same microparticle was found to elicit superior stimulation of CD8+ T cells compared to separate particles presenting activating ligands or cytokine release separately.⁹⁵ The complexity involved in engineering immune responses is highlighted by the fact that these IL-2-encapsulating aAPCs induced expansion of CD8+ T cells, but apoptosis of CD4+ T cells *in vitro*.⁹⁵ Recently, PLGA microparticle aAPCs have also been made in ellipsoidal shapes with various aspect ratios via stretching.⁹⁶ Ellipsoidal aAPCs presenting signal 1 and signal 2 stimuli improved activation of CD8+ T cells compared to spherical aAPCs, and confocal microscopy indicated that CD8+ T cells had enhanced interactions with the ellipsoidal aAPCs.⁹⁶ In an *in vivo* test of T cell activation, the ellipsoidal aAPCs extended the survival of mice to a subcutaneous melanoma tumor compared to spherical aAPCs.⁹⁶ Thus, aAPCs designed to stimulate T cells directly are a good example of how size, shape, and composition all contribute to influence the T cell response both *in vitro* and *in vivo*.

Recently, an interesting formulation of liposomes and lipid rafts enriched with signal 1 has been used to prime both CD4 and CD8 T cell responses *in vivo*. In this approach, bone marrow-derived immature DCs were first activated *in vitro* with OVA protein and LPS, and then lysed. The lipid rafts containing membrane-bound peptide/MHC-II complexes were then extracted and fused to synthetic liposomes (Figure 4b).⁹⁷ The resulting (raft)osomes were ~200-300 nm in diameter and contained high levels of OVA peptide/MHC-II complexes.⁹⁷ These materials were used in mouse immunization studies in the absence of adjuvants and were found to induce a stronger IgG antibody response compared to soluble OVA, and the subtype was mostly IgG1.⁹⁷ The (raft)osomes were also found to protect against challenge with an OVA-expressing tumor, delaying the tumor growth compared to control mice.⁹⁷ Surprisingly, IFN γ secretion from splenocytes was similar after *ex vivo* challenge with either OVA protein or OVA₂₅₇₋₂₆₄, a MHC-I-specific peptide epitope, even though neither this epitope nor the whole protein were deliberately included in the (raft)osomes during immunization of mice.⁹⁷ These results suggest that perhaps the (raft)osomes contained also processed OVA epitopes presented on MHC-I and that both the CD4+ and CD8+ T cells together contributed to the decrease in the tumor volume, although the mechanisms have not yet been fully clarified.

In addition to aAPCs, other synthetic platforms have been designed to target transferred T cells after adoptive cell therapy (ACT). A recent example consisted of liposomes manufactured using maleimide-functionalized PEG-lipids, which allowed for thiol-mediated conjugation to various proteins (Figure 4c).⁹⁸ Two ligands were tested: IL-2 fused to mouse IgG2a Fc portion (IL2-Fc), to target and stimulate activated T cells expressing the IL-2 receptor, and the F(ab)₂ portion of a monoclonal antibody against a marker specific for transferred mouse T cells (Thy 1.1).⁹⁸ It was found that both IL2-Fc and anti-Thy1.1 liposomes bound to transferred T cells *in vivo* after intravenous delivery, particularly in the spleen.⁹⁸ IL2-Fc liposomes were further assessed in a metastatic lung tumor model. Ten days after the tumor was established, mice were lymphodepleted and then treated with tumor-antigen-specific CD8+ T cells, to model clinical ACT. Simultaneous or subsequent immunization with IL2-Fc liposomes was found to boost the expansion of the transferred T cells *in vivo* compared to ACT alone or ACT with exogenous IL-2 treatment, indicating that the material-bound cytokine effectively enhanced ACT.⁹⁸ Overall, these studies exemplify the progress made in exploiting the direct interactions of biomaterials with T cells. Their findings begin to elucidate strategies and guidelines for the development of more targeted

and effective strategies to circumvent the requirement for APCs to activate and boost antigen-specific immunity *in vivo*.

3.3. Antigen-specific T cell or B cell Tolerance

As an alternative to immunoisolation, the specific engagement of immunological T cell tolerance by biomaterials has been increasingly sought. This area has been driven by the need for antigen-specific methods to induce peripheral tolerance induction to treat autoimmune diseases.^{99,100} Peripheral tolerance refers to mechanisms that decrease the responsiveness of mature circulating effector T cells that express receptors (TCRs) that have affinity for or can cross-react with self-antigens.¹⁰¹ Misregulation of antigen-specific tolerance mechanisms is known to precipitate autoimmune diseases such as multiple sclerosis and type I diabetes, allergic responses, or transplant rejection. T cell tolerance is mediated via three complementary mechanisms: anergy, deletion of autoreactive effector T cells, and induction of regulatory T cells.¹⁰¹

To elicit tolerance to self-antigens or to foreign antigens, recent approaches have exploited, mimicked, or been inspired by the natural clearance pathways for apoptotic materials.^{102,103} Getts et al. immunized mice with encephalitogenic myelin-derived peptides crosslinked to polystyrene beads or to PLGA particles to prevent and treat relapsing experimental autoimmune encephalomyelitis (R-EAE), a mouse model for multiple sclerosis (MS).¹⁰⁴ Intravenous delivery of the particles induced abortive T cell activation, and the T cells were unable to synthesize cytokines (IL-17A and IFN γ) after antigen challenge, implying that the particles promoted T cell anergy. Interestingly, the size of the particles was critical to this effect: only 500 nm beads covalently coupled to the encephalitogenic peptides were able to induce resistance and control of EAE; larger and smaller sizes were significantly less effective.¹⁰⁴ The reason for this size dependence was not explored, but it may be related to uptake of the particles by tolerogenic antigen-presenting cells in the spleen.

In another approach to promote tolerance, antigens have been engineered to bind to red blood cells, whose apoptotic debris can induce tolerance.¹⁰⁵ This technology is based on fusion of the antigen with either peptides or a single chain Fv (scFv) antibody fragment that binds to glycophorin-A, a protein found specifically on the surface of mouse red blood cells (Figure 5a).¹⁰⁵ Erythrocyte-bound antigens induced cross-presentation and antigen-specific T cell deletion, with one particular formulation inducing very high levels of PD-1, a marker of T cell exhaustion, and Annexin-V, a marker of apoptosis.¹⁰⁵ Using a diabetogenic mouse model, it was demonstrated that fusions of a pancreatic islet β -cell autoantigen peptide with the RBC-binding scFv antibody could prevent the onset of diabetes, as the infiltration and proliferation of pathogenic CD4⁺ T cells and their destruction of insulin producing islet cells were markedly reduced.¹⁰⁵

For delivering particles to APCs without activating them, thus avoiding T cell responses, a variety of strategies have been explored. Microparticles have been modified with anti-CD11c antibody, PD-2 (a peptide binding the CD11c integrin), or anti-DC205 antibodies (which target endocytic receptors).¹⁰⁶ For *in vitro* cultures of APCs, these modifications have been found to enhance uptake without eliciting cytokine secretion (Figure 5b).¹⁰⁶ When injected subcutaneously, the surface modifications enhanced uptake and trafficking to lymph nodes in mice.¹⁰⁶ These results suggest the possibility of including tolerance-inducing molecules and antigens, to induce antigen-specific T cell tolerance at the lymph nodes.

Actively inducing immunosuppressive T regulatory cells (Tregs) involves the differentiation of naive T cells to the Treg phenotype, using a combination of cytokines. For example, large-size PLGA microparticles (15-25 μ m) have been utilized *in vitro* for the controlled

release of the Treg inducing cytokines TGF- β and IL-2, plus rapamycin (the inhibitor of mammalian target of rapamycin, mTOR). The microparticle formulations were found to induce the conversion of naive T cells into functional Tregs, for both mouse and human cells (Figure 5c).¹⁰⁷ Future development of this approach may assess the effect of these formulations *in vivo* and formulate a strategy for the suppression of antigen-specific T cells.

Finally, cognate interactions between B and T cells are known to play a crucial role in the progression of autoimmune diseases, in both human and experimental animal models, and these can be modulated using biomaterials. B cells acting as APCs can contribute to the induction of autoreactive T cells through epitope spreading, and T cell help can promote B cell receptor (BCR) affinity maturation and promote isotype switching, leading to autoreactive B cell responses.¹⁰⁸ One recent example of a biomaterial designed to promote B cell tolerance employed multivalent antigen display with additional ligands for the CD22 receptor present on B cells, a member of the sialic acid binding Ig-like lectin (SIGLEC) family.¹⁰⁹ Crosslinking these receptors inhibits B cell activation.¹¹⁰ SIGLEC-engaging tolerance-inducing antigenic liposomes (STALs) were designed with conjugated immunogenic proteins and CD22 ligands on the liposomal surface, thus engaging both BCRs and CD22 receptors on the surface of B cells to induce antigen-specific B cell tolerance.¹⁰⁹ After immunization of mice with STALs, antibody production elicited by antigen challenge was greatly diminished, using either T-independent (nitrophenol) and various T-dependent antigens (OVA, hen egg lysozyme, myelin oligodendrocyte glycoprotein and human factor VIII protein).¹⁰⁹ These responses were also able to suppress antibody production against human Factor VIII, a protein used in the clinic for the treatment of hemophilia. Administration with STALS containing Factor VIII was found to protect against lethal bleeding in a mouse model of hemophilia, illustrating the strategy's future potential.

4. Conclusions and Future Outlook

In this review, we have highlighted important findings made at the intersection of nanotechnology, biomaterials, and immunology, specifically focusing on materials-focused strategies to modulate the adaptive immune responses generated by B cells and T cells. A variety of engineering approaches have attained antigen-specific antibody, CD4⁺ T cell, and CD8⁺ T cell responses, and some of the more central aspects for designing immunologically active biomaterials were discussed.

We highlighted that epitope content is perhaps the most important factor to elicit antigen-specific adaptive immunity, and the context in which the antigen is delivered determines whether the responses will induce a vigorous response or tolerance. Although the size of the material is a critical parameter to accessing the lymphoid tissues enriched in B cell and T cells, a variety of material sizes have been able to elicit some form of adaptive immunity. An important area of future research is to assess the contribution of material degradation and byproducts. In clinical use, throughout their service life many biomaterials are likely to undergo a progression of size, shape, multivalency, and association with both immune-stimulating and immune-dampening molecules. Also, although much progress has been made regarding the targeting of APCs to elicit adaptive immunity, future strategies may continually focus on targeting B cells and T cells directly as well. Some progress on artificial systems for direct T cell stimulation has been made, but many of these strategies are just beginning to be applied *in vivo*.

An additional layer of detail likely to be important in future research is a continuing development of a thorough understanding of the material parameters that contribute to the polarization of immune responses. Many immunologically active biomaterials developed to

date elicit high-affinity IgG antibodies, dominated by the Th2-polarized IgG1 subtype. A better understanding of the mechanism underlying this trend would facilitate the engineering of specialized therapies where antibody responses are not needed, when they are undesirable, or when other antibody subclasses are required. Finally, targeted strategies to address the incorporation of signal 3 (i.e. stimulation with cytokines) are important for properly controlling the polarization of T cells into various phenotypes (e.g. Th1, Th2, Th17, Treg). This will require control over the timing of release for various combinations of cytokines, their localization, their persistence in the tissue, and optimized routes for their delivery. Despite these challenges, it is clear that recent advances in the knowledge base of material-immune interactions are enabling the continual refinement of strategies to target specific phenotypes of adaptive immunity, which collectively promise to prevent and treat a range of complex diseases in the future.

Acknowledgments

Research in our group on self-assembled immunotherapies has been supported by the National Institutes of Health, grant numbers 1R21AI094444 (NIAID) and 5R01EB009701 (NIBIB), the Bill and Melinda Gates Foundation (OPP1061315), and the Chicago Biomedical Consortium with support from the Searle Funds at The Chicago Community Trust. The contents of this review are solely the responsibility of the authors and do not represent the official views of any of the funding agencies. The authors have no conflicting financial interests. We wish to thank Rebecca Pompano for assistance in writing the manuscript.

References

1. Place ES, Evans ND, Stevens MM. *Nat Mater.* 2009; 8:457–470. [PubMed: 19458646]
2. Babensee JE. *Semin Immunol.* 2008; 20:101–108. [PubMed: 18054498]
3. Badylak SF, Gilbert TW. *Semin Immunol.* 2008; 20:109–116. [PubMed: 18083531]
4. Kou PM, Babensee JE. *J Biomed Mater Res A.* 2011; 96:239–260. [PubMed: 21105173]
5. Sefton MV, Babensee JE, Woodhouse KA. *Semin Immunol.* 2008; 20:83–85. [PubMed: 18221888]
6. Moon JJ, Huang B, Irvine DJ. *Adv Mater.* 2012; 24:3724–3746. [PubMed: 22641380]
7. Smith DM, Simon JK, Baker JR. *Nat Rev Immunol.* 2013; 13:592–605. [PubMed: 23883969]
8. Leleux J, Roy K. *Adv Healthc Mater.* 2013; 2:72–94. [PubMed: 23225517]
9. Bachmann MF, Jennings GT. *Nat Rev Immunol.* 2010; 10:787–796. [PubMed: 20948547]
10. Hubbell JA, Thomas SN, Swartz MA. *Nature.* 2009; 462:449–460. [PubMed: 19940915]
11. Swartz MA, Hirose S, Hubbell JA. *Sci Transl Med.* 2012; 4:148rv9.
12. Skwarczynski M, Toth I. *Curr Drug Deliv.* 2011; 8:282–289. [PubMed: 21291373]
13. Purcell AW, McCluskey J, Rossjohn J. *Nat Rev Drug Discov.* 2007; 6:404–414. [PubMed: 17473845]
14. Pape KA, Catron DM, Itano AA, Jenkins MK. *Immunity.* 2007; 26:491–502. [PubMed: 17379546]
15. Crotty S. *Annu Rev Immunol.* 2011; 29:621–663. [PubMed: 21314428]
16. Pardoll DM. *Nat Rev Immunol.* 2002; 2:227–238. [PubMed: 12001994]
17. Demento SL, Siefert AL, Bandyopadhyay A, Sharp FA, Fahmy TM. *Trends Biotechnol.* 2011; 29:294–306. [PubMed: 21459467]
18. Trombetta ES, Mellman I. *Annu Rev Immunol.* 2005; 23:975–1028. [PubMed: 15771591]
19. Sun P, Ju H, Liu Z, Ning Q, Zhang J, Zhao X, Huang Y, Ma Z, Li Y. *Comput Math Methods Med.* 2013; 2013:943636. [PubMed: 23970944]
20. Lundegaard C, Lund O, Kesmir C, Brunak S, Nielsen M. *Bioinformatics.* 2007; 23:3265–3275. [PubMed: 18045832]
21. Stranzl T, Larsen MV, Lundegaard C, Nielsen M. *Immunogenetics.* 2010; 62:357–368. [PubMed: 20379710]
22. Nielsen M, Lundegaard C, Blicher T, Peters B, Sette A, Justesen S, Buus S, Lund O. *PLoS Comput Biol.* 2008; 4:e1000107. [PubMed: 18604266]

23. Nielsen M, Lund O, Buus S, Lundegaard C. *Immunology*. 2010; 130:319–328. [PubMed: 20408898]
24. Vita R, Zarebski L, Greenbaum JA, Emami H, Hoof I, Salimi N, Damle R, Sette A, Peters B. *Nucleic Acids Res*. 2010; 38:D854–62. [PubMed: 19906713]
25. Kim Y, Ponomarenko J, Zhu Z, Tamang D, Wang P, Greenbaum J, Lundegaard C, Sette A, Lund O, Bourne PE, Nielsen M, Peters B. *Nucleic Acids Res*. 2012; 40:W525–W530. [PubMed: 22610854]
26. Rudra JS, Tian YF, Jung JP, Collier JH. *Proc Natl Acad Sci USA*. 2010; 107:622–627. [PubMed: 20080728]
27. Rudra JS, Sun T, Bird KC, Daniels MD, Gasiorowski JZ, Chong AS, Collier JH. *ACS Nano*. 2012; 6:1557–1564. [PubMed: 22273009]
28. Rudra JS, Mishra S, Chong AS, Mitchell RA, Nardin EH, Nussenzweig V, Collier JH. *Biomaterials*. 2012; 33:6476–6484. [PubMed: 22695068]
29. Chen J, Pompano RR, Santiago FW, Maillat L, Sciammas R, Sun T, Han H, Topham DJ, Chong AS, Collier JH. *Biomaterials*. 2013; 34:8776–8785. [PubMed: 23953841]
30. Mitragotri S, Lahann J. *Nat Mater*. 2009; 8:15–23. [PubMed: 19096389]
31. Ahsan F, Rivas IP, Khan MA, Torres Suarez AI. *J Control Release*. 2002; 79:29–40. [PubMed: 11853916]
32. Choi HS, Liu W, Liu F, Nasr K, Misra P, Bawendi MG, Frangioni JV. *Nat Nanotechnol*. 2009; 5:42–47. [PubMed: 19893516]
33. Reddy ST, Rehor A, Schmoekel HG, Hubbell JA, Swartz MA. *J Control Release*. 2006; 112:26–34. [PubMed: 16529839]
34. Reddy ST, van der Vlies AJ, Simeoni E, Angeli V, Randolph GJ, O'Neil CP, Lee LK, Swartz MA, Hubbell JA. *Nat Biotechnol*. 2007; 25:1159–1164. [PubMed: 17873867]
35. Manolova V, Flace A, Bauer M, Schwarz K, Saudan P, Bachmann MF. *Eur J Immunol*. 2008; 38:1404–1413. [PubMed: 18389478]
36. Tran KK, Shen H. *Biomaterials*. 2009; 30:1356–1362. [PubMed: 19091401]
37. Sexton A, Whitney PG, Chong SF, Zelikin AN, Johnston APR, De Rose R, Brooks AG, Caruso F, Kent SJ. *ACS Nano*. 2009; 3:3391–3400. [PubMed: 19824668]
38. Kaba SA, Brando C, Guo Q, Mittelholzer C, Raman S, Tropel D, Aebi U, Burkhard P, Lanar DE. *J Immunol*. 2009; 183:7268–7277. [PubMed: 19915055]
39. Moon JJ, Suh H, Bershteyn A, Stephan MT, Liu H, Huang B, Sohail M, Luo S, Um SH, Khant H, Goodwin JT, Ramos J, Chiu W, Irvine DJ. *Nat Mater*. 2011; 10:243–251. [PubMed: 21336265]
40. Moon JJ, Suh H, Li AV, Ockenhouse CF, Yadava A, Irvine DJ. *Proc Natl Acad Sci USA*. 2012; 109:1080–1085. [PubMed: 22247289]
41. Kaba SA, McCoy ME, Doll TAPF, Brando C, Guo Q, Dasgupta D, Yang Y, Mittelholzer C, Spaccapelo R, Crisanti A, Burkhard P, Lanar DE. *PLoS ONE*. 2012; 7:e48304. [PubMed: 23144750]
42. Wahome N, Pfeiffer T, Ambiel I, Yang Y, Keppler OT, Bosch V, Burkhard P. *Chem Biol Drug Des*. 2012; 80:349–357. [PubMed: 22650354]
43. Bershteyn A, Hanson MC, Crespo MP, Moon JJ, Li AV, Suh H, Irvine DJ. *J Control Release*. 2012; 157:354–365. [PubMed: 21820024]
44. De Geest BG, Willart MA, Hammad H, Lambrecht BN, Pollard C, Bogaert P, De Filette M, Saelens X, Vervaet C, Remon JP, Grooten J, De Koker S. *ACS Nano*. 2012; 6:2136–2149. [PubMed: 22303914]
45. Black M, Trent A, Kostenko Y, Lee JS, Olive C, Tirrell M. *Adv Mater*. 2012; 24:3845–3849. [PubMed: 22550019]
46. Stano A, Nembrini C, Swartz MA, Hubbell JA, Simeoni E. *Vaccine*. 2012; 30:7541–7546. [PubMed: 23103199]
47. Stano A, Scott EA, Dane KY, Swartz MA, Hubbell JA. *Biomaterials*. 2013; 34:4339–4346. [PubMed: 23478034]
48. Swartz MA, Hubbell JA, Reddy ST. *Semin Immunol*. 2008; 20:147–156. [PubMed: 18201895]
49. Roozendaal R, Mebius RE, Kraal G. *Int Immunol*. 2008; 20:1483–1487. [PubMed: 18824503]

50. Gonzalez SF, Degn SE, Pitcher LA, Woodruff M, Heesters BA, Carroll MC. *Annu Rev Immunol.* 2011; 29:215–233. [PubMed: 21219172]
51. Clark SL. *Am J Anat.* 1962; 110:217–257. [PubMed: 13879732]
52. Gretz JE, Norbury CC, Anderson AO, Proudfoot AE, Shaw S. *J Exp Med.* 2000; 192:1425–1440. [PubMed: 11085745]
53. Itano AA, Jenkins MK. *Nature Immunology.* 2003; 4:733–739. [PubMed: 12888794]
54. Sixt M, Kanazawa N, Selg M, Samson T, Roos G, Reinhardt DP, Pabst R, Lutz MB, Sorokin L. *Immunity.* 2005; 22:19–29. [PubMed: 15664156]
55. Roozendaal R, Mempel TR, Pitcher LA, Gonzalez SF, Verschoor A, Mebius RE, von Andrian UH, Carroll MC. *Immunity.* 2009; 30:264–276. [PubMed: 19185517]
56. Jewell CM, López SCB, Irvine DJ. *Proc Natl Acad Sci USA.* 2011; 108:15745–15750. [PubMed: 21896725]
57. Kasturi SP, Skountzou I, Albrecht RA, Koutsouanos D, Hua T, Nakaya HI, Ravindran R, Stewart S, Alam M, Kwissa M, Villinger F, Murthy N, Steel J, Jacob J, Hogan RJ, García-Sastre A, Compans R, Pulendran B. *Nature.* 2011; 470:543–547. [PubMed: 21350488]
58. Puffer EB, Pontrello JK, Hollenbeck JJ, Kink JA, Kiessling LL. *ACS Chem Biol.* 2007; 2:252–262. [PubMed: 17432821]
59. Hinton HJ, Jegerlehner A, Bachmann MF. *Curr Top Microbiol Immunol.* 2008; 319:1–15. [PubMed: 18080412]
60. Tam JP. *Proc Natl Acad Sci USA.* 1988; 85:5409–5413. [PubMed: 3399498]
61. Tam JP, Clavijo P, Lu YA, Nussenzweig V, Nussenzweig R, Zavala F. *J Exp Med.* 1990; 171:299–306. [PubMed: 1688609]
62. Bachmann MF, Rohrer UH, Kündig TM, Bürki K, Hengartner H, Zinkernagel RM. *Science.* 1993; 262:1448–1451. [PubMed: 8248784]
63. Liu W, Chen YH. *Eur J Immunol.* 2005; 35:505–514. [PubMed: 15627976]
64. Jardine J, Julien JP, Menis S, Ota T, Kalyuzhnyi O, McGuire A, Sok D, Huang PS, MacPherson S, Jones M, Nieuwsma T, Mathison J, Baker D, Ward AB, Burton DR, Stamatatos L, Nemazee D, Wilson IA, Schief WR. *Science.* 2013; 340:711–716. [PubMed: 23539181]
65. Zhang S, Yong LK, Li D, Cubas R, Chen C, Yao Q. *PLoS ONE.* 2013; 8:e68303. [PubMed: 23874581]
66. Molino NM, Anderson AKL, Nelson EL, Wang SW. *ACS Nano.* 2013
67. Chackerian B, Durfee MR, Schiller JT. *J Immunol.* 2008; 180:5816–5825. [PubMed: 18424700]
68. Wilkinson BL, Day S, Chapman R, Perrier S, Apostolopoulos V, Payne RJ. *Chemistry.* 2012; 18:16540–16548. [PubMed: 23090901]
69. Crapo PM, Gilbert TW, Badylak SF. *Biomaterials.* 2011; 32:3233–3243. [PubMed: 21296410]
70. Mirmalek-Sani SH, Sullivan DC, Zimmerman C, Shupe TD, Petersen BE. *Am J Pathol.* 2013; 183:558–565. [PubMed: 23747949]
71. Allman AJ, McPherson TB, Badylak SF, Merrill LC, Kallakury B, Sheehan C, Raeder RH, Metzger DW. *Transplantation.* 2001; 71:1631–1640. [PubMed: 11435976]
72. Biswas SK, Mantovani A. *Nature Immunology.* 2010; 11:889–896. [PubMed: 20856220]
73. Ansaloni L, Cambrini P, Catena F, Di Saverio S, Gagliardi S, Gazzotti F, Hodde JP, Metzger DW, D'Alessandro L, Pinna AD. *J Invest Surg.* 2007; 20:237–241. [PubMed: 17710604]
74. Bejjani, Ghassan K.; Zabramski, Joseph. 2007; 106:1028–1033.
75. Xu H, Wan H, Sandor M, Qi S, Ervin F, Harper JR, Silverman RP, McQuillan DJ. *Tissue Eng Part A.* 2008; 14:2009–2019. [PubMed: 18593339]
76. Palmer EM, Beilfuss BA, Nagai T, Semnani RT, Badylak SF, van Seventer GA. *Tissue Eng.* 2002; 8:893–900. [PubMed: 12459068]
77. Liu L, Deng L, Wang Y, Ge L, Chen Y, Liang Z. *Int Urogynecol J.* 2012; 23:1271–1278. [PubMed: 22538554]
78. Aachoui Y, Ghosh SK. *PLoS ONE.* 2011; 6:e27083. [PubMed: 22087247]
79. Hudalla GA, Modica JA, Tian YF, Rudra JS, Chong AS, Sun T, Mrksich M, Collier JH. *Adv Healthc Mater.* 2013; 2:1114–1119. [PubMed: 23436779]

80. Rathinam VAK, Vanaja SK, Fitzgerald KA. *Nature Immunology*. 2012; 13:333–332. [PubMed: 22430786]
81. Delamarre L, Pack M, Chang H, Mellman I, Trombetta ES. *Science*. 2005; 307:1630–1634. [PubMed: 15761154]
82. Kamphorst AO, Guernonprez P, Dudziak D, Nussenzweig MC. *J Immunol*. 2010; 185:3426–3435. [PubMed: 20729332]
83. Satpathy AT, Wu X, Albring JC, Murphy KM. *Nature Immunology*. 2012; 13:1145–1154. [PubMed: 23160217]
84. Dresch C, Leverrier Y, Marvel J, Shortman K. *Trends Immunol*. 2012; 33:381–388. [PubMed: 22677187]
85. Ali OA, Huebsch N, Cao L, Dranoff G, Mooney DJ. *Nat Mater*. 2009; 8:151–158. [PubMed: 19136947]
86. Ali OA, Emerich D, Dranoff G, Mooney DJ. *Sci Transl Med*. 2009; 1:8ra19.
87. Hirosue S, Kourtis IC, van der Vlies AJ, Hubbell JA, Swartz MA. *Vaccine*. 2010; 28:7897–7906. [PubMed: 20934457]
88. Scott EA, Stano A, Gillard M, Maio-Liu AC, Swartz MA, Hubbell JA. *Biomaterials*. 2012; 33:6211–6219. [PubMed: 22658634]
89. Shortman K, Liu YJ. *Nat Rev Immunol*. 2002; 2:151–161. [PubMed: 11913066]
90. Ju X, Clark G, Hart DNJ. *Methods Mol Biol*. 2010; 595:3–20. [PubMed: 19941102]
91. Nierkens S, Tel J, Janssen E, Adema GJ. *Trends Immunol*. 2013; 34:361–370. [PubMed: 23540650]
92. Prakken B, Wauben M, Genini D, Samodal R, Barnett J, Mendivil A, Leoni L, Albani S. *Nat Med*. 2000; 6:1406–1410. [PubMed: 11100129]
93. Fadel TR, Steenblock ER, Stern E, Li N, Wang X, Haller GL, Pfefferle LD, Fahmy TM. *Nano Lett*. 2008; 8:2070–2076. [PubMed: 18547120]
94. Steenblock ER, Fahmy TM. *Mol Ther*. 2008; 16:765–772. [PubMed: 18334990]
95. Steenblock ER, Fadel T, Labowsky M, Poher JS, Fahmy TM. *J Biol Chem*. 2011; 286:34883–34892. [PubMed: 21849500]
96. Sunshine JC, Perica K, Schneck JP, Green JJ. *Biomaterials*. 2013
97. Ding Q, Chen J, Wei X, Sun W, Mai J, Yang Y, Xu Y. *Pharm Res*. 2013; 30:60–69. [PubMed: 22878683]
98. Zheng Y, Stephan MT, Gai SA, Abraham W, Shearer A, Irvine DJ. *J Control Release*. 2013
99. Miller SD, Turley DM, Podojil JR. *Nat Rev Immunol*. 2007; 7:665–677. [PubMed: 17690713]
100. Bluestone JA, Bour-Jordan H. *Cold Spring Harb Perspect Biol*. 2012; 4
101. Mueller DL. *Nature Immunology*. 2010; 11:21–27. [PubMed: 20016506]
102. Ferguson TA, Choi J, Green DR. *Immunol Rev*. 2011; 241:77–88. [PubMed: 21488891]
103. Griffith TS, Ferguson TA. *Immunity*. 2011; 35:456–466. [PubMed: 22035838]
104. Getts DR, Martin AJ, McCarthy DP, Terry RL, Hunter ZN, Yap WT, Getts MT, Pleiss M, Luo X, King NJC, Shea LD, Miller SD. *Nat Biotechnol*. 2012; 30:1217–1224. [PubMed: 23159881]
105. Kontos S, Kourtis IC, Dane KY, Hubbell JA. *Proc Natl Acad Sci USA*. 2013; 110:E60–8. [PubMed: 23248266]
106. Lewis JS, Zaveri TD, Crooks CP, Keselowsky BG. *Biomaterials*. 2012; 33:7221–7232. [PubMed: 22796161]
107. Jhunjhunwala S, Balmert SC, Raimondi G, Dons E, Nichols EE, Thomson AW, Little SR. *J Control Release*. 2012; 159:78–84. [PubMed: 22285546]
108. Salinas GF, Braza F, Brouard S, Tak PP, Baeten D. *Clin Immunol*. 2013; 146:34–45. [PubMed: 23202542]
109. Macauley MS, Pfrengle F, Rademacher C, Nycholat CM, Gale AJ, von Drygalski A, Paulson JC. *J Clin Invest*. 2013; 123:3074–3083. [PubMed: 23722906]
110. Tsubata T. *Infect Disord Drug Targets*. 2012; 12:181–190. [PubMed: 22394175]

Figure 1. Canonical pathways for particles or materials to engage immune cells

Biomaterials can induce tolerance (left) or immunogenicity (right) to an antigen depending on the incorporation and/or modulation of tolerogenic signals (for example, apoptotic debris) or pathogen- or damage-associated molecular patterns (PAMPs or DAMPs) recognized by pathogen recognition receptors (PRRs) on dendritic cells (DCs). Tolerance mechanisms can include T cell anergy, induction of T regulatory (Treg) cells, and antigen-specific deletion of effector T cells. Activation of DCs by antigen in the presence of PAMPs and/or DAMPs results in higher antigen presentation on MHC molecules, as well as increased expression of co-stimulatory molecules (CD80, CD86 and CD40). Mature DCs can then activate cytotoxic T lymphocytes (CTLs or CD8+ T cells), natural killer (NK) cells, or cytokine-producing CD4+ T helper cells (Th1, Th2 or Th17). B cells, on the other hand, can be directly activated by particles but also need input signals from DCs and T follicular helper cells (not shown) to differentiate into antibody-secreting cells.

Figure 2. Biomaterials enabling antibody responses via the multivalent surface display of antigens can span a range of size

a. Virus-like particles (VLPs) and other self-assembled proteins are non-replicating nanoparticles that mimic the shape and size of viruses. They can be composed of viral-derived non-pathogenic proteins or synthetic sequences that self-assemble into icosahedral or rod-like structures. Shown is a representative VLP nanoparticle designed for multivalent display of eOD-GT6, a rationally designed HIV immunogen.⁶⁴ **b.** Interbilayer cross-linked multilamellar vesicles (ICMVs) are prepared from the fusion of maleimide-functionalized liposomes that co-encapsulate antigen (pink) in the aqueous core, and monophosphoryl lipid A (MPLA, blue), a TLR4 ligand, in the lipid bilayers. The maleimide groups in the precursor liposomes are used for cross-linking the bilayers (orange), surface immobilization of antigen, and conjugation of polyethylene glycol groups (PEG, black) that add chemical stability to the ICMVs.³⁹ **c.** Self-assembling peptide nanofibers composed of self-assembling peptide domains (blue) chemically synthesized in tandem with an antigenic peptide (red), form nanofibers in physiological buffers. The antigenic peptides are made accessible on the surface of the fibers by means of a flexible peptide linker.²⁶ **a** reproduced with permission from Jardine *et al.*⁶⁴ Copyright 2013, the American Association for the Advancement of Science **b** reproduced with permission from Moon *et al.*³⁹ Copyright 2011, Nature Publishing Group **c** reproduced with permission from Rudra *et al.*²⁶ Copyright 2010, Proceedings of the National Academy of Sciences of the United States of America.

Figure 3. Antibody and tissue responses elicited by decellularized xenogeneic matrices (a-c)⁷¹ and self-assembled peptide materials (d-g)²⁹
a. SIS decellularized matrices elicit significant IgG responses, with IgG1 being the major isotype. **b.** In mice, non-decellularized xenogeneic tissue elicits a rejection response, with necrosis and inflammation evident (xenogeneic muscle tissue implanted subcutaneously, imaged at 28 days, stained with hematoxylin and eosin, 40× magnification); **c.** whereas decellularized SIS is well integrated into the tissue, exhibiting resolved inflammation and remodeling (SIS implanted subcutaneously, imaged at 28 days with hematoxylin and eosin, 40× magnification). **d.** Similarly, self-assembled peptide materials raise strong antibody responses (showing total IgG titers against self-assembled OVAQ11, OVA peptide delivered in alum (pOVA-Alum), self-assembled OVA-Q11 delivered in alum (OVAQ11-Alum), and soluble OVA peptide (pOVA)). **e.** Like SIS, self-assembled peptide matrices do not elicit significant inflammation when implanted into mouse footpads (gross swelling in footpads injected with OVA-Q11 in alum (left) and OVA-Q11 (right)). **g.** Histological evaluation at day 8 with hematoxylin/eosin showed significant necrosis, swelling, and inflammation for alum-adjuvanted OVA-Q11 (**f**), but no inflammation for OVA-Q11 (**g**). Scale bar 50μm for **f** and **g**.²⁹ **a-c** reproduced with permission from Allman *et al.*⁷¹ **d-g** reproduced with permission from Chen and Pompano *et al.*²⁹

Figure 4. Biomaterials designs for direct T cell targeting and stimulation

a. Artificial antigen-presenting cells (aAPCs) capable of paracrine delivery of the cytokine interleukin-2 (IL-2), and surface display of T cell activating ligands (peptide/MHC complexes, anti-CD3/CD28) elicit proliferation of CD8⁺ T cells but apoptosis of CD4⁺ T cells.⁹⁵ **b.** (Raft)osomes are liposomes that incorporate peptide-MHC II-enriched lipid rafts from membranes of DCs that were previously stimulated with protein antigens. The (raft)osomes have been utilized as anti-tumor vaccines that elicit CD4⁺ T cell priming and antibody responses that significantly delay tumor growth.⁹⁷ **c.** Liposomes conjugated with antibodies that target unique surface proteins on T cells (Thy1.1) can be used to target this population *in vivo* after adoptive cell therapy (ACT). Also, delivery of liposomes crosslinked to IL-2-Fc fusion proteins boosted proliferation of ACT transferred T cells. For conjugation, IL-Fc fusion or Thy1.1 F(ab)₂ were treated with DTT to expose thiol groups that would allow crosslinking with thiol-reactive lipids (MAL-PEG lipids) on the liposomes.⁹⁸

Figure 5. Engineering strategies for inducing T cell tolerance

a. Targeting antigen to erythrocyte-specific proteins can lead to tolerance after processing of apoptotic erythrocytes by dendritic cells (DCs). Erythrocyte-specific binding is achieved through conjugation of high affinity glycophorin-A binding peptide or through expression of an antigenic peptide fused to an engineered glycophorin-A single chain (sc) Fv fragment antibody.¹⁰⁵ **b.** Targeting of DCs with PLGA microparticles presenting surface-immobilized antibodies (anti-CD11c, anti-DEC-205) or CD11c-targeting peptides (PD-2) enhances microparticle internalization and migration by DCs without activation.¹⁰⁶ **c.** Differentiation of naive mouse or human CD4 T cells into Tregs can be achieved *in vitro* with controlled-release microparticle formulations of interleukin-2 (IL-2), transforming growth factor β (TGF- β), and rapamycin (rapa). The T cells induced using these optimized formulations expressed FoxP3 and suppressed the function of effector T cells.¹⁰⁷