PUBLISHED ONLINE: 23 OCTOBER 2013 | DOI: 10.1038/NMAT3775

nature materials

Engineering synthetic vaccines using cues from natural immunity

Darrell J. Irvine[1,2,3,4,5]*, Melody A. Swartz[6] and Gregory L. Szeto[1,3,4]

Vaccines aim to protect against or treat diseases through manipulation of the immune response, promoting either immunity or tolerance. In the former case, vaccines generate antibodies and T cells poised to protect against future pathogen encounter or attack diseased cells such as tumours; in the latter case, which is far less developed, vaccines block pathogenic autoreactive T cells and autoantibodies that target self tissue. Enormous challenges remain, however, as a consequence of our incomplete understanding of human immunity. A rapidly growing field of research is the design of vaccines based on synthetic materials to target organs, tissues, cells or intracellular compartments; to co-deliver immunomodulatory signals that control the quality of the immune response; or to act directly as immune regulators. There exists great potential for well-defined materials to further our understanding of immunity. Here we describe recent advances in the design of synthetic materials to direct immune responses, highlighting successes and challenges in prophylactic, therapeutic and tolerance-inducing vaccines.

Vaccines can induce the activation of T cells and B cells of the adaptive immune system (that is, cells that recognize and respond to a particular antigen), eliciting the differentiation of these lymphocytes into long-lived memory cells that will rapidly respond if the microbe is encountered in the future. Vaccines were first developed by injecting weakened forms of a live microbe (for example a virus or bacterium) that stimulate immune responses without inducing disease, and these 'live attenuated' vaccines can induce lifelong protective immunity. Often cited as the most effective public health intervention ever developed, successful vaccines have eliminated or greatly reduced the burden of former epidemics, including smallpox, poliomyelitis, tetanus, diphtheria and rubella, helping to increase life expectancy markedly in the developed world over the past century[1–4]. But the future impact of vaccination as a medical intervention extends beyond prophylactic immunization against infectious diseases, and the first therapeutic cancer vaccine was licensed in 2010[5]. Parallel advances in cancer immunotherapy treatments that block inhibitory receptors on T cells, such as the approval of an antibody against cytotoxic T-lymphocyte antigen 4 (CTLA-4) and promising clinical trial results with antibodies against programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1), are likely to provide additional opportunities to enhance vaccine efficacy in cancer patients[5–7]. Vaccines eliciting IgE-blocking, allergen-specific immune responses have shown promise in recent clinical trials for the treatment of allergies[8]. Intense research is also focused on vaccines that promote tolerance to self-antigens as potential treatments for autoimmune diseases ranging from diabetes to lupus and multiple sclerosis[9]. Optimism for the potential impact of new vaccine technologies coupled with improved global public health programmes led the Bill and Melinda Gates Foundation in 2010 to pledge US$10 billion over the next 10 years, supporting a 'Decade of Vaccines' to advance vaccine strategies in the poorest countries of the world.

The enthusiasm over recent successes in vaccine research and development must be tempered by recognizing the significant challenges that remain[1,2]. Vaccines against many chronic infections, including human immunodeficiency virus (HIV), malaria, tuberculosis and hepatitis C, remain important unmet needs. Starting with Jenner's original demonstration of the concept of vaccination, made before the microbial origin of infectious disease was even established, most licensed vaccines have been developed largely empirically[10], and mostly target pathogens with low mutation rates where natural primary infection can drive long-lived immunity in surviving hosts[1]. These vaccines predominantly drive the generation of neutralizing or opsonizing antibodies, which may not be readily achievable in some diseases. Thus, the translation of advances in cellular and molecular immunology to the design of new vaccines with improved efficacy remains a goal for many vaccinologists.

Rational vaccine design is challenging, above all, because of our incomplete understanding of the enormously complex human immune system. In diseases that lack successful vaccines, we often do not know what type of immune response would give the best long-term protection or therapeutic efficacy[1], such as the balance of cellular and humoral immunity, the ratio of effector to memory T cells, the functional properties of activated T cells (for example granzyme or perforin secretion, state of differentiation, and repertoire of cytokines secreted), or the breadth versus strength of specificity. Even with such knowledge, we lack clear guidelines for how to drive those particular responses. Thus, biomaterials tools should be used more extensively to probe such questions and develop a more quantitative understanding of design principles for vaccinology. Other outstanding challenges include the difficulty of designing antigens that elicit a predefined target antibody or T-cell response, and the substantial limitations of imperfect preclinical animal models[4]. Indeed, much of our understanding of immunology comes from inbred mouse strains that lack genetic and environmentally driven diversity and have significant species-related differences from humans in their immune systems.

Another considerable hurdle is that of eliciting a sufficiently potent immune response while meeting the exceptional safety standards necessary for prophylactic vaccines designed for administration to healthy (often infant) populations. Much of the current effort in vaccine science is focused on developing subunit

[1]Department of Materials Science and Engineering, Massachusetts Institute of Technology (MIT), 77 Massachusetts Avenue, Cambridge, Massachusetts 02139, USA, [2]Department of Biological Engineering, MIT, Cambridge, Massachusetts 02139, USA, [3]Koch Institute for Integrative Cancer Research, MIT, Cambridge, Massachusetts 02139, USA, [4]The Ragon Institute of MGH, MIT, and Harvard, East 149 13th Street, Charlestown, Massachusetts 02129, USA, [5]Howard Hughes Medical Institute, 4000 Jones Bridge Road, Chevy Chase, Maryland 20815, USA, [6]Laboratory of Lymphatic and Cancer Bioengineering, Institute of Bioengineering and Swiss Institute for Experimental Cancer Research (ISREC), École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland. *e-mail: djirvine@mit.edu

© 2013 Macmillan Publishers Limited. All rights reserved

Table 1 | Challenges in different types of vaccine strategies.

Type	Aim	Example disease settings	Challenges	References
Immunogenic, prophylactic	Build immune protection against future potential infectious disease to which host is naive	Successful: smallpox, polio, rubella, human papilloma virus Not yet successful: malaria, arenaviruses, HIV, hepatitis C virus, filoviruses Evolving: universal influenza vaccine (multivalents)	Rapid mutation rates (for example influenza, HIV) Shielded neutralizing sites (for example HIV) Lack of natural host immune response to control infection and thus lack of guidance for vaccine design Protective T-cell immunity needed (for example mycobacteria, parasites, some viruses) Immunodominant antigen shedding by pathogen	3, 4, 122
Immunogenic, therapeutic	Activate potent immunity against pre-existing/chronic diseased cells	Cancer, hepatitis B virus, human papilloma virus	Difficulty in overcoming immunological tolerance (cancer) Antigen downregulation or mutation T-cell exhaustion or depletion Target antigens that may also be expressed in other healthy cells Dysfunctions of immune system induced by disease	123–125
Tolerogenic, prophylactic	Eliminate (or drive immunological ignorance of) lymphocytes specific for a previously unencountered antigen	Cases where early biomarkers of autoimmune disease can be detected before disease onset (for example GAD65 antibodies circulating in young children predicts type I diabetes); therapeutic proteins such as asparaginase, factor VIII, uricase or α-glucosidase	Mechanisms of peripheral tolerance and deletion not fully understood Disease-causing antigens not always well defined	126, 127
Tolerogenic, therapeutic	Eliminate lymphocytes that are already primed for immunity against a specific antigen	Autoimmune diseases, allergies	Mechanisms to revert pre-existing immunity to tolerance are poorly understood	8, 126, 128, 129

vaccines composed of molecularly defined protein, peptide or polysaccharide antigens that are expected to meet these safety standards. Purified antigens are typically poorly immunogenic, however, and must be combined with adjuvants, materials that promote the immune response or directly instruct antigen-presenting cells (APCs) to elicit immunity against the co-administered antigen. It is in the design of adjuvants that materials science and engineering has a second fundamental, and potentially critical, role to play in the future of vaccinology, through the design of new immunostimulatory materials and in the development of delivery systems that can potentiate the immune response for safe and effective subunit vaccines.

Until very recently, the only adjuvants approved for use by the US Food and Drug Administration were aluminium salts (collectively termed alum), which were first developed in the 1920s. But the explosion in understanding of molecular pathways regulating microbe sensing by the immune system has led to a broad range of new targets for adjuvant design. As we will discuss below, tailored biomaterials offer the prospect of targeted triggering of immune sensors during vaccination, combined with control over vaccine exposure kinetics at the tissue and single-cell levels, suggesting the potential for engineering safe and greatly enhanced vaccine responses. These materials also have enormous potential as tools for understanding the complexities of immune regulation. For example, properties such as the precise coupling of antigen or adjuvant; surface features that can promote or prevent protein adsorption or activate complement; sensitivity to endosomal or lysosomal degradation after cell uptake; and size can all be precisely controlled with engineered materials and their immune outcomes compared.

The advances and challenges described above encompass the four main types of vaccine in preclinical and clinical development (see Table 1). Prophylactic vaccines are designed to trigger immunological memory in healthy populations to prevent disease on future exposure, and are often based on the induction of long-lived neutralizing antibody responses. Therapeutic vaccines seek to raise an immune response in the face of ongoing disease (for example chronic infections such as HIV or human cytomegalovirus, or cancer). These vaccines must overcome pathogen-mediated evasion of the immune response and are likely to require induction of strong cytotoxic T-lymphocyte (CTL; activated $CD8^+$ T cell) responses to deal with pathogens that have already established intracellular infections. But the mechanisms for inducing potent effector and memory T cells while avoiding T-cell depletion, desensitization or excessive inflammation remain poorly understood[1]. In the case of cancer, therapeutic vaccines may also need to overcome a variety of suppressive mechanisms used by tumours, such as immunological ignorance, exhaustion or tolerance to tumour antigens.

The counterparts to vaccines targeting prophylactic or therapeutic immunity are immunizations designed to promote prophylactic or therapeutic tolerance. 'Tolerizing' immunizations aim to induce an immunologic state of unresponsiveness to a foreign antigen (for example protein therapeutics or a food allergen) in a naive individual (prophylactic tolerance), or to induce such tolerance in a patient with ongoing autoimmune disease or allergies (therapeutic tolerance).

Each of these settings presents distinct challenges to vaccine development that synthetic materials may be well suited to help address. In this Review, we will describe recent advances in the development of biomaterials that show promise for promoting immunity or tolerance in vaccination; each case is prefaced by a brief discussion of the cellular and molecular mechanisms of the immune system motivating and inspiring the approach.

Controlling delivery of antigen and adjuvant

Synthetic materials have a key role to play in the design of vaccines that make use of evolved pathways by which the immune system recognizes and responds to microbes.

Immunological decision-making. The immune system must respond vigorously to dangerous pathogens while avoiding damage

© 2013 Macmillan Publishers Limited. All rights reserved

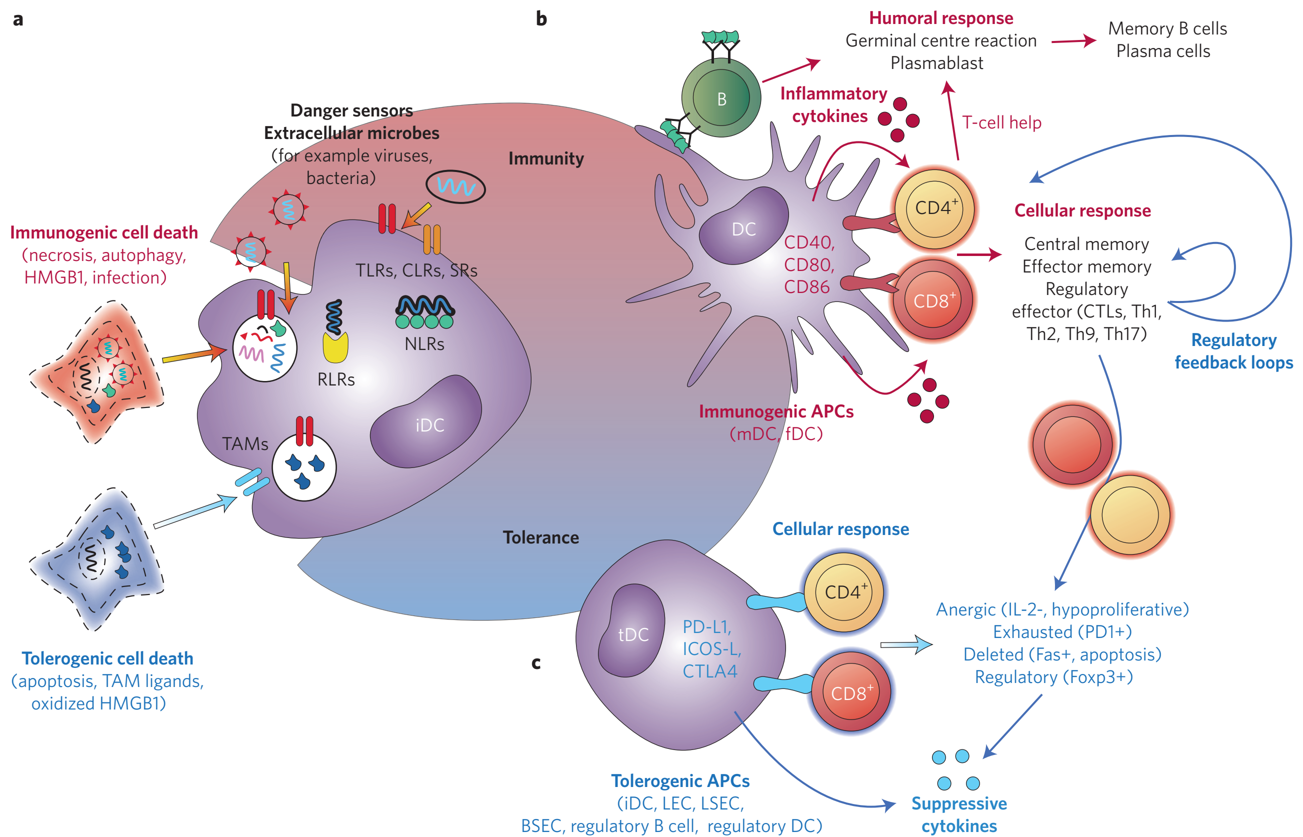

Figure 1 | Pathogen sensing by the immune system and immune context during the priming of an adaptive immune response. Dendritic cells (DC) are a central interpreter in distinguishing between foreign and self-antigens in the context of microenvironmental cues, and play an important role (along with other innate immune cells) in determining the outcome of antigen recognition by T and B cells. **a**, At steady state, immature dendritic cells (iDCs) throughout the periphery constantly sample their environment and encounter (1) immunogenic signals from infected or immunized, dying cells, accompanied by triggering of danger sensors (TLRs, CLRs, NLRs, RLRs, SRs) or (2) tolerogenic signals from dying self-cells or cellular debris generated by homeostatic turnover; these produce a continuous spectrum of output responses ranging from strong induction of effector-phase immunity to strong induction of tolerance, with the exact outcome determined by the integration of inputs by the dendritic cell. Pathogen detection occurs through a conserved suite of danger sensors and relies on detection of microbe-associated products with distinct molecular motifs[11]. Different sensors are present in endosomes (TLRs, SRs), the cytosol (RLRs, NLRs), the endoplasmic reticulum and the plasma membrane (TLRs, CLRs, SRs). Each danger sensor recognizes a different motif that is present in a class of microbes but absent from host tissues. In response to these 'danger' or tolerizing signals, dendritic cells (and other innate cells) create the immunological context for antigen recognition by secreting cytokines, expressing diverse adhesive, co-stimulatory or regulatory receptors that provide cues to responding lymphocytes. **b**, In immunogenic contexts, responding B cells can subsequently enter germinal centres to undergo somatic hypermutation, become short-lived plasmablasts, or differentiate into long-lived memory B cells or plasma cells. T cells can differentiate into effector cells or memory cells with distinct homing and functional capacities; effector cells can have diverse functions (Th1, Th2, Th17 and so on) depending on the context set by dendritic cells. Notably, regulatory feedback loops are engaged even in highly inflammatory contexts, as part of the natural control system regulating immunity, and primed effector cells can be driven to anergic/exhausted states similar to tolerance at later stages of an immune response. **c**, Peripheral tolerance is maintained by a distinct set of signals: for example, apoptotic cells that die during homeostatic turnover contain ligands that activate the plasma membrane-expressed Tyro-3, Axl and Mer (TAM) family receptor tyrosine kinases, inhibiting dendritic-cell activation and maturation. Many additional APCs also participate in tolerogenic signalling. In tolerogenic contexts, T cells are driven into several different states of non-responsiveness (anergy, exhaustion, deletion or regulatory fates) that prevent effector responses against self or harmless environmental antigens. tDC, telerogenic dendritic cell.

to healthy tissue or responses to benign environmental antigens. Therefore, the activation of adaptive immune cells through their surface antigen receptors is tightly controlled, and multiple mechanisms exist to govern their effector, memory and regulatory functions. B cells specifically sense antigens via direct binding to B-cell receptors (BCRs), whereas T-cell receptors (TCRs) recognize peptide fragments bound to self major histocompatibility complex (MHC) molecules displayed on the surface of APCs. The T and B cells are produced throughout life in the thymus and bone marrow, respectively, and genetic diversity in their antigen receptors is generated by random gene rearrangements to ensure that the host has lymphocytes capable of responding to any pathogen that might be encountered. A process known as negative selection in central tolerance leads to the death of lymphocytes with overt reactivity to self-antigens. Central tolerance does not, however, eliminate all self-reactive lymphocytes, and thus additional mechanisms (collectively termed peripheral tolerance) control cells in the periphery reactive to self- or 'safe' antigens, or to abundant foreign environmental antigens. Naive lymphocytes that receive signalling through their antigen receptor together with a variety of contextual cues develop a fate (for example anergy, effector, memory or deletion) dependent on the integration of these signals. These contextual cues include the cytokines, co-stimulatory signals and biophysical cues received by the lymphocyte in tandem with antigen

© 2013 Macmillan Publishers Limited. All rights reserved

receptor signalling; many of these signals are provided by dendritic cells, the most potent APCs during induction of primary immune responses[11,12] (Fig. 1). Dendritic cells distinguish between foreign and self-antigens by means of a conserved suite of sensor proteins that detect 'danger signals', microbe-associated products with distinct molecular motifs or signatures of cell stress or tissue damage[13]. This array of sensors is composed of several receptor families, including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs) and scavenger receptors (SRs) (Fig. 1). The immune context created by innate immune cells and dendritic cells presenting antigen determines the outcome of prophylactic, therapeutic or tolerizing immunization; thus a key role for biomaterials as vaccine agents is to help to shape this context during vaccination.

Microbes as natural micro- and nanoparticle targets. Interactions of the immune system with pathogens are shaped not only by danger signals, but also by the physical nature of microbes, which are biological microparticles and nanoparticles (Fig. 2). The surfaces of many microbes (especially viruses) have dense, multicopy displays of protein and polysaccharide motifs used to bind to target cells. But this surface patterning also promotes recognition by B cells through antigen-specific BCRs. Viruses expressing highly organized, dense arrays of proteins efficiently activate B cells, whereas the same proteins expressed at low copy number on the surface of infected cells are ineffective at triggering antibody responses[14].

The particulate nature of microbes is also exploited by the immune system to promote T-cell responses. Soluble antigens endocytosed by dendritic cells from the extracellular environment are typically degraded into short peptides and loaded onto class II MHC molecules that are surveyed by $CD4^+$ helper T-cells. A critical discovery was made by Blander and Medzhitov, who identified that the decision to present the contents of phagocytosed particulate materials on MHC II molecules depends on the presence of danger signals within the same phagosomal compartment[15]. Normally, only proteins in the cytosol of dendritic cells are degraded into peptides that are loaded onto class I MHC molecules. In contrast to soluble antigens, particulate antigens or whole pathogens internalized by dendritic cells can undergo cross-presentation[16], where particle-associated antigens are processed and loaded onto class I MHC molecules for $CD8^+$ T-cell activation by a process whose biological mechanisms are still debated. Cross-presentation can be achieved by several cell types, including (most importantly) dendritic cells, as well as (to a lesser extent) neutrophils, macrophages, and blood and lymphatic endothelial cells[17,18]. In mice, $CD8^+$ dendritic cells are the most potent subset for cross-priming of T cells[19]. A recent study of human lymphoid-resident populations of dendritic cells, however, demonstrated that, in contrast to blood dendritic cells, all lymphoid-resident human subsets of dendritic cells ($BDCA1^+$, $BDCA3^+$ and pDCs) had efficient cross-presentation capacity[20]. Cross-presentation of antigens typically follows their cell uptake by phagocytosis (receptor-mediated uptake for particles ≥500 nm) or macropinocytosis (non-receptor-mediated engulfment of particles along with fluid and solute)[21]. Encapsulation of antigens within synthetic micro- or nanoparticles can also promote their capture by APCs and avoid the rapid clearance seen with injected protein antigens[22–24]. These findings have motivated the recent focus on development of synthetic nano- and microparticles as carriers to co-deliver antigen and danger signals to APCs.

New strategies for packaging vaccines in synthetic particles. Synthetic particles surface-conjugated with antigen are especially well suited to triggering B cells[25]. On the other hand, encapsulation can lead to much higher quantities of antigen per particle, which is of interest for achieving high intracellular concentrations of antigen in APCs[26–28]. Liposomes and particles composed of biodegradable polymers such as poly(lactide-co-glycolide) (PLGA) have been studied for many years as particulate vaccine carriers, but they suffer from multiple limitations, including low levels of entrapped antigen, the potential for damage/denaturation of three-dimensional antigen structures critical for humoral immune responses and the need for organic solvents in processing.

To address these limitations, several alternative approaches and new materials have been explored. Polymer capsules formed through layer-by-layer assembly of polyelectrolytes or disulphide-bonding polymers, together with vaccine components, on sacrificial templating microspheres have been shown to promote efficient antigen uptake and presentation by human and mouse dendritic cells *in vitro* and *in vivo*[29,30]. These particles are multifunctional and allow highly efficient encapsulation of different types of cargo within the core and the layered shell compared with the limited capacity of typical solid polymeric particles. Dierendonck *et al.*[31] simplified polyelectrolyte capsule fabrication to a cost-efficient, scalable two-step process by using spray-drying instead of layer-by-layer deposition, achieving antigen encapsulation efficiency approaching 85%. Another important advance in the fabrication of particulate vaccine carriers uses the PRINT process[32], standing for particle replication in non-wetting templates. This soft lithography approach uses fluoropolymer moulds that allow large-scale fabrication of monodisperse nano- and microscale particles of diverse morphologies. PRINT is compatible with a wide variety of particulate formulations, including polymeric particles, and a first demonstration of PRINT particles as a delivery system for adsorbed influenza vaccine was recently reported[33].

Coordinating antigen and danger-signal delivery. Antigen that is associated with synthetic particles, either through conjugation to particle surfaces or encapsulation in degradable materials, can elicit cross-presentation similar to natural microbial particles (Fig. 2). The first reports of particle-triggered cross-presentation suggested a size dependence to this effect, with optimal cross-presentation triggered by particles ~3 μm in size[16,34], but this is likely to be system-dependent, as recent studies of antigen delivery using very small nanoparticles (20–50 nm) have also demonstrated effective $CD8^+$ T-cell priming, with antigen either coupled to the surface[27,28] or encapsulated within[35,36].

In addition to aiding cross-presentation of whole-protein antigens, an important recent advance has been the demonstration in small animal models that synthetic nanoparticles can elicit $CD8^+$ T-cell responses comparable to those induced by strong live microbial vaccine vectors through the co-packaging of antigen and TLR agonists as strong molecular danger signals. Moon *et al.* developed lipid nanocapsules (interbilayer-crosslinked multilamellar vesicles, or ICMVs) composed of multiple covalently crosslinked lipid bilayers surrounding an aqueous core[37]. ICMVs entrapping a protein antigen with the TLR-4 agonist monophosphoryl lipid A (MPLA) embedded in the capsule walls elicited T-cell responses that could be boosted to levels where nearly one in three T cells in the peripheral blood were specific for a single target antigen; this response was dependent on co-delivery of the TLR agonist stably incorporated in the vesicle walls. Nordly *et al.* also elicited robust CTL responses by developing a colloidally stable formulation of the TLR-3 agonist poly(I:C) complexed with cationic, gel-state liposomes adsorbed with protein antigen[38]. CTL responses elicited by this potent lipid nanoparticle vaccine exhibited polyfunctional cytokine secretion and durable memory that could be recalled 10 weeks after immunization. Zaks *et al.* similarly reported strong $CD8^+$ T-cell responses elicited by cationic liposomes complexed with antigen and various TLR agonists[39]. Although it remains to be confirmed whether such vigorous T-cell responses to protein vaccines can be elicited in

© 2013 Macmillan Publishers Limited. All rights reserved

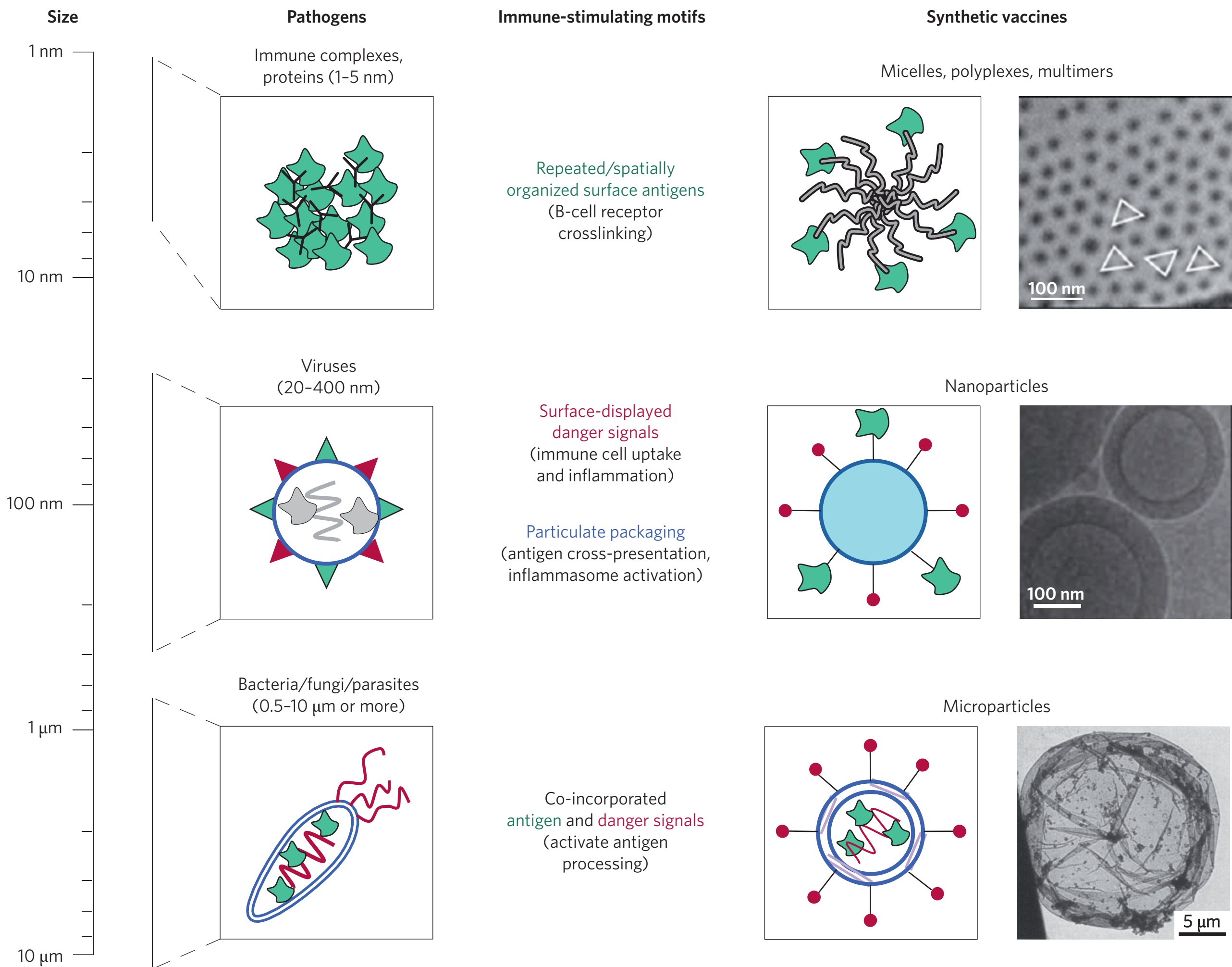

Figure 2 | Structural and compositional features of microbes and their mimicry in synthetic biomaterials-based vaccines. Microbes and microbial products are particulates spanning length scales from tens to thousands of nanometres in size, with distinct structural and chemical features that are sensed by the immune system. A rich strategy in biomaterials-based vaccines is to design nanoparticles and microparticles that mimic key features of microbes to invoke signalling pathways and immune responses similar to those elicited by native microbes, without the danger of infection or uncontrolled inflammation. Right-hand images reproduced with permission from: top, ref. 130, © 2011 Elsevier; middle, ref. 37, © 2011 NPG; bottom, ref. 131, © 2000 ACS.

non-human primates (NHP) or humans, these results demonstrate that appropriate coordination of antigen and inflammatory signals by synthetic particle vectors can markedly influence the nature of T-cell responses elicited by subunit vaccines.

Notably, vaccines comprised of danger signals co-encapsulated with antigen or loaded in separate particles have both been shown to be effective vaccines. For example, Powell *et al.* took a direct approach to the incorporation of danger signals by generating a fusion of an immunogenic, designed peptide antigen with the TLR2 agonist Pam_3Cys (ref. 40). The delivery of this fused peptide/danger signal by LbL microparticles formed a synthetic malaria vaccine candidate with greater potency and efficacy than a traditional vaccine or non-TLR2-containing LbL microparticles. By contrast, Kasturi *et al.* reported that immunization with antigen and TLR4/7 agonists encapsulated in separate PLGA particles was highly effective at inducing antigen-specific neutralizing antibodies and generated antibody-secreting cells that persisted for 1.5 years post-immunization[41]. These results are consistent with other previous work demonstrating effective vaccination achieved by adjuvants and antigens delivered in separate particles[42]. Many other groups have also observed synergistic effects by using combinations of TLR agonists in particulate vaccines[43,44].

Delivery of molecular adjuvants such as TLR agonists with micro- or nanoparticles may enhance their safety, enable the use of more potent adjuvant combinations or enable small-molecule compounds with poor pharmacokinetics to become useful adjuvants. For example, Tacken *et al.* demonstrated that the co-delivery of TLR agonists and antigens co-encapsulated in PLGA nanoparticles elicited robust antigen-specific immunity while enabling nearly 100-fold dose sparing of adjuvant, and reduced serum cytokine levels following vaccination, relative to an equivalent soluble vaccine[45]. Alternatively, the enhanced immunologic potency of particle-formulated antigens may allow lower quantities of soluble danger signals to be administered for equivalent immune responses — as illustrated by a study showing that lipid nanocapsule-formulated antigens required 250-fold less MPLA adjuvant to achieve humoral responses equivalent to soluble protein in a malaria vaccine[46]. Nguyen *et al.* screened a library of lipid materials for enhanced functional delivery of immunostimulatory RNA (isRNA) adjuvants as lipidoid–RNA nanoparticles (LRNPs)[47]. Their approach identified materials that formed LRNPs with isRNAs that were distinct from other lipid-based DNA/RNA delivery formulations (for example DOTAP, lipofectamine) in their enhanced endosomal retention, tail-chemistry-based cell targeting, and robust triggering of cellular and humoral immunity.

© 2013 Macmillan Publishers Limited. All rights reserved

Materials-based adjuvancy. The studies cited above have focused on using synthetic materials to deliver molecularly defined biological danger signals, but an exciting future direction is the design of biomaterials themselves to be direct triggers of danger sensors. Partial motivation for this approach derives from recent advances in understanding the mechanisms of action of the oldest vaccine adjuvant, alum.

Alum/antigen complexes have recently been shown to serve as a unique danger signal to dendritic cells, and a receptor-independent, direct interaction has been identified between cell-membrane lipids and crystalline compounds such as alum or monosodium urate (MSU, a product released by dying cells following alum injection)[48,49]. Alum and MSU interact with membrane lipids, causing receptor aggregation at lipid rafts and recruitment and activation of intracellular kinases. Alum induces abortive phagocytosis and promotes non-phagocytic antigen uptake, leading to endosomal processing and loading onto MHCII molecules. Furthermore, alum-mediated cell death and subsequent host-cell DNA release also promote Th2 responses and humoral immunity[49,50].

Inflammasome activation is another proposed mechanism of particulate material adjuvancy identified by *in vitro* studies. Inflammasomes are cytosolic danger sensor complexes[51] triggered following endolysosome rupture by these materials[52], or indirectly through release of crystalline MSU from stressed/dying cells[50,53]. In the case of alum, it has been shown that inflammasomes are dispensable for alum's adjuvancy *in vivo*, which instead seems to operate through the direct membrane interactions discussed above. Polymeric particles (for example PLGA and polyethylene glycol (PEG)–poly(propylene sulphide)[54]) have recently been shown to serve as direct activators of the inflammasome in dendritic cells. But there is little evidence yet that inflammasome activation affects the humoral response to particulate vaccines, and the role of inflammasome activation in the T-cell response is still unclear[55,56]. The inflammasome-triggering ability of materials internalized by innate immune cells may be a general response to internalization of particulates, as it has been observed *in vitro* with alum, silica, polystyrene particles, polymeric multilayer capsules[57] and PEG–poly(propylene sulphide) nanoparticles[54]. Synthetic particles may also trigger additional processes in antigen-presenting cells that affect immune responses: Li *et al.* recently demonstrated that tumour antigen delivery by α-alumina nanoparticles resulted in immune activation and presentation by means of induction of autophagy in dendritic cells, a process in which cells degrade and recycle their intracellular components within specialized vesicles[58]. Autophagy resulted in robust cross-presentation and T-cell priming that was able to eradicate established subcutaneous melanoma when delivered as a therapeutic vaccine.

Another strategy to activate danger sensors with biomaterials is through the design of materials that trigger complement or other innate sensing responses *in vivo*. This approach was first demonstrated with poly(propylene sulphide) nanoparticles stabilized by poly(propylene oxide-*b*-ethylene oxide) block copolymers. These nanoparticles displayed a PEG corona with hydroxyl chain ends that activated complement, providing an intrinsic danger signal to effectively act as adjuvant in the response to antigen tethered to the particle surfaces[27]. A follow-up study by Thomas *et al.* demonstrated the potential to fine-tune complement activation by manipulating particle design parameters including core thiolation, surface charge and surface carboxylation[59].

Some materials have been identified with adjuvant properties for which the specific mechanism of action remains unknown. Hydrophobicity is a property that has been proposed to dictate the adjuvancy of synthetic polymers and moieties of some microbial danger signals. For example, a systematic series of gold nanoparticles with organic ligand shells of increasing hydrophobicity elicited expression of inflammatory cytokines by splenocytes proportional to particle hydrophobicity[60]. Petersen *et al.* recently demonstrated that the activation of innate immune responses by pathogen-mimicking nanoparticles was influenced by polymer hydrophobicity and polar/hydrophobic moiety patterning[61]. Hydrophobicity has also been shown to affect particulate uptake[62]. Another example of materials with intrinsic adjuvant properties comes from work by Rudra *et al.*, who recently demonstrated strong class-switched, apparently T-cell-independent antibody responses induced by peptide antigens fused to amino acid sequences that form self-assembling fibrils *in vivo*. These fibril-forming vaccines might act as depots of antigen, be recognized by known or unknown danger sensors, or act through new mechanisms to drive the immune response[63]. Further exploration of such routes of adjuvant action may lead to new strategies for adjuvant development that move beyond the well-established classical adjuvants and danger signal pathways.

Delivering antigens and tolerogenic signals. Following many of the same design principles used for immunizing vaccines, researchers are also developing prophylactic and therapeutic vaccine strategies that can drive antigenic tolerance. Using danger-signal-free synthetic microparticles coupled to immunogenic myelin epitopes, Getts *et al.* successfully generated protective and therapeutic tolerance in an animal model of multiple sclerosis[64]. In this study, inert polystyrene and PLGA beads were used to mimic the known tolerogenic properties of apoptotic cells. The authors found that tolerance was dependent on 500-nm particle size, covalent linkage of the peptide antigens and intravenous administration, which resulted in delivery to splenic marginal-zone macrophages. Interestingly, tolerance was induced in a multifaceted manner that included T_{reg} activation, abortive T-cell activation and T-cell anergy. Lewis *et al.* have also shown that antigen-carrying particles can be functionalized with ligands for dendritic-cell-specific receptors to enable targeting of tolerogenic antigen to dendritic cells without inducing dendritic-cell activation[65].

Many groups are pursuing strategies to induce tolerance by co-delivery of small-molecule drugs with antigens. Recently, Yeste *et al.* used a PEGylated gold nanoparticle vehicle for co-delivery of the model autoimmune-inducing antigen MOG and a tolerogenic small-molecule drug targeting the aryl hydrocarbon receptor, which is implicated in tolerogenic signalling in dendritic cells[66]. They demonstrated profound induction of tolerogenic dendritic cells that primed increased numbers of $CD4^+$ regulatory T-cells, and these regulatory cells alleviated antigen-induced autoimmune encephalitis. They also demonstrated that co-delivery of the antigen and small molecule together was necessary to achieve maximum therapeutic efficacy.

In a very different approach, Tsai *et al.* designed self-peptide-MHC-coated iron oxide nanoparticles (pMHC-NP) with the goal of treating autoimmune type I diabetes (T1D) by tolerizing disease-specific $CD8^+$ T cells[67]. These particles can bind to T-cell receptors and provide direct signalling to T cells independent of APCs. Their results were robust and unexpected, demonstrating that pMHC-NPs were therapeutic in a murine model of T1D by expanding a memory subset of low-avidity regulatory $CD8^+$ T cells, instead of directly tolerizing T1D-specific T cells. The existence of this pool of regulatory cells and their ability to expand in response to pMHC-NP without co-stimulation were new immunologic findings. The therapeutic efficacy of pMHC-NP was thus attributed to expansion of regulatory cells that mediated both direct suppression of other T cells and killing of autoantigen-presenting dendritic cells. This mechanism has promising therapeutic potential because these regulatory T cells are able to suppress autoimmune responses against multiple potential auto-antigens by acting at the level of the dendritic cells.

© 2013 Macmillan Publishers Limited. All rights reserved

NATURE MATERIALS DOI: 10.1038/NMAT3775

Kinetics and biodistribution of vaccine exposure

The immune response to vaccination is a function of a given formulation's spatiotemporal profile, with the timing and localization of antigen and danger signals dictating qualitative and quantitative aspects of vaccine-induced immunity.

Shaping the response to infections and vaccination. Antigens and appropriate adjuvant signals provide the context for a developing immune response, but the timing of exposure of the immune system to these signals can be as important as the choice of vaccine components in generating the quality and magnitude of response. The role of antigen and inflammation kinetics in shaping the immune response is perhaps best typified by considering natural acute versus chronic infections.

Acute infections are often characterized by brief bursts of antigen production and inflammation induced from infected cells, which may persist initially, but decay over the course of ~1–2 weeks, coinciding with the induction of the primary immune response and clearance of the pathogen[68]. Such brief (albeit potentially strong) exposure to antigen and inflammatory cues can be accompanied by massive clonal expansion of T cells, robust generation of affinity-matured antibodies, and development of long-lasting memory T-cell and B-cell populations. By contrast, in chronic infections where antigen and inflammation are persistently produced over months or years without effective clearance by the immune response, defective lymphocyte memory, T-cell 'exhaustion', and failure of immune effector functions ensues[68,69]. This may reflect the pathogen confusing the immune system by mimicking the continuous antigen exposure of self-antigens, which normally drives tolerance.

Notably, these patterns of natural antigen/inflammation exposure during infection are quite distinct from the relatively brief exposure of lymph node cells to antigen and adjuvant molecules following traditional bolus subunit vaccine injection, where vaccine components carried by lymph are flushed through the lymph nodes within hours, and migrating dendritic cells from the injection site traffic antigen for only 1–2 days (refs 70,71). In vaccination, giving the same immunization with different kinetic patterns (achieved by repeated injections of varying vaccine doses over time) markedly alters the strength of $CD8^+$ T-cell responses to peptide vaccines: administration of exponentially increasing doses of peptide and adjuvant injected daily over the period of one week gave much stronger T-cell responses than a single-timepoint bolus injection, steady low-dose administration or an exponentially decaying administration pattern of the same total vaccine dose[72]. Antigen exposure kinetics are equally crucial at the single-cell level within APCs. Studies of antigen processing have shown that proteins are selected for antigen presentation with APCs during a very narrow window of time following internalization into endolysosomal compartments[73], indicating that antigens that are released from endolysosomally localized particle carriers after a few hours may be too late to contribute productively to antigen presentation.

Importantly, factors such as vaccine particle size, route of administration and even surface chemistry can affect the exposure kinetics through control of physiological trafficking to various tissues and cells as well as intracellular compartments. Although there is growing appreciation that these variables significantly affect the quality and magnitude of the immune response, there remains little understanding of the underlying mechanisms at play. These issues are discussed below.

Delivering vaccines into target tissues. The first step in immunization is the deposition of a vaccine into a target tissue; synthetic materials are being developed to enable vaccine delivery through non-invasive routes (skin, oral administration) and to promote vaccine uptake through mucosal surfaces (airways, gastrointestinal and reproductive tracts). Materials that are capable of protecting antigens in transit through the stomach and gut for oral vaccination have long been sought[74], but poor uptake efficiency[75] and the potential for antigens encapsulated in solid degradable polymers to be degraded[76] have remained substantial hurdles to oral vaccine development. Recently, Zhu *et al.* have reported promising results with a two-stage oral delivery system based on antigen and adjuvant-loaded PLGA nanoparticles encapsulated within microspheres composed of a pH-responsive Eudragit poly(methacrylic acid) copolymer[77]. The Eudragit microspheres served as a protective carrier that selectively dissolved only within the above-neutral pH conditions of the terminal ileum, releasing the antigen-carrying nanoparticles where they could be taken up across the intestinal epithelium. This two-stage system promoted T-cell and humoral responses that were protective against mucosal challenge with recombinant vaccinia virus in mice. Targeting of particles to antigen-transcytosing M cells overlying Peyer's patches in the gut by means of antibodies or M-cell-specific ligands may provide additional avenues to further enhance vaccines delivered to the gastrointestinal tract[78].

Materials are also being developed to promote vaccine delivery through the skin, and here many exciting advances have come in the area of microneedle skin patches designed to perforate the outer layers of the stratum corneum and painlessly deposit vaccines in the epidermis and/or upper dermis. Microneedles composed of polyvinylpyrrolidone polymerized *in situ* in moulds in the presence of an inactivated influenza vaccine allowed the formation of dissolving vaccine-loaded skin patch arrays that promoted enhanced protection of mice from influenza challenge when compared with a traditional intramuscular syringe vaccination[79]. Because the microneedles quickly dissolve upon application to the skin, these patches are strong candidates for self-administrable vaccines that would have no associated biohazard waste. Dissolving microneedles can also potently deliver synthetic nano/microparticle vaccines into skin[80]. A second recent advance has been in the development of silicon microneedle arrays that are geometrically designed to penetrate to precise depths to release coated vaccines within the epidermal layer, where Langerhans cells (the key dendritic cells of the skin) reside[81]. Finally, the ability of microneedles to carry and release complex vaccine formulations may enable currently ineffective vaccine strategies to achieve potency. DeMuth *et al.* recently described an approach for 'polymer multilayer tattooing', where microneedles coated with a quick-releasing polyelectrolyte multilayer coating deposit these vaccine-loaded films into skin. They then release DNA and adjuvants over a tunable time period, thereby greatly enhancing immunogenicity and achieving immune responses comparable to the current gold-standard process known as *in vivo* electroporation[82].

Vaccination through mucosal surfaces is of great interest to promote protection at key portals of pathogen entry, but the mucus barrier that lines the airway, gastrointestinal and reproductive tract mucosa is a formidable barrier to vaccine absorption. Mucus is composed of fibrous bundles of mucins — large, highly glycosylated proteoglycans comprising hydrophobic protein backbones decorated by a dense brush of short anionic polysaccharides. Mucus gels range from very thin layers up to 800 μm thick in portions of the gut[83], and are designed to entrap and remove particulates and microbes. Aqueous pores in the physical gels formed by mucin fibres have been estimated to be ~340 nm in diameter, but particles much smaller than this mean size can be efficiently captured by the mucin strands[84]. The high negative charge of the gel traps any positively charged particle, while providing an electrostatic barrier to partitioning of negatively charged particles. Finally, particles with exposed hydrophobic patches are efficiently captured

© 2013 Macmillan Publishers Limited. All rights reserved

by the hydrophobic domains of the protein backbones of mucin chains. Recent studies have revealed, however, that synthetic particles as large as 500 nm in size are capable of rapid diffusion through mucus if they have dense PEG coatings that simultaneously mask hydrophobic surface patches and provide near-neutral net surface charge[85,86] — thus mimicking the charge-neutral surface chemistry of mucus-penetrating viral particles. These mucus-penetrating surface chemistries enable vaccine- and drug-loaded polymer particles to penetrate to the epithelial surfaces in the lungs[26] and the reproductive tract[87,88]. Mucus-penetrating particles may enable vaccines to be delivered to mucosal dendritic cells before elimination by mucociliary clearance mechanisms. For delivery to nasal mucosa, Nochi *et al*. demonstrated an alternative approach based on amphiphilic pullulan polymers that self-assembled with protein antigens into cationic nanogels. These cationic nanoparticles adhered tightly to the nasal mucus/apical epithelial membranes for up to 2 days (soluble antigen, in comparison, was cleared from the nasal passages within hours), enabling antigen to be more effectively captured by nasal dendritic cells and promoting robust *Clostridium botulinum* or tetanus-neutralizing antibodies[89]. Because vaccination at mucosal sites often promotes mucosa-localized memory/plasma cells much more efficiently than does parenteral immunization, these approaches have great promise for enhancing protection against a variety of respiratory, reproductive tract and gastrointestinal pathogens.

Size of subunit vaccines. The size of an injected particulate affects its physiological tissue distribution and kinetics, cellular distribution (for example within the draining lymph node), cellular uptake by APCs and intracellular processing pathways (Fig. 3), thereby strongly influencing the quality and quantity of the immune response that it elicits. Within the interstitial (or extravascular) space, where particulate material is introduced either by injection or by extravasation (for example in tumours with leaky blood vessels), particles can both diffuse and convect. The Peclet number (Pe) represents a ratio of convective transport to diffusion for a given situation; when $Pe > 1$, fluid convection — which is driven in the interstitium by lymphatic drainage caused by a pressure gradient between the blood and lymphatic vessels — and its associated convective forces dominate particle transport. Both interstitial diffusion and convection are hindered, however, by the architecture of the extracellular matrix. For moderately sized proteins (for example 69 kDa albumin with a hydrodynamic radius of ~3.5 nm and diffusion coefficients measured in the range of 40–110 $\mu m^2 s^{-1}$ depending on the tissue), convection begins to become more important compared with diffusion in average interstitial flows of ~0.1–1 $\mu m s^{-1}$ (ref. 90). For typical nanoparticles 20–50 nm in diameter, convection is likely to dominate interstitial transport in most tissues; larger particles are sterically hindered by the extracellular matrix and mostly convect along heterogeneous fluid channels.

Convective flows sweep macromolecules and particles from the interstitium into lymphatic vessels that carry them to the lymph node. Lymphatic targeting is optimal for particles in the size range of 10–50 nm (refs 27,91); particles above this size can still enter lymphatic vessels, but do so at a rate that decreases with increasing particle size, owing to interstitial hindrance. Small molecules (<2 nm) may also access blood vessels, although their solute permeability is lower than that of the interstitium. Thus, nearly all injected macromolecules and nanoparticles in the size range of 10 to ~50 nm will leave the interstitial space of healthy tissues by lymphatic drainage.

Once inside lymphatic vessels, particles are transported to the lymph node, where the larger particles are taken up by subcapsular macrophages, and smaller particles or molecules can enter the B- or T-cell zones through conduits (formed by follicular dendritic cells or fibroblastic reticular cells, respectively) or the lymphatic sinuses. Some particles will not enter the lymph node, instead remaining in the subcapsular sinus and leaving by way of the efferent lymphatic vessel. The term 'lymph node retention' refers to the relative fraction of entering solute that remains in the lymph node (presumably taken up by resident cells), although this is a kinetic phenomenon. In general, particles of increasing size show increasing lymph node retention once inside the lymphatics, but target subcapsular macrophages more readily, whereas smaller particles can rapidly enter B- and T-cell zones for uptake by B cells and dendritic cells[24].

Within a few hours after injection, nanoparticles can be seen in the systemic circulation (to which they eventually drain), peaking in concentration at ~12 h post-injection[23]. There, they may concentrate in the spleen as well as liver, kidneys and lungs. A recent biodistribution study demonstrated that after intradermal injection of 25-nm polymeric nanoparticles, total accumulation of particles in leukocytes within the draining lymph nodes peaked after 1 h, whereas peak accumulation in dendritic cells, monocytes and B cells occurred between 6 and 24 h. In contrast, splenic accumulation in all cell types peaked at 24 h with a striking affinity for $Ly6c^+$ monocytic cells, particularly in tumour-bearing mice[23].

As discussed above, cellular uptake and processing pathways are also size-dependent. Antigens that are in particulate form (including micro- and nanosized carriers, exosomes, and proteins complexed with heat-shock proteins) are more likely to be processed through cross-presentation pathways than those that are in free protein form. In terms of dose, however, it should be considered that attaching antigens to particles leads to localized antigen concentration with a smaller effective concentration relative to soluble antigens, considering 'concentration' as the number of antigenic particles (or molecules) per volume (Fig. 3c). This factor should be considered when comparing immune responses to equivalent antigen dose of free or of particle-bound antigen, because the effective 'concentration' (that is, outside the cell) of particle-bound antigen is equal to the antigen concentration divided by the number of antigen molecules per particle. Overall, however, the improved targeting and processing pathways of antigens in particulate forms permits lower antigen doses with particle-bound antigen. Numerous studies have demonstrated that particulate forms of antigen drive stronger immune responses than free antigens when co-delivered with the same adjuvants, thus reducing the required antigen dose[22,27,37,46,63,92,93].

Vaccine targeting at the single-cell level. At the single-cell level, synthetic materials can also regulate vaccine delivery by controlling the intracellular timing and location of antigen/danger-signal release following vaccine uptake by APCs. Murthy *et al*. first demonstrated engineering of the timing of intracellular antigen release from synthetic particles by designing antigen-loaded hydrogel particles crosslinked by acetal linkages that were stable at neutral pH but acid-labile, providing rapid degradation and antigen release within the acidic endolysosomal compartments of dendritic cells[93]. Rapid breakdown of these gel particles into soluble polymer fragments simultaneously provided an osmotic driving force for rupture of the endosomes and release of the freed antigen into the cytosol, promoting cross-presentation of antigen to $CD8^+$ T cells. More recently, a variety of examples of endosome-disrupting polymer particles and vesicles that release antigen into the cytosol have been reported, which are triggered by the acidic conditions of the endolysosomal pathway[94–97], the reducing conditions of the endosomes[36] or external light cues[98]. It has also been shown that antigen-releasing PLGA nanoparticles internalized by dendritic cells can continuously release antigen within cells, thus providing an intracellular store that prolongs antigen presentation over several days, enhancing $CD8^+$ T-cell priming[99]. Confocal

© 2013 Macmillan Publishers Limited. All rights reserved

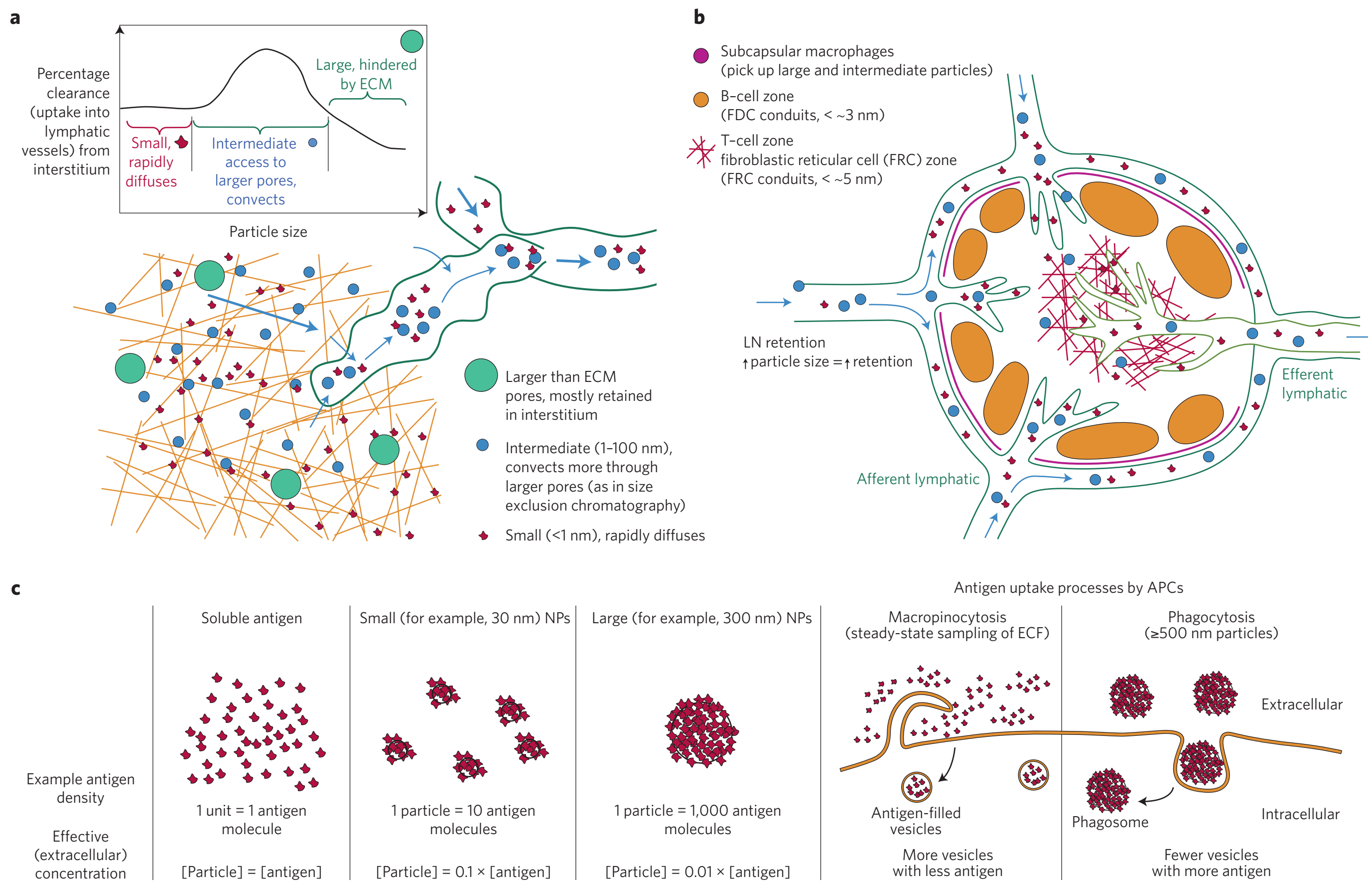

Figure 3 | Effects of particulate size on tissue, cell and intracellular targets after entry into interstitial tissue. a, After injection into the interstitium (that is, intramuscular, intradermal or subcutaneous injection, for instance), particles (whose definition here includes molecules) will disperse and convect with interstitial flow, driven by transient pressure gradients that arise from the injection as well as the natural small pressure gradient between blood and lymphatic capillaries. Very small particles (red), whose diffusion velocity is greater than convective velocity, can readily diffuse and will rapidly dilute in local concentration, which limits the effective lymphatic concentration. Larger, intermediate-sized (blue) particles have smaller diffusion speeds and furthermore are transported within the more permeable regions of the extracellular matrix (as in size-exclusion chromatography). Their transport is thus governed more by convection, and they are more efficiently directed into the lymphatic vessels. As size increases, however, steric hindrance becomes limiting, and particles that are too large (over about 500 nm, although this depends on tissue, level of hydration and experimental conditions) remain mostly trapped in the interstitial space. **b**, Once inside the lymphatic vessel, lymph node (LN) retention positively correlates with particle size. Larger (or opsonized) particles are readily taken up by subcapsular macrophages, whereas intermediate-sized particles can directly access the T-cell zone and associated dendritic cells. The B-cell zone conduits, however, which are formed by follicular dendritic cells (FDC), restrict access to particles under about 3 nm. **c**, Size also affects antigen concentration and dose upon intracellular uptake by the APC. If the antigen is a free protein, then the effective 'particle concentration' is equal to the antigen concentration, and this is also equal to the concentration within macropinocytotic vesicles after uptake. But if antigen is adsorbed or incorporated into a nanoparticle, then the concentration of antigen 'units', or particles, is less than the antigen concentration by the number of antigen molecules adsorbed per particle. Larger particles of, for example, 250 nm can contain 1,000 antigen molecules per particle, and thus reduce the effective antigen concentration 1,000-fold. On the other hand, upon uptake, antigen should be 10-fold or 1,000-fold more concentrated inside the phagosome when taken up in nanoparticulate form than in free antigen form. It is unknown how such differences in antigen delivery (that is, more vesicles with fewer antigens each, compared with fewer vesicles with more antigens each) affect cross-presentation efficiency.

microscopy suggested that some particles were not localized in acidic endolysosomal compartments, and antigen was detected directly in the cytosol, explaining these enhanced T-cell responses. In the realm of nucleic acid-based vaccines, Geall *et al.* recently reported a cationic PEGylated liposome system for intracellular delivery of self-replicating mRNA-based vaccines, which achieved greatly enhanced potency relative to naked mRNA or DNA injections[100]. As cytosolic delivery is a key objective broadly in drug delivery, other new materials developed for nucleic acid delivery (reviewed elsewhere in this issue) and additional cytosolic access strategies (for example cell-penetrating nanoparticles[101]) may be of future interest for regulating the entry of antigen into cells. In addition, controlling the kinetics of molecular adjuvant delivery into the cytosol could open up many recently discovered intracellular danger sensors (for example NLRs and RLRs[13,51]) as viable targets for vaccine immunomodulation.

Regulating antigen exposure kinetics with synthetic vaccine materials. The application of synthetic materials as controlled-release vehicles to regulate the kinetics of vaccine exposure was appreciated in the earliest studies of vaccines, and the first synthetic vaccine adjuvant, alum, was long believed to act mainly as a slow-release source of antigen, although more recent studies suggest that alum is not an effective 'depot' for many antigens and that sustained antigen release over time is not important for the immune response to this adjuvant[102,103]. But the development of controlled-release

© 2013 Macmillan Publishers Limited. All rights reserved

Table 2 | Biomaterials-based vaccine technologies in commercial development.

Company	Materials/technology platform	Disease indications	Development phase
Vedantra Pharmaceuticals	Crosslink-stabilized lipid nanocapsules	Malaria, HPV	Preclinical
CSL	ISCOMATRIX saponin derivative/cholesterol/ lipid nanoparticles	HPV, HCV, influenza	Phase 1
Isconova	ISCOM-based matrix platform, saponin derivative/cholesterol/lipid nanoparticles	Influenza, malaria, rabies, HSV-2	Phase 1
Liquidia Technologies	PRINT process for monodisperse particles	Influenza, pneumonia	Phase 1 (influenza, elderly)
NanoBio Corporation	Nanoemulsion of bacterial spores	Influenza, trivalent influenza RSV, hepatitis B virus, anthrax, smallpox	Phase 1 (influenza)
NanoTherapeutics	GelVac self-gelling polymer plant polysaccharide powders	Influenza, typhoid, norovirus, HIV	Phase 1 (H5N1 influenza)
Selecta Biosciences	Targeted polymer nanoparticles	Nicotine addiction, malaria, type I diabetes, food allergy	Phase 1 (nicotine addiction)
Novavax	RSV-F micelles	Respiratory syncytial virus (RSV), influenza, rabies	Phase 2 (RSV, influenza)
Pevion Biotech	Virosome (cell-free lipid/protein self-assembling influenza-like liposomes)	Recurrent vulvovaginal candidiasis, RSV, malaria, HIV, hepatitis C virus	Phase 1 (RVVC, HIV, hepatitis C virus); phase 2 (malaria)
Vical	Cationic liposomes, lipid/DNA poloxamer	HSV2, HIV, dengue	Phase I (Dengue); phase 2 (HIV)
Crucell (Johnson & Johnson)	Virosomes	Aluminium-free hepatitis A virus, rabies, influenza	Approved (aluminium-free hepatitis A virus, Epaxal; influenza, Inflexal V)
GSK	'Adjuvant system' family of vaccine adjuvants, including liposomal monophosphoryl lipid A/ saponin derivative QS21 (AS01B), oil-in-water emulsion with MPLA/QS21 (AS02A), MPLA adsorbed on alum (AS04)	Tuberculosis, malaria, HIV, HSV, HPV	Phase 1 (tuberculosis, HIV); phase 3 (malaria); approved (HPV)
Novartis	MF59 oil-in-water nanoemulsion; mRNA replicons in cationic liposomes	MF59, many infectious diseases; liposomal replicons initial focus on RSV	Approved (influenza, Europe); preclinical (liposomal replicons)

polymer matrices and particles that definitively provide continuous release of antigen over periods of weeks to months demonstrated the ability of slow-release formulations to robustly promote the humoral immune response[104–106]. Gupta *et al.* in particular showed that antibody responses against tetanus toxoid released from large (~50 μm) PLGA microspheres over more than 1 month *in vivo* elicited robust toxin-neutralizing antibodies, whereas the same 'empty' microspheres mixed with soluble antigen (controlling for possible inflammatory or APC-activating actions of the microspheres themselves) elicited no response[104].

Three points of note about these early studies are that (1) they generally focused on designing materials to achieve sustained release of antigen over a period of many weeks with the objective of obtaining single-shot vaccines that do not require boosting, although this pattern of antigen exposure may better mimic the setting of chronic infection that tends to ablate T-cell responses; (2) early slow-release vaccines were generally developed without the explicit addition of inflammatory molecules, and thus the type and kinetic pattern of inflammation induced by these vaccines is poorly understood; and (3) often only humoral immunity was analysed in response to these sustained-antigen release vaccines (and typically only antibody titres). In regard to the second point, Hailemichael *et al.* showed that long-lived antigen depots formed by mixing peptide vaccines with incomplete Freund's adjuvant lead to T-cell deletion rather than expansion in the absence of an added inflammatory signal (for example TLR agonists)[107].

Recently, the impact of regulating the kinetics of inflammatory adjuvant exposure rather than antigen was explored through intranodal injections of PLGA microspheres releasing the TLR-3 agonist polyI:C (ref. 108). These experiments showed that both T-cell and antibody responses were substantially amplified by persistently exposing lymph node cells to adjuvant over the course of 5–7 days, as opposed to bolus injections where the adjuvant was cleared within hours; this effect was mediated by sustained levels of dendritic cell activation in the lymph nodes over time. Cytokines have also been explored as adjuvants in vaccines, and these molecules also typically have very short half-lives (minutes) following parenteral injection. St John *et al.* demonstrated a biomimetic approach to controlling cytokine delivery in vaccination, by packaging tumour necrosis factor-α (TNF-α, a prototypical inflammatory cytokine) in zwitterionic heparin/chitosan polyelectrolyte complexes roughly 400 nm in diameter mimicking the inflammatory granules released by mast cells within minutes of stimulation in inflamed tissues[109]. These mast-cell mimics deposited in lymph nodes and exhibited a ~24-hour release of cytokine *in vitro*, presumably promoting sustained exposure *in vivo*. Nanoparticle-TNF delivery promoted a 'Th1'-like immune response (associated with better protection in many disease models such as cancer[110] and *Leishmania* infection[111]) and protection by a model influenza vaccine. Increased exposure of draining lymph nodes to vaccines is probably also achieved by other particulate vaccine carriers that are transported through lymph to deposit in the subcapsular sinus or diffuse into the lymph-filled conduits of the node, where they may deposit and act as local depots of antigen and/or inflammatory signals for days to weeks[27,46].

In an approach designed to regulate the kinetics of multiple steps in the immune response, Ali *et al.* employed biodegradable polymer scaffolds that released the cytokine granulocyte/macrophage colony stimulating factor (GM-CSF) to attract and differentiate dendritic cells, CpG DNA to activate the resulting dendritic

© 2013 Macmillan Publishers Limited. All rights reserved

cells, and tumour antigen to load the activated dendritic cells and drive an antitumour immune response[112,113]. Greatly enhanced antitumour immunity was generated by this strategy when compared with bolus injection of the same vaccine components[112]. In a related approach, Singh *et al*. demonstrated the ability of *in situ* crosslinking hydrogels carrying chemokines, plasmid DNA and siRNA-loaded microparticles, to attract dendritic cells, modulate their cytokine signalling and drive a Th1-biased immune response to plasmid-encoded antigens[114]. Altogether, these data suggest that the kinetics of antigen and inflammatory signal exposure may be equally important in controlling the immune response during vaccination, and that sustained exposure to inflammatory signals over at least ~1 week enhances both T- and B-cell responses to subunit vaccines.

Outlook

Although great successes have been achieved over the past two centuries in vaccinology, enormous challenges remain, both in our understanding of what type of immune response is needed for fighting many diseases and in how to generate those specific responses. Most of the diseases that have no available vaccine present challenging immunological problems, such as in chronic infections or cancer where natural immunity fails, or in autoimmune diseases where natural tolerance mechanisms fail. The studies summarized here illustrate how synthetic materials can be used as adjuvants or delivery systems to amplify, regulate or qualitatively alter vaccine immune responses. Additionally, these materials provide tools for further understanding the basic mechanisms controlling immunity.

What are the key challenges ahead? One such issue is the evaluation of more materials-based vaccine concepts in non-human primate (NHP) models and humans. Small animal models are important tools for proof-of-concept studies, but often fail to predict the efficacy of vaccine concepts in NHPs and humans[115,116]. For example, no vaccine strategy so far has demonstrated the ability to robustly induce $CD8^+$ T-cell responses to protein vaccines in large animal models. If the promising cross-priming of $CD8^+$ responses triggered by nanoparticle vaccines in small animals is replicated in NHPs, this would represent an important advance for vector-free vaccine development. Currently, few nanoparticle/microparticle or materials-adjuvant-based vaccines have been tested in NHPs or clinical trials, although the data generated from initial studies are encouraging[41,117–119]. The next few years will see many additional concepts undergo advanced preclinical and early clinical testing through the numerous biotechnology companies pursuing commercialization of these technologies (Table 2).

A second challenge for the growing field of immuno-biomaterials engineering is to begin analysing in deeper detail the qualitative features of immune responses triggered by advanced materials that modulate cellular or tissue targeting, innate immune stimulation and vaccine kinetics. Moving beyond simply aiming for greater expansion of antigen-specific T cells and higher antibody titres to the critical questions of immune functionality (for example, what functions do these T cells have? What is their avidity? What epitopes are recognized by elicited antibodies? What degree of somatic hypermutation have they undergone?) will be an important step in determining the ultimate impact of synthetic materials in vaccine design. It is becoming increasingly clear that qualitative features of the immune response, such as the polyfunctionality and avidity of T cells, glycosylation and subclass of antibodies, and the degree of affinity maturation of antibodies, can have a great impact on the effectiveness of vaccines[120,121]. There exists little understanding of how vaccines can be designed to affect these qualitative features. Biomaterials can play a key role in providing controlled systems to stimulate the immune system and determine what molecular levers must be pulled, how hard, and when, in order to direct the immune system to the desired state of memory. Answering these questions will be an exciting challenge at the interface of materials science and immunology in the coming years.

Received 10 February 2013; accepted 9 September 2013; published online 23 October 2013

References

1. Germain, R. N. Vaccines and the future of human immunology. *Immunity* **33,** 441–450 (2010).
2. Pulendran, B., Li, S. & Nakaya, H. I. Systems vaccinology. *Immunity* **33,** 516–529 (2010).
3. Plotkin, S. A. Vaccines: Past, present and future. *Nature Med*. **11,** S5–S11 (2005).
4. Rappuoli, R. & Aderem, A. A 2020 vision for vaccines against HIV, tuberculosis and malaria. *Nature* **473,** 463–469 (2011).
5. Mellman, I., Coukos, G. & Dranoff, G. Cancer immunotherapy comes of age. *Nature* **480,** 480–489 (2011).
6. Topalian, S. L. *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *New Engl. J. Med*. **366,** 2443–2454 (2012).
7. Brahmer, J. R. *et al.* Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *New Engl. J. Med*. **366,** 2455–2465 (2012).
8. Valenta, R. *et al.* From allergen genes to allergy vaccines. *Annu. Rev. Immunol.* **28,** 211–241 (2010).
9. Dolgin, E. The inverse of immunity. *Nature Med.* **16,** 740–743 (2010).
10. D'Argenio, D. A. & Wilson, C. B. A decade of vaccines: Integrating immunology and vaccinology for rational vaccine design. *Immunity* **33,** 437–440 (2010).
11. Steinman, R. M. & Banchereau, J. Taking dendritic cells into medicine. *Nature* **449,** 419–426 (2007).
12. Banchereau, J. & Steinman, R. M. Dendritic cells and the control of immunity. *Nature* **392,** 245–252 (1998).
13. Kawai, T. & Akira, S. The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int. Immunol.* **21,** 317–337 (2009).
14. Bachmann, M. F. *et al.* The influence of antigen organization on B cell responsiveness. *Science* **262,** 1448–1451 (1993).
15. Blander, J. M. & Medzhitov, R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature* **440,** 808–812 (2006).
16. Kovacsovics-Bankowski, M. & Rock, K. L. A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. *Science* **267,** 243–246 (1995).
17. Lund, A. W. *et al.* VEGF-C promotes immune tolerance in B16 melanomas and cross-presentation of tumor antigen by lymph node lymphatics. *Cell Rep.* **1,** 191–199 (2012).
18. Lin, M. L., Zhan, Y., Villadangos, J. A. & Lew, A. M. The cell biology of cross-presentation and the role of dendritic cell subsets. *Immunol. Cell Biol.* **86,** 353–362 (2008).
19. Den Haan, J. M. & Bevan, M. J. Constitutive versus activation-dependent cross-presentation of immune complexes by CD8(+) and CD8(-) dendritic cells *in vivo*. *J. Exp. Med.* **196,** 817–827 (2002).
20. Segura, E., Durand, M. & Amigorena, S. Similar antigen cross-presentation capacity and phagocytic functions in all freshly isolated human lymphoid organ-resident dendritic cells. *J. Exp. Med.* **210,** 1035–1047 (2013).
21. Foged, C., Hansen, J. & Agger, E. M. License to kill: Formulation requirements for optimal priming of CD8(+) CTL responses with particulate vaccine delivery systems. *Eur. J. Pharm. Sci.* **45,** 482–491 (2012).
22. Bachmann, M. F. & Jennings, G. T. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nature Rev. Immunol.* **10,** 787–796 (2010).
23. Kourtis, I. C. *et al.* Peripherally administered nanoparticles target monocytic myeloid cells, secondary lymphoid organs and tumors in mice. *PLoS One* **8,** e61646 (2013).
24. Swartz, M. A., Hirosue, S. & Hubbell, J. A. Engineering approaches to immunotherapy. *Sci. Transl. Med.* **4,** 148rv149 (2012).
25. Villa, C. H. *et al.* Single-walled carbon nanotubes deliver peptide antigen into dendritic cells and enhance IgG responses to tumor-associated antigens. *ACS Nano* **5,** 5300–5311 (2011).
26. Nembrini, C. *et al.* Nanoparticle conjugation of antigen enhances cytotoxic T-cell responses in pulmonary vaccination. *Proc. Natl Acad. Sci. USA* **108,** E989–E997 (2011).
27. Reddy, S. *et al.* Exploiting lymphatic transport and complement activation in nanoparticle vaccines. *Nature Biotechnol.* **25,** 1159–1164 (2007).

© 2013 Macmillan Publishers Limited. All rights reserved

28. Fifis, T. *et al.* Size-dependent immunogenicity: therapeutic and protective properties of nano-vaccines against tumors. *J. Immunol.* **173,** 3148–3154 (2004).
29. De Rose, R. *et al.* Binding, internalization, and antigen presentation of vaccine-loaded nanoengineered capsules in blood. *Adv. Mater.* **20,** 4698–4703 (2008).
30. De Geest, B. G. *et al.* Surface-engineered polyelectrolyte multilayer capsules: synthetic vaccines mimicking microbial structure and function. *Angew. Chem. Int. Ed.* **51,** 3862–3866 (2012).
31. Dierendonck, M. *et al.* Facile two-step synthesis of porous antigen-loaded degradable polyelectrolyte microspheres. *Angew. Chem. Int. Ed.* **49,** 8620–8624 (2010).
32. Perry, J. L., Herlihy, K. P., Napier, M. E. & DeSimone, J. M. PRINT: A novel platform toward shape and size specific nanoparticle theranostics. *Acc. Chem. Res.* **44,** 990–998 (2011).
33. Galloway, A. L. *et al.* Development of a nanoparticle-based influenza vaccine using the PRINT technology. *Nanomed Nanotechnol. Biol. Med.* **9,** 523–531 (2013).
34. Reis e Sousa, C. & Germain, R. N. Major histocompatibility complex class I presentation of peptides derived from soluble exogenous antigen by a subset of cells engaged in phagocytosis. *J. Exp. Med.* **182,** 841–851 (1995).
35. Scott, E. A. *et al.* Dendritic cell activation and T cell priming with adjuvant- and antigen-loaded oxidation-sensitive polymersomes. *Biomaterials* **33,** 6211–6219 (2012).
36. Hirosue, S., Kourtis, I. C., van der Vlies, A. J., Hubbell, J. A. & Swartz, M. A. Antigen delivery to dendritic cells by poly(propylene sulfide) nanoparticles with disulfide conjugated peptides: Cross-presentation and T cell activation. *Vaccine* **28,** 7897–7906 (2010).
37. Moon, J. J. *et al.* Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. *Nature Mater.* **10,** 243–251 (2011).
38. Nordly, P. *et al.* Immunity by formulation design: induction of high CD8+ T-cell responses by poly(I:C) incorporated into the CAF01 adjuvant via a double emulsion method. *J. Control. Release* **150,** 307–317 (2011).
39. Zaks, K. *et al.* Efficient immunization and cross-priming by vaccine adjuvants containing TLR3 or TLR9 agonists complexed to cationic liposomes. *J. Immunol.* **176,** 7335–7345 (2006).
40. Powell, T. J. *et al.* Plasmodium falciparum synthetic LbL microparticle vaccine elicits protective neutralizing antibody and parasite-specific cellular immune responses. *Vaccine* **31,** 1898–1904 (2013).
41. Kasturi, S. P. *et al.* Programming the magnitude and persistence of antibody responses with innate immunity. *Nature* **470,** 543–547 (2011).
42. Kazzaz, J. *et al.* Encapsulation of the immune potentiators MPL and RC529 in PLG microparticles enhances their potency. *J. Control. Release* **110,** 566–573 (2006).
43. Zhu, Q. *et al.* Using 3 TLR ligands as a combination adjuvant induces qualitative changes in T cell responses needed for antiviral protection in mice. *J. Clin. Investig.* **120,** 607–616 (2010).
44. Garaude, J., Kent, A., van Rooijen, N. & Blander, J. M. Simultaneous targeting of toll- and nod-like receptors induces effective tumor-specific immune responses. *Sci. Transl. Med.* **4,** 120ra116 (2012).
45. Tacken, P. J. *et al.* Targeted delivery of TLR ligands to human and mouse dendritic cells strongly enhances adjuvanticity. *Blood* **118,** 6836–6844 (2011).
46. Moon, J. J. *et al.* Enhancing humoral responses to a malaria antigen with nanoparticle vaccines that expand Tfh cells and promote germinal center induction. *Proc. Natl Acad. Sci. USA* **109,** 1080–1085 (2012).
47. Nguyen, D. N. *et al.* Lipid-derived nanoparticles for immunostimulatory RNA adjuvant delivery. *Proc. Natl Acad. Sci. USA* **109,** E797–E803 (2012).
48. Ng, G. *et al.* Receptor-independent, direct membrane binding leads to cell-surface lipid sorting and Syk kinase activation in dendritic cells. *Immunity* **29,** 807–818 (2008).
49. Flach, T. L. *et al.* Alum interaction with dendritic cell membrane lipids is essential for its adjuvanticity. *Nature Med.* **17,** 479–487 (2011).
50. Marichal, T. *et al.* DNA released from dying host cells mediates aluminum adjuvant activity. *Nature Med.* **17,** 996–1002 (2011).
51. Harris, J., Sharp, F. A. & Lavelle, E. C. The role of inflammasomes in the immunostimulatory effects of particulate vaccine adjuvants. *Eur. J. Immunol.* **40,** 634–638 (2010).
52. Hornung, V. *et al.* Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nature Immunol.* **9,** 847–856 (2008).
53. Shi, Y., Evans, J. E. & Rock, K. L. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* **425,** 516–521 (2003).
54. Ballester, M. *et al.* Nanoparticle conjugation and pulmonary delivery enhance the protective efficacy of Ag85B and CpG against tuberculosis. *Vaccine* **29,** 6959–6966 (2011).
55. Sharp, F. A. *et al.* Uptake of particulate vaccine adjuvants by dendritic cells activates the NALP3 inflammasome. *Proc. Natl Acad. Sci. USA* **106,** 870–875 (2009).
56. Demento, S. L. *et al.* Inflammasome-activating nanoparticles as modular systems for optimizing vaccine efficacy. *Vaccine* **27,** 3013–3021 (2009).
57. De Geest, B. G. *et al.* Polymeric multilayer capsule-mediated vaccination induces protective immunity against cancer and viral infection. *ACS Nano* **6,** 2136–2149 (2012).
58. Li, H., Li, Y., Jiao, J. & Hu, H. M. Alpha-alumina nanoparticles induce efficient autophagy-dependent cross-presentation and potent antitumour response. *Nature Nanotech.* **6,** 645–650 (2011).
59. Thomas, S. N. *et al.* Engineering complement activation on polypropylene sulfide vaccine nanoparticles. *Biomaterials* **32,** 2194–2203 (2011).
60. Moyano, D. F. *et al.* Nanoparticle hydrophobicity dictates immune response. *J. Am. Chem. Soc.* **134,** 3965–3967 (2012).
61. Petersen, L. K. *et al.* Activation of innate immune responses in a pathogen-mimicking manner by amphiphilic polyanhydride nanoparticle adjuvants. *Biomaterials* **32,** 6815–6822 (2011).
62. Seong, S. Y. & Matzinger, P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nature Rev. Immunol.* **4,** 469–478 (2004).
63. Rudra, J. S., Tian, Y. F., Jung, J. P. & Collier, J. H. A self-assembling peptide acting as an immune adjuvant. *Proc. Natl Acad. Sci. USA* **107,** 622–627 (2010).
64. Getts, D. R. *et al.* Microparticles bearing encephalitogenic peptides induce T-cell tolerance and ameliorate experimental autoimmune encephalomyelitis. *Nature Biotechnol.* **30,** 1217–1224 (2012).
65. Lewis, J. S., Zaveri, T. D., Crooks, C. P. II & Keselowsky, B. G. Microparticle surface modifications targeting dendritic cells for non-activating applications. *Biomaterials* **33,** 7221–7232 (2012).
66. Yeste, A., Nadeau, M., Burns, E. J., Weiner, H. L. & Quintana, F. J. Nanoparticle-mediated codelivery of myelin antigen and a tolerogenic small molecule suppresses experimental autoimmune encephalomyelitis. *Proc. Natl Acad. Sci. USA* **109,** 11270–11275 (2012).
67. Tsai, S. *et al.* Reversal of autoimmunity by boosting memory-like autoregulatory T cells. *Immunity* **32,** 568–580 (2010).
68. Wherry, E. J., Blattman, J. N., Murali-Krishna, K., van der Most, R. & Ahmed, R. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J. Virol.* **77,** 4911–4927 (2003).
69. Mueller, S. N. & Ahmed, R. High antigen levels are the cause of T cell exhaustion during chronic viral infection. *Proc. Natl Acad. Sci. USA* **106,** 8623–8628 (2009).
70. Pape, K. A., Catron, D. M., Itano, A. A. & Jenkins, M. K. The humoral immune response is initiated in lymph nodes by B cells that acquire soluble antigen directly in the follicles. *Immunity* **26,** 491–502 (2007).
71. Itano, A. A. *et al.* Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity* **19,** 47–57 (2003).
72. Johansen, P. *et al.* Antigen kinetics determines immune reactivity. *Proc. Natl Acad. Sci. USA* **105,** 5189–5194 (2008).
73. Howland, S. W. & Wittrup, K. D. Antigen release kinetics in the phagosome are critical to cross-presentation efficiency. *J. Immunol.* **180,** 1576–1583 (2008).
74. Marx, P. A. *et al.* Protection against vaginal SIV transmission with microencapsulated vaccine. *Science* **260,** 1323–1327 (1993).
75. Neutra, M. R. & Kozlowski, P. A. Mucosal vaccines: the promise and the challenge. *Nature Rev. Immunol.* **6,** 148–158 (2006).
76. Zhu, G., Mallery, S. & Schwendeman, S. Stabilization of proteins encapsulated in injectable poly (lactide-co-glycolide). *Nature Biotechnol.* **18,** 52–57 (2000).
77. Zhu, Q. *et al.* Large intestine-targeted, nanoparticle-releasing oral vaccine to control genitorectal viral infection. *Nature Med.* **18,** 1291–1296 (2012).
78. Fujkuyama, Y. *et al.* Novel vaccine development strategies for inducing mucosal immunity. *Expert Rev. Vaccines* **11,** 367–379 (2012).
79. Sullivan, S. P. *et al.* Dissolving polymer microneedle patches for influenza vaccination. *Nature Med.* **16,** 915–920 (2010).
80. Zaric, M. *et al.* Skin dendritic cell targeting via microneedle arrays laden with antigen-encapsulated poly-D, L-lactide-co-glycolide nanoparticles induces efficient antitumor and antiviral immune responses. *ACS Nano* **7,** 2042–2055 (2013).

© 2013 Macmillan Publishers Limited. All rights reserved

81. Prow, T. W. *et al.* Nanopatch-targeted skin vaccination against West Nile Virus and Chikungunya virus in mice. *Small* **6,** 1776–1784 (2010).
82. DeMuth, P. C. *et al.* Polymer multilayer tattooing for enhanced DNA vaccination. *Nature Mater.* **12,** 367–376 (2013).
83. Atuma, C., Strugala, V., Allen, A. & Holm, L. The adherent gastrointestinal mucus gel layer: thickness and physical state *in vivo*. *Am. J. Physiol. Gastrointest. Liver Physiol.* **280,** G922–G929 (2001).
84. Lai, S. K., Wang, Y. Y., Hida, K., Cone, R. & Hanes, J. Nanoparticles reveal that human cervicovaginal mucus is riddled with pores larger than viruses. *Proc. Natl Acad. Sci. USA* **107,** 598–603 (2010).
85. Tang, B. C. *et al.* Biodegradable polymer nanoparticles that rapidly penetrate the human mucus barrier. *Proc. Natl Acad. Sci. USA* **106,** 19268–19273 (2009).
86. Lai, S. K. *et al.* Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. *Proc. Natl Acad. Sci. USA* **104,** 1482–1487 (2007).
87. Cu, Y., Booth, C. J. & Saltzman, W. M. *In vivo* distribution of surface-modified PLGA nanoparticles following intravaginal delivery. *J. Control. Release* **156,** 258–264 (2011).
88. Ensign, L. M. *et al.* Mucus-penetrating nanoparticles for vaginal drug delivery protect against herpes simplex virus. *Sci. Transl. Med.* **4,** 138ra179 (2012).
89. Nochi, T. *et al.* Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. *Nature Mater.* **9,** 572–578 (2010).
90. Fleury, M. E., Boardman, K. C. & Swartz, M. A. Autologous morphogen gradients by subtle interstitial flow and matrix interactions. *Biophys. J.* **91,** 113–121 (2006).
91. Tang, L., Fan, T. M., Borst, L. B. & Cheng, J. Synthesis and biological response of size-specific, monodisperse drug–silica nanoconjugates. *ACS Nano* **6,** 3954–3966 (2012).
92. Bershteyn, A. *et al.* Robust IgG responses to nanograms of antigen using a biomimetic lipid-coated particle vaccine. *J. Control. Release* **157,** 354–365 (2012).
93. Murthy, N. *et al.* A macromolecular delivery vehicle for protein-based vaccines: acid-degradable protein-loaded microgels. *Proc. Natl Acad. Sci. USA* **100,** 4995–5000 (2003).
94. Hu, Y. *et al.* Cytosolic delivery of membrane-impermeable molecules in dendritic cells using pH-responsive core-shell nanoparticles. *Nano Lett.* **7,** 3056–3064 (2007).
95. Su, X., Fricke, J., Kavanagh, D. G. & Irvine, D. J. *In vitro* and *in vivo* mRNA delivery using lipid-enveloped pH-responsive polymer nanoparticles. *Mol. Pharm.* **8,** 774–787 (2011).
96. Haining, W. N. *et al.* pH-triggered microparticles for peptide vaccination. *J. Immunol.* **173,** 2578–2585 (2004).
97. Heffernan, M. J., Kasturi, S. P., Yang, S. C., Pulendran, B. & Murthy, N. The stimulation of CD8+ T cells by dendritic cells pulsed with polyketal microparticles containing ion-paired protein antigen and poly(inosinic acid)-poly(cytidylic acid). *Biomaterials* **30,** 910–918 (2009).
98. Vasdekis, A. E., Scott, E. A., O'Neil, C. P., Psaltis, D. & Hubbell, J. A. Precision intracellular delivery based on optofluidic polymersome rupture. *ACS Nano* **6,** 7850–7857 (2012).
99. Shen, H. *et al.* Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles. *Immunology* **117,** 78–88 (2006).
100. Geall, A. J. *et al.* Nonviral delivery of self-amplifying RNA vaccines. *Proc. Natl Acad. Sci. USA* **109,** 14604–14609 (2012).
101. Verma, A. *et al.* Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles. *Nature Mater.* **7,** 588–595 (2008).
102. Marrack, P., McKee, A. & Munks, M. Towards an understanding of the adjuvant action of aluminium. *Nature Rev. Immunol.* **9,** 287–293 (2009).
103. Hutchison, S. *et al.* Antigen depot is not required for alum adjuvanticity. *FASEB J.* **26,** 1272–1279 (2012).
104. Gupta, R. K., Chang, A. C., Griffin, P., Rivera, R. & Siber, G. R. *In vivo* distribution of radioactivity in mice after injection of biodegradable polymer microspheres containing ^{14}C-labeled tetanus toxoid. *Vaccine* **14,** 1412–1416 (1996).
105. Preis, I. & Langer, R. S. A single-step immunization by sustained antigen release. *J. Immunol. Methods* **28,** 193–197 (1979).
106. Thomasin, C., Corradin, G., Men, Y., Merkle, H. P. & Gander, B. Tetanus toxoid and synthetic malaria antigen containing poly(lactide)/poly(lactide-co-glycolide) microspheres: Importance of polymer degradation and antigen release for immune response. *J. Control. Release* **41,** 131–145 (1996).
107. Hailemichael, Y. *et al.* Persistent antigen at vaccination sites induces tumor-specific CD8+ T cell sequestration, dysfunction and deletion. *Nature Med.* **19,** 465–472 (2013).
108. Jewell, C. M., Lopez, S. C. & Irvine, D. J. *In situ* engineering of the lymph node microenvironment via intranodal injection of adjuvant-releasing polymer particles. *Proc. Natl Acad. Sci. USA* **108,** 15745–15750 (2011).
109. St John, A. L., Chan, C. Y., Staats, H. F., Leong, K. W. & Abraham, S. N. Synthetic mast-cell granules as adjuvants to promote and polarize immunity in lymph nodes. *Nature Mater.* **11,** 250–257 (2012).
110. Braumuller, H. *et al.* T-helper-1-cell cytokines drive cancer into senescence. *Nature* **494,** 361–365 (2013).
111. Reiner, S. L. & Locksley, R. M. The regulation of immunity to Leishmania major. *Annu. Rev. Immunol.* **13,** 151–177 (1995).
112. Ali, O. A., Emerich, D., Dranoff, G. & Mooney, D. J. *In situ* regulation of DC subsets and T cells mediates tumor regression in mice. *Sci. Transl. Med.* **1,** 8ra19 (2009).
113. Ali, O. A., Huebsch, N., Cao, L., Dranoff, G. & Mooney, D. J. Infection-mimicking materials to program dendritic cells *in situ*. *Nature Mater.* **8,** 151–158 (2009).
114. Singh, A. *et al.* An injectable synthetic immune-priming center mediates efficient T-cell class switching and T-helper 1 response against B cell lymphoma. *J. Control. Release* **155,** 184–192 (2011).
115. Langhorne, J. *et al.* The relevance of non-human primate and rodent malaria models for humans. *Malaria J.* **10,** 23 (2011).
116. Morgan, C. *et al.* The use of nonhuman primate models in HIV vaccine development. *PLoS Med.* **5,** e173 (2008).
117. Tsuji, K. *et al.* Induction of immune response against NY-ESO-1 by CHP-NY-ESO-1 vaccination and immune regulation in a melanoma patient. *Cancer Immunol. Immunother. CII* **57,** 1429–1437 (2008).
118. Ataman-Onal, Y. *et al.* Surfactant-free anionic PLA nanoparticles coated with HIV-1 p24 protein induced enhanced cellular and humoral immune responses in various animal models. *J. Control. Release* **112,** 175–185 (2006).
119. Otten, G. *et al.* Induction of broad and potent anti-human immunodeficiency virus immune responses in rhesus macaques by priming with a DNA vaccine and boosting with protein-adsorbed polylactide coglycolide microparticles. *J. Virol.* **77,** 6087–6092 (2003).
120. Appay, V., Douek, D. C. & Price, D. A. CD8+ T cell efficacy in vaccination and disease. *Nature Med.* **14,** 623–628 (2008).
121. Nimmerjahn, F. & Ravetch, J. V. Antibody-mediated modulation of immune responses. *Immunol. Rev.* **236,** 265–275 (2010).
122. Virgin, H. W. & Walker, B. D. Immunology and the elusive AIDS vaccine. *Nature* **464,** 224–231 (2010).
123. Crow, J. M. HPV: The global burden. *Nature* **488,** S2–S3 (2012).
124. Ma, B., Xu, Y., Hung, C-F. & Wu, T-C. HPV and therapeutic vaccines: where are we in 2010? *Curr. Cancer Ther. Rev.* **6,** 81–103 (2010).
125. Klebanoff, C. A., Acquavella, N., Yu, Z. & Restifo, N. P. Therapeutic cancer vaccines: are we there yet? *Immunol. Rev.* **239,** 27–44 (2011).
126. Harrison, L. C. Vaccination against self to prevent autoimmune disease: the type 1 diabetes model. *Immunol. Cell Biol.* **86,** 139–145 (2008).
127. Wisniewski, T. & Goni, F. Immunomodulation for prion and prion-related diseases. *Expert Rev. Vaccines* **9,** 1441–1452 (2010).
128. Linhart, B. & Valenta, R. Vaccines for allergy. *Curr. Opin. Immunol.* **24,** 354–360 (2012).
129. Sela, M. & Mozes, E. Therapeutic vaccines in autoimmunity. *Proc. Natl Acad. Sci. USA* **101, Suppl. 2,** 14586–14592 (2004).
130. Velluto, D. *et al.* PEG-b-PPS-b-PEI micelles and PEG-b-PPS/PEG-b-PPS-b-PEI mixed micelles as non-viral vectors for plasmid DNA: Tumor immunotoxicity in B16F10 melanoma. *Biomaterials* **32,** 9839–9847 (2011).
131. Caruso, F. *et al.* Enzyme encapsulation in layer-by-layer engineered polymer multilayer capsules. *Langmuir* **16,** 1485–1488 (2000).

Acknowledgements

This work was supported in part by the Ragon Institute of MGH, MIT, and Harvard, the Gates Foundation, the Department of Defense (contracts W911NF-13-D-0001 and W911NF-07-D-0004, T.O. 8) and the NIH (AI095109 and AI091693). D.J.I. is an investigator of the Howard Hughes Medical Institute. Funding was provided by the Bill and Melinda Gates Foundation and Carigest SA (to M.A.S.). We wish to dedicate this paper to the memory of Officer Sean Collier, for his caring service to the MIT community.

Additional information

Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to D.J.I.

Competing financial interests

D.J.I. is an inventor on patents licensed to Vedantra Pharmaceuticals, in which D.J.I. holds equity and is a co-founder.

© 2013 Macmillan Publishers Limited. All rights reserved