Designing spatial and temporal control of vaccine responses

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Abstract | Vaccines are the key technology to combat existing and emerging infectious diseases. However, increasing the potency, quality and durability of the vaccine response remains a challenge. As our knowledge of the immune system deepens, it becomes clear that vaccine components must be in the right place at the right time to orchestrate a potent and durable response. Material platforms, such as nanoparticles, hydrogels and microneedles, can be engineered to spatially and temporally control the interactions of vaccine components with immune cells. Materials-based vaccination strategies can augment the immune response by improving innate immune cell activation, creating local inflammatory niches, targeting lymph node delivery and controlling the time frame of vaccine delivery, with the goal of inducing enhanced memory immunity to protect against future infections. In this Review, we highlight the biological mechanisms underlying strong humoral and cell-mediated immune responses and explore materials design strategies to manipulate and control these mechanisms.

Vaccines are among the most effective medical interventions in history (FIG. 1a; BOX 1). The eradication of smallpox, the near eradication of poliomyelitis and substantial decreases in diphtheria, measles and rubella are testaments to the ability of vaccines to reduce the disease burden worldwide[1,2]. It is estimated that vaccines save 2.5 million lives worldwide per year[3]; however, diseases without effective vaccines remain. These include diseases for which vaccine development has not yet reached a clinical product (for example, HIV), but also clinically used vaccines that need to be improved (for example, influenza, tuberculosis and malaria), especially for high-risk groups such as older people or individuals who are immunosuppressed[4,5]. Moreover, the threat of new pandemic strains of viruses motivates the need for continued improvement of vaccine technologies.

The immune response to infection or vaccination depends on the complex coordination between cells across the body. The vaccine immune response occurs in multiple locations — peripheral tissues, lymph nodes and systemic circulation — each of which has its own cell composition and function. This coordinated action of immune cells requires precise spatial and temporal cues. Tissues at the interface with the outside world (for example, skin, lungs and mucosal sites) are the primary locations of infections, and therefore contain tissue-resident immune cells and are constantly patrolled by migratory immune cells. Lymph nodes downstream of the location of pathogen or vaccine exposure are called draining lymph nodes, and are key sites from the beginning of the immune response throughout the development of mature effector B cells and T cells. The blood provides an important route for innate immune cells to quickly infiltrate the site of vaccination or infection in the early immune response. After the immune response is mounted, the blood enables antibodies and memory T cells to reach infected tissue and protect the entire body. Activation of the innate immune system and migration of key cells and vaccine components to lymph nodes occurs within hours, followed by B cell and T cell maturation within days and weeks. The long-term memory response remains for months to years following vaccination, providing protection against future infection. This sequence of events is based on complex spatial and temporal control of each step, which needs to be dissected and modulated to control the immune response by vaccination (FIG. 1). Materials engineering allows the precise design of spatio-temporal cell–vaccine interactions.

In this Review, we focus on prophylactic subunit vaccines that contain specific subunit antigens from a pathogen. Subunit antigens lack pathogen-associated molecular patterns (PAMPs) necessary for innate immune cell recognition by germline-encoded pattern-recognition receptors (PRRs). Thus, immunostimulatory molecules, that is, adjuvants, are typically used to augment antigen immunogenicity and, therefore, enhance vaccine efficacy. The subunit vaccine approach affords precise selection of molecularly defined antigen and adjuvant components, which improves safety and manufacturing

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compared with vaccines comprising inactivated or attenuated whole pathogens. Yet subunit vaccines often exhibit weaker and less durable immune responses than whole pathogen-derived vaccines, which contain a complex mixture of antigen and adjuvant molecules. To better design subunit vaccines, approaches are needed to appropriately guide the immune system and enhance the potency, quality and durability of immunity.

Immunomodulatory materials have emerged as a powerful strategy for studying and influencing the immune system. Biological and synthetic materials can be engineered with diverse properties, enabling a range of delivery time frames. The efficacy of an immunomodulatory therapeutic can be improved by optimizing its spatial or temporal delivery, which can be achieved by designing materials to engage with the immune system in a complex and controlled manner[6–12].

Here, we describe key mechanisms and unknowns of the vaccine immune response. We highlight the importance of spatial and temporal cues in innate immune cell activation, the B cell response and the generation of a potent and durable adaptive immune response. We discuss material design strategies to manipulate, study and enhance the immune response by increasing innate immune cell activation, creating a local inflammatory niche, targeting delivery of vaccine components to lymph nodes and providing sustained co-delivery of vaccine components. Lastly, we give a perspective on future directions for immunomodulatory materials in vaccine delivery.

Vaccine immunity in space and time

The spatial and temporal characteristics of key events in the vaccine immune response are important when developing strategies that improve vaccine efficacy. Controlled delivery systems may intentionally or unintentionally augment these spatio-temporal responses.

Outcomes of a successful vaccine

A potent vaccine results in long-term — ideally lifelong — protection against a specific pathogen, which can be achieved by inducing both long-lasting production of neutralizing antibodies (humoral immunity) and cell-mediated immunity. Antibodies bind to antigens on the pathogen's surface to block infection of host cells by multiple mechanisms, including neutralization, complement fixation and increased phagocytosis[13,14]. Cellular immunity involves T cells that can directly kill infected cells, which eliminates the pathogen. Long-term immunity by these two mechanisms requires the maturation and activation of appropriate cell phenotypes after vaccination. Immunoengineering studies often assess the effects of delivery systems only on a small number of immunological outputs, such as cytokine production or antibody titres; however, the ultimate goal of all prophylactic vaccines is to elicit an effective memory response.

Innate immune activation and vaccine transport

At the injection site. The immune system first interacts with a vaccine at the site of injection (FIG. 1b). Vaccines are typically administered intramuscularly, subcutaneously or intradermally, with intramuscular administration most commonly used in the clinic[15]. Vaccine administration to mucosal tissues is also being explored; for example, intranasal vaccination for pathogens that primarily infect the respiratory system[16–18]. Notably, FluMist is a licensed, intranasally delivered influenza vaccine that effectively protects against H1N1 infection[19]. The majority of the vaccine response occurs in the lymph nodes; however, the site of administration influences the quantity and phenotypes of tissue-resident immune cells that initially interact with the vaccine, thus affecting the magnitude, duration and flavour of adaptive immune responses[15]. Initial innate immune cell infiltration, activation and antigen uptake at the site of injection play a crucial role in the quality of the adaptive immune response[15,20]; however, the nuances of this response have not yet been well studied in the context of vaccination[15], but can be addressed by materials engineering.

The extent of local inflammation is determined by the site of administration and the presence of adjuvants in the vaccine (BOX 2). Many immunostimulatory adjuvants mimic PAMPs present on natural pathogens to activate innate immune cells through stimulation of PRRs. Innate immune cells, such as macrophages and dendritic cells, immediately respond to adjuvants by producing cytokines that recruit cells to the site of injection, essentially leading to a spatial reorganization of innate immune cells (FIG. 1b). The activation cues dendritic cells receive and their signalling to T helper cells (T_H cells) shape the character of their response and, therefore, the outcome of the vaccine response[21,22]. Depending on the phenotype of T_H cells, distinct responses are elicited; T_H1 cells mainly establish cellular immunity, whereas T_H2 cells stimulate humoral immunity[5]. Certain adjuvants, such as unmethylated cytosine–phosphate–guanine oligodeoxynucleotide (CpG) and lipopolysaccharide (LPS), can programme dendritic cells to stimulate T_H1-type polarized responses[23], whereas other adjuvants, such as alum, result in T_H2-type polarized responses[24]. Furthermore, a comparison of the adjuvants MF59, alum, trehalose-6,6′-dibehenate (TDB), complete Freund's adjuvant (CFA) and several Toll-like receptor (TLR) agonists, including LPS (TLR4a) and Pam3CysSerLys4 (Pam3CSK4; also known as TLR1/2a), has demonstrated an adjuvant-dependent transient local inflammatory response at the injection site following intramuscular administration, leading to different levels of cytokines and cell infiltration[25]. Thus, adjuvant choice influences

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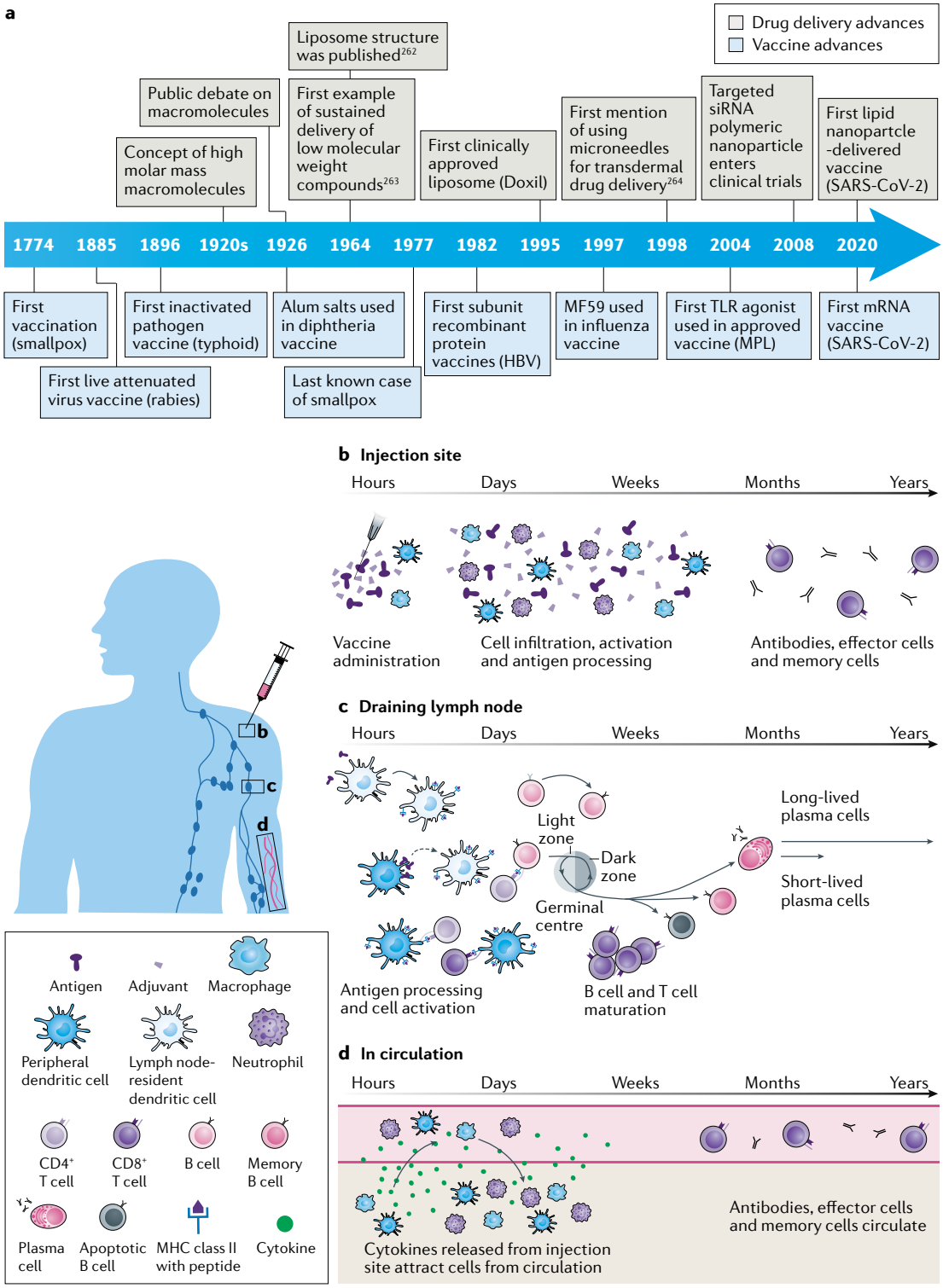


the extent of the local inflammatory response; however, it remains unclear whether these differences are caused by engagement of different receptors (for example, TLR1, TLR2, TLR4, TLR9) or whether the pharmacokinetics and bio-distribution of these physico-chemically distinct adjuvants impact the spatio-temporal characteristics of innate immune cell activation (for example, local retention and/or prolonged exposure). Unfortunately, assessment of pharmacokinetics in studies evaluating the influence of adjuvants remains limited thus far. Materials can be used to directly augment cell–adjuvant interactions by controlling the location and timing of their presentation to immune cells.

In the draining lymph node. After the initial inflammatory response at the site of injection, activated dendritic cells and vaccine components travel through afferent lymph vessels to the draining lymph nodes[21,26,27],

Fig. 1 | **Timeline of vaccine advances and vaccine immune response. a** | Timeline of major events in drug delivery and vaccine development. **b** | Following administration of a vaccine, interactions between cells and vaccine components lead to a strong and lasting response. At the site of administration, innate immune cells, such as neutrophils and antigen-presenting cells (APCs), first encounter the antigen and adjuvant. The antigen component of the vaccine is endocytosed and broken down by APCs before being presented on the APC surface major histocompatibility complex (MHC) molecules. As innate immune cells become activated, they release cytokines that attract other immune cells from the bloodstream to the site of administration. Soluble vaccine components and activated cells enter the lymphatics and travel to local lymph nodes. **c** | Maturation and development of a potent adaptive response continues in lymph nodes downstream of the vaccination site (draining lymph nodes). Early in the vaccine response, lymph node-resident phagocytic cells and migratory innate cells arriving from peripheral tissues present antigen and produce inflammatory signals to activate T cells. As the immune response develops, sites of B cell development, called germinal centres, form in the B cell zones of the lymph nodes. **d** | Immediately following vaccine administration, local innate cells release cytokines into the circulation to enable a coordinated response. These signals are crucial in triggering cell infiltration to the injection site. Following vaccination, plasma cells secrete antigen-specific antibodies, which travel through the circulatory system to tissues, where they respond immediately upon pathogen exposure. Memory T cells also use the circulatory system to inspect the body for foreign invaders. HBV, hepatitis B virus; MPL, monophosphoryl lipid A; siRNA, small interfering RNA; TLR, Toll-like receptor.

where cells that are usually present at low concentrations in the body come together and are precisely spatially organized to enhance the cell–cell interactions necessary for generating a robust immune response[26,28] (FIG. 1c). Migratory dendritic cells either present antigen directly to T cells in the lymph nodes or transfer antigens to lymph node-resident dendritic cells[29,30]. The migration of immune cells to lymph nodes can be enhanced by adjuvants[20,31]. For example, the adjuvant MF59 achieves cell recruitment to lymph nodes within 3 h of immunization, and cellular recruitment persists for up to 11 days[32]. In addition to antigen-presenting migratory dendritic cells, lymph node-resident dendritic cells are constantly scanning the lymphatic fluids to capture antigens that reach the lymph nodes by passive diffusion. Lymph node-resident dendritic cells can rapidly present antigens to T cells; however, migratory dendritic cells may be crucial for extending antigen presentation and shaping the immune response[33]. The importance of lymph node-resident dendritic cells relative to migratory dendritic cells in the vaccine response is not yet fully understood. These cell populations may provide redundancy to the immune system to ensure that tissues and the lymph are both sampled during infection. Therefore, the vaccine response benefits from promoting antigen presentation, migration of tissue-resident dendritic cells and an increase in antigen trafficking to the lymph nodes. Furthermore, it may be possible to bypass transport to the lymph nodes by using intra-nodal administration[34,35]. Indeed, direct injection of vaccine-loaded microparticles into lymph nodes enhances and prolongs dendritic cell activation and subsequent immune responses[34].

Adaptive immune maturation

The lymph node is spatially organized to enable distinct cell–cell and cell–vaccine interactions. T cell maturation occurs in the T cell zone (paracortex), whereas B cell maturation occurs in the B cell follicles, where antigen-activated B cells undergo rapid proliferation to form transient and dynamic structures, called germinal centres. Germinal centres provide structural organization for antibody affinity maturation and B cell differentiation.

T cell zone (paracortex). $CD8^+$ and $CD4^+$ T cells are activated in the T cell zone. To induce cellular immunity, dendritic cells in the lymph node directly interact with $CD8^+$ T cells in the central paracortex, providing the signals for maturation and expansion of antigen-specific cytotoxic T cells[28,36]. To become activated, naive $CD8^+$ T cells must interact with peptide–major histocompatibility complex (MHC) class I complexes on dendritic cells and receive co-stimulatory and cytokine signals. Once activated, $CD8^+$ T cells must also receive $CD4^+$ T cell help through a complex three-way interaction between T cells and $XCR1^+$ dendritic cells[37]. These interactions occur in the paracortex of the lymph nodes or the white pulp of the spleen and direct T cell fate towards short-lived effector cells or memory cells[28,37]. T cell receptor signals, co-stimulation and inflammatory cytokine levels must all remain low to induce memory T cell phenotypes[38].

To promote humoral immunity, antigen-presenting cells (APCs), such as dendritic cells and macrophages, present antigen to both $CD4^+$ T cells and B cells. B cells can also function as APCs to activate $CD4^+$ T cells, as has been observed in vaccination with virus-like particles as well as during influenza infection[39,40]. Activation of $CD4^+$ T cells occurs in clusters in the peripheral paracortex, distinct from $CD8^+$ T cell activation regions[28]. T follicular helper cells (T_{FH} cells) are a subset of $CD4^+$ T cells crucial for promoting B cell maturation, and are located close to the T cell–B cell border[30]. T_{FH} cell differentiation initially requires priming of naive $CD4^+$ T cells by professional APCs, followed by additional signals from B cells, to mature into germinal centre T_{FH} cells[36]. Following a mucosal influenza virus challenge in mice, migratory dendritic cells, specifically $CD11b^+$ conventional dendritic cells (cDC2s), have been shown to be necessary and sufficient to induce T_{FH} cell priming[30]. Studies of adjuvant effects have demonstrated their ability to influence the concentration of T_{FH} cells in the germinal centre. For example, MF59 leads to an increase in the concentration of T_{FH} cells, compared with alum, in mice immunized with a model antigen[41]. Furthermore, cytokines such as IL-2, IL-6 and IL-21 greatly impact T_{FH} cell differentiation, making them potentially valuable adjuvants for enhancing affinity selection and memory cell differentiation during germinal centre reactions[42,43]. Moreover, a distinct subset of regulatory T cells, T follicular regulatory cells, can mediate T_{FH} cell generation and regulation; however, a precise understanding of T follicular regulatory cells remains elusive[44].

B cell follicle. Antigen-specific B cell activation in the B cell follicle requires the antigen to reach the follicle as well as co-stimulation by $CD4^+$ T cells[45]. Depending on their size, antigens can traverse different routes to reach B cell follicles. Large proteins (typically greater than 70 kDa) require transport by APCs, whereas small proteins can passively diffuse through the lymph nodes[46,47].

Moreover, B cells are more efficiently activated by membrane-bound antigens, which allow receptor cross-linking[48]. Follicular dendritic cells reside in the B cell follicles and activate B cells by displaying opsonized antigens on their surface. Soluble and particulate antigens can also directly access and activate B cells in the B cell follicle[48–50]. Subcapsular sinus macrophages and migratory dendritic cells also play an important role in presenting antigen to B cells in the B cell follicles[48]. Notably, antigen availability and the relative abundance of low-frequency epitopes can influence the immune response owing to antigen-presenting naive B cells competing for limited T_H cell stimulation[51].

Class switch recombination is also an important B cell process occurring in B cell follicles. Here, antibodies of different isotypes are produced by DNA recombination, in which the antibody variable domains are maintained but the constant domain of the heavy chain is altered[52]. Thereby, the biological functions of the antibodies can be tailored, and antigenic specificity is maintained. Mature naive B cells initially express and secrete IgM, which provides immediate but limited protection against pathogens, because it is restricted to the bloodstream and has low affinity to antigens. IgM is known to be important at the onset of infection; however, increasing evidence suggests that IgM-expressing memory cells also play a role in long-term immunity[53]. Class switch recombination enables diversification of antibody effector functions by initiating B cell expression of IgG (the most common type of antibody in the blood circulation) and IgA (which plays a crucial role in the immune function of mucous membranes), and to a lesser extent IgE, as well as their various subtypes. Class switch recombination is induced by activation of multiple receptors on B cells, including the B cell receptor (BCR), CD40, TLRs, B cell-activating factor receptor (BAFFR), transmembrane activator (TACI) and cytokine receptors[52]. The exact receptors that are activated determine the outcome of class switch recombination, and the simultaneous activation of TLRs and the BCR enhances class switch recombination[54]. Class switch recombination not only determines antibody effector function but also biases the differentiation of B cells into plasma cells and memory cells[55].

Box 1 | **A brief history of vaccines**

Vaccine-like technologies were first reported in China in the tenth century. Pustules from patients with smallpox were inoculated into scratched skin to deliberately infect individuals with a less severe form of the disease. This method, known as variolation, was first applied in England by Lady Mary Montagu in 1721, and used in Africa and Europe during the eighteenth century to protect from future disease by causing a milder form of smallpox[248,249]. In 1774, with the hope of protecting his family against smallpox while avoiding the risks associated with variolation, Benjamin Jesty used cowpox pustules from cows, rather than human smallpox pustules, for inoculation[250]. This is the first example of vaccination, and indeed led to less severe side effects and provided robust protection against disease. In the late eighteenth century, Edward Jenner used this technique to vaccinate against smallpox and is renowned for popularizing smallpox vaccination[248,250]. The discovery that microorganisms cause infectious diseases heralded a new era of vaccine development, with vaccines made directly from pathogens[251]. In the late nineteenth century, Louis Pasteur discovered methods to attenuate the virulence of fowl cholera and anthrax, creating the first laboratory vaccines[252]. He then went on to develop the first rabies vaccine[252]. These vaccines are all live-attenuated vaccines, one of several classes of vaccine used in the clinic today[253].

Germinal centre. Germinal centres form within the first week of infection or vaccination, and dissipate as the immune response wanes — a process not yet fully understood[50,56]. The germinal centre has two anatomical compartments; the dark zone, where B cells proliferate and undergo somatic hypermutation; and the light zone, where antigen-driven selection favours higher-affinity B cells[56]. B cells cycle between these two compartments to drive antibody affinity maturation, eventually differentiating and exiting the germinal centre as plasma cells, long-lived plasma cells or memory B cells[57] (FIG. 1c).

The specificity of the BCR and antibodies for an antigen is primarily determined in the complementarity-determining region of the molecule that directly contacts the antigen[14]. In the dark zone, mature antigen-specific B cells proliferate and diversify their antibody genes by undergoing somatic hypermutation[56,58]. The diversity of the B cell repertoire is initially determined by stochastic selection of individual variable (V), diversity (D) and joining (J) gene segments in the early phases of B cell development, which theoretically yields millions of distinct antibody sequences[14], although the true diversity is lower and has not yet been well quantified[59]. In the germinal centre, antigen-specific sequences are further edited by activation-induced deaminase, achieving single-nucleotide substitution at a frequency of about one mutation per cell division in the variable region of the immunoglobulin loci (IgV)[58]. Depending on the antigen, BCRs undergo 10–20 rounds of somatic hypermutation[58]. For context, 40–100 mutations are necessary to elicit broadly neutralizing antibodies against HIV[60], highlighting the importance to initiate sufficiently durable germinal centre responses to enable adequate somatic hypermutation and improve the breadth and affinity of antibody responses.

Proliferation and somatic hypermutation of B cells in the dark zone is followed by positive affinity selection in the light zone[56]. Antigen-triggered BCR signalling is crucial for the selection of germinal centre B cells. BCR signalling promotes B cell transition from the dark zone to the light zone to maintain the germinal centre selection cycle. In addition, BCR signalling synergizes with T_{FH} cell-derived signals to induce key factors associated with positive selection[49,61]. Germinal centre B cells with higher-affinity receptors outcompete cells with lower affinity in terms of BCR signalling and T_{FH} cell-derived signalling, resulting in their positive selection. In the light zone, follicular dendritic cells present membrane-bound antigen in the form of immune complexes to germinal centre B cells[47]. The antigen concentration in the germinal centre must be high enough to enable these selection processes; however, it must also be sufficiently low to drive competition and commensurate selection of high-affinity B cell clones[62].

Another key role of follicular dendritic cells is to prolong intact antigen retention in the germinal centre to allow the affinity selection process to persist[56]. Longer antigen availability enhances affinity maturation by enabling more cycles of somatic hypermutation and positive germinal centre B cell selection[63–65]. Sustained delivery technologies allow investigation of the impact

Box 2 | **Adjuvant technologies**

Aluminium salt based

Alum consists of aluminium phosphate or aluminium hydroxide particles and is one of the earliest and most widely used vaccine adjuvants[254]. Alum adsorbs antigens to serve as an antigen depot and acts as a mild irritant inducing pro-inflammatory responses. Alum can also be mixed with Toll-like receptor (TLR) agonists to further enhance the immunogenicity of subunit vaccines. For example, in AS04 (REF.[255]), which is part of the human papillomavirus (HPV) vaccine Cervarix (approved in 2009), alum is combined with the TLR4 agonist monophosphoryl lipid A (MPL), and in AS37 (REF.[256]) alum is combined with the TLR7 agonist SMIP7-10.

Emulsions

Numerous oil-in-water emulsion systems have been evaluated as adjuvants. An 'incomplete' form of Freund's adjuvant (solely a mineral–oil emulsion) predominantly enhances T helper 2 cell (T_H2 cell) responses, whereas a 'complete' form (mineral–oil emulsion comprising inactivated mycobacteria) enhances T_H1 cell responses[257]. Squalene, an oil naturally occurring in shark liver, is often combined with surfactants to form oil-in-water emulsions that stimulate the body's immune response through the local production of cytokines and chemokines as well as the recruitment of innate cells. Examples of squalene-based adjuvants are MF59, used in the influenza vaccine Fluad (approved in 1997), and AS03, used in the pandemic flu vaccine Pandemrix (approved in 2009)[258].

TLR agonists

Pathogen-associated molecular patterns (PAMPs) are commonly used as vaccine adjuvants, because they activate downstream immune signalling pathways that elicit potent inflammatory cytokine and chemokine production, leading to potent innate immune responses. TLRs are located on the endosomal membrane and many recognize nucleic acids. Well-studied TLR agonists include the synthetic double-stranded RNA mimic poly(inosinic:cytidylic acid) (pIC) (TLR3)[22], the single-stranded RNA analogues imiquimod (R837; also known as TLR7) and resiquimod (R848; also known as TLR7/8), and single-stranded DNA cytosine–phosphate–guanine oligodeoxynucleotide (CpG) oligodeoxynucleotides (TLR9)[259]. CpG 1018, the most recently clinically approved adjuvant, increases the immune response by strongly activating B cells[100]. CpG 1018 is included in the hepatitis B vaccine Heplisav-B (approved in 2017) and is currently being tested in phase I clinical trials with numerous SARS-CoV-2 vaccine candidates. Lipid-containing PAMPs include triacyl lipopeptides and diacyl lipopeptides, which activate TLR1, TLR2 and TLR6 to produce pro-inflammatory cytokines. MPL and its analogues are detoxified derivatives of lipopolysaccharide (LPS) from Gram-negative bacteria, which activate TLR4 to drive secretion of type I interferons. Flagellin, the main protein component of bacterial flagella, can serve as a TLR5 agonist to induce the production of tumour necrosis factor (TNF) and other pro-inflammatory cytokines.

Other PAMPs

Nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) agonists, such as muramyl dipeptide (MDP), a fragment of the bacterial cell wall, induce pro-inflammatory cytokine responses[258]. Cyclic dinucleotides, such as 2′3′-cyclic guanosine monophosphate–adenosine monophosphate (cGAMP), are extremely potent activators of the innate immune sensor stimulator of interferon genes (STING), resulting in high levels of type I interferon production[260].

Liposomes

Liposome formulations are used in subunit vaccines and present antigen as a pathogen-sized particulate, enabling improved delivery to antigen-presenting cells (APCs) and low antigen degradation compared with non-liposome formulations. Saponin-based adjuvants, such as Quil-A, increase antibody production and stimulate cell-mediated responses without engagement of pattern-recognition receptors (PRRs)[261]. Combined with cholesterol and phospholipids, lipidic saponin nanoparticles called immune stimulating complexes (ISCOMs) produce strong and long-lasting immune responses, and mitigate saponin's haemolytic effects[22]. The AS01 adjuvant system is a lipidic suspension of saponin and MPL, and is currently used in the shingles vaccine Shingrix (approved in 2017) and the RTS,S malaria vaccine (approved in 2015)[261–264].

of vaccine exposure timing on germinal centre responses and commensurate somatic hypermutation, without the need to alter vaccine identity[65,66]. Indeed, immunization regimens continuously presenting antigen to germinal centres for a week or more, either through repeated injections or sustained-release devices, increase germinal centre B cell and T_{FH} cell responses, the number of B cell clones participating in germinal centre responses, and antibody affinity maturation and neutralizing responses, compared with the same vaccine delivered in a standard bolus[64–66]. However, the connection between the time frame of vaccine exposure, the magnitude and durability of germinal centre responses, and the extent of resulting somatic hypermutation remain elusive. Moreover, it remains unclear whether somatic hypermutation that occurs in a sequence of new germinal centres or continuously in the same germinal centre over time results in higher affinity and high-breadth antibody responses with enhanced neutralizing capabilities.

In the light zone, T_{FH} cells drive the selection and survival of high-affinity B cells by providing CD40 ligand and cytokine stimulation to B cells that present the highest quantities of antigen[49,50]. Following BCR engagement with antigen, B cells die if they do not receive T_{FH} cell stimulation[67]. This process may be inadvertently bypassed by vaccines, because certain TLR agonist adjuvants can rescue low-affinity germinal centre B cells in the absence of T_{FH} cell stimulation[68]. In addition to controlling the positive selection of high-affinity B cells, T_{FH} cells also regulate their differentiation into memory B cells and plasma cells[36]. For example, increasing CD40 ligand availability can lead to surface changes on germinal centre B cells, resulting in plasma cell fate commitment[69]. Long-lived plasma cell differentiation occurs in the germinal centre following the generation of class-switched high-affinity B cells[55,70]. T_{FH} cells provide important cues to germinal centre B cells in the light zone to induce long-lived plasma cell differentiation, including increased IL-21 and interferon-γ (IFNγ) stimulation[36,71].

Germinal centre reactions are commonly characterized in rodents using end point analysis of lymph nodes, for example, by flow or mass cytometry and bulk or single-cell sequencing techniques[66,70,72]. Alternatively, fine-needle aspiration allows sampling of lymph nodes over time for longitudinal germinal centre characterization in non-human primates and human subjects[65,73].

Systemic immunity

Humoral immunity. Long-term humoral immune protection against pathogen infection relies on long-lived plasma cells and memory B cells. Long-lived plasma cells are the first line of defence against reinfection, because they reside in the bone marrow and constitutively produce antibodies to enable an immediate reaction

to pathogen encounter[74] (FIG. 1d). Long-lived plasma cell fate determination in germinal centres is poorly understood, yet a key step in producing durable vaccine responses and a major challenge for vaccine design[75]. Indeed, current influenza vaccines do not lead to the presence of long-lived plasma cells in the bone marrow, resulting in poor durability of humoral responses[75].

Memory B cells are the second line of defence upon infection. They can be rapidly reactivated and generate a strong antibody response[76]. These cells reside throughout the body, with some remaining in circulation and others residing in specific tissues, in preparation for reactivation[76]. Memory B cells either form independently of the germinal centre (before somatic hypermutation) or from germinal centre B cells (after somatic hypermutation)[70,76,77]. Memory B cells that develop pre germinal centre primarily produce un-switched IgM isotype and low-affinity BCRs[78]. There is evidence that low-affinity B cells enter the memory B cell compartment in the germinal centre[36,79]. Upon antigen re-exposure, memory B cells expressing high levels of CD80 and PDL2 then differentiate into antibody-forming cells, whereas memory B cells with low levels of CD80 and PDL2 re-enter germinal centres[80]. More research is needed to identify the memory B cell populations most important for protection against future infections and to design approaches to specifically increase those populations. For example, accumulation of CpG, a potent TLR9 agonist, in B cell follicles can cause B cells to immediately differentiate into low-affinity short-lived plasma cells, resulting in high antibody titres with poor antibody affinity[68,81,82]. These reports suggest that controlling the distribution of certain adjuvants — perhaps CpG — may be required to produce antibody responses with the high levels of somatic hypermutation essential for certain pathogens (for example, HIV)[81].

Ultimately, both long-lived plasma cells and memory B cells provide protection through the generation of antibodies[14]. The most important antibody isotypes for pathogen clearance are IgG, which protects blood and tissues, and IgA, which protects mucosal surfaces[13]. Antibodies can be characterized by their quantity (titre), affinity, avidity, effector functions and breadth of binding epitopes, which together confer the ability to neutralize their target pathogen. Adequate antigen design ensures that the antibodies produced by the vaccine response are protective[83–86]. In addition, antibody quality can be improved by prolonging and guiding the somatic hypermutation process through controlling exposure of antigen and adjuvants to the immune system[64,65]. Importantly, antibodies produced in response to vaccination can be profoundly influenced by previous exposure to similar pathogens, which is the so-called original antigenic sin effect[87,88]. Furthermore, immunodominance — that is, only a small set of dominant epitopes on the antigen are targeted — constitutes a major challenge for creating neutralizing antibody responses against highly mutating pathogens[88]. Therefore, for challenging targets, such as HIV, the production of broadly neutralizing antibodies requires the engagement of the correct precursor B cells early in the B cell development process[86,89,90].

Cell-mediated immunity. Vaccine studies often focus on humoral immunity; however, cell-mediated immunity also plays an important and complementary role in immune protection. Effector $CD8^+$ T cells recognize pathogen-derived peptides bound to MHC class I molecules on the surface of infected cells and initiate a signal cascade to kill them. The cell-mediated response is necessary for eliminating virus-infected host cells[13,14,91], highlighting the importance of this branch of adaptive immunity for the design of therapeutic vaccines to treat an established infection. For example, a vaccine eliciting both antibody and cell-mediated responses provides enhanced protection against HIV infection in non-human primates, compared with a vaccine that only promotes one response[92]. Of note, in a natural infection, $CD8^+$ T cells often recognize epitopes from internal proteins of the pathogen[93], which is particularly beneficial for the protection against pathogens that frequently mutate, because internal proteins are typically better conserved than surface proteins[94]. Influenza infections also lead to strong tissue-resident $CD8^+$ T cell responses, which aid in the protection against future influenza infections; however, current vaccine strategies primarily rely only on humoral immunity[94].

Memory $CD8^+$ T cells are categorized as central, effector or tissue-resident memory T cells based on their location and circulation in the body following activation[95,96]. Tissue-resident memory T cells are non-circulating cells, which reside in most tissues of the body and play a key role in local immunity and recall responses[38,93]. These T cells survey their local environment for infected cells and immediately respond to pathogen exposure; by contrast, circulating memory T cells require hours to days to proliferate and migrate to infected tissues[38,93] (FIG. 1d). Thus, alternative immunization routes, such as direct administration to the relevant mucosa, have been explored to establish tissue-resident memory T cells at the typical site of infection for a given pathogen (for example, intranasal for respiratory pathogens[16,17]). Strategies to engage and enhance the cytotoxic T cell response are often explored in the context of cancer vaccines, but would also greatly benefit the development of lasting protection against infectious diseases.

Designing spatial and temporal control

Subunit vaccines based on protein antigens offer opportunities for more precise vaccine design, improved safety and manufacturing compared with inactivated or attenuated vaccines. However, subunit antigens often exhibit low immunogenicity, poor uptake and processing by APCs, and poor targeting of lymph tissues. Accordingly, immunostimulatory adjuvants are used to augment antigen immunogenicity and improve vaccine efficacy (BOX 2). These adjuvants recruit and activate innate immune cells, including neutrophils, natural killer cells, innate lymphoid cells, macrophages, monocytes and dendritic cells, and induce phenotypic maturation and the production of cytokines both at the injection site and in the lymph nodes[15,20,97]. Multiple adjuvants are often selected to mimic whole-pathogen vaccines, such as in the highly potent yellow fever vaccine (YF-17D),

which activates dendritic cells via multiple TLRs[98] and retinoic acid-inducible gene I (RIG-I)[99]. Importantly, most adjuvant molecules that have been developed against the multifarious PRRs are physico-chemically distinct (FIG. 2a), varying in molecular weight (from hundreds to several million daltons), charge (from uncharged to highly charged nucleic acids) and relative hydrophobicity. Similarly, subunit antigens substantially vary in molecular weight (FIG. 2b). Therefore, subunit vaccines are complex mixtures of physico-chemically distinct molecules. For example, Heplisav-B, a hepatitis B subunit vaccine, contains the recombinant protein hepatitis B surface antigen (HBsAg; molecular weight of ~24 kDa) and the nucleic acid oligomer TLR9a adjuvant CpG (molecular weight of ~11 kDa)[100]. Nanoparticles, antigen conjugates, self-assembled scaffolds, hydrogels or microneedles can be used as vehicles to control the location and timescale of the delivery of subunit vaccine components to the immune system to enhance many elements of the vaccine response.

Improving innate immune cell activation at the injection site

Activated innate immune cells at the injection site are crucial for initiating vaccine responses. Infiltrating APC subsets must efficiently take up the vaccine antigen and present it to $CD4^+$ and $CD8^+$ T cells. Particle constructs can be designed to enhance innate immune responses by increasing adjuvant potency and improving antigen processing (FIG. 3). Here, the route of administration is important to improve injection site reactions, because different locations have different numbers and types of resident innate immune cell (for example, the skin has more innate immune cells than the muscle) or can contain distinct physiological barriers (for example, epithelial barriers, which must be navigated following mucosal delivery)[101]. In this Review, we focus on vaccines delivered by traditional routes, such as subcutaneously and intramuscularly; however, similar principles may be applicable to mucosal vaccination, which can provide additional benefits and challenges[102,103].

Particles increase adjuvant potency. Innate cells must be activated in the right place and at the right time to trigger the maturation of APCs and the production of pro-inflammatory cytokines and chemokines, such as IFNγ and IL-12, which together stimulate downstream humoral and cellular responses[15,97]. Promising adjuvants, such as agonists for TLRs, stimulator of interferon genes (STING) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), have been designed to specifically target and activate APCs; however, many of these agonists are small molecules that freely and rapidly diffuse from the injection site into the blood, reducing their ability to prime immune cells and often causing systemic side effects, such as a cytokine storm[104–106]. To maximize the activation of APCs and other innate immune cells and minimize systemic toxicities, nanoparticles can be used to control the magnitude, bio-distribution and time frame of cytokine production by promoting innate cell uptake and/or targeting molecular adjuvants to the lymph nodes[101]. Moreover, distinct PAMPs are activated on the surface of cells (for example, TLR1, TLR2, TLR5), in endosomes (for example, TLR3, TLR4, TLR7, TLR8, TLR9) or in the cytosol (for example, RIG-I, STING), highlighting the need for targeting molecules to specific cellular compartments.

Polymer nanoparticles benefit from modularity, scalable manufacturing and biocompatibility[104,105,107–109].

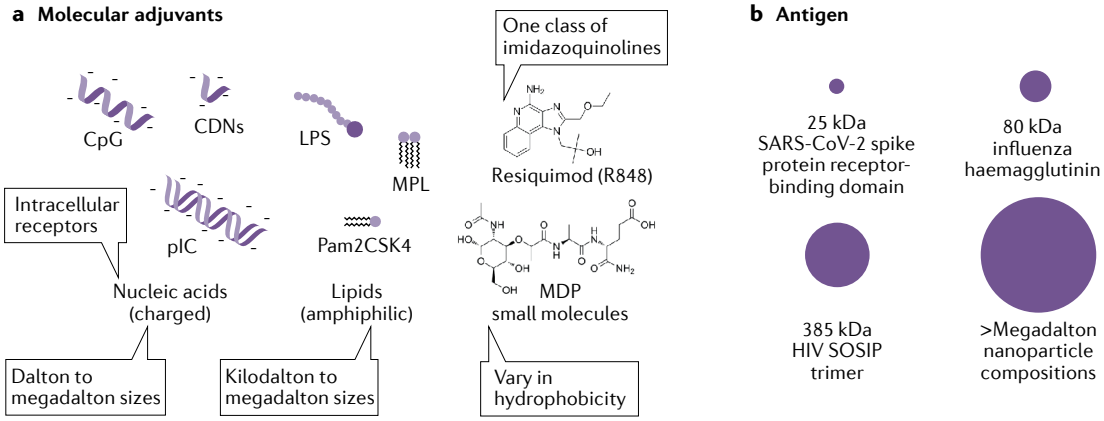


Fig. 2 | **Vaccine delivery from a chemical perspective.** Subunit vaccines are composed of antigens and adjuvants with a range of molecular weights and diverse physical and chemical properties, which affect delivery vehicle selection, encapsulation efficiencies, cargo stability, potential for co-delivery of multiple compounds and delivery characteristics. **a** | Molecular adjuvants, such as Toll-like receptor (TLR) and nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) agonists, include highly charged nucleic acids, amphiphilic lipids and small molecules with varying hydrophobicity. Nucleic acid adjuvants have similar charge densities, but can have different molecular weight; for example, cyclic dinucleotides (675 Da) and poly(inosinic:cytidylic acid) (pIC) (up to 5 MDa)[245]. **b** | Subunit antigens include small proteins, such as the SARS-CoV-2 spike protein's receptor-binding domain with a hydrodynamic size of less than 3 nm, and multivalent protein nanoparticle constructs with hydrodynamic sizes of up to 50 nm (REF.[198]). CDNs, cyclic dinucleotides; CpG, cytosine–phosphate–guanine oligodeoxynucleotide; LPS, lipopolysaccharide; MDP, muramyl dipeptide; MPL, monophosphoryl lipid A; Pam2CSK4, a synthetic diacylated lipopeptide.

Nucleic acid-based adjuvants, such as poly(inosinic:cytidylic acid) (pIC) (a TLR3 agonist)[107], CpG (a TLR9 agonist)[110] and cyclic dinucleotides such as 2′3′-cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) (a STING agonist)[111,112], can be encapsulated in nanoparticles through electrostatic interactions with cationic lipids and polymers, similar to delivery vehicles developed for small interfering RNA (siRNA) or mRNA delivery[113,114]. However, unlike siRNA or mRNA, nucleic acid adjuvant species are active either in endosomes or in the cytosol. For example, encapsulation of pIC in poly(β-aminoester) (PBAE) nanoparticles can shift cytokine production from pro-inflammatory cytokines to type I interferons through selection of different PBAE polymers[107]. The chemistry of the PBAE-based nanoparticles controls the degree to which pIC activates endosomal TLR3 or cytosolic RIG-I, with certain nanoparticle formulations enabling a more than 13-fold increase in desirable IFNα production compared with non-encapsulated pIC in mice, likely by modulating the levels of endosomal escape[107]. Similarly, endosomolytic polymerosomes can improve cytosolic STING activation by entrapped cGAMP[111]. By contrast, small hydrophobic or amphiphilic molecules, such as monophosphoryl lipid A (MPL) (a TLR4 agonist)[109], imiquimod (R837) (a TLR7 agonist)[108,109], resiquimod (R848)

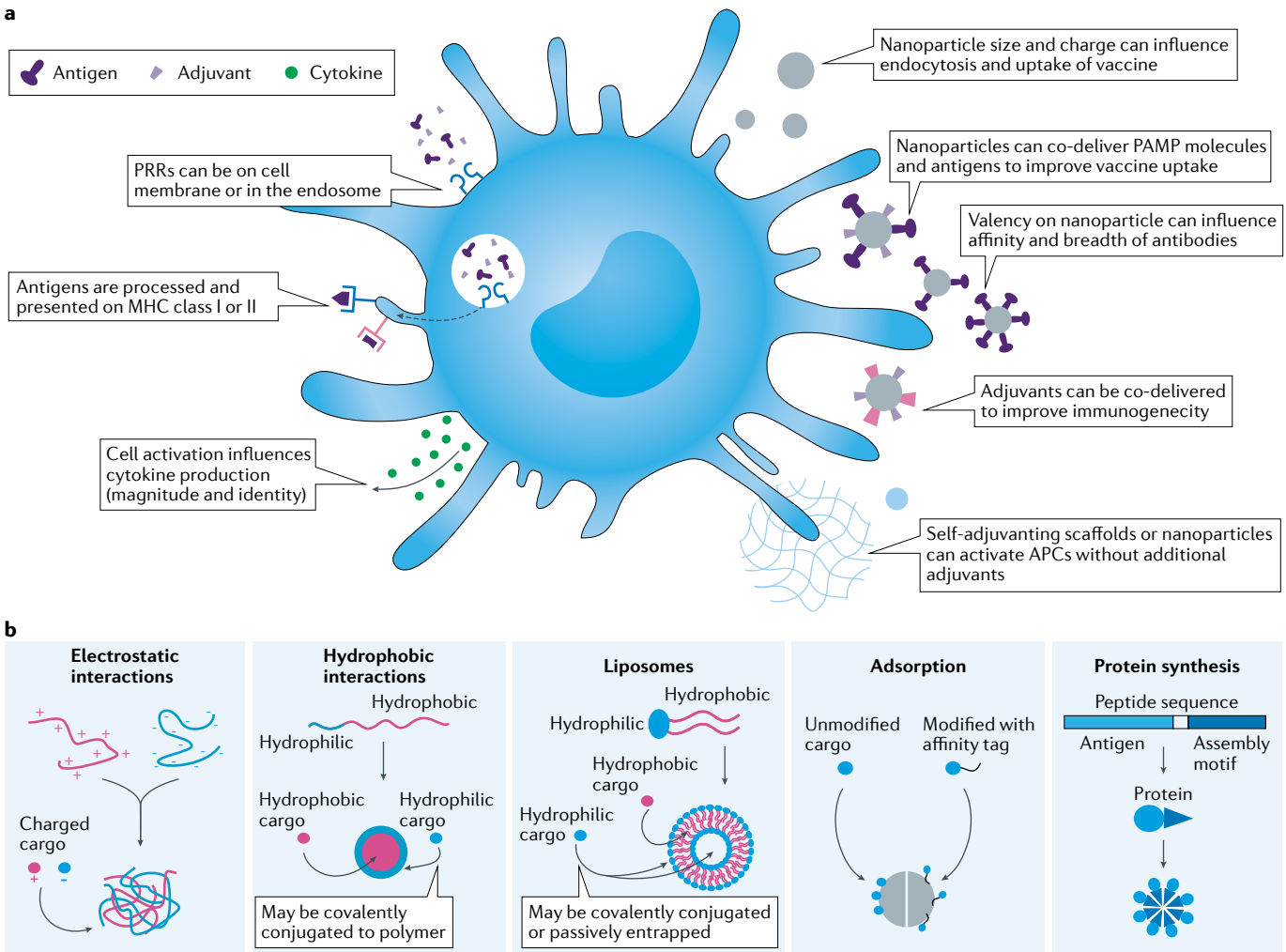


Fig. 3 | **Materials enhance innate immune cell activation. a** | Biomaterials, such as nanoparticles, microparticles and scaffolds, can be used as vehicles for the controlled delivery of antigens and adjuvants, and interact with the immune system in a spatio-temporally controlled manner. These biomaterials can be designed to enhance innate immune cell activation. Protein antigens and adjuvants, such as pathogen-associated molecular pattern (PAMP) molecules, can be co-delivered to improve antigen-presenting cell (APC) recognition and uptake of vaccine components. Nanosized particles can further improve endocytosis by APCs. Self-adjuvanted scaffolds can create a local depot to improve innate cell infiltration, resulting in increased antigen uptake by APCs. Improved antigen processing and APC activation can increase cytokine and chemokine production to improve humoral and cell-mediated adaptive immune responses. **b** | Nanoparticle strategies can be based on various materials and cargo encapsulation mechanisms. For example, highly charged species such as nucleic acid-derived adjuvants (for example, poly(inosinic:cytidylic acid) (pIC) or cytosine–phosphate–guanine oligodeoxynucleotide (CpG)) can be encapsulated by complexation with polyelectrolytes of opposite charge[107,246], whereas hydrophobic cargo, such as monophosphoryl lipid A (MPL) or Pam2CSK4 (a synthetic diacylated lipopeptide), can be encapsulated in degradable hydrophobic particles or liposomes[109]. Traditional adjuvants, such as alum particles, typically adsorb proteins and/or other adjuvants in an uncontrolled albeit multivalent fashion, and peptide assembly motifs can be leveraged for precise multivalent display of antigens and/or adjuvants on nanoparticle constructs[198]. MHC, major histocompatibility complex; PRR, pattern-recognition receptor.

(a TLR7/8 agonist)[104,105,115] and the synthetic TLR7/8a ligand 3M-052 (REFS[92,116,117]), can be encapsulated in particles made of poly(lactic-co-glycolic acid) (PLGA), *N*-(2-hydroxypropyl)methacrylamide (HPMA) or poly(propylene sulfide) (PPS) polymers.

When TLR7/8a ligands are conjugated to particles, they are efficiently taken up by APCs and induce persistent innate immune activation in lymph nodes, thereby substantially reducing systemic toxicities in comparison with soluble small-molecule TLR7/8a, which shows rapid systemic distribution[105,115]. Moreover, improved trafficking by activated dendritic cells increases activation of T_H1-type $CD4^+$ and $CD8^+$ T cell responses and leads to higher antibody titres and improved antibody affinity maturation compared with immunization with soluble TLR7/8a ligands. Similarly, presenting CpG on the surface of peptide-based ferritin particles increases the valency of this adjuvant and improves the potency of adjuvant responses[118].

For PRRs that are active on cell membranes or in endosomes (most TLRs and NLRs), multivalent display of molecular adjuvants on the surface of particles enables receptor clustering and, thus, potent innate cell activation. By contrast, the activation of cytosolic receptors (for example, STING and inflammasome) requires the release of molecular adjuvants from nanoparticles. Therefore, encapsulation strategies are needed that allow control of the timescale of release[119]. Co-delivery of multiple adjuvant molecules is possible with all these types of material and can lead to synergistic responses; however, co-encapsulation approaches have typically been restricted to physico-chemically similar molecules. Evaluation of TLR4a and TLR7a delivery by encapsulation in separate particles or in the same particle showed that synergistic effects are only induced if the adjuvants are delivered together in the same particle (thereby activating the same innate cell)[109]. Surface conjugation strategies benefit from the ability to co-present physico-chemically distinct cargoes on the same construct, imparting similar pharmacokinetics and bio-distribution, which is challenging or impossible to achieve with co-encapsulation strategies.

Particles improve antigen processing and selection. Particles can also improve antigen recognition, uptake and processing by APCs by increasing endocytosis through tuning of their size, shape and surface properties[120–123]. For example, liposome particles encapsulating trimerized gp-140 HIV antigen (BG505 MD39) improve APC recognition and antigen presentation, compared with soluble antigen, without requiring additional adjuvants[124,125]. These constructs induce higher concentrations of antigen-specific T_{FH} cells, which result in correspondingly higher-magnitude and higher-avidity antibody responses, compared with the soluble trimer[124,125]. Antigen taken up by APCs is processed and cross-presented, and thus its location on particles (for example, surface-attached or encapsulated) negligibly influences its immunogenicity. Numerous particle systems[35,122,123] have been shown to improve delivery of encapsulated subunit antigen[126,127]; for example, quaternized chitosan hydrogel microparticles encapsulating an H5N1 split virion influenza vaccine improve humoral responses and enhance $CD8^+$ T cell activation, compared with an alum control, when administered intramuscularly in mice[126]. This improvement was attributed to enhanced antigen uptake and cross-presentation by APCs owing to increased endocytosis of the positively charged particle scaffold.

Co-delivery of antigen and PAMP molecules, such as TLR agonists (for example, by encapsulation or surface presentation), improves recognition by PRRs and promotes APC maturation and antigen processing[15,97,128]. Precise co-delivery of adjuvant and antigen can be achieved using nanoparticles based on self-assembled virus-like particles, such as ferritin[118,129] and hepatitis B core antigen (HBcAg) proteins[130,131], or synthetic nanoparticles, such as liposomes[125] or degradable PLGA nanoparticles[109,128]. A diverse array of antigens has been co-delivered by nanoparticles, including ovalbumin[128], HIV BG505 SOSIP (REF.[118]), influenza haemagglutinin[109] and SARS-CoV-2 spike protein[129], leading to improved humoral and cell-mediated immune responses compared with co-delivery without nanoparticles. For example, a subcutaneous injection of ovalbumin and pIC co-encapsulated in PLGA nanoparticles led to significantly higher antigen-specific $CD8^+$ T cell priming in mice, compared with soluble and microparticle formulations of the same vaccine owing to more efficient endocytosis by dendritic cells[128]. Indeed, the improvement in T cell priming owing to higher MHC class I antigen presentation on dendritic cells promotes a more balanced T_H1 cell/T_H2 cell response. Similarly, multilayered liposomes encapsulating a hydrophilic protein antigen in the aqueous interior and the lipophilic TLR4 agonist MPL in the lipid membrane elicit stronger humoral and cellular immune responses compared with soluble antigen with MPL (14-fold increase in $CD8^+$ T cell responses)[132].

C-type lectin receptors on APCs mediate endocytosis and participate in antigen capture. Therefore, co-presentation of glycoproteins, such as mannose, on particles can improve uptake[133]. Such synthetic approaches to glycosylation benefit from easy characterization and enhanced stability over time. However, aberrant glycosylation or covalent attachment of glycopolymers to an antigen can inhibit intracellular antigen processing for MHC presentation by sterically blocking proteolysis[134]. To mitigate potentially undesirable outcomes, self-immolative linkers can be used to conjugate an antigen, for example a malaria antigen, to glycosylated synthetic polymer nanoparticles encapsulating TLR7 agonists[108]. Using this approach, antigen targeting to dendritic cells by mannose-binding receptors could be improved, resulting in a more robust humoral and cellular immunity than elicited by the antigen alone. These studies suggest that antigen release is crucial and can be controlled by encapsulation or direct conjugation using stimuli-responsive linkers.

Creating a local inflammatory niche

Biomaterial scaffolds can be applied to recruit and programme innate immune cells at the site of administration to induce effective adaptive immunity. To increase

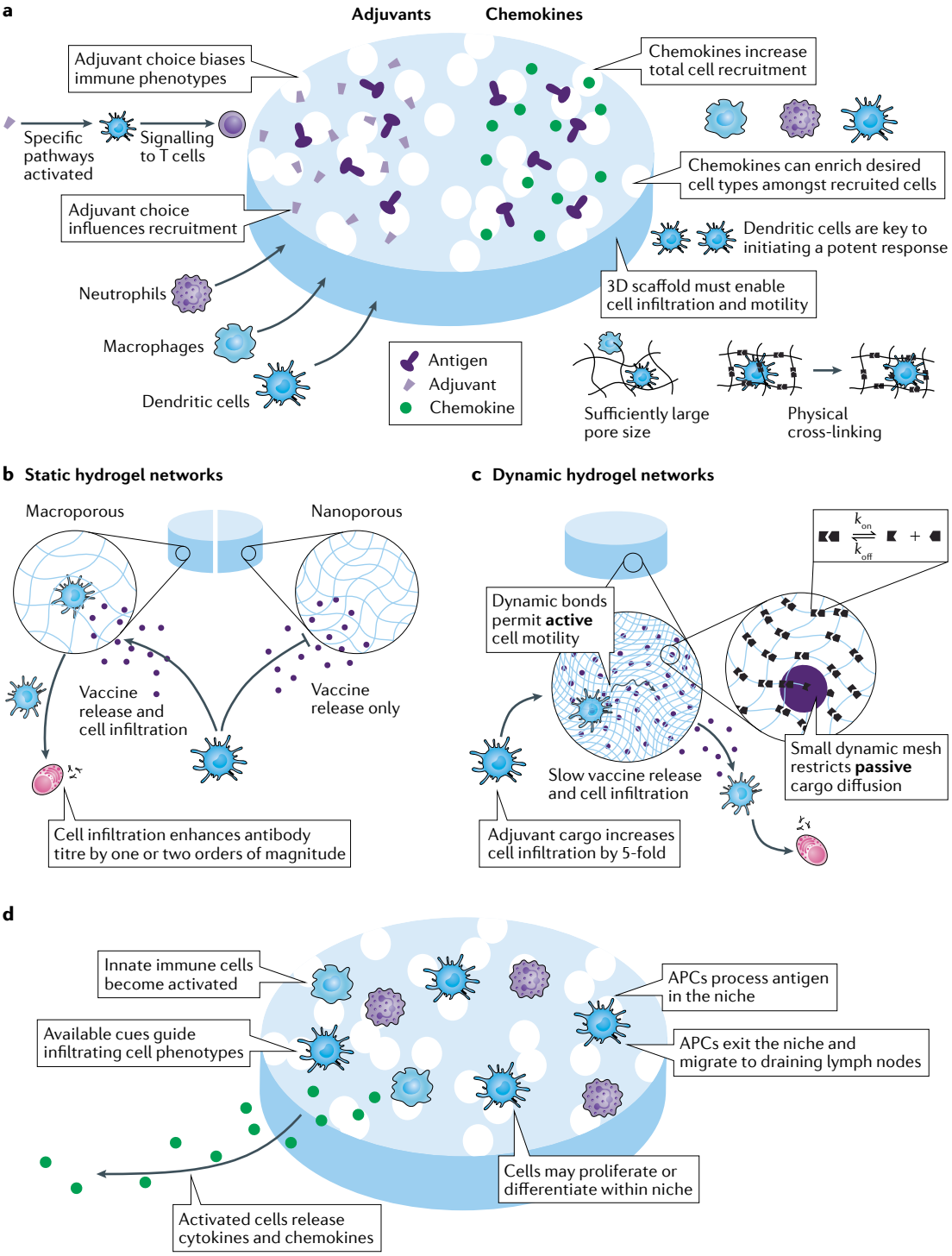
innate immune cell infiltration to the injection site, biomaterials should be easily injectable in a minimally invasive fashion, and create a 3D scaffold at the site of injection to provide space for cell recruitment (FIG. 4a). Hydrogels, self-assembled scaffolds, microparticles and microneedles can be designed to remain at the site of injection and promote immune cell infiltration to generate a local inflammatory niche. These materials provide tools to direct the immune response by attracting endogenous cells to the vaccine site, increasing antigen uptake and providing activation cues locally and rapidly[135]. Various materials have been explored for the formation of a local inflammatory niche to enhance immune responses[136]; however, the impact of specific niche properties (for example, specific innate cell populations and/or their activation profiles) and the duration of niche persistence on downstream vaccine responses are poorly understood. Studies with self-assembled scaffolds based on silica rods have indicated that the local inflammatory niche must persist for at least 7 days to achieve prolonged antibody titres, compared with a bolus control[136]. By contrast, niches created by injectable hydrogels that persist for about 2–4 weeks lead to a more durable and higher-affinity humoral immune response than a bolus control[66]. The development of tunable niche-forming materials will enable more robust investigation of the influence of vaccine identity and the time frame of niche persistence on the magnitude, duration and quality of immune responses.

Niche-forming materials. Injectable hydrogels made of polymers physically cross-linked by thermally induced phase segregation[137], triggered covalent cross-linking of functional polymers[138] or dynamic cross-linking of polymers by supramolecular interactions[66,139,140] can flow through a needle and achieve solid-like mechanics after injection. Alternatively, macroporous structures based on mesporous silica rods can form through hydrostatic interactions following injection[136,141–143], whereas supramolecular peptides[144–147] enable facile self-assembly of nanofibre constructs following injection[145]. Materials for cell infiltration need to be designed in a way that cells can easily travel through the scaffold structure. For example, surface modification of self-assembled mesoporous silica rods considerably impacts cellular infiltration, whereby polyethylene glycol (PEG)-conjugated mesoporous silica rods show increased cellular infiltration compared with unmodified rods in mice[148]. The design of constructs that maintain cell motility but also ensure prolonged retention of encapsulated vaccine cargo remains challenging (FIG. 4b). Dynamically cross-linked hydroxypropylmethyl cellulose (HPMC) hydrogels based on supramolecular polymer–nanoparticle interactions contain a transient polymeric mesh, which restricts passive cargo diffusion to ensure sustained vaccine retention within the hydrogel depot and, simultaneously, enables active cell infiltration and motility[66,139] (FIG. 4c).

To attract immune cells to a material scaffold, chemokines can be incorporated and slowly released, increasing cell migration through chemotaxis. The most commonly used chemokine is granulocyte–macrophage colony-stimulating factor (GM-CSF), which promotes chemotaxis and proliferation of dendritic cells. GM-CSF can be encapsulated as a free protein or can be conjugated to nanoparticles to prolong its release[149]. Delivery of GM-CSF increases the recruitment of immune cells, in particular dendritic cells, to the injection site[137,142,150]. Subcutaneous injection of an antigen-loaded thermosensitive hydrogel carrying GM-CSF in mice increases dendritic cell recruitment to the gel and the draining lymph nodes compared with the gel-based vaccine without the chemokine, resulting in an improved $CD8^+$ T cell response[137]. Similarly, injectable hydrogels delivering the cytokine CCL21 following subcutaneous administration preferentially recruit dendritic cells[139]. Adjuvants, such as TLR agonist ligands, also promote innate immune cell recruitment and infiltration, without requiring delivery of a chemokine[66,138]. Importantly, co-retention of adjuvants and antigens in the inflammatory niche over similar time frames appears to be crucial for improving the magnitude, durability and affinity maturation of antibody responses[151]. For example, prolonged co-retention of an influenza haemagglutinin antigen and a TLR7/8a adjuvant within an injectable hydrogel depot results in a substantial increase in antibody titre and breadth, compared with a system enabling sustained antigen retention but rapid adjuvant release[151]. Prolonged local retention of antigen may also negatively impact the immune response by attracting antigen-specific T cells to the site of injection and initiating their apoptosis owing to a lack of appropriate immune signals[152].

Niche-forming materials, such as hydrogels, can either provide a location for local inflammation or be designed to be 'self-adjuvanting', for example, self-assembled scaffolds based on mesoporous silica rods and peptide nanofibres. Here, parts of the scaffold can be taken up by infiltrating cells. For example, mesoporous silica rods can activate the NLR NLRP3, resulting in the generation of pro-inflammatory cytokines, which improve innate immune cell infiltration and subsequent immune responses in mice[148,153]. Similarly, positively charged fibres in peptide nanofibre scaffolds are more readily taken up by dendritic cells than negatively charged fibres, resulting in higher cytokine production and more potent T cell responses in mice[144,147]. The tunable multivalent display of T cell and/or B cell epitopes for West Nile virus[146], malaria[154] and influenza[155] on these self-assembled nanofibre constructs can drive robust humoral and cellular immune responses in mice[145,156,157].

Microneedles. Patches with microneedles enable painless, non-invasive and easy intradermal vaccine delivery, providing high immunogenicity owing to the presence of a high number of APCs in the dermal layer of the skin, compared with other tissues[158–160]. Solid microscopic needles that are either non-degradable (for example, metal), dissolvable (for example, hydrophilic polymers such as cellulose derivatives or sugars) or degradable (for example, silk or PLGA) can deliver vaccines into the skin[72,158,159,161–163]. Vaccine delivery with microneedle patches can increase local immune cell recruitment to the site of administration; however, microneedles cannot



really create immunological niches, because they are typically made of solid matrices that cannot be infiltrated by innate immune cells. A study in humans comparing uncoated microneedles with needles coated with an influenza vaccine showed that vaccine coating increases infiltration of skin-resident dendritic cells, called Langerhans cells, to the site of administration[164]. Thus, vaccine cargo can manipulate the migratory pattern of dermal APCs, highlighting that swellable or porous microneedles may provide a tool to create an inflammatory niche. Microneedles coated with swellable polymers and loaded with subunit vaccines promote infiltration of tissue-resident T cells into the hydrogel, allowing the study of the local immune response[165]. Therefore, microneedle platforms could also be used to generate an inflammatory niche at the site of injection to further improve the vaccine response.

Targeting vaccine components to lymph nodes

Direct intra-nodal administration can substantially enhance humoral and cell-mediated immune responses[34]. However, intra-nodal administration is

◀ Fig. 4 | **Enhancing the vaccine response by engineering an inflammatory niche.** **a** | Biomaterials, such as hydrogels and self-assembled scaffolds, can be designed to create an inflammatory niche, which persists in vivo and encourages innate immune cell engagement. Incorporating adjuvants into the vaccine formulation leads to local immune cell activation and further recruitment of cells from circulation. Encapsulating chemokines in the delivery system can increase cell infiltration into the site of administration. **b** | Engineering an inflammatory niche requires endogenous cells to migrate into the material. For cells to enter a static network, the pores of the material must be larger than the cells. Nanoporous materials do not allow cell infiltration and can lead to a decrease in humoral immunity compared with microporous structures[136]. **c** | Physically cross-linked networks with dynamic bonds permit active cell motility through a polymer mesh, even if the mesh size is smaller than the cells. If the pores in the polymer mesh are sufficiently small, passive diffusion and release of molecular cargo can be extremely slow, prolonging local retention of the cargo. Cell motility into the material is enhanced by adjuvant encapsulation[66]. **d** | Following cell infiltration into the biomaterial niche, antigen-presenting cells (APCs) may become activated by immune stimulants in the material and begin processing antigen locally. Cell phenotypes in the material are determined by the encapsulated adjuvants and chemokines, as well secreted cytokines from the infiltrating cells. APCs may exit the inflammatory niche and migrate to the draining lymph node to mediate the adaptive immune response.

technically challenging and, therefore, poorly translatable. Materials can be used to deliver vaccine components through afferent lymphatic vessels into the draining lymph nodes, providing a more readily translatable tool to enhance antigen presentation, APC maturation and lymphocyte priming in the lymph nodes[120]. Delivery of antigen and adjuvants to the draining lymph nodes can be augmented using nanoparticles or by directly modifying vaccine components, taking advantage of the binding of endogenous proteins. Passive or active transport can be leveraged by materials to reach key cells, making this a powerful strategy to overcome the challenges associated with conventional delivery methods[166].

Passive drainage of nanoparticles to lymph nodes. Lymph nodes contain a large number of immature lymph node-resident APCs, which are able to stimulate an immune response independent of peripheral migratory populations[167]. Passive targeting of vaccine components to lymph node-resident APCs, bypassing the migration of APCs from the injection site to the lymph nodes (which typically takes 24–48 h), can enable more rapid T cell activation. Thus, delivery vehicles that enable passive drainage and prolonged retention of vaccine components in the lymph nodes enable rapid stimulation of a potent immune response. By precise design of nanoparticle size[168,169], shape[170,171] and surface properties (that is, charge and hydrophobicity)[172–174], efficient lymph node targeting can be achieved, without the need for specific cell-targeting ligands.

The size of particles, such as virus-like particles[175], liposomes[174], lipidic nanoparticles[176] and polymeric nanoparticles[168,172,177], affects passive delivery to the lymph nodes[35,178] (FIG. 5). Nanoparticles that are smaller than 5 nm can immediately partition into the bloodstream and systemically circulate, whereas particles between 20 and 100 nm efficiently drain through lymphatic vessels into the lymph nodes[168,169,179], where they are taken up by lymph node-resident APCs[180] (FIG. 5a,b). For example, nanoparticles made of PPS[168,177] and PLGA–PEG[179], with diameters of ~20 nm, efficiently target the lymph nodes and are internalized by lymph node-resident APCs, following intradermal or subcutaneous administration in mice. A study evaluating 50-nm nanoparticles, administered subcutaneously in wild-type mice and *CCR7*$^{-/-}$ mice lacking migratory dendritic cell populations, showed the same percentage of nanoparticle-carrying dendritic cells in the draining lymph nodes[181], confirming that migratory dendritic cells do not play a role in trafficking these nanoparticles to the lymph nodes[181]. Furthermore, PEGylation of 50-nm polymeric nanoparticles, which increases their hydrophilicity and reduces fouling by proteins, leads to higher lymph node accumulation following subcutaneous administration in rats, compared with non-PEGylated particles, suggesting that hydrophilic modification plays a role in cell-free trafficking to lymph nodes[172] (FIG. 5a). Large particles (up to 1 μm in size) are also passively and quickly[179] transported to the lymph nodes, demonstrating that additional factors, such as biological characteristics of specific tissues and injection-induced hydrodynamic fluxes, can also influence lymphatic nanoparticle delivery[33]. Clinical alum adjuvants are also nanoparticles, which can bind to site-specifically modified antigens bearing alum-binding peptides to increase antigen uptake and retention in lymph nodes, and thus improve germinal centre formation, neutralizing antibody concentration as well as memory and long-lived plasma cell responses[182].

Albumin-binding dyes target draining lymph nodes and thus provide a strategy for the visual identification of sentinel lymph nodes following intratumoural administration[183]. Molecular vaccine cargo can also be designed to 'hitch-hike' albumin for the passive targeting of lymph node-resident APCs[173,184]. This hitch-hiking strategy increases the magnitude of T cell activation by >30-fold, compared with standard bolus administration of the antigen alone[173,184]. Similarly, an albumin-binding CpG-based amphiphile adjuvant can elicit strong antigen-specific T cell responses, high antibody titres and potent viral neutralization, when administered with the SARS-CoV-2 spike protein receptor-binding domain antigen[185]. These amphiphilic molecular vaccines afford a simple and broadly applicable strategy to increase the potency of subunit vaccine components by improving targeting to lymph nodes.

Active transport of cargo to lymph nodes by APCs. Cell-mediated transport can also be exploited for the delivery of vaccine components to lymph nodes. Here, peripheral APCs take up cargo at the site of injection and migrate through the lymphatics into the draining lymph nodes, where they activate T cells and initiate an immune response. Uptake of nanoparticle cargo by migratory APCs and subsequent transport to lymph nodes can be engineered by directly targeting APC surface receptors or by tuning nanoparticle size and surface chemistry[168,186] (FIG. 5b).

Direct targeting of tissue-resident APCs can be achieved by binding to C-type lectin receptors[187], such as CD205 (REFS[188,189]), CD40 (REF.[190]), dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)[191] and mannose receptors[192]. For example, subcutaneous administration of 1-μm particles

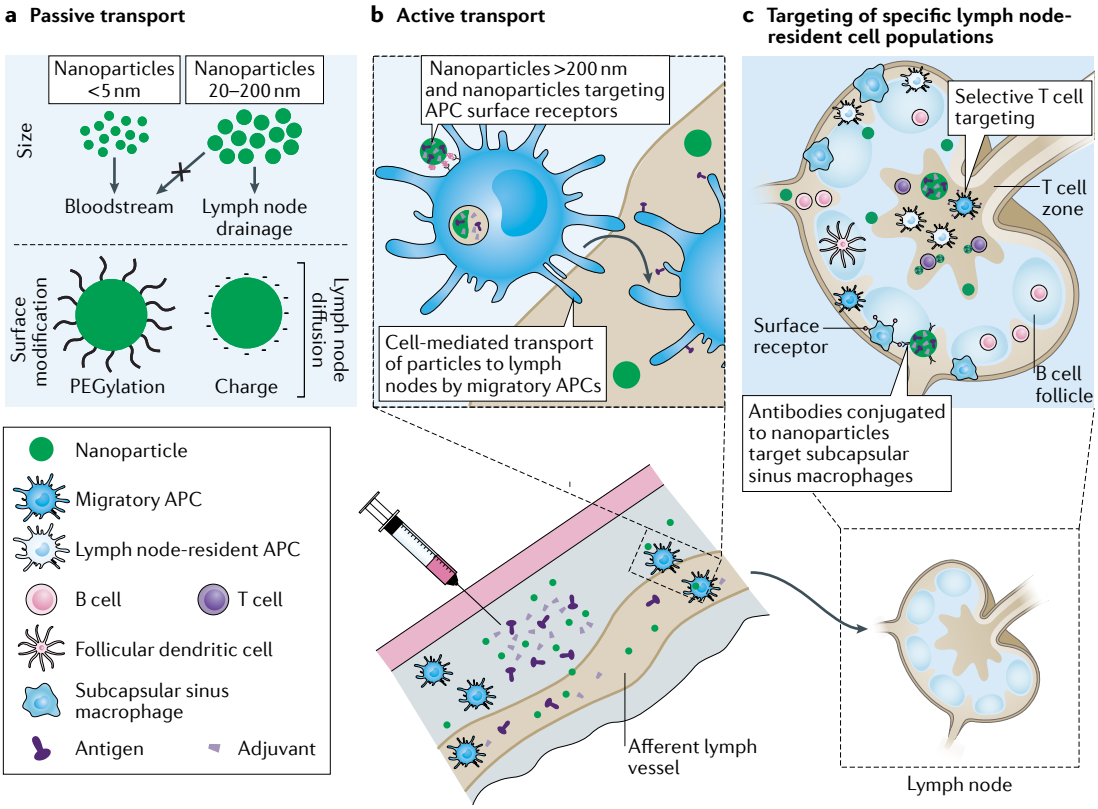


Fig. 5 | **Strategies for lymph node targeting.** Targeted delivery of antigen and adjuvant to the lymphatic system can be achieved by nanoparticle injection in the subcutaneous, muscular or intradermal space. Nanoparticle properties, such as size, surface chemistry and targeting ligands, impact drainage through the lymphatic system and interactions with antigen-presenting cells (APCs). **a** | Passive drainage is best achieved with nanocarriers of a hydrodynamic diameter between 20 and 200 nm. Further surface modifications such as PEGylation and anionic surface charge increase diffusion to the lymphatic system. **b** | Large nanoparticles are actively transported to the lymph nodes by migratory APCs. **c** | Targeting of specific cell populations within the lymph nodes, for example, subcapsular sinus macrophages, follicular dendritic cells, B cells and T cells, can be achieved through surface modification of the nanoparticles with, for example, antibodies targeting specific cell surface markers. PEG, polyethylene glycol.

conjugated with anti-CD205 monoclonal antibodies increases receptor-mediated uptake and subsequent migration of $CD205^+$ APCs to lymph nodes[188]. Subcutaneous administration of lentiviral vectors that specifically bind to DC-SIGN cell surface proteins leads to a 10-fold increase in lymph node accumulation after 72 h compared with non-targeted vectors, demonstrating increased trafficking of dendritic cells to lymph nodes[191]. Particles with diameters of 0.5–1 μm are too large to passively drain through lymphatic vessels, but can be transported to lymph nodes by migratory dendritic cells within hours[169]. Indeed, no passive draining is observed for particles of this size administered in transgenic mice lacking migratory dendritic cells ($CD11c^-$DTR/GFP). In addition, whereas only few particle-positive cells accumulate in the lymph nodes after 24 h, they persist for up to 20 days[105]. $CD11c^+CD8^-B220^-$ migratory populations account for the majority of particle uptake, confirming that large particles (for example, >200 nm) are primarily trafficked through active cell-mediated transport. As large particles are not passively transported to lymph nodes, they may form depots at the injection site, promoting local infiltration of APCs and prolonged delivery of antigen and adjuvants.

Cell-specific targeting within lymph nodes. Targeting vaccine components to specific immune cells in the lymph nodes can improve vaccine responses; however, the structure and compartmentalization of lymph nodes make access to specific cell populations challenging. Nanocarriers can be used to overcome this challenge and to spatio-temporally engineer the delivery of vaccines[35] (FIG. 5c). Directly reaching and modulating certain cell types can increase the potency of vaccine responses, thereby decreasing required doses and improving the specificity of responses.

Nanoparticle size, the presence of glycans and antigen valency all influence immunogen targeting to follicular dendritic cells, which play an integral role in B cell activation and promote robust antibody responses[47,193]. Nanoparticles of 50–100 nm are retained in follicular dendritic cell networks for many weeks, whereas smaller particles are cleared within 48 h (REF.[194]). Moreover, high antigen valency on particles and glycosylation are both necessary to increase antigen trafficking to follicular dendritic cell networks within lymph nodes[193]. For example, removal of glycosylation from HIV and influenza antigen nanoparticles results in decreased co-localization with follicular dendritic cells[193],

indicating that glycosylation triggers mannose-binding lectin-mediated immune recognition by the complement pathway, resulting in immunogen trafficking to follicular dendritic cells. Therefore, antigen design (for example, glycosylation) may influence delivery properties in addition to directing the immune response towards a neutralizing epitope. Importantly, adjustment of size, valency and/or synthetic introduction of glycans to antigen nanoparticles provide the design space for targeted delivery to follicular dendritic cells.

Nanoparticles have also been designed for multivalent presentation of antigens to improve B cell processing or selection, which impacts the magnitude and composition of B cell responses as well as the breadth of B cell affinities[195–199]. Entrapped and surface-attached antigens can both induce T cell responses; however, only surface-attached antigens are directly recognized by B cells, because encapsulated antigens must first be released to be accessible. Low-valency constructs may impose more stringent affinity selection pressure, precluding low-affinity B cell clones from germinal centre and plasma cell responses, whereas high-valency constructs enable a broad array of B cell affinities[195]. DNA origami nanoparticles can be used to systematically evaluate the impact of antigen valency and spacing on B cell activation in vitro. Using this approach, it has been shown that five antigens maximally spaced on a rigid 40-nm nanoparticle can stimulate potent B cell signalling[200]. Thus, antigen valency on nanoparticles can be used to tune the affinity and breadth of antibody responses to elicit neutralizing antibody production, which is especially important for rapidly mutating pathogens, such as HIV, influenza and coronaviruses, such as SARS-CoV-2.

Specific cell types can also be targeted by functionalizing particles with ligands. For example, subcapsular sinus macrophages, which present non-degraded antigen on their surface that is then directly taken up by B cells[201,202], can be targeted by conjugating anti-CD169 antibodies to the surface of antigen-loaded nanoparticles[35]. Vaccines can also benefit from directly targeting T cells in the lymph nodes, which can be achieved through the blood vasculature. For example, peripheral node addressin (PNAd) is strongly expressed on the surface of high endothelial venules in the lymph nodes. Monoclonal antibodies, for example MECA79, which bind to PNAd, can be conjugated to particles. Following intravenous injection in mice, these particles accumulate in draining lymph nodes, allowing selective delivery to T cells[203,204]. In particular, particles with diameter ~100 nm have longer circulation times and broad bio-distribution, compared with larger particles (2 μm), thereby enabling interactions with more T cells[204]. B cell and T cell targeting within lymph nodes can be further improved by orders of magnitude using nanoparticles that first passively diffuse to lymph nodes and then release their cargo following cleavage of a degradable linker. This platform enables timing of release and lymph node-wide distribution of molecular cargo, which can be controlled through selection of the degradation rate of the linker[205]. Spatial and temporal control of immune responses by specific particle constructs also provides opportunities for the targeting of vaccine components to specific lymphocytes.

Sustained co-delivery of vaccine components

Modulating the kinetics of vaccine exposure to the immune system can greatly influence the immune response, because many features of the vaccine response require precise temporal cues[166,206] (FIG. 6). For the humoral immune response, the germinal centre reaction is a well-studied example of a process that directly benefits from prolonged exposure (FIG. 6a). The germinal centre reaction requires many cycles of somatic hypermutation and affinity selection to yield the high degree of antibody affinity maturation required for potent and broadly neutralizing antibody responses. Moreover, it is hypothesized that sustained delivery of vaccine components better mimics the kinetics of natural infections and the conditions in which our immune system has evolved to develop a strong response.

Proof-of-concept studies evaluating sustained vaccine delivery using osmotic pumps (FIG. 6b) clearly demonstrate the benefits of prolonged exposure, compared with standard bolus administrations. Sustained release leads to more robust germinal centre responses, higher antibody titres and targeting of a more diverse set of epitopes than bolus administration of the same vaccine[65]. However, many materials capable of achieving prolonged vaccine delivery and minimally invasive dosage have not yet been studied in depth in the context of vaccine delivery. Furthermore, many sustained delivery vehicles remain at the site of injection for a long time, and thus the impact of creating a local inflammatory niche often confounds individual effects on immune responses, especially because injection site interactions are poorly understood. Nevertheless, microneedle technologies (FIG. 6c) and various depot technologies based on hydrogels or self-assembled scaffolds (FIG. 6d) have been explored for sustained vaccine delivery and shown to elicit potent, high-quality and durable immune responses.

Microneedles. Microneedle patches have primarily been developed to deliver vaccines intradermally without the aim of prolonged cargo delivery. However, several degradable polymer microneedle systems have been designed to remain at the site of administration and degrade slowly to prolong vaccine exposure[207] (FIG. 6c). Microneedles for extended cargo release have been prepared with a range of polymers, including chitosan[208], silk[209] and PLGA[207,210]. Indeed, extended intradermal vaccine exposure leads to more potent humoral and cellular immune responses, compared with intradermal bolus injections of the same vaccines[209]. For example, microneedles made of silk fibroin protein remain implanted in the skin after administration[72,209] and can provide sustained release of an HIV vaccine comprising BG505 SOSIP trimer antigen for more than 2 weeks in mice. However, the strategy of encapsulating cargo in polymer matrices is limited by cargo size; that is, cargo size influences the release rate and, therefore, microneedles cannot match the release kinetics of antigens and adjuvants of different molecular sizes[72] (FIG. 6c). However, prolonged antigen release by silk fibroin microneedles has been shown to have a

greater impact on vaccine responses than the kinetics of adjuvant release[72]. This observation may be specific to the particular adjuvant and antigen, and thus requires further investigation.

Depot technologies. Various sustained delivery technologies based on biodegradable polymers, aluminate or silicate particles and hydrogels have been developed for drug delivery applications[211]. Solid polymer microparticles, for example, made of biodegradable PLGA or polyanhydride polymers, can be used to encapsulate and slowly release subunit antigens over days to months[212,213]. However, despite their established safety and possibility of slow vaccine delivery, antigen degradation and/or aggregation during encapsulation have limited clinical translation of polymer nanoparticles thus far. Furthermore, although polyanhydrides degrade through surface erosion[214], the bulk erosion of PLGA typically results in a local pH drop, which can detrimentally impact entrapped antigen stability.

Cargo delivery kinetics from hydrogels can be defined by cargo diffusion through a static mesh; here, a small mesh size slows the diffusion and prolongs release[10] (FIG. 6d). A mesh size smaller than the cargo leads to

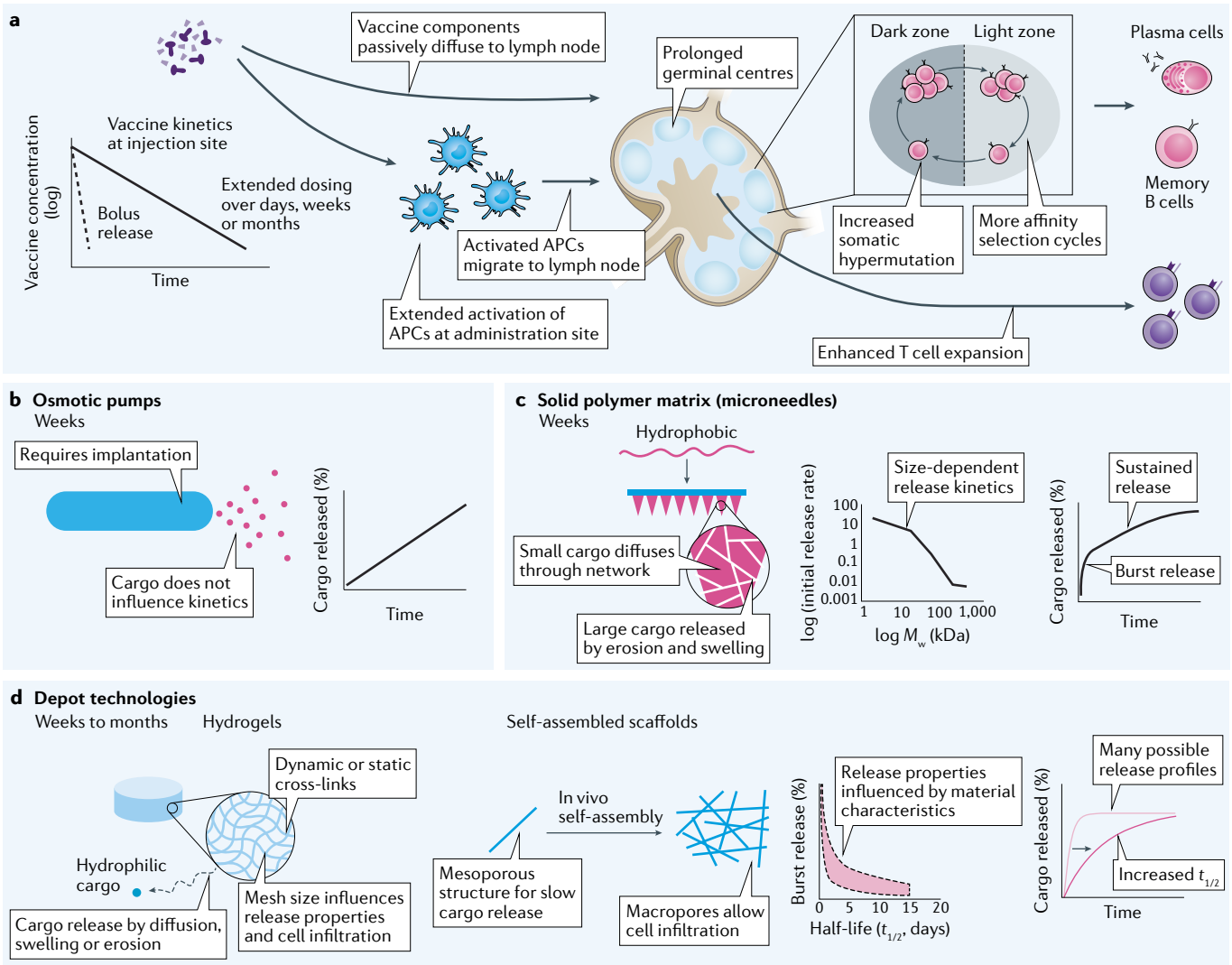


Fig. 6 | **Enhancing the vaccine response by sustained release of vaccines.** Materials can be engineered to control the temporal dynamics of vaccine exposure to the immune system. **a** | Biomaterials encapsulating antigen and adjuvant can be designed with extended release kinetics after administration. Prolonged release allows the vaccine cargo to enter lymph nodes through the lymphatics and extends activation of antigen-presenting cells (APCs) at the administration site. Activated APCs process antigen and migrate to the draining lymph nodes. Prolonged presence of vaccine components extends germinal centre reactions, which facilitate more rounds of affinity selection and somatic hypermutation, ultimately leading to a higher-quality antibody response. **b** | Osmotic pump technology enables extended vaccine release, but requires surgical implantation. **c** | Solid polymer matrices, for example microneedle technologies, can provide sustained delivery of entrapped cargo over several weeks; however, the cargo release rate is highly dependent on the cargo's molecular size and physico-chemical properties[72], limiting the type of antigens and adjuvants that can be delivered. **d** | Depot technologies, such as hydrogels and self-assembled scaffolds, provide tunable cargo release, from days to months. Passive cargo diffusion in hydrogels can be controlled through modulation of the polymer mesh size[10]. Physically entrapped cargo, whereby the cargo is larger than the mesh size, can only be released through network degradation, swelling or dynamic rearrangement. Hydrogels constructed with dynamic or degradable cross-links enable cell infiltration and formation of an inflammatory niche[247]. Self-assembled scaffolds can provide sustained cargo delivery and are inherently macroporous, enabling cell infiltration and rapid formation of an inflammatory niche[136].

entrapment of the cargo, and thus release is dependent on bulk erosion, which can cause burst release[10]. Therefore, designing hydrogels for extended release over weeks and matched release kinetics for cargo of different molecular sizes or distinct physico-chemical properties remains challenging. Antigens and adjuvants can substantially differ from a molecular perspective, making prolonged co-release of vaccine components from hydrogels difficult. A physically cross-linked polymer–nanoparticle hydrogel enables co-delivery of a model vaccine containing differently sized ovalbumin and pIC over the course of weeks, which increases the magnitude and persistence of the humoral immune response, prolongs the germinal centre reaction and improves affinity of antibodies by more than 1,000-fold compared with the same vaccine delivered in a standard saline bolus[66]. The physically cross-linked network entraps both cargoes, thereby slowing their diffusion to a similar rate to the self-diffusion of the dynamic polymer network itself[66]. Alternatively, injectable self-assembling peptides or block copolymers also allow slow release of entrapped cargo; however, such systems have not yet been thoroughly evaluated in the context of slow vaccine delivery[215–217].

Many self-assembling materials that improve the formation of a local inflammatory niche also provide mechanisms for sustained delivery, which may contribute to enhanced humoral and cellular immune responses. For example, antigen and molecular adjuvants can be loaded into the nanoscale pores of self-assembling mesoporous silica rods, enabling prolonged delivery over the course of 10 days[136,141–143]. Similarly, antigen-displaying peptide nanofibre systems have been suggested to enable extended antigen availability[154,218].

Outlook

The vaccine response is directed by precise spatio-temporal cues, which can be provided by immunomodulatory materials to improve potency, durability and quality of vaccine responses. The physio-chemical properties of nanoparticles and antigen conjugates that are similar to pathogens in structure and size can improve uptake by innate cells and transport to lymph nodes. Depot technologies and microneedles enable modulation of the innate response at the injection site as well as sustained vaccine delivery. However, collaboration of immunologists and materials engineers will be crucial to move the field of immunoengineering forward and create truly transformational vaccine technologies.

The immune response to vaccination is based on the complex coordination of multiple cell types with diverse phenotypes across multiple tissues within the body over time. Biomaterial-based controlled delivery technologies can be applied to better understand the underlying spatio-temporal cues and to elucidate fundamental immunological mechanisms[219]. Combined with proteomic[220], transcriptomic[221] or genomic characterization techniques, such experiments will enable transformational insights into immunological mechanisms[222]. In particular, a more precise understanding of germinal centre biology will enable mechanism-based design of next-generation vaccines. Moreover, adjuvant studies indicate that many adjuvants increase antibody titres, but do not alter, and in some circumstances even impair, somatic hypermutation and affinity maturation[68,81]. By contrast, sustained vaccine exposure can result in a modest increase in antibody titre, compared with standard bolus administration, but a more than 1,000-fold increase in antigen-specific affinity[66]. Therefore, future studies must evaluate not only the magnitude and durability of antibody titres but also the stimulation of germinal centre responses and extent of somatic hypermutation and affinity maturation. However, assays for characterizing somatic hypermutation and affinity maturation are challenging and time and resource intensive, highlighting the need for more straightforward methods for longitudinal characterization of germinal centre reactions. For example, systemic levels of the chemokine CXCL13 strongly correlate with germinal centre activity[223], making it a potentially useful target. In addition, design criteria need to be developed to circumvent the immunodominance of non-neutralizing antigen epitopes, 'original antigenic sin' and antagonistic tolerance observed for some pathogens, for which robust vaccines have not yet been developed (for example, HIV, influenza and malaria).

The vaccine immune response is orchestrated by a distinct sequence of signals and cellular differentiation events requiring the correct stimulation of specific cells or collections of cells in the right place and at the right time, which can be addressed by precise spatio-temporally controlled delivery strategies. The ideal vaccine would maximize antigen processing at the injection site, allow for intact antigen to efficiently reach the lymph nodes at the correct concentrations, activate APCs to express the appropriate cytokines and surface proteins to guide a protective adaptive response, prolong the germinal centre reaction to enable affinity maturation, and lead to the differentiation of memory cell phenotypes to provide long-term protection. Therefore, there is tremendous potential to leverage controlled delivery technologies to precisely control each step of the immune response. Innate immune cell activation and antigen processing by APCs at the injection site, both of which can enhance vaccine responses, are rarely studied in the vaccine context. Injection site interactions can be harnessed, for example, using targeted adjuvants or by creating a transient inflammatory niche. Importantly, vaccine studies have mainly focused on adjuvants with similar physico-chemical properties thus far (for example, CpG and pIC, which are both nucleic acid polymers, or MPL and imidazoquinolines, which are both hydrophobic); however, new materials technologies can provide opportunities to evaluate novel, synergistic pairings[224,225]. Moreover, sustained delivery technologies enable prolonged vaccine exposure, which is required to enhance germinal centre reactions. Importantly, using engineered biomaterials for mucosal administration (for example, for size-dependent trans-epithelial transport) may achieve potent and persistent immunity at mucosal sites, which are often the target of infection.

The clinical translation of vaccines is further limited by a lack of adequate models and fundamental immunological differences between species (for example, PAMP

expression on innate immune cells)[226,227]. Mice and non-human primates are commonly used to understand the effects of bio-distribution and pharmacokinetics on vaccine efficacy, which cannot be probed in vitro using human cells[228,229]. Numerous vaccine studies in mice have led to successful therapies in humans; however, there have also been several high-profile vaccine failures in the clinic. For example, in 2008, an HIV vaccine candidate, which had shown protective efficacy in non-human primate challenge studies with the humanized simian immune deficiency virus[230], resulted in increased rates of HIV infection in individuals with prior immunity against the adenovirus 5 vector used in the vaccine[231]. Therefore, it is important to elucidate possible confounding factors in the translation of vaccine technologies early in the discovery–development pipeline[227].

Importantly, vaccines need to be globally distributed, and many vaccine formulations require multiple administrations to achieve long-term protection, which may be challenging to achieve in areas with poor health-care infrastructure and a shortage of health-care professionals. Immunomodulatory materials can be designed to enable single-administration vaccines and to achieve long-term memory without the need for booster shots. For example, injectable particles and microneedle patches can be designed to provide preprogrammed burst delivery of prime and boost doses at distinct time points to incorporate a traditional immunization regime into a single administration[232]. Furthermore, microneedle technology can be applied to deliver microparticle patterns into the skin, which can act as a discrete 'tattoo' for vaccination record-keeping. This tattoo is invisible to the eye, but identifiable using semi-automated machine learning and a modified smartphone[233].

Vaccination regimens that require multiple immunizations can further elicit anti-vehicle immune responses in addition to responses against antigens. For example, many protein-based systems and viral vectors elicit strong immune responses against the delivery vehicles themselves[234], limiting their application in vaccines that require numerous immunizations. Synthetic platforms for controlled delivery of vaccine cargo are usually not immunogenic, thus allowing repeated immunization with the same formulation (that is, homologous boosting). However, PEG, which is ubiquitous in drug delivery systems, can lead to the production of anti-PEG antibodies in animal models and humans[235]. Anti-PEG responses to a delivery vehicle may simply reduce the efficacy of subsequent immunizations, but could also cause hypersensitivity reactions, which can lead to anaphylactic shock[236]. Therefore, viable alternatives to PEG need to be developed[237].

Many encapsulation technologies can also stabilize vaccine cargo, thereby reducing the requirement for cold storage, which can restrict access to vaccines[238–241]; for example, the stringent storage requirements for the SARS-CoV-2 vaccines produced by BioNTech/Pfizer and Moderna (–70 °C and –20 °C, respectively)[242].

The COVID-19 pandemic has made the importance of plug-and-play technologies evident. Such technologies enable rapid manufacturing and global access[242], and are not only crucial for prevention of SARS-CoV-2 infections but also for future pandemics. The development of new vaccines will particularly benefit from the design of generalizable platforms that are amenable to a broad array of antigens and/or adjuvants. Therefore, it is important to study the nuances of material design for immune responses; for example, the rational design of a co-delivery platform, such as microneedles or injectable depot technologies, for a specific antigen would require a more detailed understanding of how different adjuvants (for example, a TLR agonist) alter the magnitude and 'flavour' of antibody responses[66,136]. Similarly, nanoparticle-based vaccine design could be improved by clarifying the impact of the valency of antigen presentation on antibody affinity maturation and the breadth of antibody responses[195]. Finally, translational materials must be easy and inexpensive to manufacture at scale to enable urgent responses to global demand[243]. At every stage of the design process, it is important to consider whether controlled delivery technology could be simplified without sacrificing efficacy and safety[218,244].

We hope that this Review offers immunologists and biomaterial engineers insight into the immunological mechanisms that build the foundation of a strong and durable immune response, and material technologies that can be used to better control those mechanisms.

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G.A.R. and E.A.A. are listed as inventors on patent application describing sustained vaccine delivery (WO/2020/072495). The other authors declare no competing interests.

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