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Review article

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Targeted modulation of immune cells and tissues using engineered biomaterials

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Abstract

Therapies modulating the immune system offer the prospect of treating a wide range of conditions including infectious diseases, cancer and autoimmunity. Biomaterials can promote specific targeting of immune cell subsets in peripheral or lymphoid tissues and modulate the dosage, timing and location of stimulation, thereby improving the safety and efficacy of vaccines and immunotherapies. Here, we review recent advances in biomaterials-based strategies, focusing on targeting of lymphoid tissues, circulating leukocytes, tissue-resident immune cells and immune cells at disease sites. These approaches can improve the potency and efficacy of immunotherapies by promoting immunity or tolerance against different diseases.

Sections

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Key points

- In immunotherapy, choosing the right target cell, tissue and treatment duration is essential to ensure effective immunomodulation while avoiding toxicity.
- Biomaterial-mediated targeting of immune cells in lymph nodes improves the potency and efficacy of vaccines by promoting immunity or tolerance.
- Circulating migratory immune cells can be targeted to perform as living chaperones to carry therapeutics into tissues.
- Systemic administration or intratumoral injection of nanomaterials and therapeutic depots can selectively accumulate and target immune cells in tumours.
- Reducing biomaterial complexity is essential to facilitate clinical translation.

Introduction

Modulating the immune system is a pivotal treatment strategy of modern medicine. Of the top ten drugs (by sales in 2021), seven acted on the immune system, including, notably, the messenger RNA (mRNA)-based vaccines for COVID-19 (ref. [1]). Beyond its established role in vaccine development, immunomodulation has therapeutic potential for various conditions including autoimmunity and cancer, as well as inflammatory, fibrotic and infectious diseases. However, immunomodulation is a two-edged sword, as is most clearly demonstrated in the field of cancer immunotherapy, where despite inducing substantial anti-tumour immunity, systemic administration of many immunotherapy drugs has resulted in immune-related adverse events distal to tumour sites[2]. Therefore, delivering precise dosages of immunomodulatory drugs with spatiotemporal control to specific cells and tissues, while avoiding unwanted off-target stimulation, is essential to ensure a safe and effective immune response.

Targeting of immune cells can be achieved using traditional protein-engineering strategies, employing monoclonal antibodies, engineered binding proteins or recombinant native ligands for immune cell surface receptors. These approaches are at various stages of clinical development, with mono- and multi-specific antibodies being the most advanced[3–7]. However, the only cell surface protein that truly defines a lymphocyte as relevant to a particular disease is its antigen receptor, making targeting of disease-specific immune cells challenging. In addition, simply linking an antibody domain to an immunomodulatory drug is not always sufficient to achieve effective targeting, because the therapeutic payload can dominate the biodistribution of such fusions[8]. Engineered biomaterials can introduce additional functionality beyond simple binding to target cells by concentrating immunotherapy agents in specific tissue sites, controlling their release kinetics, and/or controlling their intracellular localization.

The immune system consists of well-defined regional control centres (lymphoid organs), important tissue-resident cell populations (especially at barrier tissues such as mucosal surfaces) and mobile cell populations that constantly recirculate through blood and tissues, providing challenges and opportunities as a therapeutic target (Fig. 1a). Here, we review the recent advances of biomaterials-enabled therapeutic strategies for in vivo targeted modulation of the immune system. We focus on approaches targeting immune cells and lymphoid organs, excluding direct targeting of pathogens such as viruses or bacteria, followed by a discussion on prospects and challenges for developments in this area.

Targeting lymphoid organs

Lymphoid organs coordinate the maturation and migration of immune cells while organizing and regulating immune responses (Fig. 1a). Primary lymphoid organs in adults include the bone marrow and thymus, which serve as niches for lymphocyte development. Secondary lymphoid organs — which include 600–800 lymph nodes distributed across the body, the spleen and the mucosa-associated lymphoid tissue — house and organize T cells, B cells and antigen-presenting cells (APCs). These organs serve as command centres of adaptive immunity where activation of naive B and T lymphocytes occurs (Fig. 1a,b) and are thus natural targets for vaccines and immunotherapies.

Principles of lymph node targeting

Passive targeting of therapeutics to lymphatic vessels. Lymph nodes interface with peripheral tissues through lymphatic vessels, which drain lymph fluid from all tissues and provide a conduit for immune cell trafficking. Drugs and vaccines can be targeted to lymph nodes through lymphatic uptake following parenteral injection. A major factor influencing lymphatic targeting is the physical size of the injected agents: following injection into tissue (including tumours), particles larger than 50–100 nm in diameter become trapped in the extracellular matrix (ECM), whereas particles in the size range of 5–50 nm convect with lymph into the lymphatic vessels and flow to draining lymph nodes (dLNs). By contrast, particles smaller than 5 nm partition preferentially into the blood rather than the lymph[9,10] (Fig. 2a). These approximate size ranges depend on the tissue site of injection (which can vary in ECM and lymphatic composition) and factors such as injection volume and rate (which can cause mechanical expansion of the flaps mediating entry into lymphatic vessels), especially in small-animal models, where tissue volume relative to injection volume is much smaller. Furthermore, particle shape, charge and surface chemistry can also play a part by promoting or hindering convection through the tissue and lymphatic entry[11]. Notably, capture of particles at the downstream dLN is a less studied but equally important prerequisite for lymph node modulation, as evidenced by data showing that proteins[12] and small hydrophilic nanoparticles[13] can pass through the entire lymphatic chain, reach the thoracic duct and enter the systemic circulation following a parenteral injection. In this regard, using adjuvants that promote compound entry into lymph nodes[14], or designing carriers that stimulate recognition by macrophages lining the subcapsular and medullary sinuses, can prove useful[15].

Protein nanoparticles with surface-arrayed antigens and a size optimized for efficient lymphatic uptake enhance the immunogenicity of vaccines[16–20]. Protein nanoparticle vaccines are currently in clinical trials for human immunodeficiency virus (HIV), SARS-CoV-2 and influenza, and have been shown to be safe and elicit potent neutralizing antibody responses in humans[21,22] (Table 1). Lipid nanodiscs and block copolymer micelles smaller than 50 nm also efficiently traffic and deliver compounds into lymphatics[23,24]. Cage-like nanoparticles formed by the self-assembly of saponin and lipids (termed ISCOMs) are potent vaccine adjuvants of an ideal size (about 40 nm) for lymph node targeting, which received emergency use approval in the USA against SARS-CoV-2 (clinical trial EudraCT 2020-004123-16)[25]. Another strategy to promote lymphatic uptake is to exploit albumin and lipoproteins

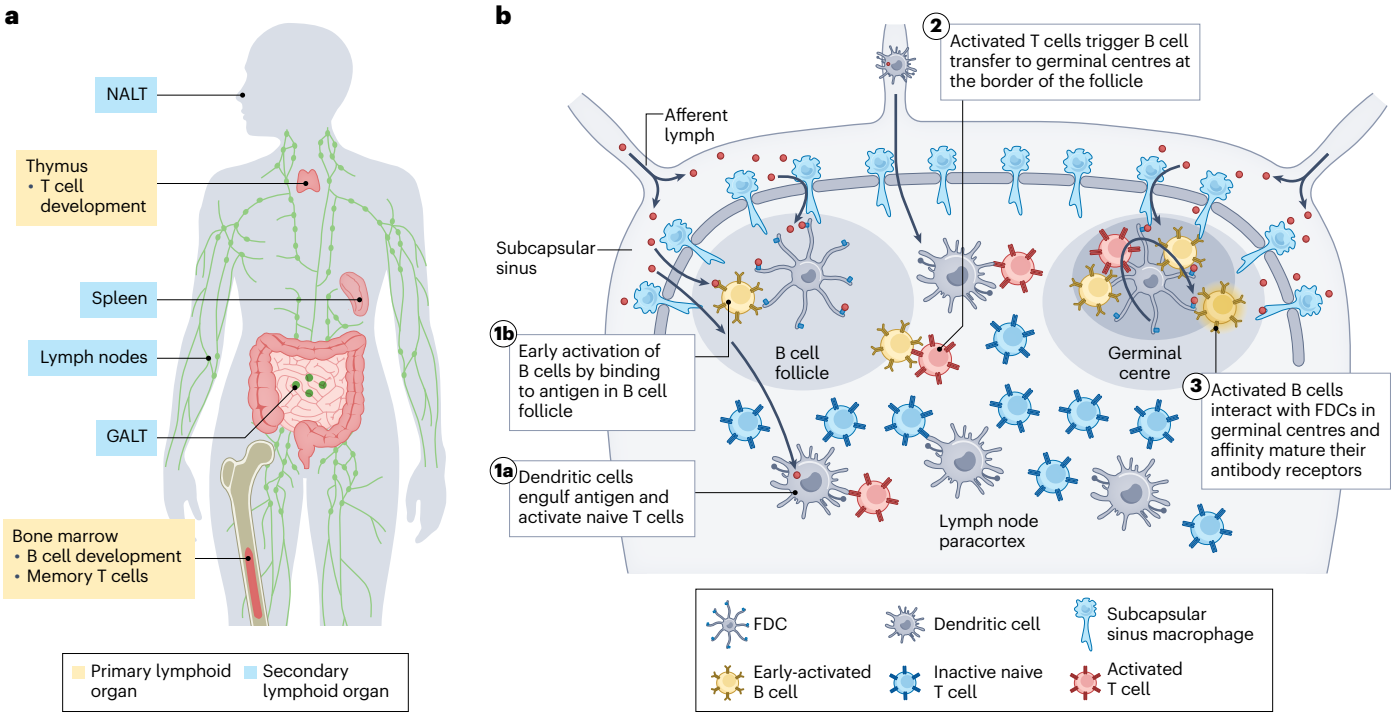

Fig. 1 | Primary and secondary lymphoid organs. a, Anatomical distribution of primary and secondary lymphoid organs. **b**, General organization of secondary lymphoid organs and sites of key interactions leading to adaptive immunity, using the lymph node as an example. Shown are orchestrated steps in the early activation of adaptive immune response in response to an antigen (orange). (1a) Dendritic cells (grey) acquire antigens trafficked into the lymph node or migrate to the lymph node from peripheral tissues carrying antigens, which they then present to naive T cells (blue) to drive T cell activation and proliferation. (1b) B cells (yellow) bind to antigens arriving in follicles, triggering initial B cell activation and proliferation. (2) Early-activated B cells receive help signals from activated $CD4^+$ T cells at the T zone–follicle border, providing signals to drive entry into germinal centres. (3) Activated B cells enter germinal centres where they undergo proliferation and somatic hypermutation to affinity mature their antibody receptor through interactions with follicular helper T cells and the antigens captured on the dendrites of follicular dendritic cells (FDCs). GALT, gut-associated lymphoid tissue; NALT, nasal-associated lymphoid tissue.

that naturally traffic from blood to lymph. Amphiphilic conjugates — consisting of peptides, proteins or small molecules linked to an albumin-binding lipid tail through a hydrophilic poly(ethylene glycol) (PEG) spacer (called amph-vaccines) — bind to endogenous albumin in the tissue following parenteral injection, improving lymph node targeting[26–29]. For example, conjugation of an albumin-binding lipid tail to a CpG oligonucleotide adjuvant resulted in a 12-fold increase in lymph node exposure following subcutaneous immunization in mice, compared with unmodified CpG[26]. This approach is currently being tested in the clinic for lymph node targeting of a cancer vaccine (NCT04853017; Table 1)[30]. Lymph node-targeting nanoparticles are also being used to deliver latency-reverting drugs in HIV cure strategies[31] and to induce immunological tolerance[32–35]. Nanoparticles composed of self-assembled proteins, synthetic bioresorbable polymers or lipid assemblies have different in vivo lifetimes and stabilities that can affect their functionality. For example, proteolysis of protein nanoparticle vaccines could lead to particle disassembly and loss of antigen multimerization. However, such effects have received limited attention to date.

Targeting lymph-node-bound migratory immune cells in peripheral tissues. Complementary to the strategy of direct lymphatic targeting, this approach leverages the natural process by which immune cells, including dendritic cells (DCs), monocytes and neutrophils, internalize antigens in peripheral tissues and transport them to lymph nodes as part of their constitutive immune surveillance (Fig. 2b). For example, subcutaneously implanted porous polymer scaffolds releasing the cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF) attract and differentiate monocytes into the DC lineage. Molecular adjuvants and tumour antigens carried by these scaffolds are taken up by the recruited cells and transported to dLNs[36,37] (Fig. 2b). This approach improved anti-tumour immunity, eliciting approximately 50% complete responses in an aggressive mouse model of melanoma and leading to a first-in-humans clinical trial of this technology (NCT01753089; Table 1). Data from this trial are not yet published, but should provide important insights into the safety of generating a strong localized inflammatory reaction at the implant site, an approach that could be extended to applications beyond cancer. To avoid implantation, injectable polymeric hydrogels[38] or suspensions of biodegradable silica particles[39] have been employed, resulting in both cellular and humoral immune responses against cancer or microbial antigens[40]. Similarly, microparticles loaded with tumour lysates as antigen and adenosine triphosphate (ATP) as a chemotaxis-inducing 'find-me' signal promoted recruitment of DCs and increased levels of mature DCs migrating to tumour dLNs, resulting in reduced tumour growth[41].

DC-attracting biomaterials are also being developed to programme tolerance rather than immunity. Dual-sized poly(lactide-*co*-glycolide) (PLGA) particles consisting of small (0.5–2.5 μm) phagocytosable microparticles loaded with the tolerogenic metabolite vitamin D3 and

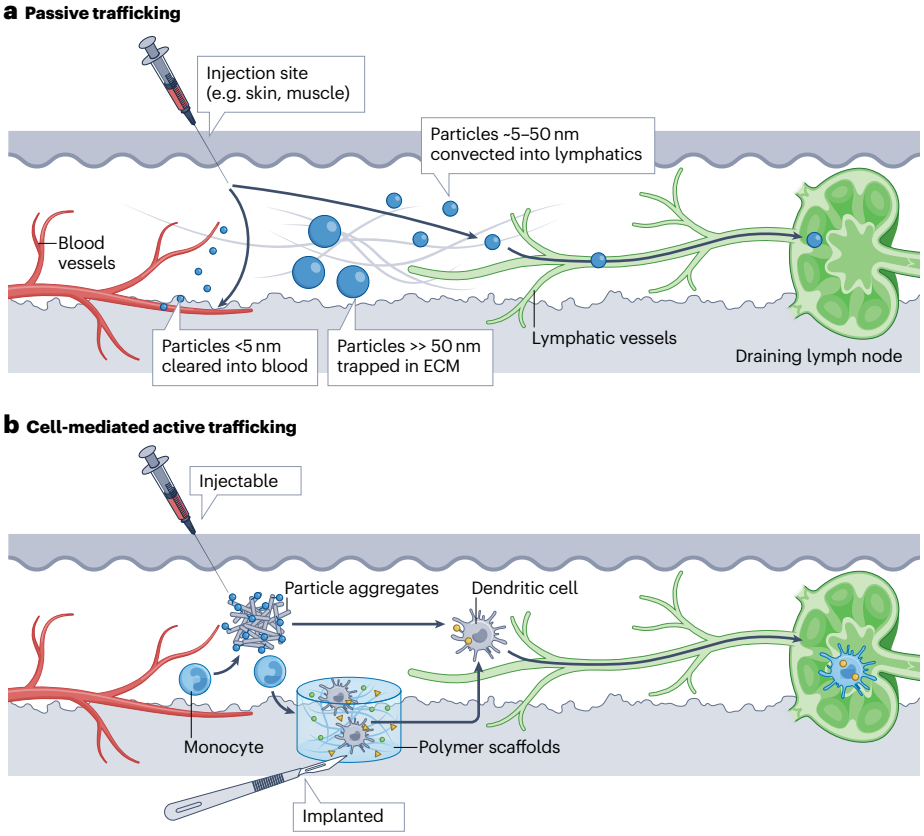

Fig. 2 | Targeting therapeutics to tissue-draining lymph nodes. a, Passive targeting of lymphatics through particle size. Particles smaller than 5 nm are preferentially cleared into the blood vasculature, whereas particles larger than 50 nm tend to become trapped in the tissue. Intermediate-sized particles (5–50 nm) exhibit preferential trafficking into lymphatic vessels. **b**, Targeting migratory leukocytes to traffic vaccines and therapeutics to draining lymph nodes. Shown are examples of injectable or implantable biomaterials that attract monocytes or dendritic cells from the local tissue and peripheral blood. The cells are then stimulated by the implanted material, triggering activation and differentiation into migratory antigen-presenting cells that carry antigens or other compounds to the draining lymph nodes. ECM, extracellular matrix.

autoantigens, combined with large (10–65 µm) non-phagocytosable microparticles designed to release extracellular transforming growth factor-β (TGFβ) and GM-CSF, prevented hyperglycaemia in a mouse model of type 1 diabetes[42] and induced antigen-specific tolerance in mouse models of multiple sclerosis and collagen-induced arthritis[43,44]. This example highlights the potential of DC-attracting approaches not only to modulate immunity, but also to programme tolerance.

Targeting niches within lymph nodes

Organization of lymph nodes. Lymph nodes are highly organized organs with defined compartments enriched in different immune cell subsets. Afferent lymph enters the subcapsular sinus (SCS) of the lymph node, a fluid space between the lymph node capsule and the lymph node parenchyma, lined by lymphatic endothelial cells and macrophages (Fig. 1b). Within the parenchyma, many of the key cell populations regulating adaptive immunity have specific niches; B cells and follicular dendritic cells (FDCs) reside in follicles arrayed around the exterior of the node, whereas T cells are localized deeper in the lymph node paracortex[45,46] (Fig. 1b). This physical segregation has an important role in orchestrating sequential steps in the immune response, so targeting distinct niches in the lymph node could offer valuable therapeutic opportunities.

Entry into lymph nodes. Under physiological conditions, substances in the lymph can pass into the parenchyma through narrow collagen conduits, or be captured and transferred into the lymph node interior by SCS macrophages[47–49] (Fig. 1b). The conduits exclude globular proteins larger than approximately 70 kDa (refs. [49,50]), although evidence suggests that this size limit can change in the presence of a live infection[51]. Vaccine adjuvants such as oil-in-water nanoemulsions and saponin nanoparticles have been used to boost entry of compounds into lymph nodes by inducing rapid death of SCS macrophages[14,52,53] (Fig. 3a). Similarly, liposomal formulations of clodronate, which is widely used to deplete SCS macrophages, improve antigen entry into lymph nodes[54]. In a two-stage delivery strategy, CpG oligonucleotides were coupled to approximately 30-nm poly(propylene sulfide) (PPS) nanoparticles through cleavable oxanorbornadiene linkers and then injected intradermally in a murine model of lymphoma[55]. The nanoparticles trafficked from the injection site to dLNs, but were trapped in the sinuses after 24 hours. By tuning the chemistry of the oxanorbornadiene linker, the CpG adjuvant payload remained bound to the nanoparticles during trafficking to the dLN, and were later released deep into the lymph node parenchyma (Fig. 3b).

Targeting specific cells and niches within the lymph node. To facilitate intra-nodal delivery, specific cell populations can be targeted within the lymph node parenchyma, in particular DCs and macrophages. For example, oral or systemic administration of rapamycin promotes tolerance by acting on DCs and myeloid cells (for example, in organ transplants), though with considerable systemic side effects[56]. Subcutaneous injection of rapamycin encapsulated in PEG-b-PPS polymer vesicles (about 100 nm) targeted the lymph nodes in a diabetic mouse model, where it was preferentially phagocytosed by DCs and other myeloid cells and induced a regulatory DC phenotype

enabling allogeneic pancreatic islet transplants to be maintained over 100 days, without side effects[57]. To drive antigen-specific tolerance, lymph node DCs were targeted using liposomes co-delivering a tolerance-driving aryl hydrocarbon receptor agonist drug together with a multiple sclerosis-related peptide in a murine multiple sclerosis model[58]. Cancer neoantigens linked to hydrophobic peptides bearing Toll-like receptor 7 (TLR7) agonists were developed to self-assemble into approximately 20-nm micelles, which efficiently trafficked from subcutaneous injection sites to dLNs, achieving uptake in 80% of lymph node DCs, and leading to anti-tumour immunity in multiple murine tumour models[59]. DC targeting has also been achieved by targeting specific receptors expressed by these cells. Following intradermal vaccination, polymer chains functionalized with antigens, TLR7 agonists and mannose moieties to target mannose receptor-positive DCs improved uptake of antigens in lymph nodes (Fig. 3c), generating antigen-specific $CD8^+$ T cell responses and increasing serum antibody concentrations (1.7- and 3.6-fold compared with the potent alternative adjuvant formulations AS01EL and poly(I:C), respectively)[60].

Table 1 | Ongoing clinical trials of immune cell- and tissue-targeted biomaterials therapeutics

Immune cell and target site	Technology	Phase	Condition or disease	Clinical trial identifier	Refs.
Migratory APCs	Polymer scaffolds carrying tumour antigen, GM-CSF and CpG as a cancer vaccine	I	Stage IV melanoma	NCT01753089	36,37
B cell follicles in lymph nodes	eOD-GT8 60mer protein nanoparticle vaccine	I	HIV and AIDS	NCT03547245	17,67,69
B cell follicles in lymph nodes	Influenza haemagglutinin ferritin nanoparticle vaccine	I	Influenza	NCT03186781	21
Lymph nodes	GBP510 SARS-CoV-2 designed protein nanoparticle vaccine	I/II, III	COVID-19	NCT04750343, NCT05007951	22
Lymph nodes	Amph-CpG7909 combined with amph-KRAS peptide antigen cancer vaccine	I	KRAS-mutated PDAC and other solid-tumour cancers	NCT04853017	26–28,30
Lymph nodes	Matrix M adjuvant in Novavax COVID-19 vaccine	Approved	COVID-19	NCT05463068, NCT05468736, NCT05112848	25
Systemic APCs	Lipid nanoparticle vaccine encapsulating mRNA encoding the viral oncogenes E6 and E7	II	Head and neck cancer	NCT04534205	78
Systemic APCs	Lipid nanoparticle vaccine encapsulating mRNA encoding the viral oncogenes E6 and E7	I, II	Advanced $HPV16^+$ cancer (head and neck, anogenital, penile or cervical)	NCT03418480	78
Systemic APCs	Liposomal RNA vaccine	I	Melanoma	NCT02410733	77,79
Systemic APCs	PLGA nanoparticles vaccine carrying NY-ESO-1 antigen and IMM60 invariant NK T cell activator	I	NY-ESO-1^+ tumours	NCT04751786	81
Spleen and liver APCs	Rapamycin-encapsulating nanoparticles	III	Chronic gout	NCT04596540	83
Splenic B cells	Liposomes carrying iNK T cell ligand	I	Treatment for graft-versus-host disease post allogeneic stem cell transplant	NCT04014790, NCT01379209	88–90
Adoptively transferred T cells	IL-15 nanogels backpacked on T cells	I	Selected solid tumours, lymphoma	NCT03815682	106
$TLR9^+$ myeloid cells	TLR9 agonist-presenting spherical nucleic acids	Ib, II	Advanced solid tumours	NCT03684785	181,182
Intratumoral lymphocytes	Lipid nanoparticles encapsulating mRNAs encoding IL-12	I	Solid tumours	NCT03946800	230
Intratumoral lymphocytes	Saline-formulated mRNA cocktail encoding single-chain IL-12, interferon-α2b, GM-CSF and IL-15 superagonist	I	Metastatic neoplasm	NCT03871348	196
Intratumoral lymphocytes	Lipid nanoparticles encapsulating mRNAs encoding OX40L, IL-23 and IL-36γ	I	Selected solid tumours, lymphoma	NCT03739931	195,198
Intratumoral lymphocytes	Lipid nanoparticles encapsulating mRNA encoding OX40L	I/II	Relapsed and refractory solid tumours, lymphoma and ovarian cancer	NCT03323398	195

AIDS, acquired immunodeficiency syndrome; amph, amphiphilic; APC, antigen-presenting cell; CpG, unmethylated cytosine–guanine dinucleotide; GM-CSF, granulocyte–macrophage colony-stimulating factor; HIV, human immunodeficiency virus; HPV, human papillomavirus; NK, natural killer; NY-ESO-1, New York oesophageal squamous cell carcinoma 1; PDAC, pancreatic ductal adenocarcinoma; PLGA, poly(lactide-co-glycolide); SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TLR, toll-like receptor.

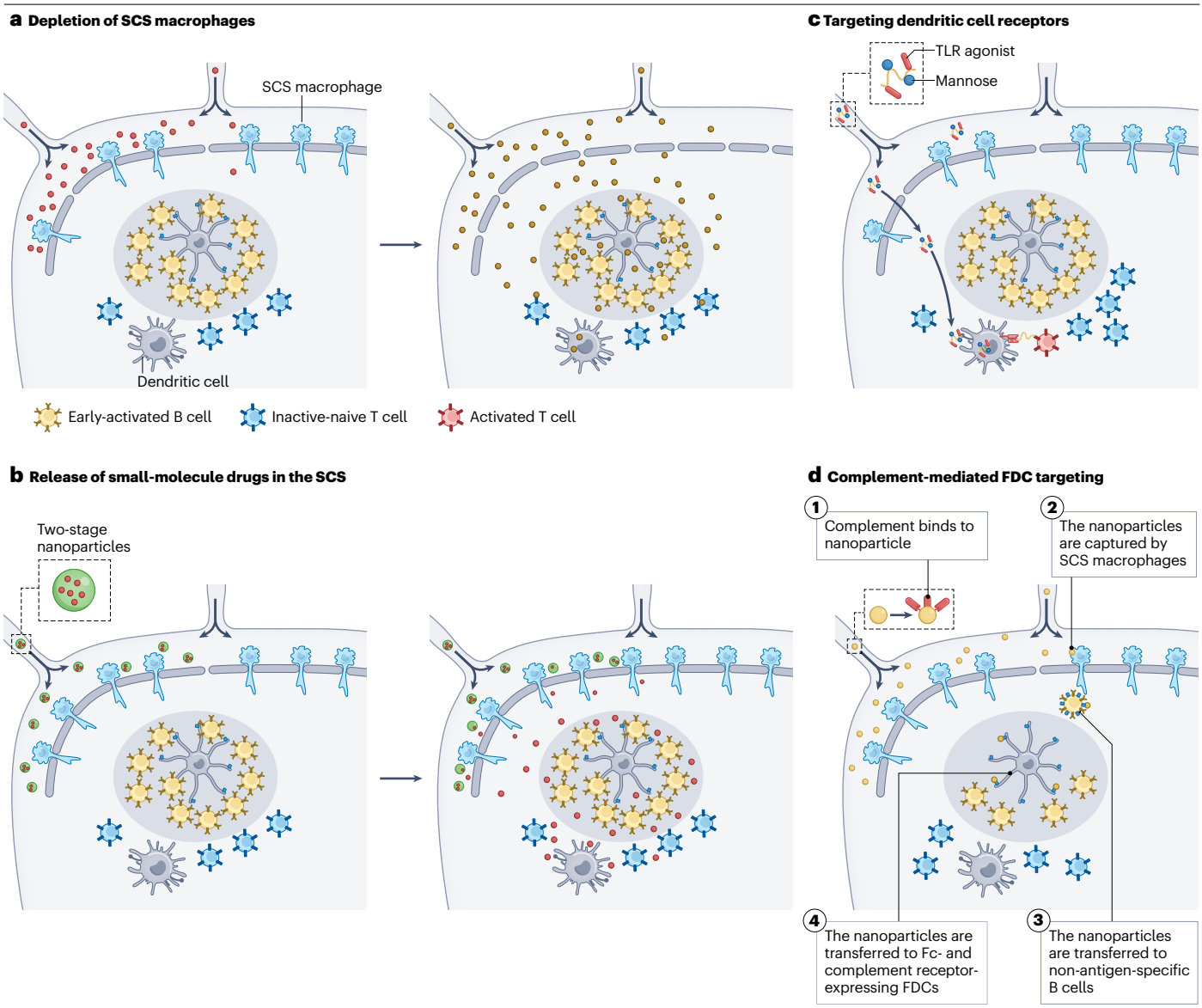
a Depletion of SCS macrophages
SCS macrophage
Dendritic cell
c Targeting dendritic cell receptors
TLR agonist
Mannose
Early-activated B cell
Inactive-naive T cell
Activated T cell
b Release of small-molecule drugs in the SCS
Two-stage nanoparticles
d Complement-mediated FDC targeting
1 Complement binds to nanoparticle
2 The nanoparticles are captured by SCS macrophages
4 The nanoparticles are transferred to Fc- and complement receptor-expressing FDCs
3 The nanoparticles are transferred to non-antigen-specific B cells
e
Endogenous albumin
CAR ligand
PEG spacer
CAR-ligand amphiphile
1 CAR-ligand amphiphiles bind to endogenous albumin and are trafficked to the lymph node
Lymph node
2 CAR-ligand amphiphiles are inserted into the plasma membrane of macrophages and dendritic cells
3 Ligand-decorated dendritic cells co-stimulate CAR T cells with ligands and cytokines
CAR-ligand amphiphiles
Cytokines
Costimulation
CAR T cell
Costimulatory receptor
CARs
Adhesion receptor

Fig. 3 | Targeting lymph node-resident cells and lymph node subregions. **a**, Promoting therapeutic entry into lymph nodes through depletion of subcapsular sinus (SCS) macrophages. Vaccine adjuvants (red) induce rapid death of SCS macrophages, leading to enhanced entry of antigens and other compounds (gold) into lymph nodes. **b**, Polymer nanoparticles (green) efficiently traffic into lymphatic vessels (20–30 nm) but are too large to efficiently penetrate the lymph node paracortex. They chemically release small-molecule payloads (red) that rapidly permeate throughout the node. **c**, Soluble polymers and polymer nanoparticles conjugated with ligands for receptors expressed by specific target cell types (such as dendritic cells) are taken up by antigen-presenting cells in lymph nodes and co-deliver vaccine antigens or dendritic cell-activating compounds (for example, TLR agonists). **d**, (1) Nanoparticles activate complement, (2) are captured by SCS macrophages (3) and transferred to non-antigen-specific B cells through complement receptors. (4) These nanoparticles are then transferred to follicular dendritic cells (FDCs), which express high levels of complement and fragment crystallizable (Fc) receptors. **e**, (1) Ligands for chimeric antigen receptor (CAR) T cells linked to albumin-binding poly(ethylene glycol) (PEG)-lipid moieties traffic from an injection site and bind to endogenous albumin. These compounds are then transferred to lymph nodes (2) where they are inserted into the plasma membrane of macrophages and dendritic cells. Subsequent encounter of CAR T cells with the ligand displayed on the surface of dendritic cells (3) will lead to CAR T cell tandem stimulation by natural costimulatory receptors and cytokine signals provided by the ligand-decorated dendritic cells.

These studies highlight the potential of co-delivering antigens and adjuvant compounds to the same cell through physical conjugation or encapsulation within the polymer carrier, a widely adopted strategy[59,61,62]. Moreover, the physical chemistry of antigen and adjuvant compounds, together with selective design of nanoparticle carriers to enable release into DCs upon arrival in the lymph nodes, are important to consider to ensure optimal T cell priming and functional polarization[62–64]. For example, autoimmune-related peptide antigens coupled to 10-nm quantum dots trafficked from subcutaneous injection sites to dLNs, where they accumulated in macrophage receptor with collagenous structure (MARCO)-positive macrophages[65]. MARCO$^+$ cells have been associated with immune tolerance and expanded regulatory T (T_{reg}) cells, contributing to disease control in a mouse model of multiple sclerosis[65]. Therefore, targeting these lymph node APC populations seems promising for promoting both immunity and tolerance. Of note, in this work, quantum dots were used owing to their bright fluorescent properties, but are known to release toxic metals over time[66]. In general, an important question to address with any polymer-based or nanoparticle-based DC-targeting strategy is whether the material is effectively captured in the dLN chain following injection, or whether it passes through the thoracic duct and enters the systemic circulation[13], raising potential toxicity concerns, in particular for potent immunostimulants.

B cell follicles are another important niche regulating humoral immune responses. Nanoparticles engineered to activate the complement system can target antigens or therapeutics to B cell follicles. For example, nanoparticles bearing a high density of mannose-containing glycans are recognized by the innate immune protein mannose-binding lectin, which triggers complement deposition on the particles. Upon arrival to the lymph nodes, SCS macrophages transfer complement-decorated particles to migratory B cells in the lymph node parenchyma, which in turn pass the particle to FDCs[53,67] (Fig. 3d). For vaccines, this strategy is glycan density dependent, amplifies the germinal centre and serum antibody responses and can be engineered into synthetic particles to promote follicle localization[67,68]. A protein nanoparticle vaccine that triggers this FDC targeting process completed a phase I trial (NCT03547245) and demonstrated 97% efficacy in initiating broadly neutralizing antibody B cell lineages in humans[69]. In principle, particles triggering complement activation through natural immunoglobulin M (IgM) or the alternative pathway should also be capable of such FDC localization. However, this trafficking is size-dependent; complement-decorated particles smaller than 15 nm were internalized and cleared by FDCs, whereas particles 50 nm or larger were retained on FDC dendrites for several weeks, resulting in improved antibody responses[70]. An important parameter to consider when designing carriers targeting B cells is that rigid particles carrying multivalent copies of an antigen are more effective than flexible polymers in triggering B cell activation[71].

T cells are another key cell type in lymph nodes, located in the paracortex and interfollicular regions (Fig. 1b). Polymer nanoparticles coated with DC-derived plasma membrane fragments and conjugated with anti-CD3 antibodies showed efficient accumulation in dLNs, activated and expanded CD8$^+$ T cells, and, in combination with anti-PD1 therapy elicited anti-tumour immunity[72]. To provide vaccine-like stimulation of chimeric antigen receptor (CAR) T cells in lymph nodes, albumin-binding 'amph-vaccine' molecules were designed carrying CAR ligands. The lipid tails of these conjugates inserted into cell membranes of DCs and macrophages in the dLN, enabling activation of CAR T cells in the lymph node and improving anti-tumour activity[73] (Fig. 3e).

Targeting lymph nodes, spleen and liver

Unlike live infections, current vaccines fail to elicit sufficient CD8$^+$ T cell responses in humans, which has motivated the field to explore new ways to prime cellular immunity[74]. Vaccines usually act locally on a chain of lymph nodes draining the injection site, such as a muscle[75]. However, this means that reacting immune cells are confined to a few lymph nodes, resulting in competition for limited resources (for example, cytokines or antigens), which, theoretically, could restrain the eventual systemic immune response. One hypothesis is that systemic administration of antigens to many lymph nodes could alleviate such resource constraints and enable more potent immune priming. A key consideration is that adjuvant and antigen signals need to be delivered together to ensure that immunity, rather than tolerance, is elicited. Moreover, intravenously administered materials are rapidly opsonized and captured by macrophages in the liver, spleen and bone marrow, in particular particles larger than 100 nm. By contrast, particles smaller than 5 nm are rapidly cleared through the kidneys[76]. Therefore, to systemically target lymphoid organs, opsonization-resistant (for example, PEGylated) particles in the range of 5–100 nm would be ideal, although this size dependency could be altered if circulating immune cells take up the particles and actively transport them into systemic lymph nodes. For example, intravenous administration of negatively charged interferon-inducing lipid nanoparticles carrying antigen-encoding mRNA efficiently targeted DCs in the spleen and peripheral lymph nodes as well as myeloid cells in the liver and bone marrow, resulting in a substantial antigen-specific T cell response (30–60% of the total CD8$^+$ T cell compartment), and consequent tumour rejection in aggressive murine tumour models[77] (Fig. 4). These findings spurred ongoing clinical trials of mRNA lipid nanoparticles in

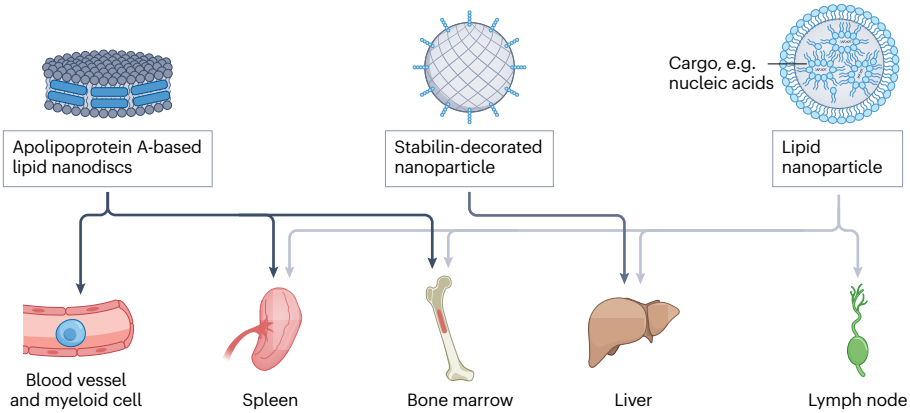

Fig. 4 | Targeting systemic lymphoid organs. Nanoparticle carriers can target lymphoid organs systemically, including lymph nodes, spleen, bone marrow and tolerogenic antigen-presenting cells in the liver. For example, apolipoprotein A-based lipid nanodiscs are preferentially taken up by myeloid cells in the liver, spleen and bone marrow. Stabilin-functionalized nanoparticles are efficiently scavenged by endothelial cells in the liver. Anionic lipid nanoparticles target antigen-presenting cells in the liver, spleen, lymph nodes and bone marrow.

multiple cancers, with promising interim results including objective response rates in 6 out of 17 patients with advanced melanoma[78,79] (NCT04534205, NCT03418480 and NCT02410733; Table 1). Similarly, intravenous vaccination with self-assembled polymer nanoparticles carrying cancer neoantigen peptides and small-molecule TLR7/8 agonists primed a larger TCF1$^+$ stem-like antigen-specific T cell population compared with parenteral vaccination with the same material, leading to improved anti-tumour immunity[80]. These examples highlight the potential beneficial effects of systemic immunization on the immune response. Following these principles, a clinical trial in the Netherlands is currently evaluating intravenously administered PLGA-based nanoparticles, delivering a tumour antigen together with a small molecule as innate immune activator designed to promote DC activation through invariant natural killer (NK) T cells (NCT04751786, Table 1)[81].

APC populations in the liver and spleen are known to have important roles in systemic tolerance, and are therefore being targeted for tolerance and autoimmunity. For example, intravenously administered biodegradable PLGA nanoparticles carrying rapamycin were efficiently captured by DCs in the spleen, and elicit a T_{reg} cell-generating, tolerizing phenotype in these cells that blocked generation of cellular and humoral responses against co-administered antigens[82]. Following this approach, a clinical trial aimed at blocking anti-drug antibody responses against recombinant uricase in gout patients demonstrated promising efficacy and safety data in humans (NCT02648269)[83]. Similarly, intravenous administration of biodegradable polymer nanoparticles coupled to autoantigens for tolerance induction led to efficient uptake in tolerance-promoting MARCO$^+$ APCs in the spleen and liver, fostering protection in mouse models of diabetes and other autoimmune conditions[84,85]. Subcutaneously injected diabetes autoantigens coupled to hyaluronic acid polymers were delivered to both disseminated lymph nodes and spleen, promoting systemic expansion of CD4$^+$ T cells with an immunosuppressive phenotype[86]. Intravenous injection of biodegradable PLGA nanoparticles loaded with a model antigen and functionalized with stabilin to target scavenger receptors expressed by liver sinusoidal endothelial cells resulted in an almost exclusive accumulation in the liver, providing protection from airway-induced allergic inflammation[87] (Fig. 4). As a final example, liposomes were administered intraperitoneally to deliver α-galactosylceramide to marginal zone B cells in the spleen. Presentation of the ligand to invariant NK T cells promoted the development of T_{reg} cells and tolerogenic DCs in the spleen of mice[88]. This treatment suppressed graft-versus-host disease following bone marrow transplant[89] and is now moving into early-stage clinical trials (NCT04014790 and NCT01379209; Table 1)[90]. In these examples, different materials were used to target antigens and stimuli to relevant immune cells. An important but understudied parameter is how the duration of cargo release in recipient target cells affects their tolerizing effect. For example, loaded PLGA particles could be engineered to release antigens over weeks, whereas fast-degrading surface-conjugated particles or liposomes could provide rapid release following endocytosis. Whether this kinetics will have a substantial impact remains unknown.

Targeting immune cells in bone marrow

The bone marrow is a primary lymphoid organ for haematopoiesis and myelopoiesis, as well as a site housing a large pool of memory T cells[91] (Fig. 1a). Myeloid precursors, in particular, have received attention because they drive a process known as 'trained immunity'. This phenomenon occurs when innate immune cells or their progenitors receive certain microbial stimuli (including Bacillus Calmette–Guérin bacteria, *Candida albicans* yeast, β-glucan or peptidoglycan[92]) that drive epigenetic changes rewiring their metabolic and functional state[93,94]. When induced in progenitor cells, these epigenetic modifications are passed on to their progeny and confer a prolonged and elevated state of responsiveness to microbial stimuli in monocytes, macrophages and NK cells. Therapeutic induction (or suppression) of trained immunity is now being considered as a promising approach to treat diverse diseases[92]. For example, intravenous injection of apolipoprotein-A1-based lipid nanodiscs carrying the minimal peptidoglycan muramyl dipeptide preferentially accumulated in myeloid cells and their precursors in blood, spleen and tumours in a mouse model of melanoma, resulting in enhanced cytokine production and increased efficacy of checkpoint blockade therapy[95] (Fig. 4). Such innate immune modulation could be a powerful complement to therapeutic strategies focused on boosting adaptive immunity.

Targeting circulating leukocytes

Many immune cells continuously recirculate between secondary lymphoid organs, blood and tissues, patrolling for foreign antigens. The ability of immune cells in blood to home in on disease sites forms the basis of adoptive cell therapies, where engineered immune cells are infused intravenously into patients and subsequently arrive at targeted distal sites. These cells can be targeted while in the blood to serve as chaperones to transport drugs into disease sites.

‘Backpacking’ cells

Adoptive cell therapies based on the injection of autologous cells engineered to have disease-specific functions is a steadily expanding approach for immunotherapy[96,97]. To date, the most clinically successful example is CAR T cell therapy, where lymphocytes isolated from cancer patients are transduced to express a CAR that enables recognition and selective killing of tumour cells. CAR T cells are then expanded ex vivo and re-infused into the patient[98]. So far, six different CAR T cell products have been approved for haematological malignancies, with several ongoing clinical trials to extend CAR T therapy to solid tumours and infectious diseases[99–101]. Adoptive cell therapies are also being developed using natural or transgenic T cell receptor (TCR)-expressing T cells, NK cells, macrophages, T_{reg} cells (for autoimmune disease and transplant tolerance) and other immune cell populations[96]. Adoptively transferred cells benefit from supporting stimulation to maintain their function or promote their expansion and survival; however, as with other immunomodulators, systemic co-administration of supporting drugs can be toxic owing to non-specific effects on endogenous immune cells.

To provide sustained and localized stimulation, therapeutic carriers (‘backpacks’) have been directly attached to the surface of donor cells prior to infusion (Fig. 5a). This strategy has been used to load tumour-specific T cells and T_{reg} cells with cytokines, small-molecule drugs or chemotherapeutic agents[102–105]. Early studies showed that lipid-nanoparticle backpacks carrying cytokines such as IL-15 remained on the cell surface for extended periods, inducing potent autocrine stimulation and proliferation of adopted antigen-specific T cells in vivo[102,105]. Backpacking protein nanogels onto T cells enabled a more controlled release of supporting cytokines in response to altered cell surface reducing activity after TCR or CAR stimulation in mice[106]. This strategy restricted donor cell stimulation to tumours and tumour-dLNs (where the antigen is encountered), improving therapeutic efficacy and

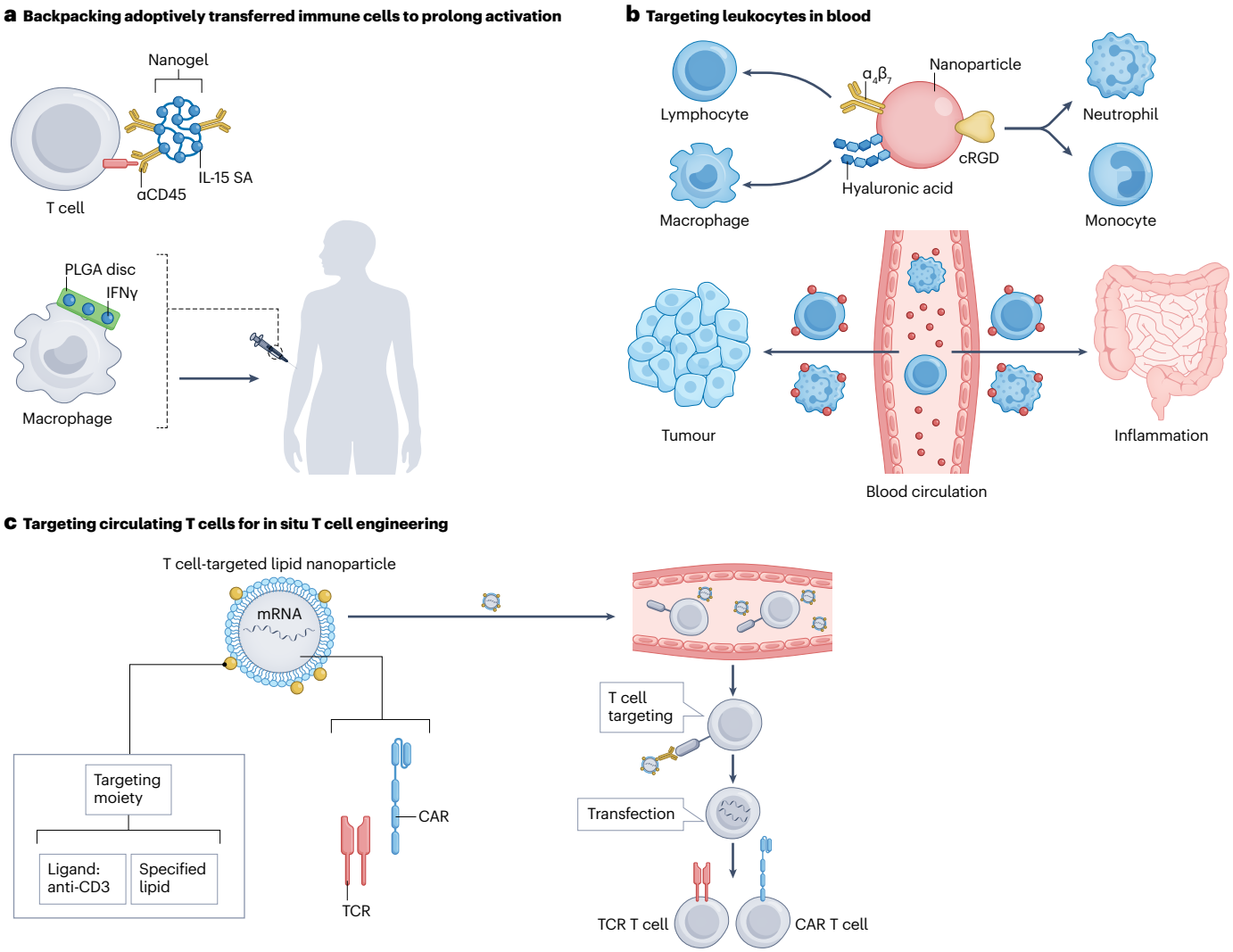

Fig. 5 | Targeting circulating immune cells. a, ‘Backpacking’ cells by attaching drug-releasing materials to cells ex vivo prior to adoptive transfer. **b**, Nanoparticles functionalized with targeting moieties can bind to circulating immune cells in blood, which then traffic into different tissue sites with the drug carrier. **c**, T cell-targeted nanoparticles deliver nucleic acid payloads (DNA or RNA) to circulating lymphocytes, resulting in the expression of chimeric antigen receptors (CARs) and other immunomodulatory gene payloads in situ. cRGD, cyclic arginine–glycine–aspartic acid; IFN, interferon; IL-15 SA, interleukin-15 super agonist; PLGA, poly(lactide-*co*-glycolide); TCR, T cell receptor.

safety[106]. Interim results from a phase I clinical trial of this backpacking approach in cancer patients (NCT03815682; Table 1) showed that only one-tenth the amount of administered IL-15 was detectable in the blood despite backpacking three times the maximum tolerated dose of free systemic fragment crystallizable (Fc)-fused IL-15 (ref. [107]) onto T cells, leading to stable disease in 10 out of 17 patients[108]. A limitation of this approach is that the therapeutic payload is, by definition, finite, and will be diluted over time as T cells proliferate in vivo. However, it could still provide immediate autocrine stimulation following adoptive transfer, enabling cells to successfully engraft and initiate tumour rejection. Owing to the cytokine carrier being directly conjugated to the cell membrane, the concentration of newly released cytokines at the cell surface is substantial, and it has been estimated that the IL-15 nanogels will continue to stimulate T cells through at least seven cell divisions before stimulatory capacity is lost[106].

Backpacking approaches have also been developed for innate immune cells; discoid polymer particles carrying interferon-γ (IFNγ) adhered to the surface of macrophages without inducing phagocytosis, resulting in persistent polarization into an 'M1' phenotype that promoted tumour rejection in a murine breast cancer model[109]. Importantly, the use of a discoid morphology was crucial to avoiding phagocytosis of the backpack[110] (Fig. 5a).

Targeting immune cells in blood

Targeting circulating cells that traffic drugs into tissues. Targeting immune cells in the blood is an attractive strategy to circumvent physical barriers in tissues. For example, lymphocytes populating Peyer's patches and mesenteric lymph nodes express well-defined adhesion and chemokine receptors ($\alpha_4\beta_7$ integrin and CCR9, respectively[111,112]) that direct their homing to these sites (Fig. 5b). Intravenous administration of lipid-coated polymer nanoparticles loaded with an HIV antiretroviral drug and surface-functionalized with antibodies against $\alpha_4\beta_7$ promoted uptake in gut lamina propria cells, which transported the particles to the gut of mice[113]. Functionalizing small interfering RNA (siRNA)-loaded lipid nanoparticles with recombinant mucosal vascular addressin cell adhesion molecule 1 (MAdCAM1), the ligand recognized by the high-affinity conformation of $\alpha_4\beta_7$, increased the particles' specificity to gut-homing lymphocytes and thus resulted in silencing of IFNγ in a murine model of colitis[114].

Nanoparticles have also been used to target circulating immune cells that home into tumours or sites of inflammation. Intraperitoneally injected hyaluronic acid-polyethyleneimine hybrid nanoparticles carrying miR-125b (a microRNA known to induce an anti-tumour 'M1' phenotype in macrophages[115]), were taken up by these cells, which then migrated to lung tumours and repolarized tumour-associated macrophages towards an M1 phenotype. We note that choosing the correct administration route is important to avoid unexpected transduction of macrophages in the liver or bone marrow. Intravenous administration of liposomes or lipid nanoemulsions conjugated with arginine–glycine–aspartic acid (RGD) peptides (known to bind to α_v integrins, which are highly expressed by monocytes and neutrophils) led to rapid uptake by these cells in the blood, which subsequently carried the particles to tumours in mice[116] (Fig. 5b). The same approach enabled the delivery of neuroprotective drugs across the blood–brain barrier to sites of ischaemia in mice[117]. Thus, targeting of immune cells in the blood is a promising strategy to hitchhike drugs into disease sites.

Antigen-specific targeting of circulating T cells. Targeting of antigen-specific T cells in the blood has been pursued to enhance adoptive cell therapy and promote protective T cell responses. For example, in mouse models of autoimmunity, intravenous injection of iron oxide nanoparticles coated with disease-relevant peptide–major histocompatibility complexes (pMHC) induced expansion of $CD4^+$ or $CD8^+$ T cells with a regulatory phenotype[118,119]. Intravenously injected class II pMHC-displaying nanoparticles can further expand T_{reg} cells to control liver autoimmune diseases without systemically suppressing immunity in other organs[120]. For cancer applications, artificial APCs were generated by conjugating biodegradable PLGA-poly(β-amino-ester) (PBAE) microparticles with pMHC and anti-CD28 antibodies[121]. Intravenous administration of these particles expanded antigen-specific cytotoxic $CD8^+$ T cells in vivo, and, in combination with checkpoint blockade therapy, increased the median survival time by 31% compared with single checkpoint blockade therapy in a mouse model of melanoma[121]. The same strategy was used to develop tolerogenic particles that promoted the expansion of forkhead box P3 ($FOXP3^+$) T_{reg} cells, which, after a single intravenous injection, resulted in a 20% increase of these cells in lymph nodes compared with untreated mice[122].

Targeting circulating T cells for gene delivery in vivo. CAR T cell therapy is having a substantial clinical impact against haematological malignancies, but its patient-specific nature and the complex manufacturing process of transducing T cells ex vivo limits its widespread application[123]. A potential alternative is to genetically modify T cells in vivo. Engineered viral vectors are being developed for this purpose[124,125]; however, viral vectors raise concerns of strong immune reactions and toxicity[126–128]. Another option is to use synthetic nanoparticle gene-delivery vectors. Intravenously injected poly(β-amino ester) nanoparticles carrying transposon DNA encoding a CAR, which targets T cells through anti-CD3 antibody fragments, proved safe and effective in mouse models of leukaemia[129] (Fig. 5c). Similarly, in vivo mRNA delivery to T cells generated transient CAR T cells capable of eliminating fibrosis-promoting fibroblasts in models of heart failure[130]. We note that immune cells (including T cells) are notoriously resistant to conventional transfection methods, so it is important to identify more effective lipid–polymer compositions for nucleic acid delivery[131–133]. CAR T cells have also been produced in vivo using an implantable macroporous alginate scaffold, which provides a contact interface for T cells and retroviruses and facilitates vector-mediated CAR gene transfer[134]. Subcutaneous implantation of these scaffolds seeded with human peripheral blood mononuclear cells and CD19-encoding retroviral particles reduced ex vivo cell-processing times to a single day, compared to 2–4 weeks for conventional CAR T cells, while maintaining similar anti-tumour efficacy[134].

Targeting tissue-resident cells

Some innate and adaptive immune cell populations permanently reside in tissues and provide functions such as immediate immune defence at barrier tissues, local production of protective antibodies and tissue-resident immune memory. In addition, these cells infiltrate sites of tissue damage, inflammation and tumours, making them important therapeutic targets for disease treatment.

Delivery at mucosal barriers

Several immune cell populations reside at mucosal barriers, such as the skin, the airways, and the gastrointestinal and reproductive tracts. DCs serve as sentinels of pathogen entry by sampling foreign antigens, whereas tissue-resident memory lymphocytes and plasma cells provide frontline adaptive immune protection[135,136], and, meanwhile,

mucosa-associated lymphoid tissues, such as the Peyer's patches and nasal-associated lymphoid tissue (NALT), serve as secondary lymphoid organs that rapidly respond to antigens entering through the mucosae[137]. These tissues are important targets for vaccination, because antigens taken up in mucosa draining lymphoid tissues prime lymphocytes that home back to mucosal tissue sites[135]. Moreover, mucosa-resident immune cells can be preferential targets of infection (for example, gut-resident memory $CD4^+$ T cells in HIV infection), making them interesting therapeutic targets for viral latency-reverting drugs against HIV[31,138]. A key challenge in targeting immune cells at these sites is that mucosal barriers have evolved to block incoming pathogens and foreign materials efficiently[139,140].

One promising strategy for effective drug delivery across mucosal surfaces is to exploit a natural bidirectional transport pathway for crossing the epithelial barrier. Following endocytosis in epithelial cells, immunoglobulin G (IgG) and albumin bind to the neonatal fragment crystallizable receptor (FcRn) in endosomes, triggering transcytosis and release at the apical surface[141]. Fusing therapeutic proteins with Fc domains or albumin promotes uptake at the mucosal surface[142] (Fig. 6a). Similar to the backpacking approach, protein or peptide antigens linked to PEG-lipids (amph-vaccines) associate with albumin in the airway fluid and 'hitchhike' through the mucus across the epithelial barrier in an FcRn-dependent manner[28,143]. Peptide amph-vaccines that were administered into the lungs primed robust lung tissue-resident memory T cells that enhanced vaccine protection against respiratory viral or tumour challenge[28]. Alternatively, intranasal administration of protein amph-vaccines amplified germinal centre responses in the NALT, promoting higher mucosal antibody titres in both mice (100–1,000-fold increase) and non-human primates (10-fold increase), compared with unmodified proteins[143].

Biomaterials are also being developed to target immune cells at sites of mucosal inflammation. For example, conjugation of the hydrophilic ECM polymer hyaluronic acid with the natural gut antioxidant bilirubin led to the formation of nanoparticles 80–400 nm in size. After oral administration in mouse models of colitis, these nanoparticles were efficiently taken up at the inflamed epithelium by macrophages expressing the hyaluronic acid receptor CD44, leading to polarization of these cells toward an anti-inflammatory phenotype[144] (Fig. 6a). Interestingly, this treatment caused an increase in microbial diversity in the microbiome, which promoted epithelial healing and inflammation reduction.

Targeting immune cells in tumours

Systemic targeting of immune cells in tumours. Instead of targeting circulating immune cells destined to migrate into tumours, there is also interest in delivering drugs directly to tumour-resident immune cells. For many years, biomaterials scientists have sought to target tumours by exploiting the enhanced permeation and retention (EPR) effect, a phenomenon first described in animal models in the late 1980s in which large proteins or nanoparticles accumulate in tumours owing to their hyperpermeable vasculature and reduced lymphatic clearance[76] (Fig. 6b). However, the efficiency of EPR-based tumour targeting remains low and penetration of nanoparticles deep into tumours is often poor[145,146]. Therefore, new approaches are being pursued to facilitate targeting of immune cells in tumours. For example, the stimulator of interferon genes (STING) pathway, a cytosolic danger sensor in host immune cells[147], has gained particular interest. However, its natural ligands, cyclic dinucleotides (CDNs), are labile and are poorly taken up by cells in vivo. Conjugating PEGylated lipid nanodiscs with CDN prodrugs enabled penetration into solid tumours, activating DCs to drive T cell-mediated tumour rejection[148]. Passive nanoparticle uptake in tumours can be further exploited to localize immune cell activation to tumours. For example, intravenously injected PEGylated gold nanorods accumulated in tumours and were then irradiated by a near-infrared laser to induce photothermal heating of the tumour-resident nanorods[149]. This heating activated thermally responsive gene cassettes in co-administered engineered T cells and induced localized CAR or cytokine expression in mice[149]. However, the clearing mechanism of gold nanoparticles remains unclear, because these materials are not resorbable in vivo[150] (Fig. 6b). Nanoparticles have also been designed to target T cells in tumours through conjugation with antibody fragments specific to T cell receptors. For example, PD1-targeted PLGA–PEG-based nanoparticles were detected on intratumoral T cells within 1 hour of intravenous injection and enabled pronounced therapeutic modulation of tumours through delivery of a small-molecule TLR7/8 agonist[151].

One factor limiting nanoparticle dissemination deep into the tumour parenchyma is the propensity of nanoparticles to be captured by tumour-associated macrophages residing near blood vessels[152–154]. This affinity is now being exploited to reprogram tumour myeloid cells into a pro-immune phenotype using polymeric or lipid carriers loaded with immunomodulators such as TLR7/8 agonists and colony-stimulating factor 1 receptor inhibitors[155,156]. As another example, polymer nanoparticles carrying mRNA targeted to myeloid cells through di-mannose moieties reprogrammed tumour-associated macrophages towards an M1 phenotype[157]. Although promising, these approaches will need to assess the potential side effects of non-specific uptake by macrophages in the liver, spleen and bone marrow, given the tendency of nanoparticles to be captured by macrophages in these organs[158,159].

In addition to capture by macrophages, abnormal tumour vasculature limits dissemination of nanoparticles and immune cells in the tumour microenvironment (TME). Inhibiting proangiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietin 2 (ANG2) (at low to intermediate doses) normalizes tumour vasculature and perfusion, thereby improving the efficacy of anticancer treatments, including immunotherapy[160–165]. For example, tumour biopsies from breast cancer patients treated with an anti-VEGF antibody in a phase III clinical trial (NCT00546156) showed increased infiltration of $CD4^+$ T cells, $CD8^+$ T cells and mature DCs[166]. Moreover, combining antiangiogenic agents with immunotherapies, including cancer vaccines and immune-checkpoint inhibitors, enhanced tumour infiltration of effector immune cells and improved therapeutic efficacy in murine cancer models[167–174].

Intratumoral delivery of biomaterials for immune cell targeting. Biomaterials can also be directly injected into the tumour. Importantly, to ensure efficacy, the delivered payload needs to remain in the tumour (or tumour-dLNs) after injection. Conjugation of immunomodulatory payloads to antibodies or engineered proteins that bind to tumour cell surface antigens[175] or ECM components such as collagen[176–178] improves safety and efficacy compared with intratumoral or peritumoral administration of free immunotherapy drugs. However, these approaches suffer from short-lived tumour stimulation (typically only a few days or less) because these agents are internalized by tumour cells or released out of the TME[176]. Biomaterial-based treatments can extend the window of drug exposure and improve efficacy from single injections, which is important for treating deep visceral lesions that require surgical access. For example, IL-12 bearing a phosphoserine peptide tag enables stable high-avidity binding to aluminium

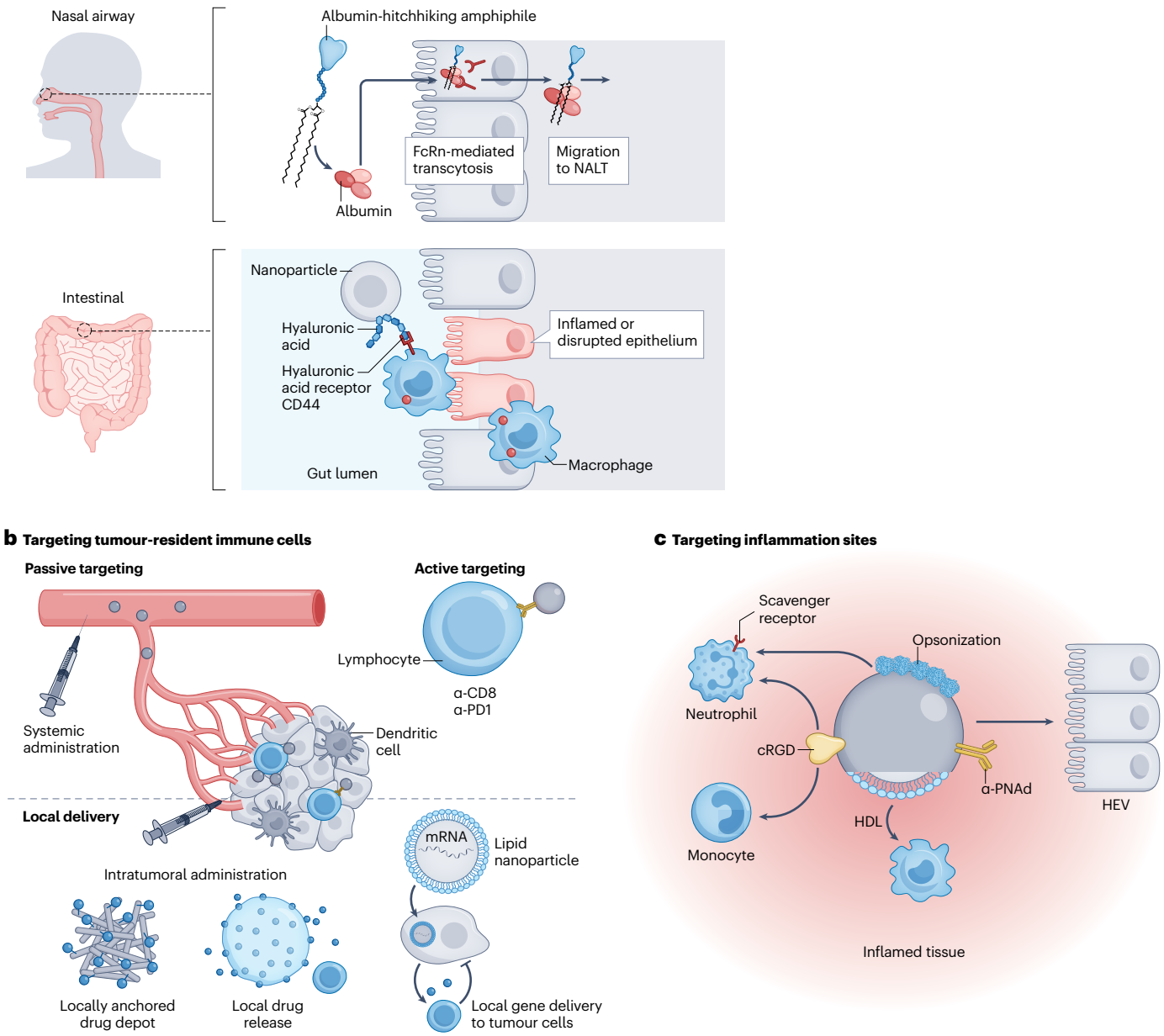

Fig. 6 | Targeting tissue-resident immune cells. a, Albumin is used as a chaperone to transport vaccines across the epithelial barrier in the nasal and respiratory pathways through the neonatal fragment crystallizable (FcRn) receptor. Nanoparticles functionalized with hyaluronic acid are phagocytosed by macrophages and other myeloid cells at sites of intestinal inflammation in the gut. **b**, Systemically injected nanoparticles passively accumulate in tumours through the enhanced permeation and retention effect, or functionalized nanoparticles actively target cells such as $PD1^+$ lymphocytes. Alternatively, biomaterials can be directly injected into tumours to locally present or release immunostimulatory agents in the tumour microenvironment or promote gene delivery to tumour cells for localized expression of immunomodulatory factors. **c**, Nanoparticles can be modified with targeting moieties or opsonization-triggering components to bind to the endothelial cells of high endothelial venules (HEVs) of the inflamed draining lymph nodes or target different innate immune cells that home to sites of inflammation. cRGD, cyclic arginine–glycine–aspartic acid; HDL, high-density lipoprotein; NALT, nasal-associated lymphoid tissue; PNAd, peripheral node addressin.

hydroxide (alum), a common clinical vaccine adjuvant[179]. Intratumoral injection of cytokine-loaded alum particles allowed drug retention in tumours for more than 2 weeks, resulting in pronounced regressions and complete responses in several tumour models in mice (Fig. 6b). Similarly, intratumoral injection of spherical nucleic acids, liposomal nanoparticles with surface-conjugated oligonucleotides as ligands for TLR9, led to strong immunomodulatory activity[180]. In a phase Ib/II clinical trial, as of the data cut-off date (1 July 2021), out of 14 patients,

2 had a complete response and 1 had a partial response, and side effects were mostly limited to injection site reactions and flu-like symptoms[181,182] (NCT03684785; Table 1). Another promising approach consists of in situ vaccination using viral nanoparticles derived from the virus cowpea mosaic virus, which shifts the immune composition of the TME towards a pro-inflammatory profile[183,184].

Biomaterials are also being considered as depots for the local release of immunotherapy payloads to the TME (Fig. 6b). For example, chitosan microparticles have been developed that, following intratumoral injection, released IL-12 over 1–2 weeks and elicited complete tumour regression in 80–100% of animals in murine cancer models[185,186]. Highlighting its synergistic potential, in pancreatic cancer models in mice, intratumoral injection of IL-12-loaded polymer microspheres at 24 hours following local stereotactic body radiation therapy resulted in increased TME reprogramming, systemic T cell responses and anti-tumour efficacy compared with IL-12-loaded microspheres or radiation-only treatments[187]. Hydrogels releasing innate immune-stimulating small-molecule drugs, such as TLR7/8 or STING agonists, decreased tumour recurrence when implanted at surgical resection sites in mouse models of breast and lung cancer[188]. Sprayable fibrin hydrogels releasing an anti-CD47 antibody at tumour resection sites stimulated macrophage phagocytic activity while neutralizing the acidic pH at the tumour site, triggering myeloid cells to eliminate residual tumour cells[189].

Biomaterials have also been used to deliver and locally stimulate adoptively transferred T cells in tumours. For example, intratumoral implantation of alginate hydrogels loaded with polyclonal tumour-specific T cells or CAR T cells, cytokines and immunostimulatory antibodies, led to enhanced tumour elimination in mouse models of breast and ovarian cancer[190]. In the adjuvant setting, hyaluronic acid hydrogels encapsulating CAR T cells together with stimulatory factors for localized immunostimulation implanted into the tumour bed following surgical resection protected against tumour recurrence and inhibited distant tumour growth[191]. In another report, scaffolds made of nitinol metal, a material routinely used as cardiovascular stents, were functionalized with T cell-stimulatory antibodies and loaded with CAR T cells for peritumoral implantation. In a mouse model of non-resectable ovarian cancer, these scaffolds eradicated tumours in 70% of animals and extended the average tumour survival time by 2.7-fold compared with untreated controls[192]. These described materials have different in vivo lifetimes and might require surgical retrieval. Clinical implications are still unclear because none of these approaches have entered clinical testing yet.

Engineered materials can also be used to increase uptake and promote cytosolic delivery of therapeutics in immune cells following localized delivery to tumour sites. For example, intratumoral administration of endosome-disrupting polymersomes delivering CDNs into phagocytes improved STING activation, indicated by an 11-fold decrease in melanoma growth rate and complete tumour rejection in one-third of the mice[193]. Similarly, intratumoral injection of CDN-loaded lipid-calcium phosphate nanoparticles surface-treated with phosphatidyl serine promoted CDN uptake by myeloid cells and DCs in the pleural space in a mouse model of malignant pleural effusion[194]. mRNA also requires cytosolic delivery to immune cells (Fig. 6b). Intratumoral injection of mixtures of lipid nanoparticles delivering mRNA encoding cytokines and membrane-bound T cell costimulatory receptor OX40L led to pronounced immune activation and complete tumour regression in 50% of animals bearing subcutaneous hepatoma tumours[195]. Similarly, intratumoral injection of saline formulations or polymer nanoparticles delivering mRNAs encoding selected potent cytokine combinations (for example IL-12, GM-CSF, IL-15 and IFNα) led to robust tumour regressions and induction of systemic T cell responses that regressed even distal untreated lesions[196,197]. Interim data from a follow-up phase I clinical trial showed that lipid nanoparticles encapsulating mRNAs encoding OX40L, IL-23 and IL-36γ, in combination with a PD1 immune checkpoint inhibitor, resulted in increased levels of proinflammatory cytokines in both plasma and tumour biopsies (NCT03739931; Table 1)[198]. These lipid nanoparticle formulations were shown to primarily transduce cancer cells and myeloid cells. Another approach is to deliver mRNA directly into tumour-infiltrating T cells; lipid nanoparticles incorporating ionizable lipids transfected around 5% and 10% of tumour-infiltrating $CD4^+$ and $CD8^+$ primary T cells, respectively, following intratumorual injection in a murine melanoma model, and, when used to deliver mRNA encoding OX40 in combination with systemic administration of anti-OX40 agonist antibodies, resulted in 60% tumour rejection in a murine lymphoma model[131].

Targeting sites of inflammation

Immune cells are central to the pathology of inflammatory diseases, therefore, targeting them at sites of inflammation is a potentially effective strategy for disease treatment. Phagocytes are involved in generating persistent inflammation, thereby providing a first-line target choice for drug development[199]. For example, polymersomes, vesicles formed by the self-assembly of amphiphilic block copolymers, were efficiently taken up by neutrophils through scavenger receptors[200]. Using pH-responsive polymersomes that disrupt endosomes in response to acidification of these compartments, cytosolic delivery of cyclin-dependent kinase inhibitor *R*-roscovitine induced neutrophil apoptosis and suppressed inflammation in a sterile injury zebrafish model. Immune cells can also serve as chaperones to concentrate nanocarriers at sites of inflammation and angiogenesis; for example, intravenous administration of nanoemulsions functionalized with cyclic RGD peptides that bind to $\alpha_V\beta_3$ integrins expressed on inflamed blood vessels rapidly adhered to circulating neutrophils and monoyctes in a mouse model of acute wound-derived inflammation within minutes. These nanoparticles also directly bound to the endothelium of inflamed vessels at later times, suggesting transfer from leukocytes to endothelial cells[201]. Neutrophils are known to effectively capture opsonized particles in the blood, a process exploited to target these cells at sites of lung inflammation using nanoparticles of different size, charge and composition, provided they are rapidly opsonized with complement upon injection[199] (Fig. 6c). Intravenously injected synthetic high-density lipoprotein nanoparticles carrying the mTOR inhibitor efficiently targeted myeloid cells and blocked the expression of inflammatory cytokines by macrophages infiltrating heart allografts, resulting in long-term allograft survival[202]. Interestingly, intravenously injected empty PLGA nanoparticles were reported to be taken up by monocytes and neutrophils in the blood, leading to their reprogramming into an anti-inflammatory phenotype and localization to sites of spinal cord injury, promoting a pro-regenerative milieu that enabled axon recovery[203].

Biomaterials are also being explored to modulate adaptive immune responses at tissue transplant sites. For example, biodegradable polymer microspheres releasing TGFβ promoted T_{reg} cell development in vitro, and promoted T_{reg} cell infiltration into allogeneic pancreatic β-cell transplants for treatment of an in vivo diabetes model[204]. In a rat model of vascularized hindlimb allogeneic tissue grafting, local

Box 1

Defining optimal immune cell targets for disease modulation

Modulation of the immune system can only be effective if the targeted cells and their responses are well understood. For example, different phenotypic and functional states exist for antigen-specific T cells. Inactivated precursors, early activated cells (including short-lived and memory precursor effector cells), activated effector cells, memory cells and memory stem cells and 'exhausted' effector cells[231] have been defined, all with different functionalities and ability to respond to immunostimulation. 'Stem-like' $CD8^+$ T cells, in particular, have characteristics of both memory cells and more activated cells[213–215,232,233], and express specific transcriptional and protein signatures (for example, T cell factor 1 ($TCF1^+$), $PD1^+$) and self-ligand receptor of the signalling lymphocytic activation molecule 6 ($SLAM6^+$)), which are important modulators of anti-tumour immune response. Fundamental studies identifying these important, but possibly rare, cell types will be required to develop targeted immunotherapies and vaccines.

administration of microspheres releasing TGFβ, rapamycin and IL-2 promoted induction and expansion of T_{reg} cells at the implant site, leading to long-term tissue engraftment[205].

Lymph node draining sites of disease are also modulated by upstream inflammation. For example, high endothelial venules (HEVs) are specialized blood vessels of the lymph nodes that express the lymphocyte homing protein peripheral node addressin (PNAd), which supports naive lymphocyte entry into lymph nodes. Notably, PNAd expression is upregulated in lymph node draining sites of chronic inflammation[206]. Exploiting this process, intravenously injected PLGA microparticles carrying the immunosuppressive drug tacrolimus and functionalized with a PNAd-targeting antibody enabled accumulation of the drug at lymph nodes draining the transplanted tissues[207]. As microparticles are too large to passively transport across the endothelium, this finding suggests particle accumulation on the luminal surfaces of the HEVs, followed by drug diffusion into the tissue across the endothelial barrier. Building on these findings, PNAd-targeted PLGA nanoparticles were found not only to bind to the HEVs of inflamed dLNs, but were also further transported into the lymph node parenchyma, leading to substantially prolonged allograft survival in a model of MHC-mismatched heart tissue transplantation[208] (Fig. 6c).

Outlook

Biomaterial-mediated targeting of immune cells and tissues is starting to have clinical impact, with great promise for the future of vaccines and immunotherapies. However, there remain important fundamental aspects of immunology to be understood, and a number of technological and translational challenges must be solved if these technologies are to reach their full potential.

For example, there is still much to learn about the dynamics of immune cells during disease and treatment. The reciprocal trafficking of lymphocytes from lymph nodes to blood and tumours, as well as between tumours, has received limited attention[209,210]. However, this information is essential for the design of targeted immune-oncology therapeutics that will 'hit' cells at a desired location or time point in their life cycle. The role of stem-like $CD8^+$ T cells in diseases, such as autoimmunity[211], infections[212] and cancer[213,214], is gaining particular attention, because these cells appear to play an important part as responders to immunotherapies such as checkpoint blockade[212,215], by producing progenies that are important effector cells (either in lymph nodes or directly at peripheral tissue sites). These cells are promising targets for immunotherapy, but how they can be generated and expanded in vivo remains poorly understood (Box 1).

Interactions of biomaterials with the immune system also need further assessment. The SARS-CoV-2 pandemic revealed that lipid nanoparticles used for mRNA delivery have direct adjuvant activity in vaccines[216–218] by triggering inflammatory cytokine production in lymph nodes; however, the exact mechanisms remain to be elucidated. Other biomaterials, for example biodegradable polymers such as PLGA, are generally considered passive scaffolds or drug-release matrices; however, lactic acid produced by PLGA hydrolysis is known to be immunomodulatory[219–221], and PLGA particles promote a pro-regenerative phenotypic state when phagocytosed by innate immune cells[203]. These effects can promote tolerance in conditions such as autoimmune disease[222], but would be undesirable in other settings, for example in cancer immunotherapy.

One reality that the field of biomaterials-based immune therapies must face is that complex multicomponent formulations requiring the production of multiple recombinant proteins and exotic multi-step chemistries using different clinical-grade materials are unlikely to be clinically translated owing to the prohibitive cost and complexities involved in the good manufacturing practice (GMP) production of such systems. To ensure clinical translation, 'less is more' should be a motivating mantra. This reality is evident by looking at the technologies undergoing clinical trials (Table 1). Furthermore, technologies are often confined to the expertise of individual laboratories. Sharing methods in the field will enable broader experimental validation and testing in different disease models.

The success of lipid-nanoparticle-delivered SARS-CoV-2 mRNA vaccines provides an important proof of concept in humans, but there is much room for improvement to realize their full potential. Despite important advances over the past 20 years, lipid nanoparticles remain substantially worse than biological vectors, such as viruses, at transfecting cells. Systemic or intratumoral injection of lipid nanoparticles to deliver mRNA to T cells transfects only 2% or less of T cells[129,196]. In addition, what makes lipid nanoparticles suitable for packaging nucleic acids (for example, their charge) also makes them prone to opsonization and non-specific binding to cells and matrices. Moreover, allergic reactions elicited in a small proportion of individuals with current PEG-stabilized lipid nanoparticle formulations represent a potential safety issue[223,224]. Despite the clinical success of lipid nanoparticles as delivery materials for mRNA and siRNA, other materials, such as endosome-responsive polymers[225–227], can outperform lipid nanoparticles for nucleic acid delivery in some cell types. Furthermore, combinatorial library studies of lipid nanoparticle compositions have revealed that lipid nanoparticle formulations can be tuned to target specific tissues and immune cell types without even the need for specific antibody or other binder-based targeting[199,228,229]. Materials with improved delivery efficiency will increase the utility of mRNA, as well as DNA and CRISPR-based gene-editing systems.

Despite existing knowledge gaps and challenges, the use of biomaterials to enable cell-specific and organ-specific targeted immune modulation is an important and exciting area of research and clinical development, which could unlock the full potential of immunotherapies for different diseases.

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Author contributions

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Competing interests

D.J.I. is an inventor on patents related to albumin hitchhiking (discussed under 'Principles of lymph node targeting'), nanoparticle modification of T cells (discussed under 'Backpacking' cells') and alum-binding cytokines (discussed under 'Intratumoral delivery of biomaterials for immune cell targeting'). These patents have been licensed to Elicio Therapeutics, Repertoire Immune Medicines and Ankyra Therapeutics, respectively, and D.J.I. holds equity in these companies. The remaining authors declare no competing interests.

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