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Engineered polysaccharides for controlling innate and adaptive immune responses

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Abstract

Therapeutic interventions can be designed by exploiting the immune system's ability to initiate specific responses to various stimuli. However, specific T cell activation, which is a key target for vaccines and immunotherapies, remains challenging. Polysaccharides derived from microbial cell walls are promising immunomodulators that interact with pathogen-recognition receptors (PRRs) on dendritic cells and macrophages, triggering robust immune responses for modulating T cell function and activating effector or regulatory pathways. In this Review, we discuss the role of polysaccharides as pathogen-associated molecular patterns (PAMPs) recognized by PRRs and their immunomodulatory potential for biomedical applications. We examine the engineering aspects of polysaccharides, investigating their potential in vaccine, immunoadjuvant, immune-modulation and drug-delivery applications and highlighting their immune-activating or immune-regulatory functions. We also explore how trained immunity can be induced by polysaccharides to trigger immune responsiveness upon re-encountering pathogens. By leveraging materials engineering principles, polysaccharides can offer a platform for effective vaccines and immunotherapies against autoimmune and other diseases.

Sections

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Key points

- Current subunit vaccines often lack immunogenicity compared to traditional whole-microbe vaccines, requiring immune modulators to enhance antigen immunogenicity and efficacy.

- Polysaccharides can serve as pathogen-associated molecular patterns (PAMPs) that interact with pathogen-recognition receptors (PRRs) on dendritic cells and macrophages, triggering robust immune responses.

- The immunological activities of polysaccharides depend on their origin, type and structure, and can thus be optimized for their rational design.

- Engineered polysaccharides, with diverse structural and immunological characteristics, offer a versatile platform for biomedical applications, demonstrating potential in immunotherapy against autoimmunity, vaccine delivery and induction of trained immunity.

Introduction

Understanding how the immune system decides what type of immune response to initiate is crucial in developing vaccines and immunotherapies. Current subunit vaccines based on protein and peptide antigens offer precise immune modulation and safety, but they are typically less immunogenic than whole attenuated or inactivated microbe vaccines, thus requiring immune modulators that can augment the immunogenicity of antigens[1] by inducing, persisting, magnifying or steering specific immune responses. In particular, T cells, which are involved in many pathologies, including cancer, infection and autoimmunity, are a key target for vaccines and immunotherapies. However, it remains difficult to prime T cells to generate and maintain effector and memory T cells for the treatment of cancer or infection, and to induce tolerogenic T cells for the treatment of autoimmune diseases. Moreover, the innate immune system — particularly dendritic cells (DCs) and macrophages — plays an important part in the induction of T cell immunity through the engagement of pathogen-recognition receptors (PRRs). Recognition and binding of PRRs to their ligands initiate innate immune responses and induce cellular modifications that have profound effects on antigen uptake, processing and presentation to T cells. Furthermore, the fate of the T cell response depends on the context of PRR signalling, that is, the type, timing and magnitude of the PRR's engagement with its respective ligand[2]. Therefore, elucidation of PRR-mediated immune responses, distinct downstream signalling pathways and their underlying molecular mechanisms could provide targets and strategies for the treatment of diseases, including cancer, infections and autoimmune diseases.

Importantly, polysaccharides derived from the microbial cell wall serve as an immune-modulating molecular pattern, termed a pathogen-associated molecular pattern or PAMP, that is recognized by PRRs on innate immune cells as a first-line-of-defence mechanism. In addition to microbial polysaccharides, polysaccharides from plants, insects and synthetic polysaccharides can function as PAMPs. Thus, polysaccharides are promising candidates for immune modulation; in particular, β-glucan functions as a Dectin-1 ligand to induce immune memory functions among innate immune cells. This phenomenon, termed 'trained immunity', manifests greater responsiveness if innate immune cells re-encounter pathogens[3,4]. We note that epigenetic modifications and metabolic reprogramming have important roles in polysaccharide-induced trained immunity[5].

Natural and synthetic polysaccharides have been used as immune modulators in clinical trials[1,6,7]. However, naturally derived polysaccharides are often heterogeneous and difficult to obtain with high purity. In addition, the underlying mechanisms of their immunological activities are not yet fully understood, hampering their rational development as immunoadjuvants or immunosuppressants.

In this Review, we discuss PRR–PAMP mechanisms with a focus on using polysaccharides as PAMPs (Box 1). From a materials engineering viewpoint, we highlight the capabilities of polysaccharides as immune modulators for the induction of effector and memory or tolerogenic T cells and explore their utility for applications in vaccines and immunotherapies. We further examine how our expanding knowledge of polysaccharides and materials engineering can be harnessed for the design of vaccines or immunotherapies against various diseases (Fig. 1).

Polysaccharide–PRR interactions

The host immune system orchestrates innate and adaptive immunity to protect against invading pathogens. A crucial first step is to quickly recognize microbes and distinguish whether they are harmful, foreign microbes that need to be eliminated or commensal microbes that are beneficial to the host. The innate immune system provides this rapid surveillance, using germline-encoded PRR proteins[8]. Upon recognition of danger signals derived from either foreign pathogens (PAMP) or endogenous sources, such as dying cells (producing damage-associated molecular patterns (DAMPs)), PRRs trigger immunogenic signal transduction pathways and initiate innate immune responses with diverse molecular and cellular actions, including changes in gene expression for cytokine and chemokine production, deployment of killing factors that induce bactericidal enzymes, toxic chemicals (such as reactive oxygen species, ROS) and antimicrobial peptides (for example, defensins), and activation of the complement system to tag (or opsonize) the microbe surface with the complement protein C3b, marking it out for destruction — all of which coordinate the early innate immune response. Innate immune cells can further promote the activation of adaptive immune responses by T cells and B cells to more efficiently eliminate the infection in a specific, controlled and durable manner through cellular and/or humoral responses[9]. The main effector mechanisms of adaptive immunity include the production of blocking antibodies by B cells, neutralization by opsonization or complement-mediated and phagocyte-mediated killing, and direct killing of infected host cells by cytotoxic T cells. In addition, adaptive immunity provides long-term memory function for protection against re-infection by the same antigen[10]. In particular, DCs and macrophages are antigen-presenting cells (APCs) that are specialized for processing and presenting foreign antigens for the induction of adaptive immunity. Because of their essential role in host immune protection, APCs can be found in many anatomical locations, including in the skin (Langerhans cells) and in the lining of the nose, lungs, stomach and intestines, as well as the peripheral lymphoid organs. Thus, understanding PAMP–PRR interactions, especially in DCs and macrophages, can provide important insights into modulating innate and adaptive immune responses.

There are four major families of PRRs: Toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors
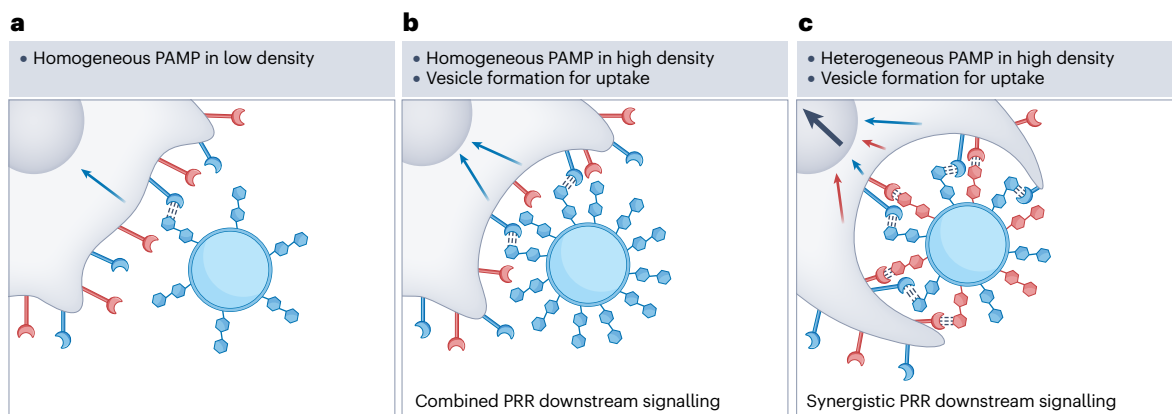
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Box 1 | Multivalent PAMP–PRR engagement for immune modulation

Natural and synthetic polysaccharides possess immunomodulatory characteristics that make them promising immune modulators; however, their low immunogenicity has been a major hurdle to their clinical use[151]. Pathogen-associated molecular pattern (PAMP)–pathogen-recognition receptor (PRR) engagement activates innate immune cells to promote the differentiation of a diverse subset of adaptive immune cells in a specific manner. In addition, each immune cell subset expresses a different set of PRRs at different levels depending on the disease. The nature, timing and strength of PRR signalling have a substantial effect on the resulting T cell response, providing the basic principles of immune modulation for developing a potent immune modulator.

The density and combination of PAMP display affect the simultaneous engagement of different classes of PRR, termed multivalent PAMP–PRR engagement, modulating the repertoire, magnitude and longevity of immune responses. High PAMP display density typically increases the number of PAMP–PRR interactions and may lead to combined intracellular downstream signalling and enhanced cellular uptake through vesicle formation (see Box 1 figure, panels **a** and **b**). PAMP–PRR interactions can also be enhanced by increasing the diversity of PAMP molecules. Each PRR exhibits distinct levels of binding strength and specificity, highlighting the dynamic and crucial effect of combining multiple polysaccharides in eliciting precise immune responses. Heterogeneous PAMP–PRR interactions may trigger multiple intracellular signalling pathways simultaneously, resulting in a synergic immune response (see Box 1 figure, panel **c**).

Therefore, multivalency can be exploited to trigger a specific immune response; for example, multivalent surface properties of mannan nanocapsules can be harnessed to engage multiple immune receptors, including Dectin-2 and Toll-like receptor 4, resulting in potent activation of T helper cell 17 responses, thereby increasing the recruitment of antitumour immune effectors within the tumour microenvironment and highlighting the potential of this strategy for cancer immunotherapy[87]. Similarly, HIV gp120 high-mannose oligosaccharides can be used for multivalent display[196], and mannosylated chitosan–DNA particles can be applied for gene delivery to dendritic cells[197]. Furthermore, mannosylated polyanhydride nanoparticles[198] and mannosylated liposomes can target antigen-presenting cells and increase antigen presentation as well as antitumour responses[199,200].

a
- Homogeneous PAMP in low density

b
- Homogeneous PAMP in high density
- Vesicle formation for uptake

Combined PRR downstream signalling

c
- Heterogeneous PAMP in high density
- Vesicle formation for uptake

Synergistic PRR downstream signalling

(NLRs)[11]. Of these, TLRs and CLRs are involved mainly in the recognition of polysaccharides (Table 1).

Here we focus on polysaccharides that trigger PRR pathways associated with foreign, pathogenic microbes. Polysaccharides may also be associated with commensal bacteria and exploited to improve gut microbiota, as prebiotics to induce anti-inflammatory, anti-cancer, antioxidant and antimicrobial effects or as intervention strategies for metabolic disorders that are affiliated with dysbiosis[12]. However, the in vitro culture of polysaccharide-producing microorganisms remains challenging, and the rational design of specific microbiome-modulating strains may require meta-analysis-based approaches, including next-generation sequencing and metagenomics.

Toll-like receptors

TLRs are transmembrane receptors with leucine-rich repeats that can recognize lipids, nucleic acids, polysaccharides and lipopeptides derived from Gram-positive and Gram-negative bacteria, fungi and viruses[13,14].

Humans have ten different TLRs (TLR1 to TLR10) expressed on the membrane of the cell surface (TLR-1, 2, 5 and 6), endosomes (TLR-3, 7, 8, 9 and 10) or both (TLR-4)[11]. Mice have twelve TLRs (TLR1 to TLR9 and TLR11 to TLR13), and the biological functions of TLRs are conserved between mice and humans. TLRs trigger distinct gene expression by interferon (IFN)-β-dependent and/or nuclear-factor (NF)-κB-dependent transcription, regulating several cellular processes, such as survival, proliferation and secretion of type-I interferon and other inflammatory cytokines[13]. Here, signalling is transduced by adaptor molecules that contain Toll/interleukin 1 receptor (TIR) domains, such as myeloid differentiation primary response 88 (MyD88) and Toll/interleukin 1 receptor domain-containing adapter-inducing interferon-β (TRIF)[13]. MyD88 or TRIF are recruited by TIR-domain-containing adaptor protein (TIRAP) and TRIF-related adaptor molecule (TRAM), respectively[13,14].

TLR2 and TLR4 can both recognize polysaccharides; in particular, TLR-2 recognizes peptidoglycan, lipoarabinomannan, phospholipomannan, zymosan and β-glycan from fungal pathogens as well
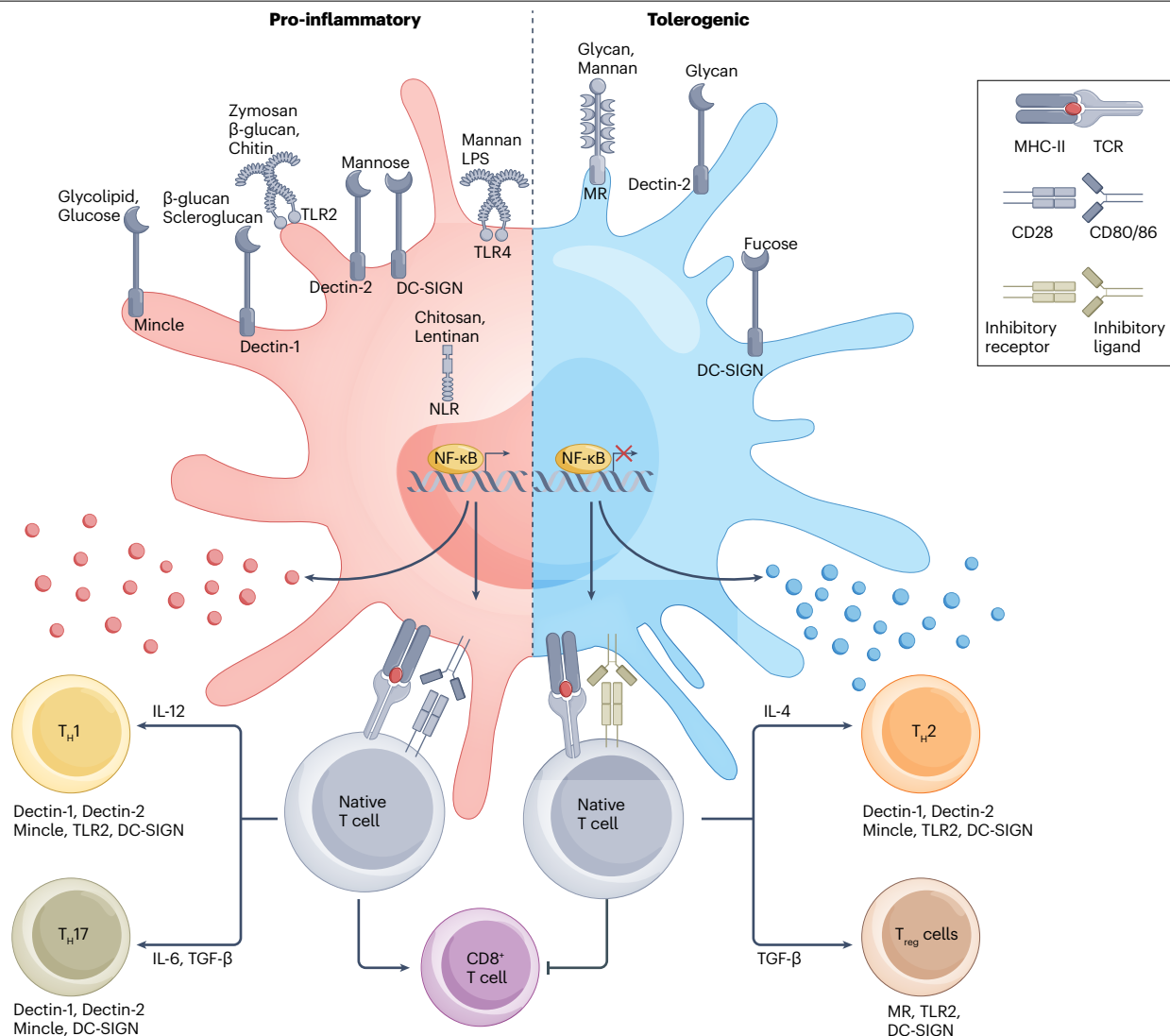
Fig. 1 | Targeting pathogen-recognition receptors on dendritic cells can shape specific T cell-mediated responses. Soluble polysaccharides interact with individual pathogen-recognition receptor (PRRs), whereas nanostructured polysaccharides can engage multiple PRRs simultaneously. Antigens are phagocytosed, processed and presented on the major histocompatibility complexes MHC-I or MHC-II to antigen-specific CD8+ or CD4+ T cells, respectively. Activated dendritic cells (DCs) upregulate co-stimulatory molecules and secrete cytokines that provide activation signals to T cells. Activated CD4+ T cells differentiate into distinct effector subtypes, depending on the cytokine milieu: T helper cell 1 (T_H1) requires secretion of the interleukin IL-12 or the interferon IFN-γ; T_H2 requires IL-4; T_H17 requires IL-6 or IL-23; and regulatory T (T_{reg}) cells require IL-10 or transforming growth factor (TGF)-β. A red DC indicates an immune-activation response; a blue DC refers to an immune-suppressive response. DC-SIGN, dendritic-cell-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing non-integrin; LPS, lipopolysaccharide; MR, mannose receptor; NLR, nucleotide-binding oligomerization domain (NOD)-like receptor; NF-κB, nuclear factor-κB; TCR, T cell receptor; TLR, Toll-like receptor.

as Gram-positive and Gram-negative bacteria, inducing MyD88-dependent signalling pathways for NF-κB activation, which leads to the production of proinflammatory cytokines, including IL-6, IL-12p40 and TNF[13,14]. TLR4 primarily recognizes lipopolysaccharide (LPS) from Gram-negative bacteria[15], in addition to fungal mannan and other polysaccharides from plants, algae, microorganisms and animals[13]. TLR4 is unique in that it can induce both MyD88 and TRIF pathways for simultaneous NF-κB activation and type-I IFN production, mounting strong immune responses[14].

C-type lectin receptors

CLRs are transmembrane receptors with various calcium-ion-dependent lectins that share one or more carbohydrate-binding domains. Receptor signalling of CLRs is dependent on a noncanonical immunoreceptor tyrosine-based activation motif (ITAM) that recruits Syk tyrosine kinase and subsequently induces NF-κB activation, which triggers phagocytosis, release of proinflammatory cytokines and T cell differentiation[16]. CLRs can act either alone or in collaboration with other CLRs, TLRs and interferon receptors to induce cellular signalling

Table 1 | Interactions of pathogen-recognition receptors and polysaccharide-based pathogen-associated molecular patterns

PRR	PRR class	Cells expressing PRR	Polysaccharide PAMP (origin)	Immunological functions	Therapeutic implications	Refs.
Toll-like receptors (TLRs)	TLR2	Macrophages DCs Epithelia	Peptidoglycan (Gram-positive and Gram-negative) Lipoarabinomannan (mycobacteria) Phospholipomannan (*Candida*) Zymosan (fungi) β-glycan (fungi) Chitin	NF-κB activation leading to production of IL-8 Production of IL-10 and TNF	NA	14,181
	TLR4	Macrophages DCs Epithelia	Lipopolysaccharide (Gram-negative bacteria) Mannan (fungi)	NF-κB activation leading to production of proinflammatory cytokines (IL-8) Type-I interferon production Induction of type-1 adaptive immune response	Vaccine adjuvants for hepatitis B and human papillomavirus Vaccine adjuvants for non-small-cell lung cancer therapy Immunotherapies for allergic rhinitis	13,14
C-type lectins	Dectin-1	Macrophages DCs Endothelia Epithelia Smooth muscle cells	β(1,3) and β(1,6)-linked glucans (fungi) Scleroglucan	Phagocytosis Cell maturation Respiratory burst Cytokine/chemokine production (IL-6, IL-12) T_H1 and T_H17 cell differentiation CTL priming ROS production	Anticancer effects in many cancers Hepatic fibrosis and neoplastic transformation therapeutics	13,16,17, 30,182
	Dectin-2	Myeloid DCs Plasmacytoid DCs Monocytes Macrophages B cells Neutrophils	High mannose (fungi)	Cytokine production (TNF, IL-1, CXCL-1, or IL-6) T_H1 and T_H17 cell differentiation	Antifungal effects Autoimmune disease	16,26,183
	MINCLE	Myeloid DCs Monocytes Macrophages TAMs	α-mannose, glucose (*Malassezia* spp., mycobacteria, fungi)	Induction of proinflammatory cytokines (TNF, IL-10) CXCL2 production Neutrophil recruitment	Infection, TAM mincle/ Syk/NF-κB pathway targeted for cancer immunotherapy Pro-tumorigenesis, autoimmunity and sterile inflammation	16,31,33
	Mannose receptor	Myeloid DCs Macrophages	High mannose, fucose and sulphated sugars (mycobacteria, fungi, viruses, parasites)	CDC42, RHoB, PAKs and ROCK1, phagocytosis Antigen presentation	Targeting for drug and therapy delivery to macrophages for cancers and infectious diseases	16,37,38
	DC-SIGN	Myeloid DCs	High mannose and fucose (mycobacteria, fungi, viruses, parasites)	Upregulation of TLR-induced IL-10 production Induction of T_H1, T_H2, T_H17 and T_{reg} cell differentiation Inhibition of T_H1 cell differentiation Induction of T_{reg} cells Antigen presentation IL-10, TGF-β, and IL-27	Targeting to prevent antitumour immune suppression Immune tolerance (T_{reg} cell induction)	16,43
Nod-like receptors (NLR)	NLRP3 inflammasome	BMDM DC Human macrophages	Chitosan Lentinan (*Lentinula edodes*)	Production of IL-10, TNF Induction of T_H1 responses and IgG2	Vaccine adjuvants for inducing cellular immunity	184–186

BMDM, bone-marrow-derived macrophage; CDC42, cell division cycle 42; CTL, cytotoxic T lymphocyte; CXCL1/2, chemokine (C–X–C motif) ligand 1/2; DC, dendritic cell; IL, interleukin; Ig, immunoglobulin; NA, not applicable; NF-κB, nuclear factor-κB; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin-domain-containing-3; PAMP, pathogen-associated molecular pattern; pDC, plasmacytoid dendritic cell; PAK, P21-activated kinase; PRR, pathogen-recognition receptor; RHoB, Ras homolog family member B; ROCK1, Rho-associated coiled-coil kinase 1; Syk, spleen tyrosine kinase; TAM, tumour-associated macrophage; $T_H1/2/17$, type 1/2/17 T-helper cell; ROS, reactive oxygen species; TNF, tumour necrosis factor.

for polarization of T helper (T_H) cells into T_H1, T_H2, T_H17 and regulatory T cell (T_{reg}) subtypes. A number of CLRs have been identified for the recognition of polysaccharides (Table 1).

Dectin-1 (also known as glucan receptor) mainly recognizes β-glucan from fungal species. Dectin-1 engagement can trigger the activation of NF-κB and nuclear factor of activated T cells together with TLR2 or through the CARD9 signalling adaptor, resulting in the production of ROS, inflammatory cytokines and chemokines, such as tumour necrosis factor (TNF), chemokine (C–X–C motif) ligand 2 (CXCL2), and the interleukins IL-1β, IL-2, IL-6, IL-12 and IL-23, for T_H1 and T_H17 cell differentiation[16–18]. Langerhans DCs recognize β-glucan and trigger a T_H17 cell response against cutaneous fungal infection[19], whereas subepithelial DCs induce both systemic T_H17 and T_H1 immunity[20]. Dectin-1 activation by β-glucan can also abrogate an aberrant T_H17 cell response associated with pathogenic hyperinflammatory disorders through the tight regulation of IL-1β production[21]. The interaction between Dectin-1 and β-glucan has been well identified, providing valuable insights into PAMP–PRR interactions[22]. Interestingly, Dectin-1 signalling is activated only by the particulate form of β-glucan and not by the soluble or linear forms, which is ascribed to the nanoclustering of the receptors with the particulate β-glucan into synapse-like structures[23]. Dectin-1 further cooperates with TLR2 to enhance cytokine responses in macrophages[24] and directs several distinct signalling pathways to shape T_H cell responses[25].

Dectin-2 recognizes mannose structures from fungal cell walls and mannose-capped lipoarabinomannan from mycobacterial leprae to induce NF-κB activation in pair with the Fc receptor common γ signalling chain (FcRγ). Dectin-2 signalling leads to TLR-independent production of cytokines and chemokines, such as TNF, IL-1, CXCL-1 or IL-6, and results in NF-κB p50–p65 activation[16]. Dectin-2 is implicated in T_H17 differentiation owing to the production of T_H17-inducing cytokines, such as IL-6, IL-1β and IL-23 (ref. 26), and may also direct T_H2 immune responses upon allergen exposure[27–29].

Macrophage-inducible C-type lectin (Mincle) can sense a range of glycolipids, α-mannosyl-containing carbohydrate structures and glucoses from fungal pathogens[16]. Mincle can direct immune suppression in pancreatic ductal adenocarcinoma in mice[30], and plays a part in protective immunity as part of innate immune cells, including DCs, macrophages and neutrophils. The signalling pathways and immunological functions of Mincle are similar to those of Dectin-2, that is, the production of proinflammatory cytokines and chemokines, such as TNF, IL-10 and CXCL2, through NF-κB activation paired with FcRγ and specifically, the induction of a Caspase recruitment domain family member 9 (CARD9)-dependent signalling pathway[16]. Mincle-induced CXCL2 expression promotes neutrophil infiltration to the site of infection[31]. Mincle has also been linked to tumour progression, autoimmunity and sterile inflammation in mice[32]. It is highly expressed on M2-type tumour-associated macrophages (TAMs), and the Mincle/Syk/NF-κB signalling pathway is important for promoting tumour development, which, upon silencing, inhibits tumour progression in mouse models of invasive lung carcinoma and melanoma (B16F10)[33]. Depending on the context and immune cell subtypes, Mincle directs T_H1 (ref. 34), T_H2 (ref. 35) or T_H17 (ref. 36) immune responses, and can engage PAMPs and DAMPs; however, the specific molecular mechanisms of Mincle that lead to either anti-inflammatory or pro-inflammatory immune responses remain to be investigated.

The mannose receptor (MR) (also known as CD206) has two carbohydrate binding domains that can recognize glycans with high mannose and fucose repeating units as well as sulphated sugars from a variety of mycobacteria, fungi, viruses and parasites[37]. MR is involved in clearing pathogens through phagocytosis by DCs and macrophages[16,37]. MR expressed on macrophages has been targeted for drug delivery or for inhibition in infectious diseases, such as tuberculosis and cancers, including in a mouse model of peritoneal metastasis and human gastric cancer[38]. In particular, MR is strongly expressed by immunosuppressive M2-type TAMs that are abundant immune cells in tumours, constituting up to half of total cell numbers in some murine tumour models[39]. Therefore, depletion or reprogramming of TAMs through MR-targeted drug delivery may prevent immune suppression and improve anti-cancer immune responses[40].

DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) (also known as CD209) recognizes mannose and fucose repeating units on a variety of mycobacteria, fungi, viruses and parasites, inducing T_H1, T_H17 or T_H2 immunity[16]. DC-SIGN was initially identified as a DC marker; however, it has also been found in tissue-resident macrophages, including TAMs, inflammatory adipose tissue macrophages and colorectal mucosal macrophages[41]. DC-SIGN promotes antigen uptake, processing and presentation to T cells, which are functional hallmarks of APCs[42]. DC-SIGN binds to intercellular adhesion molecule 3 (ICAM-3) on T cells with high affinity, thereby mediating the transient adhesion of APCs for promoting T cell proliferation[43,44]. DC-SIGN signalling triggered by mannose induces pro-inflammatory cytokine secretion through RAF1 activation for both T_H1 and T_H17 (refs. 20,45), whereas fucose signalling directs T_H2 (ref. 22) or tolerogenic T_{reg} cells[46], depending on the subsets of DCs and macrophages, or co-ligation of different PRRs.

Polysaccharide-mediated PRR activation in immune cells

DCs, macrophages, neutrophils and monocytes express a large repertoire of PRRs. Other cell types, including epithelial cells, eosinophils, mast cells and platelets, also express PRRs and contribute to the innate immune defence. The differential expression of PRRs by innate immune cells underlies their specialized functions in how these subsets react to pathogenic signals. DCs and macrophages are the major innate immune cells that orchestrate innate and adaptive immunity in response to polysaccharide-mediated activation of PRRs.

PRR activation in dendritic cells

A highly organized immune network has evolved to protect against diverse classes of pathogens. Upon engaging PAMPs through PRRs, DCs undergo maturation, up- and down-regulate their surface receptors and migrate to secondary lymphoid organs. DCs also process antigens and present epitopes on major histocompatibility complex (MHC) class I or class II molecules, depending on the nature of the antigen. PAMPs facilitate the transport of antigen-loaded MHC molecules onto the plasma membrane, where they are recognized by T cell receptors (Fig. 1). Naive CD4+ T cells recognize antigen epitopes presented by MHC II and undergo activation and differentiation into distinct subsets of effector CD4+ T_H cells, playing a primary part in shaping a distinct cytokine milieu for a specific adaptive immune response. Different T_H cell phenotypes exhibit specific immunological functions. Interferon-γ (IFN-γ)-secreting T_H1 cells are effective in eliminating intracellular infections, driving the activation of macrophages, natural killer (NK) cells and cytotoxic T lymphocytes. T_H2 cells increase the expression of MHC class II molecules by APCs, promote activation, proliferation and differentiation of B cells towards plasma cells, and direct immunoglobulin-E-mediated and eosinophil-mediated immune

responses[47]. T_H17 cells are associated with defence against extracellular fungi or bacteria by the production of T_H17-associated cytokines[48]. By contrast, T_{reg} cells suppress T cell responses to maintain homeostasis and self-tolerance.

PRR–PAMP interactions have a fundamental role in the induction of T_H responses for protection against pathogens. In particular, PRR–PAMP signalling in DCs dictates T_H cell differentiation into distinct effector subpopulations through cytokine signals (Fig. 1). Upon PRR activation, DCs upregulate CD80 or CD86 co-stimulatory molecules or inhibitory ligands and secrete characteristic cytokines for the activation and differentiation of T cells. Dectin-1 or Dectin-2 activation leads to the secretion of IL-6, IL-1β and IL-23 for T_H1 or T_H17 differentiation; extracellular TLR4 or TLR5 and intracellular TLR3, TLR7 or TLR9 induce T_H1 via the IL-12p70 cytokine; and DC-SIGN, TLR2 or TLR6 induce T_{reg} cells via IL-10 and TGF-β[22]. Thus, targeting specific PRRs can dictate T cell immune responses.

DCs express different surface receptors and PRRs depending on their anatomical location and immunological function. For example, epidermal Langerin+ DCs promote cytotoxic immune responses and can be targeted via DEC-205. Human dermal CD1α+ DCs express macrophage galactose-type C-type lectin (MGL), MR, DEC-205 and DC-SIGN. Of these, MR and DEC-205 are the best characterized with respect to APC targeting[49]. In particular, DEC-205 is highly expressed on DCs in the T cell zone of lymphoid organs and can be targeted for antigen presentation on DCs[50–52]. In addition, intracellular routing by DEC-205 is more efficient in presenting antigen to CD4+ T cells[49], as compared to MR. Targeting these receptors activates DCs and drives T_H cell proliferation[53]. CD14+ dermal DCs express high levels of DC-SIGN and are important for the generation of follicular T_H cells for antibody production[54]. Thus, distinct immune responses can be elicited by targeting specific DC subsets via their PRRs.

PRR activation in macrophages

Dysregulation of macrophage-mediated innate immune responses plays a key part in many pathologies. Macrophages may originate from erythro-myeloid progenitors, haematopoietic stem cells or circulating monocytes, and display different phenotypes in response to the distinct tissue microenvironments in which they reside. In particular, macrophages exhibit two distinct phenotypes, M1 and M2, each with specific functions and marker expression profiles (Fig. 2). The M1 phenotype is predominantly associated with phagocytosis, antitumour immune responses and tissue damage, characterized by the expression of CD80, CD86, TLR4 and inducible nitric oxide synthase (iNOS)[55–57]. Conversely, the M2 phenotype participates in immunosuppressive functions, tissue repair, angiogenesis and tumour promotion, marked by the expression of CD204, CD163, CD206 and Arginase 1 (Arg1)[55–57]. M1 phenotypes can be induced by exposure to IFN-γ and/or LPS and are considered pro-inflammatory, whereas M2 phenotypes are induced by IL-4, IL-13 and IL-33, and are considered anti-inflammatory.

Macrophages possess various PRRs, including MR (CD206), scavenger receptors, TLR2, TLR4 and TLR9, Dectin-1, Dectin-2, DC-SIGN (CD209) and Mincle[58]. Among these, Mincle[59], Dectin-1 (ref. 60), TLR4 (ref. 61) and NLR[62] can polarize macrophages to the M1 phenotype, whereas MR[59] can polarize macrophages to the M2 phenotype. However, the underlying mechanisms of action and corresponding PRR–PAMP pairs are not yet well understood, with the exceptions of β-glucan–Dectin-1 and mannan–MR. The immunological roles of Dectin-1 and MR in macrophages have been studied in cancer, infection, heart, respiratory, intestinal and neurological disorders[63,64].

Dectin-1 recognizes β-glucan in the fungal cell wall[65] and plays an important part in antifungal defence by promoting phagocytosis and nitric oxide and cytokine production[66]. In mice, Dectin-1 is expressed on various types of resident macrophage, including alveolar macrophages, Kupffer cells, intestinal macrophages and splenic macrophages[67,68]. Dectin-1 is also expressed on human monocytes and macrophages[68]. In addition, M2 macrophages express high levels of Dectin-2 (ref. 69).

Mannan binds to MR, which is predominantly expressed by M2 macrophages and upregulated in response to IL-4. Several types of tissue-resident macrophages express MR in mice and humans, including cardiac-resident macrophages, peritoneal macrophages, adipose tissue and skin macrophages[70,71]. MR on these macrophages is implicated in several immunological functions, such as anti-inflammatory cytokine production[72], profibrotic activity[73] and interaction with gut microbiota[74].

Mincle and Dectin-1 recognize α-glucan, inducing an oxidative burst in activated peritoneal macrophages[75]. TLR4 on macrophages is activated by chitosan oligosaccharides, resulting in statistically significant increases in phagocytic functions and production of nitric oxide and TNF[76]. Interestingly, chitin is a size-dependent stimulator of IL-17A and IL-17AR expression in macrophages, a response dependent on TLR2 and MyD88 (ref. 77).

MR is strongly expressed by immunosuppressive M2-type TAMs and could thus be targeted for antitumour treatments. The tumour microenvironment undergoes multiple developmental stages during tumour growth, thus affecting macrophage polarization. Typically, inflammatory M1 macrophages first infiltrate the tumour microenvironment, which, as the tumour progresses, programs macrophage polarization into the immunosuppressive M2 phenotype to avoid immune surveillance and support tumour growth. To reverse this immunosuppressive environment, PRRs, such as TLR2, TLR3, TLR4 and TLR9 (refs. 78–81) and Dectin-1 (ref. 59), can be activated on M2 phenotype macrophages to trigger their polarization to an M1 phenotype. Alternatively, TAMs can be targeted using MR ligands to delete or reprogram TAMs into the M1 phenotype with antitumour activity[82]. MR binds mannose-containing structures and enhances the uptake of mannosylated antigens, which may be exploited in the design of M2 macrophage-targeting systems.

Rationale engineering of polysaccharides

The immunological activities of polysaccharides are influenced by their origin, type, carbohydrate composition, conformation, molecular weight, presence of functional groups (for example, acetyl and sulfate groups) and degree of branching. In addition, instability of polysaccharides may induce undesirable inflammatory activity by nonspecific activation of PRRs, leading to immune-related adverse effects, such as chronic inflammation and autoimmune diseases[83]. Importantly, the immunogenicity of polysaccharides can be enhanced by modulating their molecular weight, increasing the complexity of PAMP composition and enabling multivalent PRR engagement (Fig. 3a). In general, the immunogenicity of a polysaccharide is influenced by its molecular weight. Polysaccharides with a molecular weight below 1,000 daltons are typically non-immunogenic, whereas polysaccharides exceeding 6,000 daltons are immunogenic[84]. Additionally, the more molecular structural complexity the higher the immunogenicity of polysaccharides[84]. Accordingly, branched or heterogeneous polysaccharide structures may show greater immunogenicity, compared with linear or homogeneous polysaccharides. Thus, investigating such variations in
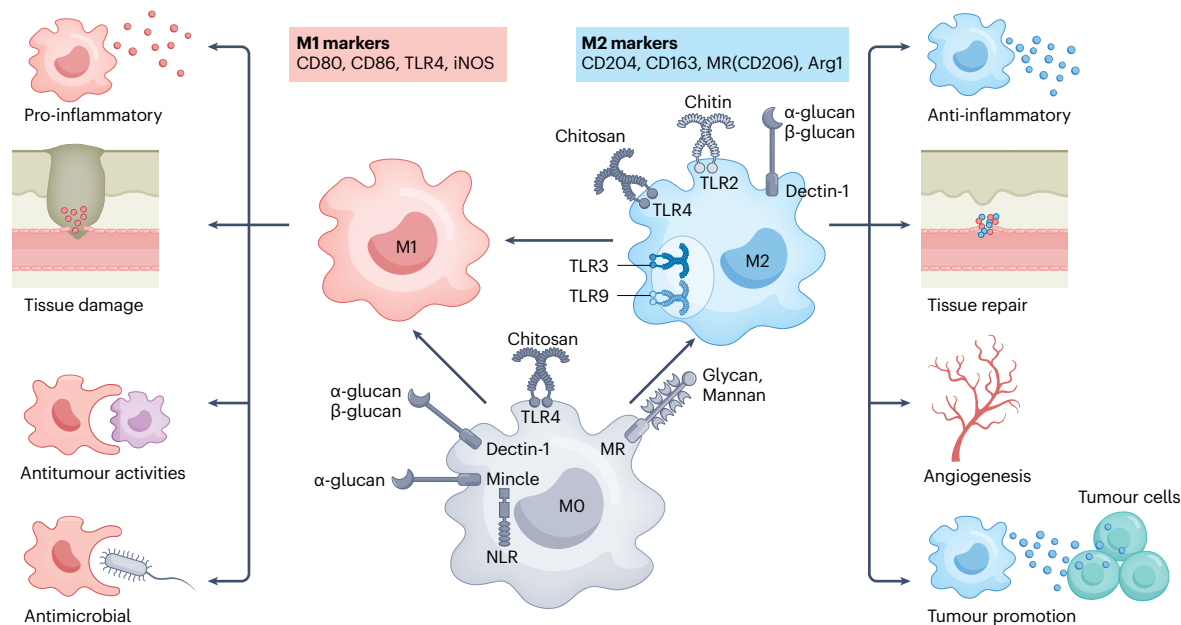
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Fig. 2 | The biological and immunological roles of macrophages. The M1 macrophage phenotype exerts pro-inflammatory effects, contributes to tissue damage, exhibits antitumour activities and displays antimicrobial properties, whereas the M2 phenotype demonstrates anti-inflammatory effects, promotes tissue repair, facilitates angiogenesis and supports tumour progression. Mincle, Dectin-1, TLR4 and NLR polarize non-activated M0 macrophages to the M1 phenotype, and mannose receptor (MR) polarizes M0 macrophages to the M2 phenotype when activated. In addition, repolarization from M2 to M1 phenotype is mediated by TLR2, TLR3, TLR4, TLR9 and Dectin-1. Polysaccharides bind to specific pathogen-recognition receptors (PRRs) on the different macrophage phenotypes. The red macrophage indicates an immune-activation response; the blue macrophage refers to an immune-suppressive response. Arg1, arginase 1; iNOS, inducible nitric oxide synthase; NLR, nucleotide-binding oligomerization domain (NOD)-like receptor; TLR, Toll-like receptor.

immunogenicity may contribute to our understanding of polysaccharide immunology. Moreover, multivalency plays a considerable part in PAMP–PRR interactions (Box 1). Formulation of polysaccharides into particulates allows the simultaneous engagement of multiple PRRs, thus increasing their immunogenicity[85–87]. Therefore, polysaccharides can be rationally designed to induce specific immune responses.

Physical, biological and chemical modifications

The physicochemical properties of polysaccharides, and consequently their immunological performance, can be improved through physical, biological and chemical modifications. Physical modification methods include ultrasonic disruption, microwave exposure, radiation-induced treatment and cleavage of glycosidic bonds of high-molecular-weight polysaccharides with external energy to degrade them into smaller fragments to improve solubility and structural flexibility. In particular, the molecular weight of a polysaccharide regulates its interaction with receptors, immune cells and organs, and may range from tens to thousands of kilodaltons[88]. Therefore, the immunomodulating activities of polysaccharides are related to their molecular weight, with a higher molecular weight typically inducing a stronger immune response[89,90] (Fig. 3b). The molecular weight influences uptake efficiency, retention, degradation and adsorption kinetics in cells and organs, especially in the gastrointestinal tract, thereby inducing varying immune responses[91,92]. For example, β-glucans with a low molecular weight of 5,000–10,000 Da are typically immunologically inactive[93], whereas high-molecular-weight β-glucans affect phagocytosis by immune cells and antimicrobial activities, including the production of TNF and IL-12

(ref. 92). However, lentinan and schizophyllan, which are β-(1,3)-glucans extracted from *Lentinus edodes* and *Schizophyllum commune*, respectively, trigger similar immune responses in a murine sarcoma 180 model regardless of their molecular weight[94]. In addition, a yeast β-glucan with low molecular weight exhibits a higher immunostimulating effect than the same β-glucan with high molecular weight[95]. Furthermore, polysaccharides can be degraded by enzymatic catalysts that can hydrolyse glycosidic chains. Physical and enzymatic methods lead to the degradation of polysaccharides, inducing conformational changes while retaining their basic compositions[96].

Soluble polysaccharides typically do not activate the complement, whereas polysaccharides immobilized on surfaces acquire complement-activating properties, enhancing interactions with phagocytes[97]. This activation is influenced by polysaccharide type and conformation and various physical and chemical modifications. For example, complement activation by dextran and chitosan can vary depending on their morphology or length[97]. Moreover, increasing the density of a glucose-based glycopolymer on nanoparticle surfaces augments complement interaction[98]. Conversely, hydroxyl (OH) substitutions on cellulose membranes reduce complement activation[99].

Chemical modifications of polysaccharides with functional groups, such as carboxymethylation[100,101], sulfation[102], phosphorylation[103], acetylation[104] and alkylation[101,105], can induce alteration in both conformation and composition to improve the structural diversity and biological and immunological activities of polysaccharides. For example, acetylation imparts hydrophobicity for the formation of hydrophobic pockets, and sulfation adds negative charges to improve

aqueous solubility. The sulfated polysaccharide chondroitin sulfate is used in the clinic for therapeutic treatment of articular cartilage osteoarthritis[106].

Formulations of engineered polysaccharides

Polysaccharides can be formulated into nanoparticles to enable them to traverse narrow capillaries, evade rapid phagocyte clearance and prolong their presence within the bloodstream. Furthermore, in the form of nanoparticles, polysaccharides can infiltrate cells and tissue interstices, facilitating their targeted delivery to specific organs, such as the liver, spleen, lung and lymph nodes. In addition, controlled-release characteristics can be implemented by exploiting biodegradability and sensitivity to factors, such as pH, ions and temperature, to improve drug efficacy and reduce toxic side effects[107]. The degree of control over these responses can be finely tuned by manipulating nanoparticle size, the specific type of polysaccharide and the level of physicochemical complexity. Notably, particulates with fungus-like sizes ranging from 2 μm to 10 μm have the potential to increase their interactions with PRRs during
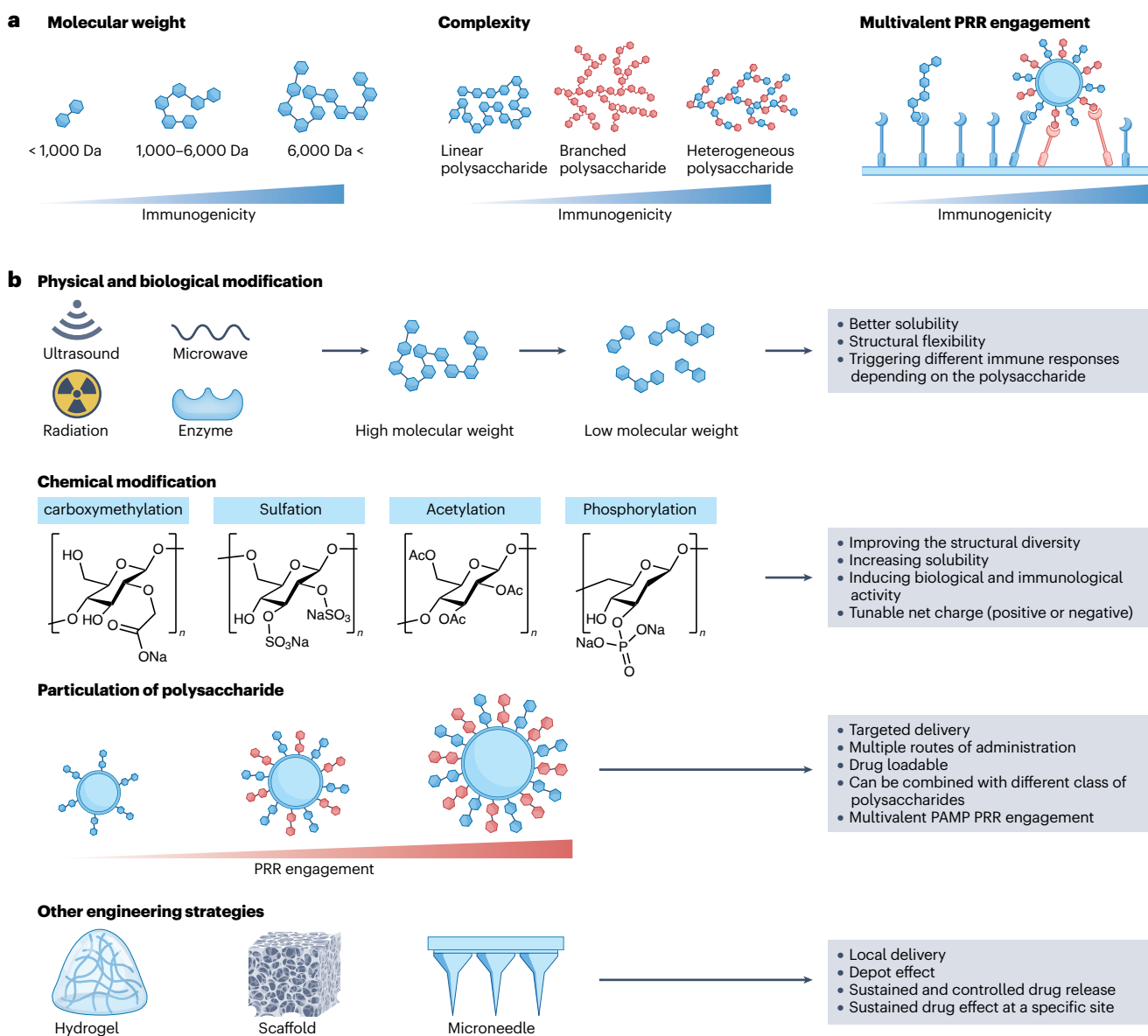

Fig. 3 | **Engineering the immunogenicity of polysaccharides. a**, High-molecular-weight polysaccharides exhibit high immunogenicity, whereas low-molecular-weight polysaccharides induce tolerogenic immune responses. Complexity in the composition and structure of carbohydrates and the degree of branching increase the immunogenicity of polysaccharides. Particulation of polysaccharides facilitates multivalent pathogen-recognition receptor (PRR) engagement for enhanced immunogenicity. **b**, Engineering strategies of polysaccharides. Physical and biological modification mainly affect the molecular weight, whereas chemical modification increases the chemical complexity to induce different immune responses. Particulation offers many advantages for the application of polysaccharides. Hydrogel, scaffold and microneedle formulations provide scalable systems. Da, dalton; PAMP, pathogen-associated molecular pattern.

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PRR engagement to augment the initiation of an immune response[108]. Furthermore, hydrogels can be incorporated to achieve desired spatial and temporal characteristics for the controlled release of loaded therapeutic agents, including small-molecule drugs, macromolecular drugs and cells[109]. The tunable physical properties and degradability of hydrogels, coupled with their ability to protect drugs from degradation, make them a valuable tool with which to control drug release[109]. Hydrogels can further provide cues that emulate natural immune niches, thereby enabling localized modulation of cell fate and behaviour[110]. In addition, transdermal delivery systems based on microneedles enable local immunotherapy, exploiting biological and/or remotely initiated stimuli for controlled drug release[111]. Crucially, these multifaceted engineering strategies may be integrated to evoke desired immune responses.

Engagement of multivalent PRRs

CLRs differ from other receptors owing to their specificity for glycans and their preference for binding multivalent ligands and densely expressed epitopes. Carbohydrate–protein interactions are usually of low affinity, which can be overcome by multivalent display of the receptor and/or ligand[112]. In addition, multivalency and epitope density contribute to the discrimination between self and non-self[113,114]. Indeed, data from competition assays for several receptors revealed that glycans carrying multiple carbohydrate epitopes have a higher ability to bind to these receptors than do monosaccharides[115], and multivalency and configuration also have an effect on the binding capacity via DC-SIGN and MGL and subsequent cellular activation[116,117].

Overall, higher glycan density increases lectin affinity and binding kinetics, which may be related to the 'bind and slide' mechanism[118], in which lectins constantly bind to and dissociate from the glycan epitope. By increasing the number of glycan epitopes along the surface, the lectin–epitope complex becomes longer and of higher affinity. This theory can be applied to soluble lectins, such as galectins. Alternatively, lectins containing multiple carbohydrate recognition domains may align to allow simultaneous binding of multiple epitopes on a multivalent ligand in a face-to-face interaction[118]. This concept is proposed to underlie the binding mechanism of DC-SIGN, which forms a tetramer on the surface of DCs, thereby increasing the affinity for its ligands mannan and gp120 (refs. 119,120). Additionally, DC-SIGN becomes organized into nanoscale clusters within lipid rafts, further supporting ligand binding and internalization by DCs[121]. Finally, in the interaction between highly mannosylated structures and DC-SIGN, additional secondary binding sites on the carbohydrate recognition domain are present and further increase the binding affinity of multivalent epitopes[119]. Knowledge of binding and activation requirements can thus be exploited to target anti-cancer vaccines to DC-SIGN[117]. Similar targeting strategies could be applied to other CLRs, which may provide the opportunity to target different APC subsets, such as Langerhans cells[122].

Soluble polysaccharides allow various administration routes, including intravenous, oral and subcutaneous routes, whereas insoluble particulates may be limited to local immune modulations[89]. We note that soluble and particulate glucans induce distinct immune responses by interacting with different PRRs, representing a unique mechanism by which to discriminate PAMP structures[123]. Several factors, such as the type and accessibility of PRRs and the availability and display pattern of PAMPs, influence PAMP–PRR interactions. Multivalent interaction to simultaneously engage multiple PRRs is a crucial factor for PAMP molecules to induce robust T cell response via receptor clustering and subsequent cellular signalling cascades[108]. More importantly, the association of several heterogeneous PRRs can enhance signalling intensity.

Dectin-1 and TLR2, for example, physically associate with one another to recognize glucans and consequently augment immune responses[124]. Particulate polysaccharides can be beneficial for such multiple receptor interactions. For example, glucan-coated particles with varying sizes of 50 nm, 200 nm or 500 nm in diameter result in different levels of ROS generation in macrophages[23], suggesting that the surface ligand arrangement on the particles considerably influences PRR engagement and immune signalling. Particles 1–2 μm in diameter are more effectively taken up by macrophages than larger-sized particles[125]. By contrast, large β-glucan microparticles (200 μm in diameter) stimulate DCs more efficiently than smaller β-glucan microparticles 1–5 μm in diameter, as assessed by the secretion of IL-1β, IL-6 and IL-23 (ref. 126). In addition to size, the shape and conformation of polysaccharides (for example, hypha or yeast form) affect the induction of immune responses by polysaccharides[127]. Therefore, particulate forms of polysaccharides allow multivalent PAMP–PRR interactions for immune activation.

Biological fate of polysaccharides

Polysaccharide degradation can occur in biological media through hydrolysis or oxidation, induced by chemical or enzymatic mechanisms. Polysaccharides are biodegradable to an intrinsically variable extent with different types of degradation. Endogenous polysaccharides, such as hyaluronic acid and alginate, can be degraded by enzymes, whereas exogeneous polysaccharides derived from pathogens are not subjected to enzymatic degradation. The route of administration can also affect the biological fate of a polysaccharide, including adsorption, digestion and excretion. Following oral administration, polysaccharides in the stomach absorb water, swell and become solubilized either completely or partially in the digestive fluid, followed by bacterial fermentation within the large intestine[128]. The majority of digested carbohydrate subunits of polysaccharides are absorbed in the small intestine. However, some polysaccharides resist hydrolysis in the stomach and small intestine and transit to the large intestine for excretion. In some cases, indigestible but fermentable polysaccharides are metabolized by microbes in the gut to generate diverse products or microbiome metabolites, such as short-chain fatty acids[129]. Notably, upon oral administration, β-glucans can directly interact with the gastrointestinal mucosa to enter the systemic circulation through rapid transfer by epithelial cells, macrophages and DCs in the gut[130]. Plasma β-glucan levels can then reach up to ~3 ng ml^{-1} after 14 days of daily oral administration of 5–6 mg of β-glucans in rats[130,131].

At the cellular level, macrophages are the primary cells for polysaccharide uptake and digestion[132]. Macrophages digest yeast β-glucans in vitro into fragments that can be detected as soon as after 3 days of culture and increase for up to 14 days. The fragments maintain immunological activity to prime co-cultured innate immune cells, such as macrophages, neutrophils and granulocytes, for antitumour efficacy[132,133]. However, the β-(1–3) glycosidic backbone of yeast glucan cannot be digested in the mouse stomach. Instead, the glucan backbone enters the proximal small intestine, where it is captured by macrophages, digested into small fragments, and transported to bone marrow and the endothelial reticular system, suggesting that the β-glucan fragments may be the active immunological components of yeast glucan[133,134].

Engineered polysaccharides for biomedical applications

With diverse structural and immunological characteristics, polysaccharides can be applied in drug delivery and as immune-modulating therapeutics. Efficient and safe polysaccharide-based systems exhibit

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controlled and sustained immunological activities by provoking innate immune responses through the engagement of PRRs via multivalent display of PAMPs along with improved solubility, systemic circulation and targeted delivery of cargo molecules.

Immune tolerance and autoimmunity

Polysaccharides have been examined for applications in immune tolerance and autoimmunity (Table 2). In particular, T_{reg} cells are a potent regulator of various immune responses. T_{reg} cells are phenotypically and functionally plastic and can modulate the immune system either by downregulating their response to overcome immunosuppression in cancer or by improving their response to attenuate autoimmunity, organ transplantation and chronic inflammation. DCs influence T_{reg}-cell homeostasis with their tolerogenic functions, depending on the maturation stage[135], tryptophan catabolism by indoleamine 2,3-dioxygenase (IDO)[136], the capture of antigens from dying cells, or

self- and environmental antigens[137]. Thus, understanding PRR–PAMP mechanisms that are associated with T_{reg}-cell maintenance are essential for the design of T_{reg}-cell-based immunotherapy.

Tolerogenic responses can be mediated by TLR2 (refs. 138,139). TLR2 agonists can induce suppressor cytokines and enhance activation, proliferation and immunosuppressive functions of natural T_{reg} cells[140]. Stimulation of splenic DCs with the TLR2 agonist zymosan, mainly composed of yeast β-glucan, results in the expansion of T_{reg} cells[138]. In line with this, TLR2 ligands from staphylococcal cell walls impede superantigen-induced CD8$^+$ T cell activation and prevent toxic shock syndrome with IL-10 production[141]. Yeast-cell-wall-derived mannan/β-1,6-glucan-containing polysaccharides can induce functionally active T_{reg} cells and suppress T_H1 differentiation of effector T cells through cyclooxygenase-2 signalling in DCs[142]. Upon activation, Dectin-1 and TLR2 mediate T_{reg}-cell induction and T_H1 suppression, respectively, leading to potent immunosuppressive effects in experimental colitis

Table 2 | Applications of polysaccharides in immune tolerance and autoimmunity

Polysaccharide (source)	PAMP	Pathway details	In vitro and in vivo results	Refs.
Zymosan (yeast)	TLR2 Dectin-1	ERK MAPK activated	Increased DC-secretion of IL-10 with little or no IL-6 or IL-12(p70) Increased splenic F4/80$^+$ macrophage-secretion of TGF-β Induction of antigen-specific T cell tolerance in vivo upon injecting zymosan with antigen into mice	138
Mannan/β-1,6-glucan yeast)	TLR2 Dectin-1	Cyclooxygenase-2-mediated	Induction of functional activation of T_{reg} cells Suppression of T_H1 differentiation Protection against disease in experimental colitis and experimental autoimmune encephalomyelitis models	142
Polysaccharide A (*Bacteroides fragilis*)	TLR2	NA	Induction of IL-17 suppression Differentiation of naive CD4$^+$ T cells into T_{reg} cells Upregulation of T_{reg}-cell secretion of IL-10 and TGF-β2 Therapeutic effect in experimental colitis and experimental autoimmune encephalomyelitis models	143,187–189
Cell surface β-glucan/galactan (*Bifidobacterium bifidum* strain PRI1)	TLR2	DC-dependent	Induction of regulatory DCs that produce high levels of inhibitor cytokines TGF-β1 and IL-10 De novo generation of peripherally generated T_{reg} cells; amelioration of inflammatory colitis in experimental colitis model	144
Fucoidan (brown seaweed)	TLR4	Inhibit NF-κB signalling pathway	Reduction of T_H1 proinflammatory cytokine levels (IL-1, IL-2, IL-6 and IFN-γ) Elevation of T_H2 anti-inflammatory cytokine levels (IL-4, IL-10 and TGF-β) Generation of T_{reg} cells Prevention of diabetic nephropathy related to spontaneous diabetes in spontaneous diabetes rat model	147
α-glucan (*Mycobacterium tuberculosis*)	DC-SIGN	Phosphorylation of p65 serine residue 276 Acetylation of lysine residue 310 of NF-κB	Induction of IL-10 secretion in LPS-primed DCs	190
Mannose-capped lipoarabinomannan (*Mycobacterium tuberculosis*)	DC-SIGN	Acetylation of p65 subunit of NF-κB	Induction of IL-10 secretion in LPS-primed DCs	190

DC-SIGN, dendritic-cell-specific intercellular adhesion molecule-3-grabbing non-integrin; ERK, extracellular signal-regulated kinase; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; PAMP, pathogen-associated molecular pattern; $T_H1/2$, type-1/2 T-helper cell; TLR, Toll-like receptor; TGF-β, transforming growth factor-β; T_{reg} cell, regulatory T cell.

and experimental autoimmune encephalomyelitis models[142]. Moreover, bacterial polysaccharides, such as polysaccharide A (derived from the gut commensal *Bacteroides fragilis*), can induce T_{reg} cells in a TLR2-dependent manner; here, polysaccharide A promotes differentiation of naive CD4$^+$ T cells into T_{reg} cells and increases T_{reg}-cell secretion of IL-10 and TGF-β2, leading to strong efficacy in murine models of colitis and experimental autoimmune encephalomyelitis[143]. Cell-surface β-glucan/galactan polysaccharides of the *Bifidobacterium bifidum* strain PRI1 ameliorate inflammatory colitis in a murine model of experimental colitis and induce T_{reg} cells specific for dietary antigens in mice[144], showcasing its possibilities to be applied for the treatment of allergies.

TLR4 interacts with polysaccharides of various structures and is mostly activated or inhibited through the MyD88-dependent signalling pathway[145]. TLR4 can enhance innate immune responses and cytokine production upon polysaccharide recognition, and TLR4-activating polysaccharides have been used for cancer immunotherapy[146]. In addition, fucoidan, a polysaccharide derived from brown seaweed, suppresses TLR4, promotes immune tolerance and decreases disease development in non-obese diabetic mice[147]. Here, fucoidan reduces T_H1 proinflammatory cytokine levels, elevates T_H2 anti-inflammatory cytokine levels and generates T_{reg} cells.

Chitosan-modified *Phellinus igniarius* polysaccharide poly(lactic-co-glycolic acid) (PLGA) nanoparticles[148] alleviate colitis symptoms in the treatment of inflammatory bowel disease, significantly decreasing the levels of pro-inflammatory cytokines (for example, IFN-γ, IL-23, IL-17A and MPO) and preventing intestinal inflammatory damage in a murine inflammatory bowel disease model. Alginate microcapsules, synthesized with pectin having a low degree of methyl-esterification, inhibit TLR2/1-mediated proinflammatory responses and improve islet transplantation in streptozotocin-induced diabetic mice[149]. The capsules may reduce DAMP-induced, TLR-dependent NF-κB activation through competitive binding. Encapsulating rat islet xenografts in the pectin-alginate capsules prolongs graft function, enhances glycaemic control, attenuates production of pro-inflammatory cytokines (such as TNF, IL-6 and GRO-α) and promotes release of IL-10.

Vaccine delivery

Natural polysaccharides possess inherent immunomodulatory properties, biocompatibility, biodegradability, low toxicity and high safety, rendering them well suited for deployment as vaccine adjuvants[150–152] (Table 3). However, polysaccharide vaccines have been limited by their ineffectiveness in maintaining the production of memory B cells[153], and their poor immunogenicity necessitates periodic boost doses[154]. To improve vaccine efficacy, polysaccharides can also be conjugated to protein carriers[154], nanoparticles, hydrogels and conjugates. However, vaccine manufacturing with conjugated polysaccharides remains challenging owing to their intricate purification procedures, their heterogeneous composition, the presence of impure cellular components, and their non-reproducible protein-conjugation chemistry[155]. Therefore, commercially purified or synthetic polysaccharides are mainly explored for vaccine purposes.

In particular, nanoparticles are the appropriate size for delivery to the lymphatic system, enable controlled loading, prolong the half-life of antigens and permit flexible routes of administration, resulting in potent immune activation and targeted delivery with low doses, thereby reducing the risk of toxicities or non-targeted effects. For example, hyaluronic acid-coated nanoparticles loaded with doxorubicin and atyrosine kinase inhibitor have been explored as an in situ cancer vaccine[156], provoking immunogenic cell death and transforming

tumour tissue into an autologous vaccine reservoir. This prompts systemic tumour-specific immune responses, including DC maturation, enhancement of M1-like TAMs, activation of cytotoxic and helper T cells, and establishment of immunological memory in murine 4T1 and B16F10 tumour models[156]. Similarly, targeting of the mannose receptor expressed on macrophages and DCs with mannose and the mucosal adhesion property of chitosan[157] can be combined in a nanoparticle vaccine to increase antibody production in mice and protect Syrian hamsters against SARS-CoV-2. Positively charged and highly soluble trimethylated chitosan can further be used to load multiple malaria antigens onto nanoparticles[158]. This nanoparticle vaccine elicits robust T cell and humoral responses against the *Plasmodium falciparum* circumsporozoite protein (CSP), ultimately conferring long-lasting protection against malaria infection in mice, possibly owing to a structural resemblance between the chitosan nanoparticle and the *P. falciparum* CSP sugar. Nanoparticles, composed of chitosan with varying low molecular weight and displaying sodium alginate, can be loaded with a measles antigen[159]. This vaccine formulation safeguards the antigen in acidic stomach conditions, prompting systemic and mucosal immune responses in orally immunized mice, with lower-molecular-weight chitosan correlating with higher levels of measles-specific IgG and IgA antibodies. Moreover, alginic-acid-coated chitosan nanoparticles enable oral DNA delivery, inducing antigen presentation in the Peyer's patches and suppressing tumour growth in a murine orthotopic 4T1 breast cancer model[160]. Chitosan can also be applied to create hydrogels that target infections, such as *C. psittaci*[161] and Zika virus[162] infection.

Mannan and dextran nanocapsules have been explored in an mRNA cancer vaccine, resulting in different antitumour immune responses[86]. The mannan mRNA vaccine activates DCs in a Dectin-2-dependent and TLR4-dependent manner, leading to a higher level of antitumour effect compared to the dextran counterpart in a murine model of melanoma. Mannan[163] and hyaluronic acid[164] can also be conjugated to model antigen ovalbumin (OVA) to increase OVA-specific IgG production in mice. Nanoparticles composed of glycosylated immunogens lead to mannose-binding lectin (MBL)-dependent and complement-dependent accumulation of antigens in lymph node follicles, augmenting germinal centre responses in mice[165]. Alternatively, co-delivery of OVA with *Angelica sinensis* polysaccharide, which consists of glucuronic acid, glucose, arabinose and galactose, stimulates sustained humoral and cellular immune responses in mice[166]. Soluble inulin microparticles encapsulating antigens also induce robust T_H2-type antibody titres against the delivered antigen[167], and delta inulin-based adjuvants delivered with antigen peptides induce seroprotection against influenza in humans[7] as well as antigen-specific T cell immunity and protection against *M. tuberculosis* infection in mice[168].

Trained immunity

The concept of immune memory has long been considered a defining feature of adaptive immunity. However, innate immune cells, such as monocytes, macrophages and NK cells, also acquire memory-like features after pathogenic stimuli, a phenomenon termed 'trained immunity' or 'innate immune memory'[169] (Fig. 4). Trained immunity enables the host to induce an enhanced innate immune response against the same or different microorganism upon a secondary infection[3]. This was first reported in mice, showing that attenuated *Candida albicans* can protect them against *C. albicans* of the same strain as well as against other bacteria[170]. Furthermore, exposure to a low dose of *C. albicans* confers protection against the reoccurrence of *C. albicans* in mice deficient of functional T and B cells, demonstrating innate immune-mediated memory that is dependent on macrophages and monocytes but not

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Table 3 | Applications of polysaccharides in vaccine delivery

Polysaccharide (source)	Target disease	Antigen	Formulation	In vivo efficacy	Ref.
Hyaluronic acid (biotechnological origin)	NA	OVA	Conjugate	Production of IgG1, IgG$_{2a}$ and IgG$_{2b}$ subclasses Increased IgG titres compared to other tested compounds, especially adjuvants similar to those used clinically (such as alum and AddaVax)	164
Hyaluronic acid (NA)	Cancer	TAAs	Nanoparticle	Strong tumour suppression in the 4T1 tumour model Persistent immunological memory generated by induction of central-memory T cells and effector-memory T cells	156
Chitosan (shells of *Pandalus borealis*)	Infection (SARS-CoV-2)	Receptor-binding domain protein	Nanoparticle	Increased sIgA antibody levels compared to controls Induction of memory T cells of the T$_H$1 phenotype that secrete IFN-γ, IL-2 and TNF in response to antigens	157
Chitosan (NA)	Infection (*Chlamydia psittaci*)	Ultraviolet-inactivated *C. psittaci* elementary bodies	Hydrogel	Increased levels of the T$_H$1-type cytokines IFN-γ, IL-2 and IL-12 upon intranasal mucosal immunization compared to intramuscular systemic delivery Increased lymphocyte proliferative response and reduced shedding of *C. psittaci* from the affected mucosa	161
Chitosan (NA)	Infection (Zika virus)	Live and inactivated Zika virus	Hydrogel	Fast and robust reaction of anti-Zika-virus IgG compared to the control group Significant increases in central-memory (CD44$^+$ and CD62L$^+$) and effector-memory (CD44$^+$ and CD62L$^-$) T cells among CD4$^+$ and CD8$^+$ T cells	162
Chitosan (NA)	Malaria	*Plasmodium falciparum* malaria parasite blood-stage apical membrane antigen 1	Nanoparticle	Strong antigen-specific humoral responseIncreased levels of CSP-specific IFN-γ-secreting T cells compared to other adjuvant groups (ISA72 and 7DW8-5) Protection against *Plasmodium falciparum* circumsporozoite protein (PfCSP)/*Plasmodium yoelii* sporozoite challenge as shown by the absence of parasites in the blood	158
Chitosan (NA)	Measles	Measles antigen	Nanoparticle	Elevated levels of antigen-specific IgG in the blood and sIgA titres in the intestinal mucosa, respectively, with lower-molecular-weight chitosan nanoparticles correlating with higher titres	159
Chitosan (NA)	Cancer	TCL	Nanoparticle	Higher endogenous DC maturation induced by mannose–chitosan–TCL nanoparticles compared to controls Induction of antitumour immune responses Robust CTL responses against tumours compared to TCL alone	191
Chitosan and alginic acid (NA)	Cancer	Legumain DNA plasmid	Nanoparticle	Enhanced antigen presentation in the intestinal Peyer's patches compared to controls Reduced tumour size and improved survival rate of tumour-bearing mice compared to controls	160
Mannan (NA)	Infection (malaria)	OVA, CSP	Conjugate	Induction of superior CSP-specific CD4$^+$ and CD8$^+$ T cell responses compared to other leading adjuvants and formulated CSPs Augmented CSP-specific IgG specificity for a broad range of CSP epitopes and reduction in malaria parasite burden in primary human hepatocytes	163
Mannan (NA)	Infection (SARS-CoV-2, IAV)	SARS-CoV-2 spike, IAV haemagglutinin	Nanoparticle	Induction of lymph node innate responses and neutralizing antibodies with broad epitope specificity to viral glycoprotein antigens	192
Mannan (*Saccharomyces cerevisiae*) and dextran (NA)	Cancer	mRNA encoding OVA	Nanoparticle	Upregulation of antigen-specific IFN-γ$^+$CD8α$^+$ and IFN-γ$^+$CD4$^+$ T cells and significantly enhanced tumour infiltration of CD8α$^+$ T cells compared to controls Activation of dendritic cells and infiltration of NK cells	86
High-mannose glycans (kifunensine-induced)	NA	HIV eOD-60mer, model I53-50 immunogens	Nanoparticle	Induction of MBL- or complement-mediated follicular accumulation, serum antibody responses, and antigen-specific germinal centre B cells	165
Pullulan, dextran (NA)	Cancer	OVA	Nanogel	Induction of OVA-specific CTL and antibodies Enhancement of OVA delivery to draining lymph nodes compared to OVA administered alone and effective interaction with Langerin$^+$ CD103$^+$ dendritic cells	193

Table 3 (continued) | Applications of polysaccharides in vaccine delivery

Polysaccharide (source)	Target disease	Antigen	Formulation	In vivo efficacy	Ref.
Angelica sinensis polysaccharide (NA)	NA	OVA	Nanoparticle	Elevated levels of OVA-specific serum IgG with increases in both T_H1-associated IgG2a and T_H2-associated IgG1 levels compared to controls	166
				Increased serum levels of T_H1 cytokines (IFN-α and IFN-γ) and T_H2 cytokines (IL-6 and IL-10) compared to controls	
Inulin (dahlia tubers)	NA	OVA	Microparticles	Enhanced levels of IgG-total and IgG-1 antibody titres compared to controls	167
Delta inulin (Advax, NA)	2009 H1N1 influenza	Recombinant haemagglutinin	Microparticles	Increased seroprotection rates compared to immunization with recombinant haemagglutinin alone	168
Delta inulin (Advax, NA)	*Mycobacterium tuberculosis* infection	CysVac2 (*M. tuberculosis* antigen)	Microparticles	Instigated antigen-specific polyfunctional (IFN-γ⁺TNF⁺IL-2⁺) CD4⁺ T cell populations and IFN-γ responses	168
				Induced protection in *M. tuberculosis*-infected mice as demonstrated by reduced lung *M. tuberculosis* colony-forming unit counts compared to unvaccinated mice	

CSP, circumsporozoite protein; CTL, cytotoxic T lymphocyte; DNA, deoxyribonucleic acid; HIV, human immunodeficiency virus; IAV, influenza A virus; IFN, interferon; Ig, immunoglobulin; mRNA, messenger ribonucleic acid; MBL, mannose-binding lectin; NA, not applicable; NK, natural killer; OVA, ovalbumin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sIgA, secretory immunoglobulin A; TAA, tumour-associated antigen; TCL, tumour cell lysates; $T_H1/2$, type 1/2 T helper cell; TNF, tumour necrosis factor.

on T or B cells[171]. In addition, T cell-independent, NK-cell-mediated protection of mice against infection of murine cytomegalovirus has been demonstrated[172]. Trained immunity relies on sustained epigenetic reprogramming[173] by a primary pathogen, which triggers protective immune responses against a range of primary and secondary pathogens either related or unrelated to the primary pathogen.

Dectin-1, the main β-glucan receptor, is crucial for triggering the Dectin-1/AKT/mammalian target of rapamycin (mTOR)/hypoxia-inducible factor-1α (HIF-1α) signalling pathway that leads to activation of glycolysis and eventually trained immunity[5,174]. β-glucan can trigger Dectin-1 signalling and induce epigenetic and functional reprogramming of monocytes and macrophages, such as histone H3 trimethylation at Lys4 (H3K4me3), H3 acetylation at Lys27 (H3K27ac) and upregulation of glucose and amino acids involved in cellular metabolism[5,171]. β-glucan can induce trained immunity and reverse immunoparalysis, as shown in an ex vivo culture of human monocytes obtained from healthy volunteers who had been intravenously challenged with LPS[175]. Although exposure to high levels of LPS can reprogram innate immune cells and engender an immune tolerance that prevents downstream pathways, β-glucan can reverse that tolerance at the epigenetic level back to a responsive state[4]. Furthermore, LPS can trigger the production of itaconate through the expression of immune-responsive-gene-1 in monocytes and block succinate dehydrogenase activity, potentially leading to immunoparalysis[4,175]. However, β-glucan treatment inhibits immune-responsive-gene-1 expression and preserves the functions of succinate dehydrogenase, re-establishing the immunocompetence of cells and exerting a long-term upregulation of innate immune functions. β-glucan-induced trained immunity may also contribute to protection against *M. tuberculosis* infection in macrophages through nitric oxide production and the triggering of calcium influx, which activates nuclear factor of activated T cells (NFAT) signalling and results in the enhancement of gene transcription upon secondary stimulation[146]. Moreover, yeast-derived whole β-glucan particles can induce trained immunity in pro-metastatic macrophages to control tumour metastasis by sphingolipid-synthesis-pathway-mediated mitochondrial fission[176]. Therefore, β-glucan may be therapeutically employed to target innate immune cells in conditions such as cancer and infectious diseases.

Polysaccharide–PAMP interactions can induce epigenetic and metabolic changes for trained immunity, which can have implications for the pathology of various diseases. Trained immunity can be either inflammatory or tolerogenic and can thus be exploited for the treatment of cancer, infection, organ transplantation, autoimmune disease and allergy. In addition, immunotherapeutic agents could be developed for immune-compromised populations.

Outlook

Polysaccharides can serve as potent PAMPs, inducing various PRR–PAMP-dependent immune-signalling mechanisms. In particular, polysaccharides can be engineered to function as immune modulators and prompt effector (memory) or tolerogenic immune responses for applications in vaccines and immunotherapies. The combined effort of innate and adaptive immunity contributes to the protection against invading pathogens, with the innate immune system being primarily responsible for the recognition of pathogens using germline-encoded PRR proteins[8]. This recognition of pathogens, such as PAMP or DAMP, enables the initiation of early innate immune responses through modification of gene expression for cytokine and chemokine production, deployment of killing factors, and marking of microbe surfaces for elimination[9]. Such functions of innate immunity are pivotal in promoting the activation of adaptive immune responses involving T and B cells for more efficient protection against pathogens. Thus, targeting innate immune cells is crucial for harnessing robust immune responses.

As a first-line-of-defence mechanism, PRRs on innate immune cells — in particular, on DCs and macrophages — initiate innate immune responses through recognition of polysaccharides derived from microbial cell walls[3,4]. Importantly, the type, timing and magnitude of PRR signalling strongly affects the outcome of the T cell response. Multivalent display of the receptor and/or ligand addresses the limitation of low-affinity carbohydrate–protein interactions[112]. Multivalency and epitope density also aid in the recognition of self and non-self molecules[113,114]. This engagement of multiple PRRs through the multivalent display of PAMPs enables polysaccharide-based systems to induce robust T cell responses and exhibit controlled and sustained immunological activities. However, the heterogeneity and impurity of
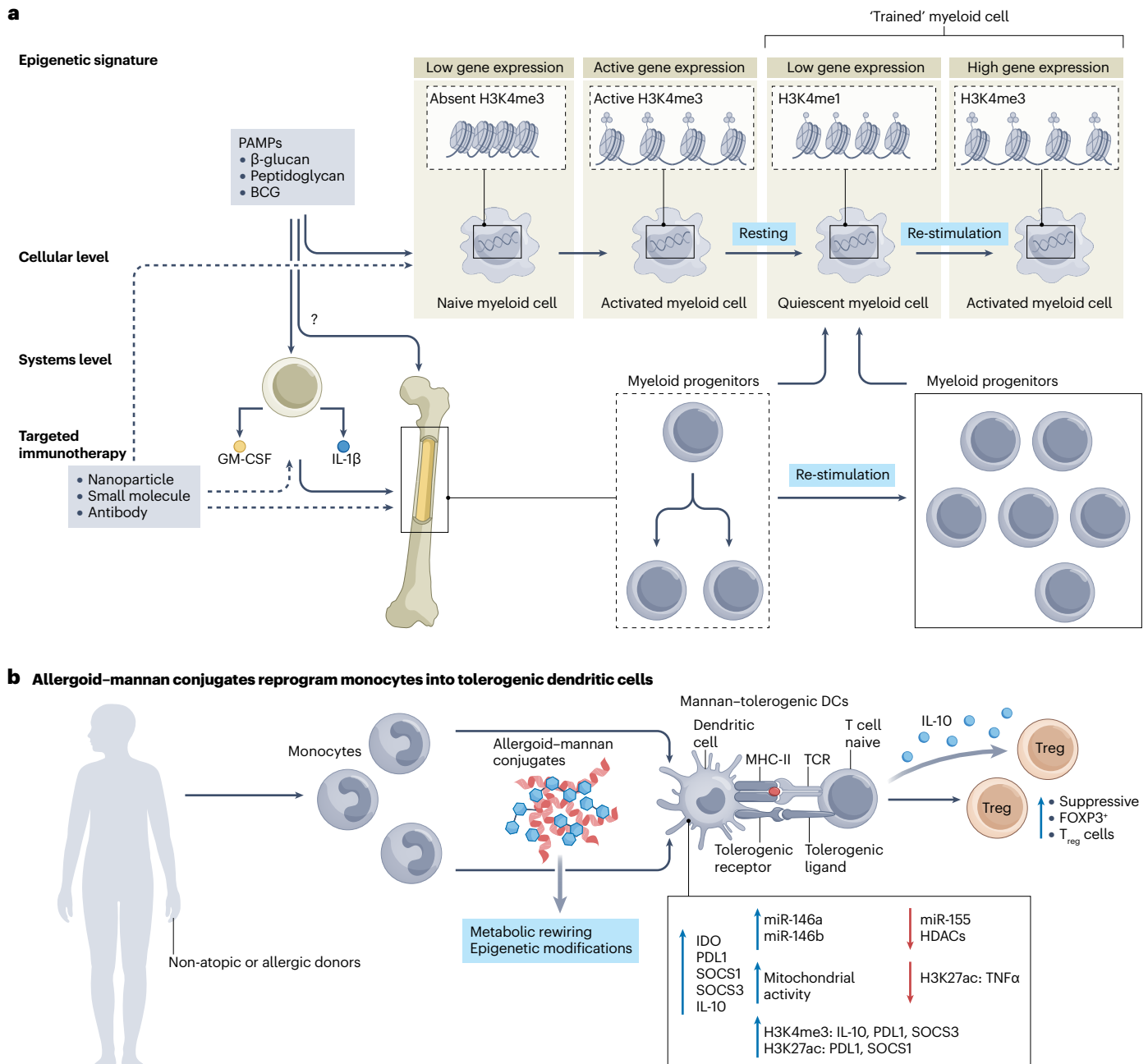
Fig. 4 | Trained immunity is regulated by metabolic and epigenetic reprogramming of innate immune cells. a, Innate immune activation by pathogen-associated molecular patterns (PAMPs), infection and vaccines lead to histone modifications and functional reprogramming of myeloid cells, including monocytes, macrophages and natural killer (NK) cells, which is termed 'trained immunity' or 'innate immune memory'. Initial activation of gene transcription is accompanied by the acquisition of specific chromatin marks, which are only partially lost after elimination of the stimulus. The enhanced epigenetic status of innate immune cells, illustrated by the persistence of histone marks, such as mono-methylation of lysine 4 on histone H3 (H3K4me1), results in a stronger response to secondary stimuli upon rechallenge. At a systemic level, involving the full haematopoietic system in mammals, bone marrow progenitors can be stimulated to produce 'trained' myeloid cells for a prolonged time period, thereby providing a compelling framework for durable therapeutic interventions. **b**, Mannan-allergen conjugates trigger mannose receptor (MR) and dendritic cell (DC)-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing non-integrin (DC-SIGN) to induce tolerogenic DC-mediated regulatory T cells (T_{reg} cells) for allergen-specific immunotherapy. Non-oxidized mannan purified from *Saccharomyces cerevisiae* can be conjugated to polymerized allergens to trigger MR and DC-SIGN, reprogramming monocyte differentiation into tolerogenic DCs for T_{reg}-cell induction via metabolic and epigenetic reprogramming. BCG, bacillus Calmette-Guérin; GM-CSF, granulocyte macrophage colony-stimulating factor; H3K4me1, histone H3 trimethylation on lysine 4; HDAC, histone deacetylase; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; PDL1, programmed cell death ligand 1; SOCS, suppressor of cytokine signalling; TCR, T cell receptor. Part **a** reprinted from ref. 194, Springer Nature Limited. Part **b** reprinted with permission from ref. 195, Mosby.

naturally derived polysaccharides pose a challenge to their translation as immune modulators, particularly as the underlying mechanisms of their immunological properties are yet to be fully understood[114]. However, these challenges may be addressed by engineering the physiochemical properties of polysaccharides, including carbohydrate composition, conformation, molecular weight, functional groups and degree of branching.

Controlling the interaction between polysaccharides and complement is crucial and varies depending on the intended application. For example, activating complement through polysaccharide interaction is desirable in vaccines. Conversely, for applications aiming to extend blood circulation time, inhibiting complement activation is preferable. Therefore, engineering strategies to modulate complement activity, the rate and route of in vivo dosing, and dosage form of the polysaccharide formulation need to be carefully considered.

Physical modifications resulting in the degradation of polysaccharides improve their solubility, structural flexibility and immunological activity by inducing conformational changes while maintaining their fundamental compositions[89,90]. In addition, chemical modifications of polysaccharides with functional groups can improve their structural diversity, as well as biological and immunological activities, by inducing both conformational and compositional change[101,103–105].

Engineered polysaccharides may be particularly suitable for targeting trained immunity developed by innate immune cells. Through sustained epigenetic changes induced by primary pathogens[175], macrophages and monocytes can generate immune responses against other pathogens, both relevant and irrelevant to the initial primary pathogen. Polysaccharide–PAMP immune modulators can reprogram this epigenetic memory to bolster resistance against a broad spectrum of infections, creating a long-lasting antiviral state in the host's immune system. In particular, β-glucan can activate Dectin-1 signalling and trigger epigenetic and functional changes in monocytes and macrophages[5,171] to reverse immunoparalysis[175], protect against infections[146] and control tumour metastasis[176].

In addition to enhancing antigen presentation to DCs by targeting DEC-205 with antibodies[50], antigen presentation to immune cells can also be improved by conjugating monosaccharides to non-polysaccharide polymers. For example, conjugating mannose and a TLR7 agonist to an antigen amplifies antigen presentation to T cells by DCs, triggering CD8+ and CD4+ T cell responses and further expanding antigen-specific memory B cells in mice[163]. Furthermore, antigens modified with polymeric forms of N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc) can target antigen-presenting cells in the liver, increasing antigen presentation and inducing antigen-specific tolerance to prevent T cell-mediated diabetes in the BDC2.5 T cell adoptive transfer mouse model of type 1 diabetes[177]. Although the underlying mechanisms remain to be elucidated, promiscuous interactions between GalNAc/GlcNAc and multiple C-type lectins expressed by hepatic antigen presenting cells[177] may contribute to antigen presentation in the liver and immune tolerance.

Polysaccharides provide a versatile platform for immune modulation and chemical modification, but many obstacles remain to be overcome for their clinical translation. From the chemical point of view, comprehensive investigations of the structure–function relationships of polysaccharides are needed, and standardized protocols should be established for the extraction, purification and structural characterization of polysaccharides in a reproducible manner. In addition, the specific type of polysaccharide used for immune modulation should be better reported in the literature[178], and non-purified polysaccharide-rich extracts containing other compounds (for example, polyphenols, proteins or LPS) should be avoided because their compositions are poorly defined[179,180]. Moreover, polysaccharides often exist in multiple types and compositions and thus, contamination of one polysaccharide by another is plausible if extraction and purification protocols are not optimized, interfering with the interpretation of data. In addition to β-glucan and mannan, other PRR–PAMP pairs and their underlying mechanisms should be further investigated.

Polysaccharides can be engineered to affect specific immune responses by modulating PRR engagement and/or the transduction of specific downstream immune-signalling pathways, which can be controlled by the physicochemical properties of the polysaccharide. In particular, the molecular conformation and higher-order structure may be important determinants for PRR binding. Therefore, determining the primary structure and structure–activity relationships of polysaccharides will provide valuable insights into the engineering of polysaccharides for immunological applications and expedite their clinical development.

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Author contributions
S.S. outlined the manuscript. J.N., A.K., K.K., J.J.M. and S.S. discussed content, researched data and wrote the manuscript. J.H.M., J.B. and M.P. helped with the figures and prepared the tables. All authors reviewed and edited the manuscript.

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