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Immunomodulatory natural polysaccharides: An overview of the mechanisms involved

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ABSTRACT

Maintenance of homeostasis is the primary function of the immune system. It detects the threats posed by pathogens, cancer, and tissue damage and mounts an immune response. The immunological memory dispensed by the immune system prevents or minimizes a second attack by the pathogen. Owing to the advent of new technologies, the comprehension of the immune system has widened, thus opening new horizons to modulate the immune system against any threat. Modulating the immune system with several biological and synthetic molecules mainly targets the innate and adaptive immune system to mount responses against several types of cancers, pathogenic infections, and autoimmune diseases. Polymers are widely used in several biological applications. The various characteristics of polymers, including molecular weight and tunable properties to modulate certain biological functions, have rendered them an ideal candidate for developing state-of-the-art therapeutic interventions. Extensive studies by various researchers across the globe have unraveled the potential of several synthetic and natural polymers to exhibit immunostimulatory activities. These polymers act as pattern recognition receptors (PRR) agonists to activate and engage the immune system. Despite polymeric nanoformulations and scaffolds being used to ferry molecular cargo, targeted delivery to specific cells, and interact with biological molecules, the innate potential of the polymers to influence the immune system is still little understood. From this perspective, this review discusses the very commonly used natural polysaccharides, which are also polymers, and their innate immunomodulatory potentials, mostly focusing on the molecular pathways influenced by the polysaccharides in the immunomodulation process. Further, the challenges associated with the application of polysaccharides and the potential solutions have also been discussed.

1. Introduction

The body's potential to recognize and combat against infections and diseases caused by microorganisms and other cell-intrinsic factors is termed as immunity [1]. The primary function of the immune system is to perform the policing of the human body, check for any immunological threats, and defend against pathogens, toxins, and cell damage. This function of the immune system is essential to maintain homeostasis. Anatomical barriers such as skin provide the initial barrier of defense against invading antigens. Still, instances where the anatomical barriers are breached lead to the kindling of the body's innate immune response. This causes the migration of immune cells that secrete an array of cytokines and chemokines at the site of damage. The secreted cytokines and chemokines play the role of a signal that activates the body's adaptive immunity. Both innate and adaptive immune responses together bring about immunity against a pathogen or any danger posed against the system. The compounds/agents that work alongside the immune system to stimulate or suppress the host's response are called immunomodulatory agents. Factors like the administration route, dosage, administration time, and site of action influence the functions of immunomodulatory compounds [2].

Several polymers have been identified to be excellent immunomodulators. The influence of polymers on innate and adaptive immunity has been a topic of study for years now. The first investigations were conducted by Goebel and Avery, who demonstrated that the conjugation of proteins and carbohydrate polymers in a pneumococcus vaccination altered the host's immunological response [3,4]. Studies in the 1960s identified hydrophilic polymers like alginate, methylcellulose, and poly-

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(ethylene glycol) could be outstanding candidates for drug excipients, whereas polystyrene and poly-(vinyl alcohol) do not provide great support for biological applications [5,6]. Studies have proved the possibility of synthesizing polymers with precise chemistry for the targeted delivery of several proteins, oligonucleotides, or other small molecules. These polymers also showed controlled biodistribution, immune response, and controlled release profile [7–10]. The applications of various polymers in the field of biomedicine, such as scaffolds, gene therapy, drug excipients, drug delivery, and anti-microbial coatings, are owing to the advancement in the technology of controlled polymerization with refinement in the monomer design [11]. From the perspective of clinical immunology, polymers are essential components in transfection reagents for CAR T-cell generation, gene therapies, and vaccine formulations containing liposomes and nanoparticles [10,12–15].

Polymers are well-suited candidates for novel therapeutic development. Polymers' ability to assemble into micro- and nanostructures, their high molecular weight and tunable properties, their potential to deliver cargo, interact with biological molecules, and cell sub-set specific targeting render them a remarkable ability to act as a modulator of the immune system [15–17]. Nevertheless, the polymers' innate ability to modulate the immune system has opened avenues to study their adverse or immunostimulatory effects once administered in therapeutic approaches [14]. Peptidoglycans from bacteria and single-stranded DNA are naturally occurring polymers that act as immunogens. They play a crucial role in distinguishing self-molecules from non-self-molecules or self-molecules that are damaged [18–20]. There are several advantages of using polymeric adjuvants to using small molecule adjuvants. For instance, alteration of pharmacokinetics, immune receptor binding with enhanced affinity and avidity, and enhanced cellular uptake can be observed with polymeric adjuvants compared with small molecule adjuvants [21–23].

Several natural polysaccharides, which are also polymers have innate immunomodulatory abilities. From this perspective, this review is focused on the immunomodulatory properties of the commonly used natural polysaccharides *per se*. The molecular pathways exploited to bring about the immunomodulatory effects in the hosts have also been briefly discussed.

2. Immune system

Immunity is the body's fundamental, significant, and intricate armor to confer protection against pathogens, environmental pollutants, tissue damage, and cancer. This excellent job of surveillance is carried out by the *Immune system* - a network of organs, tissues, cells, and soluble mediators defending the integrity of the body against any insult. The key feature of the immune system is the capacity to differentiate between self (own cells, tissues, organs) and non-self (foreign molecules, microbes), maintaining homeostasis and tolerance.

The immune system helps in the development of immunological memory by mounting a counterattack against any invading pathogen, tissue damage, or cancer. This, in turn, curtails the intensity of a second assault, thus maintaining homeostasis. The immune system is a complex framework consisting of several organs and cells. The bone marrow and the thymus make up the primary lymphoid organs, whereas tonsils, peyer's patches, lymph nodes, and spleen make up the secondary lymphoid organs. The immune cells develop and mature in primary lymphoid organs and reside in secondary lymphoid organs over an individual's lifetime [24].

2.1. Types of immunity and key players

Immunity in humans is conferred by two categories: innate and acquired immunity. Both systems work in synchrony to play the role of immune surveillance in the human body. As the name implies, innate immunity is the immunity our body has acquired over generations in the form of genetic code with little modification because of evolution stored in the germline [25]. Innate immune systems include physical barrier components (skin, mucous membrane), chemical barrier components (sweat, oil, saliva, stomach acid, tears, mucus), cellular components (basophils, mast cells, eosinophils, NK- cells (natural killer cells), dendritic cells, monocytes, neutrophils, and macrophages), enzymatic components (lysozymes) and several soluble components secreted by the cells (cytokines, chemokines, complements, etc.). T-cells and B-cells are crucial players of the adaptive immune system. Unlike the innate immune system, where the receptor-related information is encoded in the genes by inheritance, adaptive immune system receptor information is not genetically embedded. Rather, it is produced because of genetic recombination during lymphocyte development [26]. The first to respond and react to intruders is the innate immune system. No matter whether the antigen is well-known or novel, the innate immune system always mounts a sluggish and incomplete response when defending against intruders. The adaptive immune system helps maintain the body's immunological memory by keeping track of antigens that are already encountered and the ones that are new, for which the immune memory must be developed [27].

The danger signals released by the microorganism, PAMP (pathogen-associated molecular patterns) or the dying cell DAMP (damage-associated molecular patterns) after the insult are sensed by the innate immune system, that triggers an activation cascade and secretes inflammatory cytokines to counteract the situation [28–30]. The first immune cells to react are neutrophils, which travel to the location of the infection or tissue injury and devour it, secreting proteases, neutrophil traps, and inflammatory molecules to clear the pathogen by a process called NETosis [31,32]. In turn, monocytes are recruited by neutrophils, which differentiate into macrophages that secrete soluble factors and are responsible for phagocytosis of the pathogen. In this process of pathogen clearance, macrophages initially acquire a pro-inflammatory phenotype to clear pathogen and cell debris, then eventually adopt an anti-inflammatory phenotype to help tissue repair and homeostasis [33].

The action of T-cells and B-cells, responsible for protection from re-infection, is influenced by innate immune cells [1,31]. The key players in adaptive immunity, T-cells and B-cells, begin their development in the bone marrow; however, B-cells mature there while T-cells move to the thymus to complete their maturation. B- and T-cells recognize the antigen by means of specific receptors, namely B-cell receptor (BCR) and T-Cell receptor (TCR), respectively. At any point in time, there are around $\sim 10^{13}$ to 10^{18} different B- and T-cell clones in the body, with various antigen specificities [34,35]. The B-cells function by secreting antibodies that neutralize the microbe's function and promote their phagocytosis [15]. Based on the phenotype and functions, T-cells are classified into $CD4^+$ and $CD8^+$ T-cells. B-cell and $CD8^+$ T-cell functioning are enhanced by the signal cues offered by $CD4^+$ T-cells, which are also called helper cells. $CD8^+$ T-cells, otherwise known as killer cells, are actively involved in the destruction of infected cells by secreting lytic enzymes [1].

2.2. The bridge between innate and adaptive immunity

Despite there being two distinct immune systems, they never work independently; instead, they always work together. The APCs (antigen-presenting cells) of the innate immune system act as a bridge between the innate and adaptive immune systems by capturing, processing, and presenting the antigen fragments to the lymphocytes of the adaptive immune system. Among the various APCs, dendritic cells mainly carry out processing and presentation of the antigens [29]. Dendritic cells act as pickets; they are responsible for antigen presentation, where the antigen is presented to the T-cells as short peptides via the major histocompatibility complex (MHC). Further, the MHC molecules can be categorized based on the type of antigen they present and the sub-type of T-cell to which they present the antigen. MHC-I molecules present intracellular antigenic peptides to $CD8^+$ T-cells, and MHC-II molecules present peptides from exogenous antigens to $CD4^+$ T-cells. Also, DCs

(dendritic cells) are capable of cross-presenting exogenous antigens through MHC-I to prime $CD8^+$ T-cells [36,37]. The activated DCs, after their encounter with the antigen, carry the antigenic peptides on their surface and move to lymph nodes to prime the T-cells. The activated DCs act as APCs and T-cells interact with the antigen presented through MHC-I or MHC-II with the advent of co-stimulatory molecules CD8 and CD4 present on the T-cell surface. The resulting T-cell responses can be classified into two types. The first is $CD8^+$ cytotoxic T-lymphocytes mediated response (CTL) that clears infected or cancerous cells, and the second is $CD4^+$ T-helper cells mediated response that involves the secretion of immune mediators thereby enhancing $CD8^+$ and B-cell responses.

Further, Th1 and Th2 cells are the two categories of T-helper cells that aid in CTL-mediated killings and B-cell maturation, respectively. Meanwhile, B-cells can interact with the antigens (proteins, carbohydrates, etc.) via B-cell receptors (BCRs). Interaction of naïve B-cells with antigens, when mediated by Th2 cells, produces cytokines that will help in secreting antibodies as well as in the phagocytosis of the antigen. However, when induced by an antigen with repeating subunits, the B-cells mature and produce antibodies independent of T-cell stimulation. Isotype class switching of antibodies from the regular IgM and IgD to specialized IgG2 and IgA with enhanced affinity and specialized functions is achieved by employing appropriate cytokines [38]. The concomitant functioning of innate and adaptive immune systems is essential to eliminate intruders with minimal or no damage to the host. Fig. 1 depicts the overview of the immune system.

3. Immunomodulation

Various therapeutic approaches to modify immune response to combat threats to the body is immunomodulation. Immune response accrual is essential for preventing infections in case of immunodeficiency and battling against established infections and cancer. The main aims of immunomodulation are: 1. Reduction of immune system responses in hyperactivated immune systems (inflammatory disease conditions, transplant rejection, and autoimmunity) or 2. Persuasion of the immune system to combat the threat in case of a hypoactive immune system against any infections or cancers [39]. Several agents, such as antigenic proteins, peptides, polymers (natural and synthetic), drugs, extracellular matrix components, and nucleic acids, are used as immunomodulatory agents. Immunomodulatory agents perform their functions by modulating the various cell surface and intracellular surface receptors BCR, TCR, and receptors on the surface of dendritic cells. As these signaling cascades play a vital role in the immune mechanism, modulating the same with several agents will regulate the strength, direction, and tenacity of the immune cell responses [40].

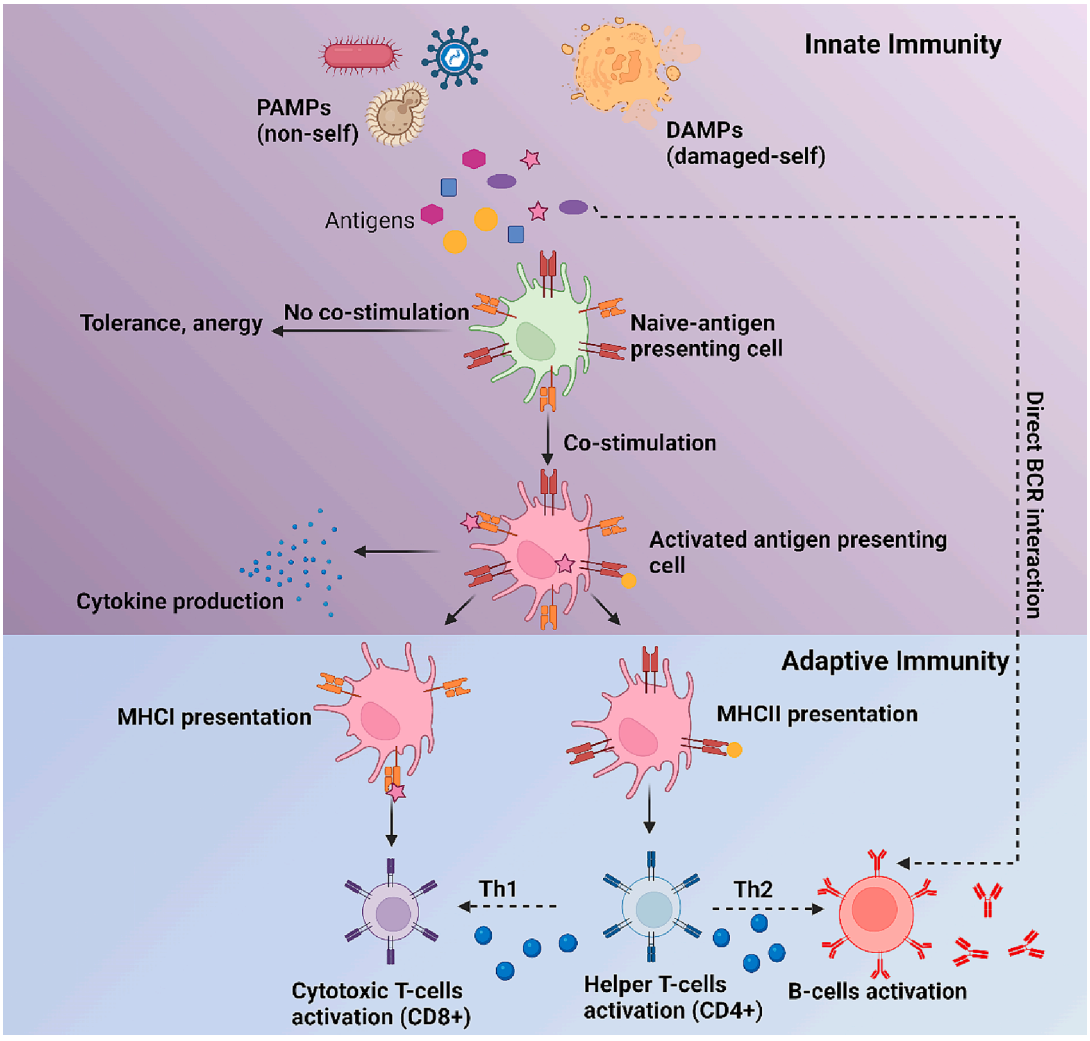

Fig. 1. Overview of various components of the adaptive and innate immune system. The antigen-presenting cells (APCs) recognize "damaged-self" (DAMPs) and "non-self" (PAMPs) molecules in case of loss in h ome ostasis through MHC-I and MHC-II and PRPs. The activated APCs secrete cytokines and present the antigens via MHC-I and MHC-II to the adaptive immune system. B-cells and T-cells constitute the adaptive immune system ($CD4^+$ and $CD8^+$). T-cells have TCRs and B-cells have BCRs to recognize the antigens on MHC complexes to elicit the destruction of invading pathogens.

4. Immunomodulatory polysaccharides

4.1. Fucoidan

Fucoidans are sulfated polymers with L-fucose and sulfate ester groups being the major components. They are copious in brown seaweeds and other marine species' cell walls. Their overall composition depends on the species from which fucoidan is isolated, their habitat, collection season, and extraction methods [41]. Structural backbone of fucoidan is generally made up of alternating α-(1–3) and α-(1–4) linked L-fucopyranose or repeating α-(1–3) linked L-fucopyranose residues, with the fucosyl being mono- or distributed along with acetate and/or sulfate groups on C-2 and C-4 or rarely in a position [42,43]. Additionally, several monosaccharides, proteins, and uronic acid residues could also be a part of the fucoidan main chain to form the structure [44]. Fig. 2 shows the structure of fucoidan. Fucoidans from marine algae have found their application in various biological activities and therapeutics. Fucoidan has been the subject of decades of research, which has revealed its many benefits, including anti-microbial, antiviral, antioxidant, anti-inflammatory, anti-diabetic, and immunomodulatory effects [45,46].

4.1.1. Fucoidan - an immunomodulatory agent

Fucoidan's immunomodulatory properties are well studied and reported. The association between the structure and immune regulatory potential of fucoidan is still debated. However, the immunomodulatory properties of fucoidan have been attributed to the existence of particular functional groups in the fucoidan. The study by Khil'chenko and the group reported that the sulfate and acetyl groups present in the fucoidan lowered the release of inflammatory cytokines, thus playing a significant role in determining the immunomodulatory properties of fucoidan [48]. *In vitro* studies demonstrated the activation of macrophages and DCs by fucoidan via scavenger receptor-A (SR-A) [49,50]. Jin *et al.*, studied fucoidan's potential to be an adjuvant *in vivo*. Fucoidan, when administered systemically, enhanced the expression of co-stimulatory molecules CD86, CD80, and CD40 in spleen DCs with the production of pro-inflammatory cytokines such as IL-6 (IL-Interleukin), IL-12, and TNF-α (TNF-Tumor necrosis factor) and promoted DCs maturation. Additionally, fucoidan increased the IL-12-dependent generation of T-cell subsets like cytotoxic (Tc1) and helper (Th1) cells. Furthermore, fucoidan, when used as an adjuvant alongside OVA antigen, upregulated MHC-I & II on DCs. They also caused the proliferation of CD4 and CD8 T-cells. Mice immunized with fucoidan as an adjuvant were shielded from tumor formation when injected with B16-OVA tumor cells [51]. Fucoidans isolated from *Undaria pinnatifida* amplified NK cells and T-cell activation along with the enhanced synthesis of pro-inflammatory cytokines in HSV-1 infected mice. Induction of phagocytosis by macrophages and B-cell blastogenesis was observed after treatment with fucoidan *in vitro* [52]. Zhang *et al.*, compared fucoidans isolated from four different species of algae: *Fucus vesiculosus, Macrocystis pyrifera, Ascophyllum nodosum,* and *Undaria pinnatifida,* for their immunomodulatory effects. The production of TNF-α, IL-6 and, IL-8, which are inflammatory cytokines, was enhanced after treatment with all the different types of fucoidan. *Undaria pinnatifida* and *Macrocystis pyrifera*-derived fucoidans deferred human neutrophil apoptosis even at very low concentrations, but *Ascophyllum nodosum* and *Fucus vesiculosus* derived fucoidans delayed the process at higher concentrations. *M. pyrifera*-derived fucoidans was concluded to be the most effective of all four owing to its potential to delay neutrophil apoptosis, T-cell response, mouse NK-cell activation, memory T-cell generation, and maturation of DCs [53]. *In vivo* studies of *Undaria pinnatifida*-derived fucoidan on immune-suppressed animals revealed the recovery of immunity by activation of T-cells and NK cells by fucoidan [54].

Effects of fucoidan from *Nizamuddinia zanardinii* studied by Tabarsa's group on NK-92 cells, and RAW264.7 cell line showed increased secretion of TNF-α, NO (nitric oxide), IL-1β, and IL-6. Also, TNF- α, and INF-γ were released because of MAPK (Mitogen-activated protein kinases) signaling pathways, NK cells, and NF-κβ (Nuclear factor kappa beta) activation [55]. Similarly, *Ascophyllum nodosum* and *Fucus vesiculosus* derived fucoidan activated signaling pathways NF-κB and AP-1, which caused NO and cytokine production in RAW264.7 cells [56].

4.1.2. Influence of molecular weight on the immunomodulatory properties of fucoidan

The influence of the molecular weight of fucoidan on immunomodulatory functions by activating signaling cascades has been well studied and reported. An intriguing study by Bi and co-workers showed that fucoidan of low molecular weight (LMWF) had better activity compared to fucoidan of high molecular weight (HMWF). Concentration-dependent induction of NO release, TNF-α, IL-6 secretion, and iNOS expression and stimulation of signaling pathways like NF-κB and MAPK was observed with LMWF treatment [57]. Similarly, fucoidan derived from *Stichopus chloronotus* activated RAW264.7 cells via TLR-2 (TLR – Toll-like Receptor) and TLR-4 and the downstream NF- κβ signaling pathway [58]. A similar study by Li *et al.* recently showed that LMWF stimulated DC cells both *in vitro* and *in vivo*. LMWF increased $CD4^+$ proliferation and $CD8^+$ T-cells *in vitro* and enhanced the DC maturation and migration both *in vitro* and *in vivo*. This has been attributed to the activation of TLR-4 and the downstream MAPK and NF-κβ signal cascades. Furthermore, LMWF *in vivo* , boosted immune response by inducing immune cell proliferation and enhancing the immune organ index [59]. Hwang and their group studied LMWF's effects on innate and adaptive immunity. The results revealed that dietary supplementation with LMWF activated innate and adaptive immunity and enabled protection against stimulation by *Mycoplasma pneumoniae* antigen. After giving mice LMWF orally, they also demonstrated enhanced phagocytic activity, splenocyte proliferation, and NK cell activity associated with the innate immune response. Also, increased serum levels of INF-γ, IL-2, and IL-4 secretion by splenocytes, Ig-A, and Ig-G content, and regulators of adaptive immune response were observed. After being challenged with antigen, LMWF treatment increased IgM and IgG content in the serum of mice [60]. Fucoidan derived from *Cladosiphon okamuranus* increased the proliferation of immune cells, IL-2, serum antibodies (IgA, IgG, IgM), phagocytes and decreased IgE, IL-4, and IL-5, indicating its immunomodulatory effect by both cellular and humoral immunity *in vivo* [61].

4.1.3. Fucoidan in cancer immunomodulation

Fucoidan's role in anti-cancerous immunomodulation has been

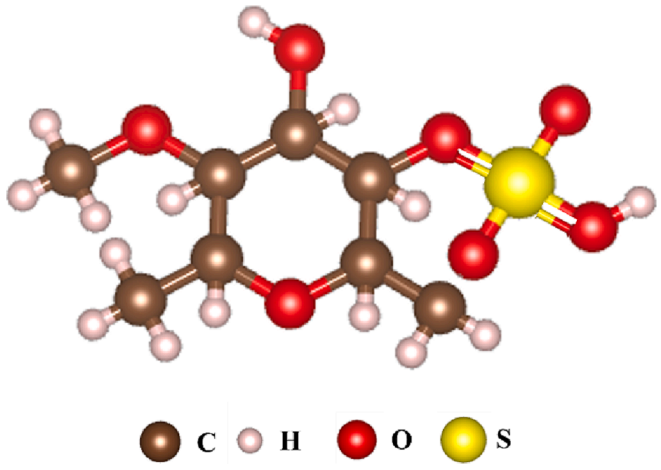

Fig. 2. 2D Structure of Fucoidan. The structure was created using VESTA [47]. (National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 129532628. https://pubchem.ncbi.nlm.nih.gov/compound/Fucoidan-_A-natural-extract-algae-mixture).

discussed in this section. Literature has reported the anti-tumor ability of fucoidan in connection with the molecular weight, monosaccharide ratio, bound sugar residues, and sulfate groups [62]. Reports have shown that fucoidan with excess sulfur groups exhibited enhanced antitumor activity compared to normal fucoidan [63]. But on the contrary, *Sargassum hornery*-derived fucoidan, devoid of sulfur, showed better antitumor activity than that of 16.9% sulfur-containing fucoidan [64]. Hence, it is evident that the immunoregulatory effect of fucoidan is not only dependent on its biological structure but also on various other factors like monosaccharide ratio, the type of sugar residues present, and so on. Zhang's group has studied the intranasal administration and mucosal adjuvant effect of fucoidan. When administered intranasally, fucoidan derived from *Ecklonia cava* (ECF) enhanced the activity of immune checkpoint inhibitors. ECF, when administered intranasally, enhanced the activation of DCs, NKs, and T-cells in mediastinal lymph nodes of mice, wherein the activation of NKs and T-cells were facilitated via DCs. ECF also increased the anti-cancer activities mediated by anti-PD-L1 (immune checkpoint inhibitors) antibodies against melanoma and carcinoma tumors in mice [65]. Also, ECF barred the CT-26 carcinoma cell infiltration into the lungs of BALB/c mice by activating splenic DCs and NK cells [66]. Fucoidan from *Laminaria japonica* induced inflammatory cytokine synthesis, activated NK cells, and T-cells. Also, it enhanced the effect of the PD-L1 antibody against Lewis lung carcinoma [67]. However, a recent study to understand the cancer inhibitory activity of fucoidan along with immune checkpoint inhibitor drug Nivolumab revealed that fucoidan *per se* activated PBMCs (peripheral blood mononuclear cells) and imposed cytostatic effects on PC3 prostate cancer cells, but did not influence cell killing [68]. Recently, the inhibitory effects of fucoidan on cancer development were studied using the inflammation-related colorectal cancer model. Fucoidan's anti-inflammatory effect was evaluated by lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. Colorectal cancer (CRC) cells treated with fucoidan showed inhibition of the proliferation, cell cycle arrest at the G0/G1 phase, and induction of apoptosis. *In vivo*, fucoidan intervention showed reduced tumor incidence in inflammation-related CRC model mice with a reduction of inflammatory markers such as TNF-α, IL-6, and IL-1β in colon tissues [69]. The specific effect of fucoidan on M2 macrophages in the tumor environment has been reported by Sun *et al.* Fucoidan adversely affected CCL2 chemokine secreted by M2 macrophages, which in turn affected the tumor cell migration and T_{reg} cell recruitment. Further, the anti-tumor property of fucoidan was attributed to its ability to suppress NF-κB activity, that is associated with reduced tumor growth and tumor surveillance [70].

4.1.4. Immunomodulation by fucoidan in dermal issues

Fucoidan has also been studied for its role in treating atopic dermatitis (AD). *Cladosiphon okamuranus*-derived fucoidan (COP) ameliorated AD-associated symptoms by inhibiting mast cell degranulation, eosinophil infiltration, epidermal hyperplasia, and synthesis of cytokines related to AD. The reduction in epidermal hyperplasia was identified to be linked to the downregulated expression of IL-22 [71]. Another study demonstrated that topical administration of COP controlled systemic immunity by increasing tolerogenic dendritic cells (Tol-DCs), which stimulated T-cell development and decreased the number of Th22 and memory B cells. Here, COP ameliorated AD by increasing TGF-1β secreting T_{reg} cells and suppressed Th2-mediated immunity [72]. The *in vitro* and *in vivo* immunomodulatory effects of Fucoidan have been listed in Table 1 & Table 2, respectively.

4.2. Chitosan

Chitin, the most prevalent biopolymer after cellulose, is present in the exoskeletons of various fungi, insects, and crustaceans. It is deacetylated to produce chitosan, a cationic polymer also known as (1–4)-linked 2-acetamido-2-deoxy-D-glucose (N-acetyl glucosamine) polymer [75,76]. Fig. 3 depicts the structure of chitosan. Chitosan's role as an anti-bacterial, anti-fungal, anti-cancer, anti-inflammatory, and immunomodulatory agent has been well reported [77–79]. The functional groups present in chitosan are mainly responsible for its biological activities. The amino/acetamido group at C-2 and the primary & secondary hydroxyl groups at C-3 & C-4 positions, respectively, mainly influence the biological activities of chitosan such as antimicrobial, antifungal, immune-enhancement, antitumor, and antioxidant activities [80]. This section will mainly focus on the innate immunomodulatory properties of chitosan and the biological pathways influenced by it.

Table 1
In vitro immunomodulatory effects of Fucoidan.

Source	Cell line used	Observed effects	Reference
Fucus vesiculosus *Macrocystis pyrifera* *Ascophyllum nodosum* *Undaria pinnatifida*	Neutrophils from humans	Enhanced synthesis of TNF-α, IL-6, and IL-8 by neutrophils	[53]
Nizamuddinia zanardinii	Murine macrophage RAW 264.7	Considerable increase in NO, IL-6, IL-1β, and TNF-α, secretion; Activated NK cells, MAPKs, and NF-κβ pathways with TNF-α and INF-γ release	[55]
Laminaria japonica *Laminaria cichorioides* *Fucus evanescens*	Human Embryonic Kidney cells. HEK293-null, HEK293-TLR2/CD14, HEK293-hTLR5, and HEK293-hTLR4/CD14 MD2	Activation of TLR2 followed with activation of NF-κβ pathways in *L. japonica* fucoidan. *F. evanescens* fucoidan and *L. cichorioides* fucoidan activated TLR-4 and subsequently activation of NF-κβ pathways are registered for *L. japonica* fucoidan *F. evanescens* fucoidan, and *L. cichorioides* fucoidan	[73]

4.2.1. Anti-tumor immunomodulation of chitosan – Pathways involved

Immunomodulatory properties of chitosan and its derivatives associated with anti-tumor effects have been very well explored by the researchers, and their anti-tumor activity is mainly attributed to two cascades – (i) cascades affecting the cancer cell directly and (ii) cascade of immunomodulation. The former is intercellular, whereas the latter is extracellular.

Chitosan - effects on cancer cells

In the case of intercellular interaction of chitosan, the effect could be biochemical or cytogenic in nature. The biochemical nature involves the apoptosis of cancer cells by induction of caspase-3 and other growth-inhibiting pathways [81,82]. In line with these pathways, when treated with very viscous chitosan, three human malignant cell lines RPMI-7951, A375, and SK-ME-L28 exhibited a decrease in malignancy, in conjunction with the activation of apoptosis in all but A375 [82]. Similarly, chitosan promoted apoptosis and inhibited the human bladder tumor cells 5637 growth [81]. Chitosan affects the cytogenetic pathways by inhibiting the cell cycle phases. In cells exposed to chitosan, fewer cells entered the S-phase of the cell cycle, less DNA was synthesized, tumor suppressor genes like p21 were upregulated, cyclin-dependent kinase-2 (cdk-2), cyclin A, and proliferating nuclear cell antigen (PCNA) were downregulated [83]. This mechanism was observed in chitosan with a molecular weight of 23.99 kDa that inhibited human hepatocellular carcinoma cells (HepG2) [84].

Chitosan immunomodulation in cancer

Chitosan exhibits its anti-cancerous effects by virtue of modulating the immune system of the host by influencing several pathways:

Table 2
In vivo immunomodulatory effects of Fucoidan.

Source	Animal model	Dose and treatment modality	Observation	Reference
Undaria pinnatifida	Immunosuppressed BALB/C mice with 5-flurocil inoculated with herpes simplex virus-1	10 mg/day for 7 days – oral treatment	Enhanced NK cells activation	[52]
Undaria pinnatifida	UVB irradiated BALB/c mice skin	500 mg/kg for 12 days – oral administration	Reduced INF-γ levels post-irradiation, decrease in leukocyte migration, and skin edema. No significant effects were observed in TNF-α, NF-κβ, IL-4, and IL-6.	[74]
Fucus vesiculosus	C57BL/6 mice	10 mg/kg peritoneal injection of a single dose	Spleen and serum levels of TNF-α and IL-6 significantly increased	[51]
Undaria pinnatifida	C57BL/6 mice	50 mg/kg for 3 days intraperitoneal injection	Only *M. pyrifera* fucoidan activated and increased the maturation of NK cells.	[53]
Ascophyllum nodosum				
Fucus vesiculosus				
Macrocystis pyrifera				

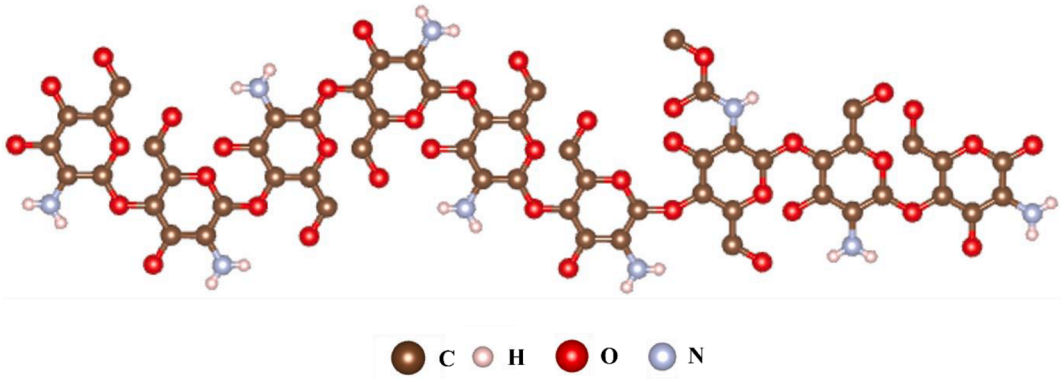

Fig. 3. 2D Structure of Chitosan. The structure was created using VESTA [47]. (National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 71853. https://pubchem.ncbi.nlm.nih.gov/compound/Chitosan).

biochemical, cytological, tumor angiogenesis, and metastatic pathways. A study by Le *et al.*, revealed that chitosan selectively inhibited the pyruvate kinase pathway in cancerous cells and hindered glucose uptake, eventually decreasing the ATP levels by reducing the glycolytic activity of adenocarcinoma cancer cells [85]. Chitosan being a polycation, acted on the highly negative surface of the cancer cells and elevated the nitrogen monoxide levels to inhibit excessive cell division [86,87]. Also, chitosan with polycationic charge and higher molecular weight caused the rupture of cancer cells by interacting with them extracellularly [88]. Further, chitosan and derivates hindered the tumor-induced angiogenesis and metastasis. Murata *et al.*, showed that sulfated chitin of molecular weight 24 kDa impeded the neovascularization in C57BL/6 mice inoculated with BI6-BL6 cells [89].

Role of chitosan in activating DCs-STING pathway

Studies have shown that chitosan induced cytokine secretion from NK cells and activated macrophages in a phagocytosis-dependent manner [90,91]. It is evident from recent research studies that chitosan induced the maturation of DCs and Type-1 interferon responses by virtue of various receptors of innate immunity [92–94].

Carrol and colleagues showed that chitosan triggered APC maturation, expression of co-stimulatory molecules, and Th1-biased adaptive responses via the STING (stimulator of interferon genes) pathway. Chitosan exposure increased the amount of mitochondrial DNA in the cytosol by stressing out the mitochondria and producing reactive oxygen species (ROS). Due to this, the STING pathway was activated, resulting in the generation of Type-1 interferon and Th1-biased responses [93]. The synthesis of cyclic dinucleotides bound to STING in the endoplasmic reticulum was triggered by the binding of cGAS to the host nucleic acid, activating the cGAS-STING pathway. Additionally, STING relocated to the perinuclear Golgi after interacting with either IKK or TBK-1, which activated NF-β and IRF-3 transcription factors, respectively [95]. Both *in vitro* and *in vivo* experiments have demonstrated that chitosan can activate the latter downstream pathway by promoting the production of type I INF instead of pro-inflammatory cytokines, demonstrating the adjuvanticity of chitosan [94].

Chitosan's impact on inflammasome activation has been well investigated [96,97]. Inflammasomes are cytosolic, multi-protein complexes consisting of NLRP3, Caspase-1, and ASC – the adaptor protein. Inflammasome activation happens in two steps, with the initial step involving the NLRP3 inflammasome complex formation, followed by the enhanced pro-IL-1β expression. The second step witnesses the caspase-1-mediated cleavage of pro-IL-1β to its biologically active form. The role of chitosan in activating the inflammasomes can be attributed to its ability to mediate several mechanisms like potassium ion efflux, reactive oxygen species (ROS) generation, and lysosomal destabilization [97,98]. Prevention of IL-1β release owing to chitosan treatment was observed when K^+ efflux and the gradient essential for the efflux were inhibited.

Further, the role of ROS was identified by pre-treating the cells with mitoTEMPO, a ROS inhibitor. The results showed the inhibition of chitosan-induced-IL-β secretion. Prevention of acidification of lysosomes by drugs such as bafilomycin A and chloroquine led to the destabilization of lysosomes that, in turn, played an essential role in inflammasome activation by chitosan [97]. Chitosan was found to secrete IL-12, a Th1 polarising cytokine, along with the Th-17 polarising cytokine IL-23 when injected intraperitoneally with TLR9 agonist CpG. Inflammasome activation was identified to be one of the key signaling pathways

involved in inducing Th-17 response. This could be taken as a base to consider chitosan as an alternative to alum which inhibited IL-12 secretion [99]. Secretion of type-I interferon and activation of inflammasomes by chitosan is a lysosomal acidification-dependent process. Research work has shown that the prevention of lysosomal acidification prevented chitosan's cytosol entry; instead chitosan was confined to intra- and extracellular vesicles. Intriguing results have been identified where type-I interferon secretion and lysosomal disruption to a minimal level were observed with low-concentration chitosan treatment. But high concentration chitosan treatment led to lysosomal acidification followed by inflammasome activation with the type I IFN responses suppressed [100]. The uptake of chitosan into the cells is mediated by phagocytosis [98], but ambiguity still surrounds the potential involvement of any specific cell surface receptors in the process. However, several proteins like lectin galectin-3 [101], type II transmembrane protein FIBCD1 [102] and CLR RegIIIγ [103] have been found to have an affinity towards oligonucleotides of chitin and chitosan. Studies have demonstrated the involvement of the Dectin-1 receptor in recognizing chitin [104–106] and its role in chitosan uptake by DCs is widely being studied. Fig. 4. gives an overview of the pathways involved in the immunomodulation by chitosan.

4.3. Alginate

Alginic acid (alginate), a copolymer of (1–4)-linked β-D-mannuronate (M) and α-L-guluronate residues (G), is an anionic, bio-

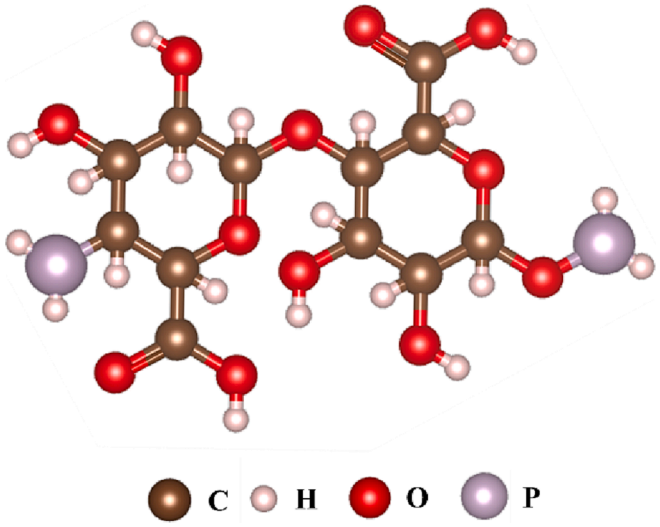

Fig. 5. 2D Structure of Alginate. The structure was created using VESTA [47]. (Ref. National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 131704328. https://pubchem.ncbi.nlm.nih.gov/compound/Alginate).

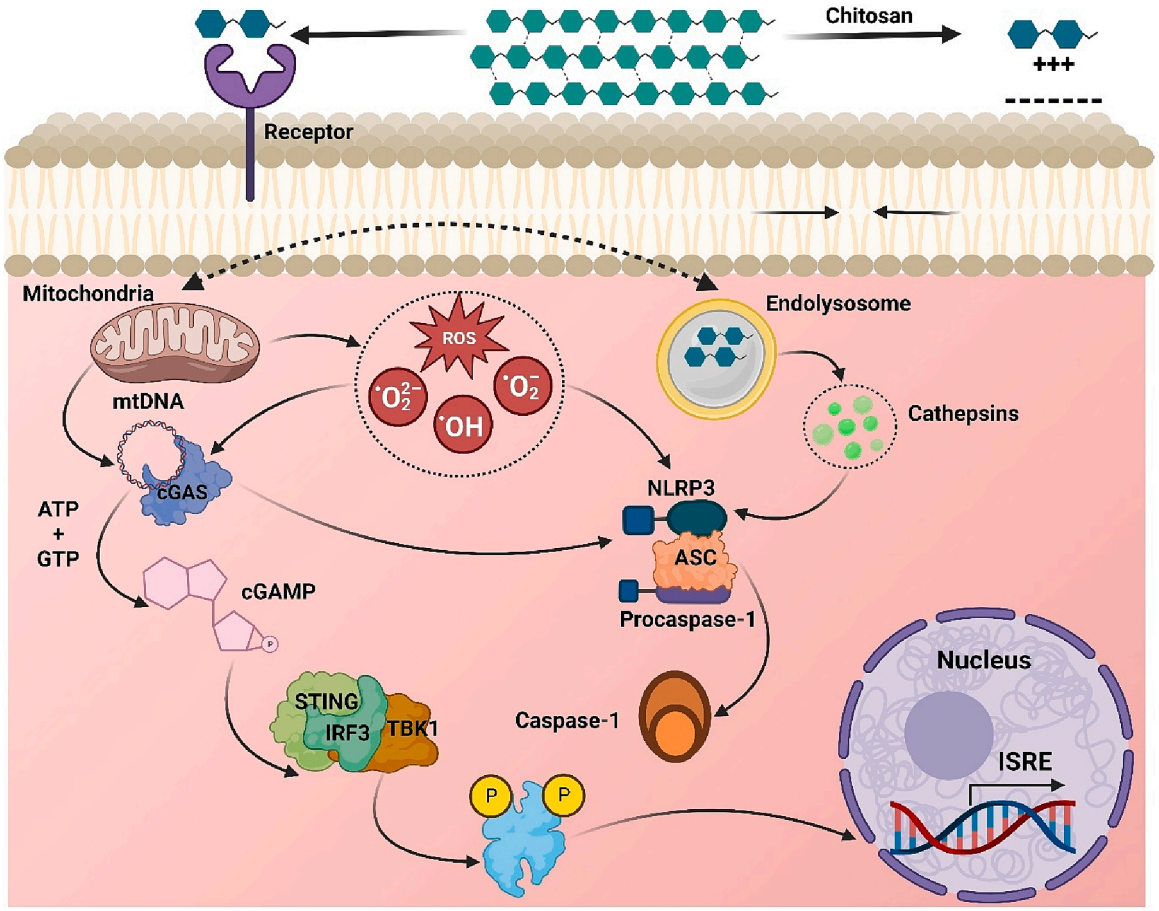

Fig. 4. Pathways involved in the immunomodulation by chitosan. Chitosan causes receptor clustering and further downstream signaling, or will get internalized by endocytosis. Chitosan causes the release of mtDNA and ROS from mitochondria either through direct interaction after internalization or down stream signaling mechanisms. ROS and mtDNA together activate the cGAS-STING pathway. Inflammasome activation mediated by chitosan could be attributed to the activity of cathepsin and ROS. Yet the accurate mechanism is not explored. Abbreviations: mtDNA-Mitochondrial DNA, GTP-Guanosine triphosphate, ATP-Adinosine triphosphate, cGAS-Cyclic GMP-AMP synthase, STING- Stimulator of Interferon Genes, IRF3- Interferon regulatory transcription factor, NLRP3- NACHT, LRR and PYD domains- containing protein 3, TBK1-TANK binding kinase 1, ASC-Apoptosis-associated speck-like protein containing CARD.

adhesive polysaccharide obtained from the cell walls of algae. Fig. 5 shows the structure of alginate. Alginate on its own is water-insoluble, and hence, sodium-alginate is the most used form in various biomedical applications. Reports have attributed the immunomodulatory effects of alginate to the G and M content present. The specific epitope on alginate responsible for TLR activation is still ambiguous in alginate. Yet it has been observed that neither 100% M alginates nor alginates with G blocks caused the activation. 0.05–0.30 fraction of single G residues were found to be essential for immune activation in HMW alginate, suggesting the need for a flexible epitope chain with an appropriate length of alternating MG sequence [107]. Further, it has been reported that a high M block containing alginate exhibited excellent immunomodulatory properties owing to the activation of macrophages and the resulting synthesis of cytotoxic factors and cytokines [108]. Reports have suggested the ability of alginate to stimulate monocytes and macrophages [109] and mostly alginate used in multiple formulations plays an essential role in stimulating phagocytosis as it promotes the adhesion of the formulation to DCs. Several studies have demonstrated the immunomodulatory capacity of alginate and alginate oligosaccharide (AOS) (degradation product of alginate) through the induction of cytokine activities [110]. AOS induced the secretion of interleukins like IL-6, IL-9, IL-13, IL-1α, and 1L-1β, along with oligomer structure-dependent secretion of several cytokines such as granulocyte colony-stimulating factor (GCSF), TNF-α, eotaxin, granulocyte–macrophage colony-stimulating factor (GM-CSF), and regulated upon activation normal T expressed and secreted (RANTES) in RAW V264.7 cells [111]. In line with the *in vitro* studies, *in vivo* studies in mice models have shown the ability of AOS to induce cytokine production. AOS at a concentration of 70 mg/kg, when administered intraperitoneally, influenced the secretion of various cytokines like keratinocyte-derived chemokine (KC), IL-12, RANTES, GCSF, IL-6, and MCP-1 [112,113]. A study by Yang and co-workers showed that sodium alginate influenced the NF-κβ signaling pathways and thereby resulted in the synthesis of cytokines such as TNF-α, IL-6, IL-12, and IL-1β in RAW264.7 macrophages [114].

Furthermore, alginate was given orally to mice with the sarcoma 180 tumor, and this resulted in a suppression of tumor growth and an observed enlargement of the spleen in the treated animals. This modulated the immune system of the host to produce the anti-tumor effects [115]. The unsaturated oligosaccharide of alginate activates the macrophages to induce immune-inflammatory responses. Guluronate oligosaccharide (GO), the enzymatically depolymerized, unsaturated alginate induced TNF and ROS synthesis in RAW264.7 cells and enhanced nitric oxide synthesis by upregulating the expression of nitric oxide synthase, thereby conferring immunomodulatory effects [116]. Investigations have revealed the involvement of several signaling cascades in the activation of macrophages induced by GO. Akt/NF-κβ and Akt/mTOR are the cascades influenced by GO for the activation of macrophages. GO's immunoregulatory role on macrophages is recognizing GO by the TLR4 surface receptors. TLR4, in turn, phosphorylates the PI3K, which in turn phosphorylates Akt. Further phosphorylation of I-κβ by phosphorylated Akt results in the nuclear translocation of NF-κβ. Phosphorylated Akt also activates mTOR and p70 S6 kinase. Each of these components influences macrophage activation either individually or has a synergistic effect.

The activation of macrophages by GOs is not only via TLR4 receptor-dependent downstream pathways. MAPK, one of the crucial downstream signaling pathways of TLR4, is phosphorylated by GOs. This phosphorylation further activated all the components of MAPK, like ERK (Extracellular signal-regulated kinase), JNK (Jun N-terminal kinase), and p-38 [110,113]. Consequently, activation of these components resulted in the transcription of genes responsible for NO and TNF-α synthesis. Interestingly, it was found that GOs binding to TLR4 caused the phosphorylation of JNK and not ERK and p-38, proving the involvement of several signaling pathways in macrophage activation by GOs.

Furthermore, studies have shown the ability of GOs to increase the phagocytic potential of macrophages by upregulation of FcγR I and FcγR II (Fcγ receptor) on the surface of macrophage [117]. However, GOs activated not only TLR4 but also TLR2 in RAW264.7 cells [116]. It should be emphasized that GOs can trigger pro- and anti-inflammatory responses in RAW264.7 macrophages, indicating that its impact on the cells is not limited to one pathway. Fig. 6. shows the pathways activated by alginate during macrophage activation [110].

4.4. β-Glucans

Prominently found on the cell walls of bacteria, fungi, cereals, and algae, β-glucans are polysaccharides with prominent biological effects [118]. Polymers of short- or long-chain, made up of glucose units linked to β-(1,3) or β-(1,4) polymers, makeup that may be branched or unbranched [119,120]. β-glucan's immunomodulatory activities are highly influenced by the ability to form secondary structures and to confer various structural forms, thereby affecting several mechanisms to elicit the immunomodulatory activity [121]. Fig. 7 represents the structure of β-glucans. The factors influencing the immunomodulatory ability of β-glucans are complex and have not been unraveled completely. However, extensive research has proved the influence of factors such as molecular weight, size, solubility, conformation, backbone structure, and sidechain on their immunomodulatory properties. During the advent of fungal infections, β-glucans are the major PAMPs recognized by the body and elicit various immune responses in the system [122]. Specific interaction of β-glucans with different pattern recognition receptors like Dectin-1 selected scavenger receptors, complement receptor 3 (CR3), and lactosylceramide (LacCer) caused downstream signal transduction in macrophages, NK cells, monocytes, neutrophils, and dendritic cells causing immunomodulation in the hosts [123,124]. The specific receptor interaction of β-glucans is cell-specific. For instance, the interaction of β-glucans with macrophages was Dectin-1 mediated, whereas neutrophil interaction was mediated by CR3. The binding of β-glucans to the lectin in CR3 of phagocytes and NK-cells resulted in the activation of receptors leading to cytotoxicity against target cells opsonized by iC3b, which included tumors also [125,126]. Dectin-1 binding of β-glucans evoked downstream signaling pathways that, in turn, provoked ROS generation, several cytokine productions, phagocytosis, and microbial destruction [127–129]. "Training" of monocytes achieved by the previous administration of β-glucans in mice resulted in the development of innate immunological memory in mice and protected it from *Escherichia coli* re-infection [130,131]. This can be attributed to epigenetic modifications and metabolic reprogramming [132–134]. Studies have proved that β-glucans of lower molecular weight do not elicit a heightened immune response, whereas innate immune memory was induced by particulate β-glucans [135,136].

4.4.1. Influence of structural properties on immunomodulation

The immunomodulatory properties of β-glucans are influenced by structural characteristics such as molecular weight, branching, charge, and solubility [137,138]. But there are still ambiguities related to the structural aspects influencing biological activities.

One of the key elements affecting the immunomodulatory potential of β-glucans is their molecular weight. Glucans with higher molecular weights have better immunological effects, perhaps due to the ease of their detection by receptors on the immune cells owing to their structural stability [139]. β-(1,3)-glucan isolated from *Grifola frandosa* with higher molecular weights had the highest immunomodulatory activity as opposed to lower molecular weight glucans [140]. Generally, low molecular weight glucans are considered inactive; however, the study by Brown and Gordon showed the cytokine-dependent immunomodulation of low molecular weight glucans, unlike high molecular weight glucans that directly induced the phagocytic, anti-microbial, and cytotoxic activities of immune cells [141]. However, the controversy around the induction of immunomodulation by higher and lower molecular weight β-glucans is still on. For instance, there was no difference observed in

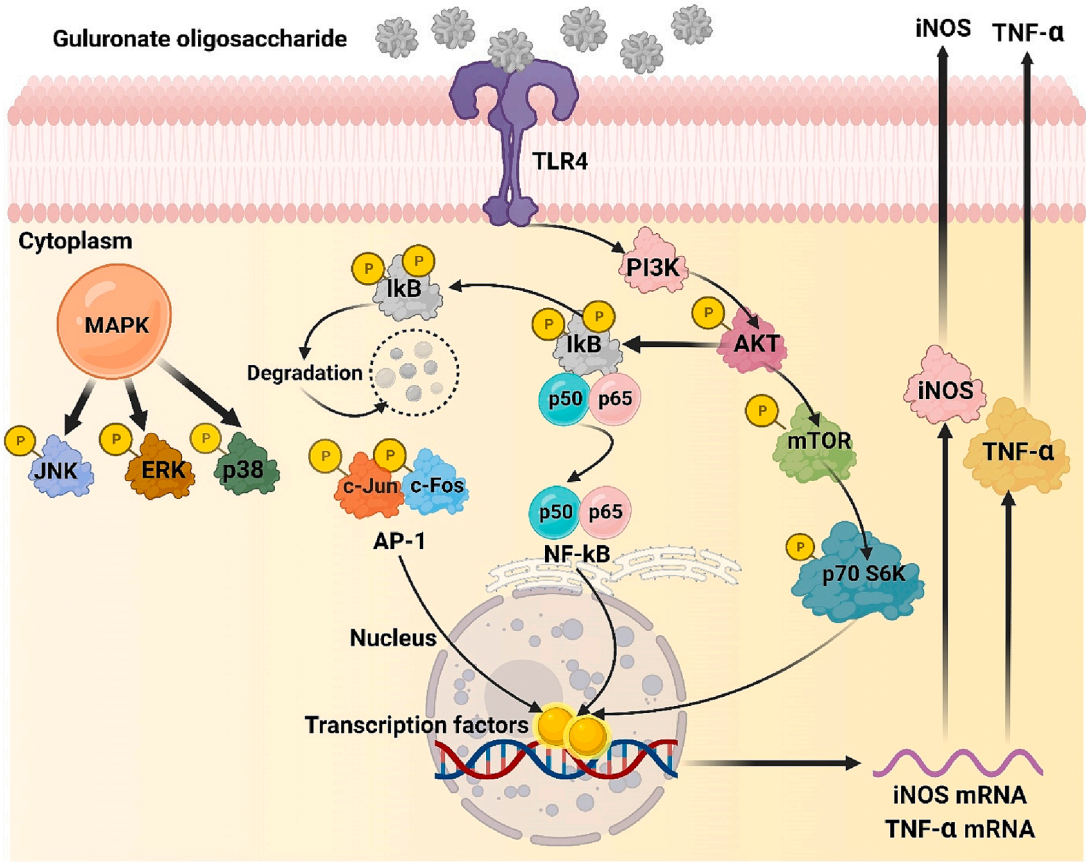

Fig. 6. Signaling pathways activated by alginate-derived glucuronate oligosaccharides during macrophage activation. The guluronate oligosaccharide (GO) binds to the TLR receptor to activate downstream pathways like Akt/NF-κβ, Akt/mTOR, and MAPK. Phosphorylation of PI3K and the subsequent phosphorylation of Akt leads to the further phosphorylation of I-κβ resulting in the nuclear translocation of NF-κβ. Similarly, phosphorylated Akt also activates mTOR and p70 S6 kinase. Further, GO binding to TLR also phosphorylates MAPK, that in turn phosphorylates downstream components like ERK, JNK, and p-38 to transcribe genes responsible for NO and TNF-α synthesis.

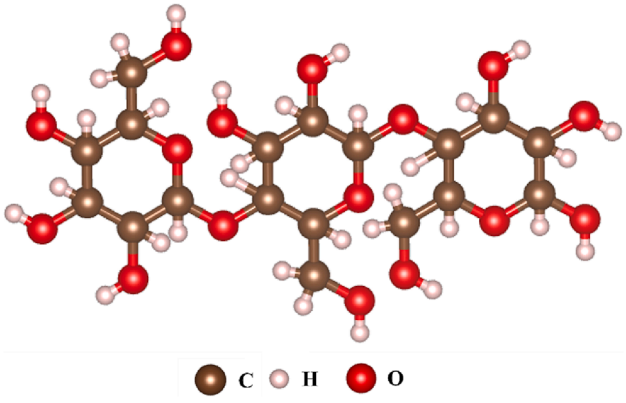

Fig. 7. Structure of β-glucan. The structure was created using VESTA [47]. (Ref. National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 439262. https://pubchem.ncbi.nlm.nih.gov/compound/beta-Glucan).

anti-tumor effect against sarcoma 180 cells (murine cancer cells) between the high molecular weight and low molecular weight β-(1,3)-glucans, lentinan, and schizophyllan isolated from *Lentinus edodes* and *Schizophyllum commune* respectively [142]. Similarly, the *in vivo* experiments by Li *et al.*, showed that low molecular weight β-glucans from yeast conferred better antioxidant and immunomodulatory effects as compared with the high molecular weight counterparts [143].

Molecular structures such as backbone, side chain, and conformation also determine the immunomodulatory effects of β-glucans. The importance of backbone structure influencing the immunomodulatory property of β-glucans has been studied by Adams *et al.*, and the results showed that Dectin-1 binding efficiency depended on the β-(1,3)-D glucopyranosyl backbone. Furthermore, β-(1,3)-D-glucan oligosaccharides with unbranched and shorter glucose subunits were left unrecognized by Dectin-1 [144]. Similarly, non-β-linked glucans and glucans with D-glucopyranosyl linear residues with a mixed β-(1,3) and β-(1,4) linkages were not recognized by Dectin-1 [145], indicating the importance of sidechain and backbone structure on immunomodulatory property of β-glucans.

In this perspective, studies have proved the importance of the glucose side chain in immunomodulation. Lower macrophage activation was found in glucans with side chains containing one glucose molecule, whereas glucan molecules with a higher number of glucose residues in the side chain showed better macrophage activation [146]. Glucans with branching efficiencies of 0.2–0.33 have been found to be the most effective immunomodulators [122]. Despite these studies, there are exceptions to these findings. For instance, *Poria cocos*-derived branched β-glucan showed no anti-tumor properties, but the debranched polymer showed anti-tumor properties [147]. This could be attributed to the steriochemical hindrance in branched glucans, with each other causing reduced receptor binding [148]. This disparity has not been addressed entirely and requires further clarification.

Glucan's ability to be single, triple helix, and random coil structures are dictated by molecular weight, sugar residue conformation, and inter- and intra-chain hydrogen bonding [149]. The immunomodulatory effect

of these structures concerning their efficiency in inducing immunomodulation is still a debate. Single-helix glucans showed reduced tumor suppression ability compared with triple-helix glucans [150]. But on the contrary, a study by Saito and co-workers has proved the higher bioactivity of single-stranded β-(1,3)-D-glucans than helix structured β-glucans [151]. This inconsistency in literature studies suggests the presence of biological systems in the host that recognizes glucans with different conformations, thus indicating a need to study the aspects of glucan conformation and bioactivity in depth.

Soluble and insoluble β-glucans both stimulate the immune system. But soluble and particulate β-glucans are recognized by different receptors. Dectin-1 receptors recognized particulate β-glucan whereas soluble β-glucans were activated by complement and CR3-dependent pathways for their anti-tumor activities [152]. A report by Goodridge *et al.*, has shown that Dectin-1-mediated macrophages are induced only by Dectin-1-particulate β-glucan interactions by forming a phagocytic synapse [136]. Fig. 8. shows the various machines involved in combating anti-tumor, anti-fungal, and development of immunological memory by β-glucan [153].

4.5. Mannans

Mannans are a well-studied natural polymer with immunostimulatory properties. (β1 → 4)-D-Man*p*, (β1 → 3)-D-Man*p* or (α1 → 3)-D-Man*p*, (β1 → 2)-D-Man*p*, and (β1 → 6)-D-Man*p* or (α1 → 6)-d-Man*p* are the immunostimulatory components of mannan backbone [154–162]. Fig. 9 illustrates the structure of mannans. As in the other natural polymers, conformation has a significant role in deciding the immunomodulatory activity of mannans as well. For instance, (β1 → 6)-D-Man*p*-rich galactoglucomannans have a random coil conformation and are highly immunostimulatory [161]. But mannans with triple-helix

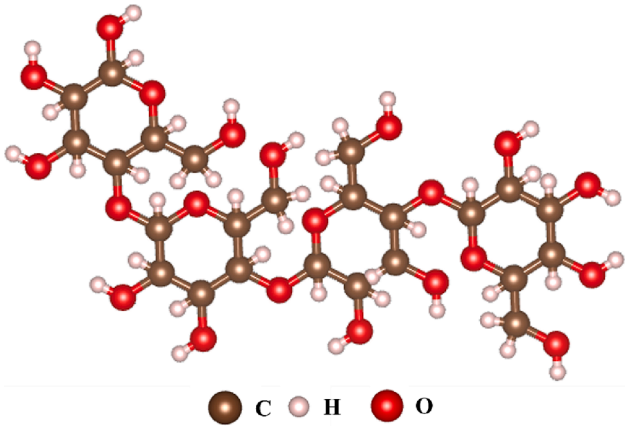

Fig. 9. Structure of Mannans. The structure was created using VESTA [47]. (Ref. National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 25147451. https://pubchem.ncbi.nlm.nih.gov/compound/Mannan).

conformation rich in (β1 → 2)-D-Man*p* with (β1 → 3)-D-Man*p* branch showed significantly less immunostimulatory activity [156] than randomly coiled galactoglucomannan [161]. Unlike other polymers, the molecular weight of mannans is not as appropriate as other structural features in influencing the immunomodulatory profile of mannans. Studies have shown that (β1 → 4)-D-Mannans with molecular weight 10MDa have better immunostimulatory activity when compared to low molecular weight mannans [163,164]. But on the contrary, studies have shown that most of the (β1 → 4)-D-Mannans with immunomodulatory activities were of the molecular weight range 5–400 kDa [165,166].

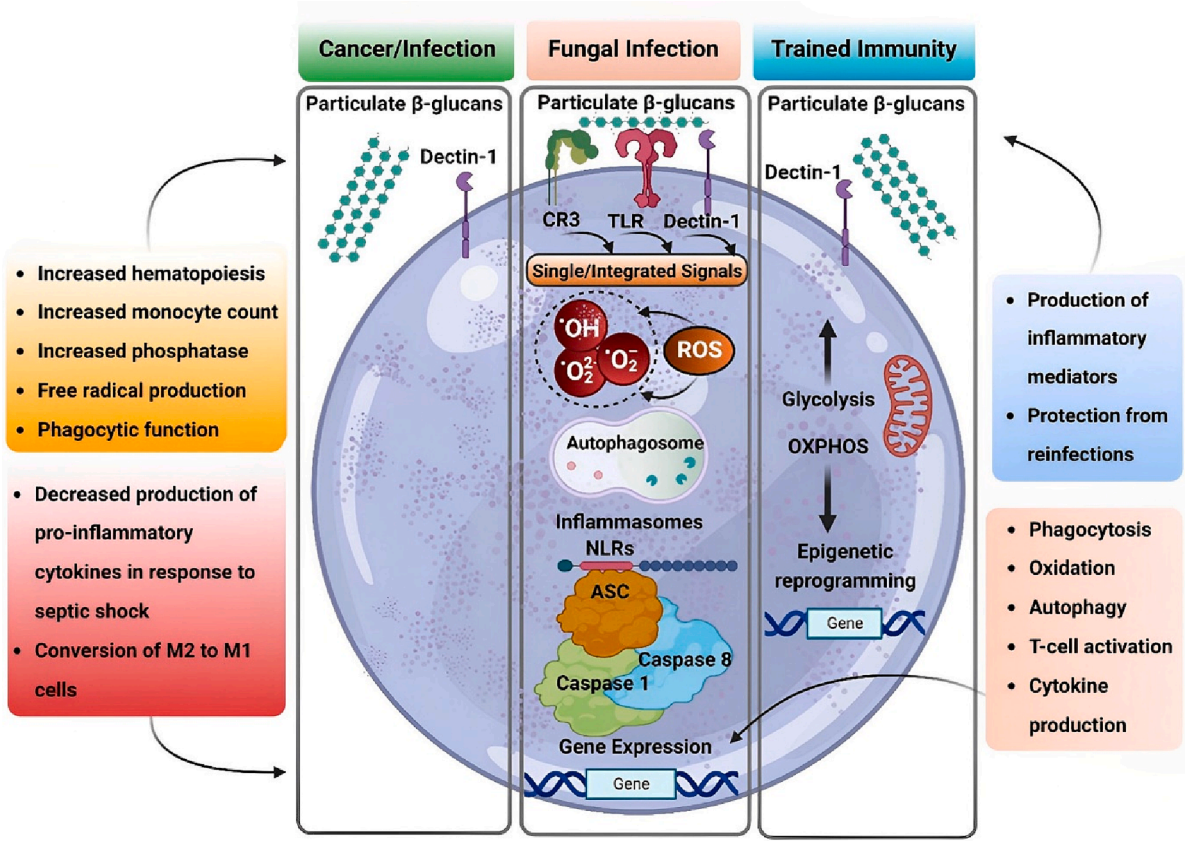

Fig. 8. Overview of the immunomodulation by β-Glucans in case of cancer, fungal infections, and development of immune memory. The binding of β-glucans to CR3, Dectin-1 and TLR evoked downstream signaling pathways and caused ROS generation, cytokine productions, phagocytosis, and microbial destruction. The binding of β-glucans to Dectin-1 also caused epigenetic modifications and metabolic reprogramming resulting in the development of trained immunity.

These reports were consistent with other mannans like (α1 → 6)-, (α1 → 3)- and (β1 → 3)-D-mannans that have immunomodulatory properties [156,162]. This discrepancy pertaining to the effects of molecular weights of mannans on immunomodulatory properties is yet to be studied and unraveled well. Acetylation in mannans influence their immunostimulatory properties. It was reported that acetylated-(β1 → 4)-D-Mannans showed better immunostimulatory activity as opposed to the non-acetylated mannans that had no immunostimulatory properties [160,165,166] The influence of sulfation on mannans' immunostimulatory potential has been well reported. It has been found that chemically sulfated mannans had a better effect than natively sulfated mannans [157,167]. The mannans with similar structures and different branching have been found to show other immunomodulatory effects. A (β1 → 3)-D-linked sidechain mannan linked to a (β1 → 2)-D-mannan backbone had a better immunostimulatory effect than (β1 → 6)-D-Man*p* residues rich mannans, with (1 → 4)-D-Gal*p* branches [156,161]. However, further studies on the impact of branching on the immunostimulatory activities of mannans are essential for a better understanding of their mechanism.

5. Challenges

Studying the innate immunomodulatory properties of synthetic and natural polymers is a novel field that might hold many exciting surprises for the research community to explore and exploit. This review mainly focuses on natural polysaccharides and their immunomodulatory effects; however, the areas that could be suitable for identifying new polymers with immunomodulatory potential have been discussed. The commonly targeted receptors of the innate immune system, like TLRs by polymers, have been very well explored. There is a pressing need to identify novel receptors that will deploy the immunomodulation with different mechanisms and desired effects. For instance, polymerization with N-carboxy anhydride was used to target Siglec and Dectin-1 [178,179]. This addition of functional groups to polymeric agonists confers them to target various receptors and ferry several molecules. Advancement in immunological research has unraveled new receptor targets for immunotherapy. For instance, the binding of F-actin-myosin to the DNGR-1 receptor (CLEC9A or CD370) on MHC-1 led to the identification of its involvement in the cross-presentation of antigen to $CD8^+$ T-cells [180]. Polymeric antagonist developmental strategies to target these receptors will aid in appropriately targeting a specific subset of cells with increased specificity. Table 3. Shows the natural polysaccharides and their specific binding receptors/proteins.

Additionally, the discovery of new polymers with immunomodulatory properties may greatly benefit from the use of machine learning, high-throughput screening, and computational methods [181]. Future developments in functional polymers will be made possible through machine learning. In contrast, computational approaches will pave the way for modeling and understanding the interaction of the polymer with biological receptors like PRR or proteins. To achieve the full immunomodulatory potential of a polymer, the polymer must reproduce the biological system's structural complexity, which can be attained by approaches to predict the protein structure and ligand-receptor binding [182–184]. Baker and co-workers have deployed the computational prediction methods and have synthesized biopolymers with specific three-dimensional topologies. Additionally, similar techniques were employed to create peptide vaccines that closely resembled the original virus and caused powerful T- and B-cell responses in a RSV (respiratory syncytial virus) model [185–187]. Machine learning, along with high-throughput screening, will aid in driving the discovery of polymers with the ability to modify innate immunity by binding to the receptors and/or can control the adaptive immune response [188,189]. Also, introducing structural complexities in synthetic polymers analogous to the biological polymers would aid in better binding with immune receptors, which would be an intriguing area of study to understand receptor-polymer interactions.

While immunomodulation by polymers is a boon on the one hand, on the other hand, the possible toxicity or the immunological reactions elicited by the polymer should be well studied. Hence screening of the material for any undesirable immunological responses is essential, and the lack of such techniques to screen them keeps us glued to imprecise *in vitro* and *in vivo* assays, which is the main hindrance in the translational aspects of polymeric materials. The polymers used for the immunomodulatory activities must be endotoxin-free. Gel clot Limulus amoebocyte lysate (LAL) assay is the most used assay to detect endotoxin contamination, and this works by the principle of coagulogen protein clotting on interaction with endotoxins. But the specificity of the test here is ambiguous as the polymer interactions usually cause non-specific clotting of coagulogen; hence there is a pressing need to develop alternative testing strategies [190,191]. In this regard, HEK TLR4 receptor cell lines, when incubated with polymers, resulted in an accurate and rapid identification of endotoxin contamination. Acid treatment, heat treatment, or extraction of endotoxin pollutants can all be used to remove endotoxins from a material [190]. After the removal of endotoxin, the polymer should be tested for its *in vitro* immunostimulatory potential by several assays, out of which estimation of IL-1β production, activation of signal transduction pathways of NF-κβ and IFN, and cellular toxicity assessments would be the better methods for polymers, as reported by previous studies [191–194]. Table 4. shows the comparison between natural and synthetic polymers.

Further, early innate immunity activation is marked by gene expression of NF-κβ and IFN and utilizing this scenario; multiplexed cytokine panels can be replaced by reporter cell lines that are genetically encoded [192]. The results of toxicity studies and IL-1β secretion and immunotoxic cell death can be assessed and quantified by assays such as ELISA. Thus, this combined immunological compatibility testing, along with computational and high throughput studies, will pave the way for developing new polymers with immunomodulatory properties.

6. Conclusion

The immune system dutifully protects the body from threats that pose a severe risk to homeostasis. The immune cells present in the body are critical players in the process of immune surveillance. The activation and suppression of the immune cells, corresponding to the threats faced, help the body maintain homeostasis. Polymers have been used to

Table 3
The natural polysaccharides and their binding receptors/proteins.

Polysaccharide	Binding Receptor/Protein
Fucoidan	Scavenger Receptor-A, TLR-2, TLR-4, Ly-49, CLEC-2, OCIL, L-selectin, P-selectin, Extracellular matrix proteins [168]
Chitosan	Mannose receptor, Lectin receptor with mannose specificity, CD14, TLR4, CR3 [169–172]
Alginate	TLR-2, TLR-4 [116]
β-Glucans	Dectin-1, CR3, TLR, LacCer [123,173,174,175]
Mannans	CD206 [176], Dectin-2 [177]

Table 4
Comparison between natural and synthetic polysaccharides [195,196].

Properties	Natural Polysaccharides	Synthetic Polysaccharides
Toxicity	Less toxic	Toxic
Synthesis	Easily available but has high extraction costs	Complicated and expensive synthesis process
Chemistry	Structurally more complex	Can be designed with specific groups
Immunomodulatory effects	Depends on the side chain, backbone, molecular weight, size, and solubility	Size, shape, surface features, chemical functionality, molecular weight, and physiochemical properties influence the immunomodulatory effects

modulate the immune system. Natural and synthetic polymers have been used in nanoformulation or scaffold forms to study their immunomodulatory potential. Natural polymers are abundant in nature and possess the innate immunomodulatory potential to modulate the immune system when used alone and as adjuvants in vaccines. Little is known about the targeted receptors and molecular pathways that the polymers alter because of a lack of substantial research to understand the mechanism behind immunomodulation. This review discussed the various immunological pathways exploited by some of the commonly used natural polymers to elicit a host's immune response.

Nevertheless, a complete understanding of the molecular pathways and molecules involved is still ambiguous. The toxicity associated with using natural polymers is still a void that must be addressed to develop a successful immunomodulatory agent. In this perspective, research to develop simple and cost-effective assays to understand the toxicity profile of the polymers is a pressing need. Further research to identify novel receptors for immunomodulation with increased specificity is the need of the hour. High-throughput screening, computational methods, and machine learning must be deployed to identify novel polymers with immunomodulatory activities and their target receptors. It is past time to examine every polymer's complete immunological profile to comprehend the existing polymeric systems and to build or identify new polymers with immunomodulatory capabilities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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