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Review Article

A review of the immunomodulatory activities of polysaccharides isolated from *Panax* species

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ABSTRACT

Panax polysaccharides are biopolymers that are isolated and purified from the roots, stems, leaves, flowers, and fruits of *Panax* L. plants, which have attracted considerable attention because of their immunomodulatory activities. In this paper, the composition and structural characteristics of purified polysaccharides are reviewed. Moreover, the immunomodulatory activities of polysaccharides are described both *in vivo* and *in vitro*. *In vitro*, *Panax* polysaccharides exert immunomodulatory functions mainly by activating macrophages, dendritic cells, and the complement system. *In vivo*, *Panax* polysaccharides can increase the immune organ indices and stimulate lymphocytes. In addition, this paper also discusses the membrane receptors and various signalling pathways of immune cells. *Panax* polysaccharides have many beneficial therapeutic effects, including enhancing or activating the immune response, and may be helpful in treating cancer, sepsis, osteoporosis, and other conditions. *Panax* polysaccharides have the potential for use in the development of novel therapeutic agents or adjuvants with beneficial immunomodulatory properties.

1. Introduction

The ginsengs have been a crucial source of natural medicines throughout human history. The common ginsengs are *Panax ginseng* (PG, also known as ginseng or Korean ginseng), *Panax quinquefolium* (PQ, North American ginseng), *Panax notoginseng* (PN, Sanchi ginseng or Chinese ginseng), and *Panax japonicus* (PJ, Japanese ginseng or Bamboo ginseng).

The chemical components of *Panax* species include ginsenosides, polysaccharides, polyacetylenes, proteins, peptides, amino acids, organic acids, vitamins, fat-soluble molecules, and other components [1]. *Panax* polysaccharide exhibits multiple biological activities including antioxidant activity [2], anti-fatigue activity [3], anticancer activity [4], anti-hyperglycemic activity [5], cytotoxicity inhibition [6], immunomodulation activity [7], gut microbiota regulation [8], and anti-septicaemic activity [9]. Among which, immunomodulation activity in *Panax* species has gained more attention due to the activity was closely related with extraction and isolation way, monosaccharide constituents, molecular weight, and linking mode of monosaccharide.

In recent years, multiple authors have reviewed the therapeutic activities of ginseng polysaccharides from Panax species, many of which are related to the regulation of the immune response. For example, Ghosh et al [10] summarised the structural characteristics and immunoregulatory properties of polysaccharides from PQ, including related binding receptors and signal transduction pathways. Many studies have been carried out on PG. For example, Sun [11] investigated the chemical components and biological effects of polysaccharides from the roots, leaves, and fruits of PG; Zhao et al [12] reviewed its polysaccharide structure, biological activity, and purification methods; and Ji et al [13] discussed the relationship between polysaccharide structure and bioactivity. Guo et al [14] conducted a detailed review of natural components with specific biological activities.

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In this article, we review the immunomodulatory activities of polysaccharides from *Panax* species, including their chemical compositions, functions, and the mechanisms for their immunoregulatory effects. We aim to provide a scientific basis for further studies regarding structure-activity relationships of *Panax* polysaccharides.

2. Composition and structural characterisation

Panax polysaccharide is a biopolymer composed of complex monosaccharide chains rich in L-arabinose (Ara), L-rhamnose (Rha), D-galactose (Gal), D-mannose (Man), D-galacturonic acid (GalA), D-glucuronic acid (GlcA), D-xylose (Xyl), and D-galactose (Gal) residues, which are linked by glycosidic bonds and form complex macromolecular structures [15]. The molecular weights range from 3.1 kDa to 9,700 kDa; distinct physicochemical properties and bioactivities have been observed [11]. Many types of *Panax* polysaccharides have been isolated from ginseng fruits, leaves, stems, flowers, and roots. While *Panax* polysaccharides are found mainly in roots, Cui et al [16] extracted a neutral polysaccharide known as WGFPN from flowers, and Zhang et al [17] and Hwang et al [18], isolated the polysaccharides PGP-SL and GS-P from stems and leaves, respectively. Wang et al [19] obtained GFP1 polysaccharide from fruits. Polysaccharides from *Panax* species differ in their molecular weights, properties, monosaccharide compositions, and structures (see Table 1). Thus far, studies of polysaccharides have focused mainly on PG, PQ, and PN; few studies have investigated PJ, *Panax vietnamensis*, or *Panax pseudoginseng*.

Panax polysaccharides are divided into neutral and acid polysaccharides. Neutral polysaccharides comprise dextran and arabinogalactan; acid polysaccharides comprise pectin-containing rhamnose and homogalacturonan [14]. The starch-like polysaccharides consist of 6-branched α-D-(1,4)-glucans, as well as 3-branched α-D-(1,6)-glucans and α-D-(1,3)-glucans [16,20]. Cui et al [16] reported that the backbone of the WGFPN polysaccharide consisted of small (1→4)-β-D-galactan branches and large (1→6)-β-D-galactan branches decorated with α-L-1,5-Araf and t-α-L-Araf residues at O-3; moreover, trace amounts of 1,4-linked Glcp, -Galp, -Glcp and -Manp residues might be attached in the form of side chains to (1→4)-β-D-galactan at O-6 or (1→6)-β-D-galactan at O-3. Using 1D and 2D nuclear magnetic resonance analyses, Li et al [20] demonstrated that the GPNE-I polysaccharide backbone contained a glucan with →4)-α-D-Glcp-(1→ and →4,6)-α-D-Galp-(1→ residues and an arabinogalactans (AG) domain, and that GPNE-II contained a glucan with t-, 3-, 4-, 6- and 4, 6-Glcp. The pectin mainly consists of GalA, Gal, Ara, and Rha [21], with trace amounts of Glc, Xyl, Man, and GlcA [22,23]. These monosaccharides are organised into distinct structural domains, including the homogalacturonan (HG), rhamnogalacturonan (RG)-I and RG-II, and AG-II domains [10,18,24–26]. HG is a linear chain composed of 1,4-linked α-D-GalpA residues, some of which undergo methylation at C-6 and partial O-acetylation at C-3 or C-2 [27]. The RG-I backbone consists of [→2)-α-L-Rhap-(1→4)-α-D-GalpA-(1→] repeat units, and the side chains contain AG-I and AG-II. WGPA-2-RG fractionated from PG is a typical RG-I pectin [25]. RG-II structurally differs from the repeating units of RG-I and is composed mainly of 1,4-linked α-D-GalpA residues containing unusual sugars such as Api, Dha, KDO, AcAce, MeXyl, and AcMeFuc [28]. Shin et al [24] demonstrated that GS-P is an RG-II polysaccharide, because KDO and Dha were found only in RG-II in plant-originated polysaccharides. In addition, arabinogalactan-type polysaccharides can be divided into types I and II. The protein-bound arabinogalactan RG-CW-EZ-CP-8 contains Ara (64.3 mol%) and comparatively low amounts of Gal and GalA (10.3 mol% and 12.3 mol%) [29]. AG-I consists of (β1→4)-D-Galp linear units as the backbone, and α-L-Araf and β-D-Galp units branch along the backbone at C-3. AG-II is composed mainly of (1→3)-linked β-D-Galp and (1→6)-linked β-D-Galp residues, with 3-Galp, 6-Galp, or Ara as the side chain (connected at position 6). Polysaccharides isolated from PQ (e.g., AGC1) contain AG-II because of the presence of 3-Galp, 6-Galp, and 3,6-Galp residues [26].

Immunomodulatory polysaccharides are compounds that interact with the immune system and strengthen host reaction-specific processes [30]. Studies regarding immunomodulatory polysaccharides have focused mainly on glucans, pectic polysaccharides, and arabinogalactans. The immunostimulatory activities of these polysaccharides are influenced by their source, molecular weight, properties, monosaccharides, glycosidic linkages, functional groups, and branching characteristics. Zheng et al [23] reported that RG-I pectin exhibited more potent induction of lymphocyte proliferation, compared with HG-rich pectin. AG is an effective immunomodulator, and the AG chain plays an important role in promoting nitric oxide (NO) production and lymphocyte proliferation. Li et al [20] reported that GPNE-I (containing a glucan domain and AG-I/II) stimulated lymphocyte proliferation more effectively, compared with GPNE-II (containing only a glucan domain); this suggested that the existence of AG domains is required to stimulate lymphocyte proliferation. Zhang et al [25] confirmed these results using bioactivity assays. Based on these reports, we conclude that *Panax* polysaccharides have regulatory effects on the immune system.

3. Immunomodulatory activity of *Panax* polysaccharides *in vitro*

In recent years, many polysaccharides isolated from *Panax* species have been reported to exhibit immunomodulatory activity in different models [31]. *In vitro* studies showed that *Panax* polysaccharides activated macrophages and promoted DC maturation, while stimulating the complement system (Table 2).

3.1. Activation of macrophages by Panax polysaccharides

Macrophages are produced by monocyte differentiation and play a unique role in the immune system where they activate the innate immune response [16]. Macrophages activated by *Panax* polysaccharides can neutralise foreign matter, infected microorganisms, and tumour cells by increasing the production of NO and cytokines, cell proliferation, and phagocytosis. WGFPN polysaccharide from PG flowers promotes the release of NO in RAW264.7 macrophages at a dose of 200 μg/mL [16]. WGPA-UH-N1 polysaccharide substantially enhances NO production in peritoneal macrophages at a dose range of 0.1–1.0 mg/mL [32]. Similarly, the polysaccharides RGP1/2, GS-P, WGPA-2-RG, RG-CW-EZ-CP-8/4, RGAP, GMP, AGC1, PPQN, WPS-1/2, AGC3, PPQA2/4/5, and AEP-2 can stimulate NO release in mouse peritoneal macrophages or RAW264.7 cells. Cytokines are low-molecular-weight peptides that include ILs, tumour necrosis factor (TNF), IFN, and granulocyte-macrophage colony-stimulating factor (GM-CSF). RG-CW-EZ-CP-8/4 and AGC3 polysaccharides increase the production of the anti-inflammatory cytokines IL-12 and IL-10, respectively, as well as the proinflammatory cytokines IL-6, TNF-α, and GM-CSF [10,29], suggesting that the body has a self-regulating mechanism to maintain homeostasis. In RAW264 macrophages, the AGC1 polysaccharide induces the secretion of IL-6, TNF-α, monocyte chemoattractant protein-1, and GM-CSF; the PPQN polysaccharide induces cytokines such as IL-6, IL-1β, and TNF-α [26,33]. Similarly, WGFPN, RGP1/2, PPQA2/4/5, and AEP-2 induce the secretion of proinflammatory cytokines in macrophages. Choi et al [34]

Table 1
Source and Structure of Polysaccharides From *Panax* Species

Panax L.	Name	Source	Molecular weight/Da	Property	Monosaccharide composition	Structure	Ref.
PG	WGFPN	flower	1.1×10^4	neutral	Gal: Ara: Glc: Man = 78:14.3:5.2:2.5	(1→4)-β-D-galactan and (1→6)-β-D-galactan.	[16]
	WGPN	root		neutral	Glc: Gal: Ara = 95.3:3.3:1.3	Starch-like glucans	[53]
	FGWP	root	6.4×10^5		Gal: Ara: Glc: Rha: GalA = 9.2:10.1:60.6:1.8:17.8	significantly higher protein content	[54]
	FGEF-C	root			Gal: Ara: Glc: Rha: GalA = 10.4:10.5:62.2:2.1:14.3	relatively high amounts of glucose	
	FGEF-A	root			Gal: Ara: Glc: Rha: GalA = 12.6:10.9:50.9:1.8:23.1	significantly higher ratios of arabinose, galactose, and galacturonic acid, and lower ratios of glucose	
	FGEF-CA	root	6.4×10^5		Gal: Ara: Glc: Rha: GalA = 16.5:11.4:45.8:3.2:22.3	contained high amounts of rhamnose, arabinose, galactose, and galacturonic acid, with low amounts of glucose	
	RGP1	root		neutral	Ara:Glc:Gal = 0.02:0.88:0.1	backbones composed of glucose	[61]
	RGP2-1	root	2.16×10^4	neutral	Rha:Ara:Glc:Gal = 0.02:0.1:0.77:0.11	backbones composed of glucose	
	NGP	root	5.04×10^5	neutral	Glu	O-α-(1→6)-D-Glucan	[44]
	GPNE-I	root	8.03×10^4	neutral	Glc = 1	a heteropolysaccharide consisting mainly of a glucan domain and type I and II arabinogalactans (AG-I and AG-II)	[20]
	GPNE-II	root	3.15×10^4	neutral	Glc:Gal:Ara = 7.0:1.9:1.0	a glucan consisting of (1→4)-α-D- Glcp backbone and (1→3)-α-D-Glcp and (1→6)-α-D-Glcp branches.	
	GS-P	leave	1.02×10^4	acidic	Rha: Fuc: Ara: Gal: GalA = 1.02: 0.31: 1.44: 1.18: 3.73	pectic polysaccharide consisting of 15 different monosaccharides, with an RG-II polysaccharide component	[18,24]
	GFP1	fruit	1.4×10^5	acidic	Rha: Ara: Gal: Glc = 1.1: 3.2: 6.1: 2.0	backbone (1→ 6)-linked-Galp, (1→3,6)-linked-Galp and (1→3,6)-linked-Glcp residues, with terminal (1→)-linked-Ara for -Rhap attached to O-3 position of (1→3,6)-linked-Galp and (1→3,6)-linked-Glcp	[19]
	WGPA-2-RG	root	1.1×10^5	acidic	Ara: Gal: Glc: Rha: GalA: GlcA = 40.9:44.4:2.9:4.1:5.3:2.0	arabinogalactans containing RG-I domains	[25]
	WGPA-UH-N1	root	1.7×10^4	acidic	Glc = 97.5	α-(1→6)-D-Glucan	[32]
	RG-CW-EZ-CP-8	root	1.47×10^5		Ara: Fuc: Rha: Man: Glc: Gal: GlcA: GalA = 64.3: 0.1:1.6: 1.3: 9.8: 10.3: 12.3: 0.4	Ara-rich polysaccharide such as arabinan or arabinogalactan backbone partly branched with acidic polymers	[29]
	RG-CW-EZ-CP-4	root	1.41×10^5	acidic	Ara: Fuc: Rha: Man: Glc: Gal: GlcA: GalA = 19.8: 0.3: 4.2: 1.6: 4.0: 29.9: 38.6: 1.7	pectic-like polysaccharide mainly composed of linear galacturonan as well as relatively small amount of galactan, arabinan, and possibly arabinogalactan	
	RGAP	root		acidic	acidic sugars: neutral sugars = 56.9:28.3		[39,62]
	Ginsan	root	1.5×10^5	acidic	glucopyranoside: fructofuranoside = 5:2	composed of α-(1→6) glucopyranoside and β-(2→6) fructofuranoside	[45,60]
	APG	root		acidic			[57,63]
	GMP	marc	1.238×10^4		Gal: Ara: Glc: Rha = 22.6:21.9:14.8:5.8	pectic-like polysaccharide	[7]
	PGP-SL	stem leave					[17]
PQ	PPQ	root	5.4×10^4	netural	Glc: Gal = 2.1:1	homogeneous glucogalactan	[64]
	AGC1	seed	5.2×10^3	netural	Gal: Ara: Xyl: Glc: Rha: Man = 60.093:19.165:11.363:6.298: 1.548: 0.79	arabinogalactan type II polysaccharides with a highly branched galactan 3-β-D-Galp residues, short side chains of 6-β-D-Galp residues	[26]
	PPQN	root	3.1×10^3	netural	Glc: Gal = 1:1.15	glucogalactan a neutral polysaccharide	[33]
	WPS-1	root	1.54×10^6	netural	Ara: Rha: Man: Gal: Glc = 21.1:2.3:2.6:18.7:55.2	consisting mainly of (1→6)-α-d-Glcp and (1→5)- α-L-Araf	[65]
	WPS-2	root	1.41×10^4	netural	Ara: Rha: Man: Gal: Glc = 27.9:1.7:2.9:20.7:46.8		
	SPS-1	root	3.62×10^5	acidic	Ara: Xyl: Man: Gal: Glc: GalA: GlcA = 22.3:6.9:9.2:28.6:15.9:13.6:3.5	consisting mainly of (1→6)- α-d-Glcp, (1→4)-α-D-Manp, (1→5)- α-L-Araf, β-D-Galp and β-D-xylose. SPS-2 and SPS-3 contained O-acetyl groups; WPS-1 and SPS-3 contained (1→4)- β-D-Rhap	
	SPS-2	root	9.7×10^6	acidic	Ara: Xyl: Man: Gal: Glc: GalA: GlcA = 14.2:5.3:7.9:22.5:25.3:16.9:7.9		
	SPS-3	root	5.12×10^5	acidic	Ara: Rha: Xyl: Man: Gal: Glc: GalA: GlcA = 19.2:2.1:9.6:12:15.2:11.5:26.3:4.1		
	AGC3	seed	4.81×10^3 3.214×10^4	acidic	Ara: Rha: Glc: Gal: GalA: GluA = 7.8: 8.1: 2: 74.3: 6.8: 1	pectic rhamnogalacturonan I polysaccharide	[10]
	PPQA2	root	2.3×10^4	acidic	Ara: Rha: Man: Gal: Glc: GalA: GlcA = 8.0:4.0:2.9:7.2:12.5:26.6:38.8	homogeneous polysaccharides contained O-acetyl groups	[66]
	PPQA4	root	1.2×10^5	acidic	Ara: Rha: Man: Gal: Glc: GlcA = 19.7:5.1:8.1:23.9:41.3:2.0	homogeneous polysaccharides	
	PPQA5	root	5.3×10^3	acidic	Ara: Rha: Man: Gal: Glc: GalA: GlcA = 8.5:3.2:5.3:10.8:32.4:15.5:24.4	homogeneous polysaccharides contained O-acetyl groups	
	AEP-2	root		acidic	Ara: Man: Gal: Glc: GalA = 1.03:0.76:1.68:3.02:3.65	contain in excess of 80% (by weight) poly-furanosyl-pyranosyl-saccharides	[67]
PN	PPN	root					[68]
	PF3111	root	6.85×10^5		Gal: Glc: Man: Ara: Xyl = 3.5:10.8:3.5:1.0:2.3	heteroglycans	[51]
	PF3112	root	3.7×10^4		Gal: Glc: Man: Ara: Xyl = 2.9:5.3:2.8:1.0:2.1		
	PBGA11	root	4.5×10^4		Gal: Glc: Man: Ara: Xyl = 3.1:4.2:5.3:1.0:3.2		

(continued on next page)

Table 1 (*continued*)

Panax L.	Name	Source	Molecular weight/Da	Property	Monosaccharide composition	Structure	Ref.
	PBGA12	root	7.60×10^5		Gal: Glc: Man: Ara: Xyl = 2.5:7.2:4.3:1.0:8.1		
	PNPS-0.3	residue	7.6655×10^4	acidic	Rha: Ara: Gal: Glc: N-Acetyl-D-Glc: GalA = 15.5:25.2:33.3:4.5:4.4:17.1	mainly consisted of a backbone of →4)- α-D-GalAp-(1 → 4-β-L-Rhap-1 → 4)-β-D- Galp-(1 → residues, with an α-L-Araf-1 → 5)-α-L-Araf-(1 → branch connecting to the backbone at O-3 of →4-β-L-Rhap-1 →	[43]
	1MD3-G2	root	1.14×10^6		Rha: Ara: Xyl: Gal: GalA = 2: 14: 29: 45: 10	with a slightly higher content of 4-galactan and HGA, and a lower content of arabinan.	[49]
	1MD3-G3	root	2.8×10^5	acidic	Rha: Ara: Xyl: Gal: GalA: GlcA = 2: 12: 41: 26: 16: 3	a much lower content of galactan and arabinan, but higher amounts of GAX and HGA.	
PJ	PSPJ	rhizome	3.98×10^4	neutral	Glc: Gal: Rha: Ara: Xyl: GalA: GlcA = 53.41: 13.62: 1.1: 0.99: 0.82: 25.02: 5.03	4-O-linked α-D-galacturonic acid, (1→4)-linked-α-D-galacturonic acid, 1, 3-linked β-D-galactose	[59]
	PJPS	rhizome		neutral	Ara: Glc: Gal	heteropolysaccharide containing pyranose ring	[52]

reported that the synergistic effects of RGAP polysaccharide and IFN-γ induced IL-1, IL-6, and TNF-α production, enhancing macrophage function. Phagocytosis is a basic function of macrophages. Enhancing phagocytic activity is a notable characteristic of macrophage activation and an important way to enhance immunological activity. Polysaccharides such as WGFPN, RGP1/2, WGPA-2-RG, GMP, WPS-1/2, and SPS-1/2/3 can enhance the phagocytic activity of macrophages.

Many studies have suggested that *Panax* polysaccharides activate macrophages by recognising macrophage surface receptors known as pattern recognition receptors (PRRs). Similar to proteins and enzymes, polysaccharides have "active sites" for one or more oligosaccharide fragments [35]. Macrophage PRRs have been widely reported by Jiang et al [35], Li et al [31], Schepetkin et al [36], and Ghosh et al [37]. They include TLRs, complement receptor 3, scavenger receptor, NOD2, MR, and dectin-1. Activation of macrophage receptors by *Panax* polysaccharides initiates a cascade of intracellular signals. The mitogen-activated protein kinase signalling pathway is required for macrophage activation. Kim et al [29] reported that RG-CW-EZ-CP-8 polysaccharide bound to macrophage PRRs and substantially induced the phosphorylation of three major mitogen-activated protein kinases (JNK, ERK, and p38 kinase), stimulating the production of NO and cytokines. NF-κB is a transcriptional nucleoprotein factor that plays an important role in macrophage activation and regulates the expression of inflammatory genes. Treatment of macrophages with RGAP polysaccharide and recombinant IFN-γ induces the activation of NF-κB and iNOS-NO and increases p65 NF-κB protein synthesis [34]. However, Ghosh et al [26] observed a slight increase in phosphorylated p65 (Ser536) after 4 h of AGC1 treatment, indicating a partial role for the NF-κB pathway in the immune stimulation response. NOD2 is a member of the NOD-like receptor family and a critical regulator of immunity; it can be activated by muramyl dipeptide to mediate protective immunity [38]. The acidic polysaccharide AGC3, containing RGI-type pectin, activates the NF-κB (p65/RelA) and mitogen-activated protein kinase (p38) signalling pathways in RAW264.7 cells. AGC3 binds NOD2 and possibly TLR4, resulting in crosstalk between these signalling pathways [10]. RGAP polysaccharide, isolated from Korean Red Ginseng, induces nuclear transcription factors such as NF-κB, AP-1, STAT-1, ATF-2, and CREB, thereby increasing NO production [39]. ERK and JNK are the most important signalling enzymes for RGAP. Therefore, RGAP can activate macrophages by activating the transcription factors NF-κB and AP-1, as well as their upstream signalling enzymes ERK and JNK.

Macrophage enzyme activity can also reflect the functional status of the analysed macrophages. Stimulation of peritoneal macrophages using the polysaccharide GMP significantly enhances lysosomal phosphatase activity. Lim et al [7] suggested that GMP could activate macrophages by modulating lysosomal enzyme activity, thereby affecting the ability of lysosomal enzyme to respond appropriately to foreign agents and increasing the proportion of phagocytic macrophages (Fig. 1).

3.2. *Induction of DC maturation by Panax polysaccharides*

DCs are powerful antigen-presenting cells that stimulate naïve T cells and initiate immune responses [40]; they were first discovered by Steinman in 1972 [41]. Mature DCs are capable of recognising, processing, and presenting antigens to T lymphocytes, thus activating the adaptive immune response [42]. After maturation induced by *Panax* polysaccharides, DCs express the specific co-stimulatory molecules cluster of differentiation (CD)40, CD80, CD86, and major histocompatibility complex (MHC) II. MHCII plays an important role in antigen presentation, while CD80 and CD86 are required for T cell activation [43]. CD40 can trigger extremely high levels of IL-12 expression, which is required to activate CD4+ T cells. Furthermore, increased IL-12 production enhances the CD4+ T cell population, as well as the communication between DCs and CD4+ T cells [44].

Panax polysaccharides modulate the immune function of DCs through direct recognition and binding of DC receptors, and through indirect pathways. For example, by regulating other immune cells (e.g., macrophages), *Panax* polysaccharides exert synergistic effects that regulate the entire immune system. BMDC pattern recognition molecules include TLRs, scavenger receptor, MR, and dectin-1 [35]. Liu et al [43] reported that PNPS-0.3 polysaccharide binds to BMDCs through multiple PRRs; TLR4 acts as the main receptor, while TLR2 and MR act as secondary receptors. This upregulates the expression levels of MyD88, IKKβ, p-p65, total p65, and NF-κB, thereby activating the NF-κB pathway to participate in the immune regulation of BMDCs. *Panax* polysaccharides also regulate immune function via the DC–CD4+ T cell pathway. Meng et al [44] showed that the NGP polysaccharide markedly enhances BMDC maturation and function, as well as the expression of MHCII, CD80, CD86, CD83, CD40, and IL-12. This upregulates antigen presentation, induces active CD4+ T cells, and strengthens the DC–CD4+ T cell pathway. In addition, IFN-γ secreted by CD4+ T cells activates macrophages, intensifying the immune response. Kim et al [45] demonstrated that ginsan, a PG polysaccharide,

Table 2
Effects and Mechanisms of Polysaccharides From *Panax* Species

Panax L.	Name	Methods	Models	Effects	pathways	Endotoxin test	Ref.
PG	WGFPN	*in vitro*	RAW264.7 macrophage cells	NO (+), TNF-α (+), IL-6(+), IL-1β (+), IFN-γ (+), phagocytic index (+)	phagocytosis	Yes	[16]
		in vivo	cyclophosphamide (CTX)-induced immunosuppressed mice	body weight (+), spleen indices (+), thymus indices (+), abolish the inhibition effect of CTX on the phagocytic uptake capacity of macrophage			
	WGPN	*in vivo*	Sarcoma-180 (S180) tumor-bearing mice	spleen weights (+), lymphocyte proliferation (+), NK cell activity (+), NO (+), TNF-α (+), phagocytic index (+)	phagocytosis		[53]
	FGWP FGEF-C FGEF-A	*in vitro*	RAW264.7 macrophage cells	IL-6 (+), IL-12 (+), TNF-α (+), FGEP-CA ≥ FGEP-A > FGEP-C > FGWP		Yes	[54]
	FGEF-CA	*in vivo*	cyclophosphamide (Cy)-induced BALB/c mice	spleen indices (+), thymus indices (+) lymphocyte proliferation (+), WBC count (+), NK cell activity (+), IL-2 (+), IL-6 (+), IFN-γ (+)			
	RGP1 RGP2-1	*in vitro*	RAW 264.7 macrophage cells	NO (+), TNF-α (+), neutral red phagocytosis (+)		Yes	[61]
	NGP	*in vitro*	murine BMDCs	IL-12 (+), phagocytosis (−), MHC II (+), CD80 (+), CD86 (+), CD83 (+), CD40 (+), IL-12p70 (+), TNF-α (+), T cell proliferation (+)	DCR, DC-CD4+T cell pathway	Yes	[44]
	GPNE-I GPNE-II	*in vitro*	spleen cells	lymphocyte proliferation (+), GPNE-I > GPNE-II			[20]
	GS-P	*in vivo*	SPF BALB/c mice (immunized with OVA, OVA + GS-P, OVA + FCA, OVA + FIA, OVA + FIA + GS-P)	IL-12 (+), TNF-α (+), IgG1 (+), IgG2b (+), Th1-type (IL-2, IFN-γ, GM- CSF) (+), Th2-type (IL-10) (+), T cell proliferation (+), IgE (−)		Yes	[18,24]
		in vitro	Splenic lymphocytes and macrophages from BALB/c mice	IL-12 (+), TNF-α (+), splenocytes proliferation			[18,24]
		in vivo	BALB/c mice	NK cell activity (+)			
	GFP1	*in vivo*	LLC-bearing mouse model	spleen and thymus weight (+), lymphocytes proliferation (+), NK cell activity (+), IL- 2 (+), IFN-γ (+), the ratio of CD4+/CD8+ (+)		Yes	[19]
	WGPA-2-RG	*in vitro*	normal mice spleens, macrophages	lymphocytes proliferation (+), NO (+), phagocytosis (+)		Yes	[25]
	WGPA-UH-N1	*in vitro*	male ICR mice spleens, macrophages	B cell proliferation (+), NO (+)			[32]
	RG-CW-EZ-CP-8 RG-CW-EZ-CP-4	*in vitro*	Balb/c and C3H/He female mice macrophages, Peyer's patches	BM cell proliferation (+), IL-6 (+), GM-CSF (+), NO (+), TNF-α (+), IL-12 (+), RG-CW-EZ-CP-8> RG-CW-EZ-CP-4	PRRs MAPK		[29]
	RGAP	*in vitro*	mice peritoneal macrophages	NO (+), IL-6(+), IL-1(+), TNF-α (+),	NF-κB pathway	Yes	[34]
		in vitro/ *in vivo*	ovariectomized rats	NK cells activity (+), iNOS (+),		Yes	[58]
		in vitro	RAW264.7 cells	NO (+), iNOS mRNA (+), NF-κB, AP-1, STAT-1, ATF-2, and CREB	TLR2, TLR4, Dectin-1, NF-κB, AP-1, EPK, JNK,		[39]
		in vitro/ *in vivo*	female BALB/c mice macrophage, spleen cell	iNOS (+), CD3CD4, CD3CD8, CD45R/B220 (−), CD11b (+)			[62]
	Ginsan	*in vivo*/ *in vitro*	BALB/c mice Sepsis model, peritoneal macrophages	TNF-α (+), IL-1β (+), IL-6 (+), IFN-γ (+), IL-12 (+), IL-18 (+), IL-10 (−), phagocytosis (+)	TLR 2, CR, SR, JNK, P38 MAPK, NF-κB		[60]
		in vitro	C57BL/6 mice bone marrow-derived DCs	IL-12 (+), IL-10 (+), TNF-α (+), CD86 (+), CD4+ T lymphocytes proliferation (+), IL-4 (+), IFN-γ (+)	DC-CD4+T cell pathway	Yes	[45]
	APG	*in vivo*	SJL/J Mice EAE	IFN-γ (+), IL-17 (+), TNF-α (+), T cell proliferation (+)			[63]
		in vivo	C57BL/6 female mice EAE	IFN-γ (+), IL-17 (+), IL-1β (+), CD25+ cells (−), Tregs (+), Foxp3 (+)	TLR, TCR, Foxp3		[57]
	GMP	*in vitro*	BALB/c mice peritoneal macrophages	lysosomal phosphatase activity (+), phagocytic index (+), H_2O_2 (+), nitrite (+), cell viability (+)	lysosomal enzyme activity, phagocytosis	Yes	[7]
	PGP-SL	*in vitro*	murine spleen lymphocytes	lymphocyte proliferation (+), IL- 2 (+), IL-2 mRNA (+), Ca^{2+} (+)	TCR/CD3, Ca^{2+}-CN-NFAT-IL-2 pathway		[17]
PQ	PPQ	*in vivo*	Lewis lung carcinoma model in C57BL/6 mouse	thymus and spleen indices (+), IFN-γ (+), IL-10 (−), IL-2 (+)	Th1 response		[64]
	AGC1	*in vitro*	RAW 264.7 murine macrophages	IL-6, TNF-α, MCP-1, GM-CSF, NOS2 gene expression, iNOS, NO, cell proliferation, complement fixation activity	TLRs, CR, SR, lectin receptors, NF-κB pathway	Yes	[26]
	PPQN	*in vitro*	RAW264.7 macrophages	NO (+), TNF-α (+), IL-6(+), IL-1β (+)			[33]
	WPS-1 WPS-2 SPS-1 SPS-2 SPS-3	*in vitro*	male Jilin mice	B cell proliferation (+), phagocytosis: SPS-3> SPS-1, NO: SPS-3 > SPS-1 > WPS-1 >WPS-2, SPS-3 > SPS-1> WPS-1> WPS-2 > SPS-2			[65]
	AGC3	*in vitro*	RAW264.7 murine macrophage cells	IL-6 (+), TNF-α (+), GM-CSF (+), MCP-1(+), IL-10 (+), NO (+), primary murine splenocyte proliferation	TLR, NOD2, C-type lectin, NF-κB (p65/	Yes	[10]

(continued on next page)

Table 2 (*continued*)

Panax L.	Name	Methods	Models	Effects	pathways	Endotoxin test	Ref.
					RelA), MAPK (p38) pathways		
	PPQA2 PPQA4 PPQA5	*in vitro*	RAW264.7 murine macrophages	IL-6 (+), TNF-α (+), NO (+)			[66]
	AEP-2	*in vitro*	RAW264.7 cells	IL-6 (+), TNF-α (+), NO (+), lymphocyte proliferation (+)			[67]
PN	PPN	*in vivo*	H22 tumor-bearing mice	CD4+ T-cells (+), IL-2 (+),	DC-CD4+T cell pathway	Yes	[68]
	PF3111 PF3112 PBGA11 PBGA12	*in vitro*	mouse peritoneal macrophages	anticomplementary activity: PBGA12> PF3112, IFN-γ (+), TNF-α (+)	classical and alternative pathway		[51]
	PNPS-0.3	*in vitro*	BALB/c mice BMDCs and T cells	CD40 (+), CD80 (+), CD86 (+), MHC II (+), TNF-α (+), IL-12 (+), CD4 (+), CD8 (+), CD69 (+), INF-β (+)	TLR 4, TLR 2, and MR TLR4/TLR2-NF-κB pathway DC- T cell pathway	Yes	[43]
	1MD3-G2 1MD3-G3	*in vitro*	Human PMNs/PBMC	complement-fixing activity		Yes	[49]
PJ	PSPJ	*in vivo*	mice bearing H22 hepatoma cells	thymus/spleen indexes (+), splenocyte proliferation (+), NK and CD8+ T cells cytotoxic activities (+), TGF-β (−), IL-10 (−), PEG2 (−), M2-like polarization of TAMs (−), IFN-γ (+), IL-2 (+), IL-4 (−),	COX-2/PGE2 signaling pathway	Yes	[59]
	PJPS	*in vivo*	kunming mice	phagocytosis (+), IgM (+), auricle swelling degree (+), thymus index (+), spleen index (+)		Yes	[52]

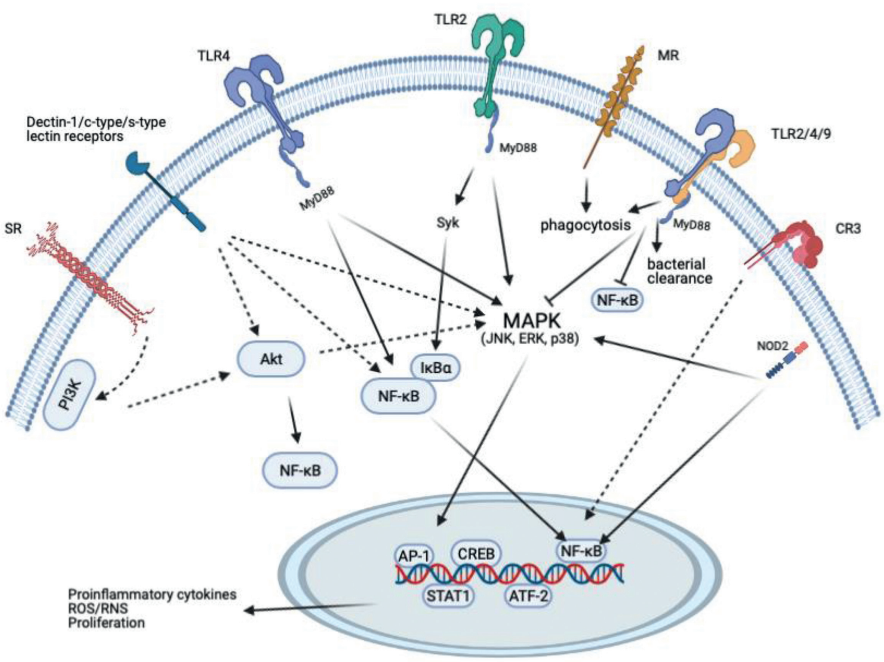

Fig. 1. Signaling pathways on macrophages activated by *Panax* polysaccharides.

regulated the immune response through the DC−CD4+ T cell signalling pathway, where CD86 acted as a costimulatory molecule. Treatment with PNPS-0.3 polysaccharide significantly increased the proportion of CD4+ and CD8+ T cells in BMDC/T cell co-culture, indicating that PNPS-0.3 polysaccharide can influence immune regulation by means of the DC−CD4+ T cell pathway [43]. Therefore, BMDC maturation upregulates antigen presentation and effectively initiates the CD4+ T cell response, while IFN-γ secretion activates macrophages, enhancing the overall immune response (Fig. 2).

3.3. *Activation of complement system by Panax polysaccharides*

The complement system consists of nine components, C1−C9, of which C1 has three subunits, C1q, C1r, and C1s. With the exception of C1q, most components are present in serum in the form of enzyme precursors, which can exert bioactivity only after activation by antigen-antibody complex or other factors [46]. Activation of the complement system (e.g., classical activation, alternative activation, and lectin activation) plays an important role in the body's defence functions, as well as regulation of immune system function and immunopathological processes [47]. The classical pathway is

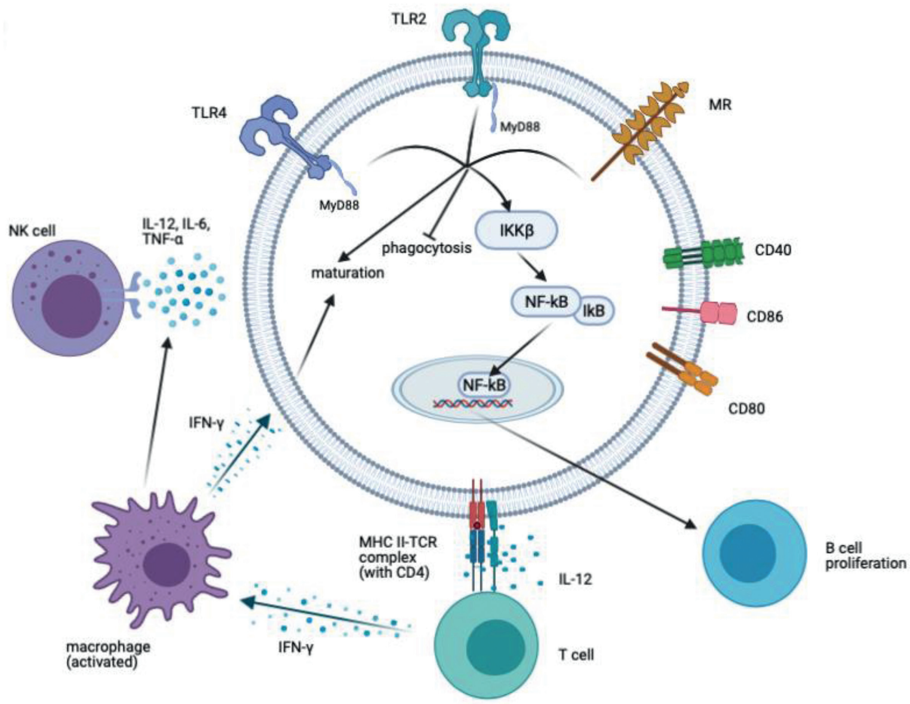

Fig. 2. Signaling pathways on DC activated by *Panax* polysaccharides.

initiated through an immune complex formed by the binding of an antibody (IgG1, IgG2, IgG3, or IgM) to the corresponding antigen; it also requires the activation of C1, C4, and C2. The alternative pathway differs from classical activation in that it is directly activated by C3. The main activators of the mannose-binding lectin pathway are pathogenic microorganisms containing N-galactosamine or mannose. Lectins directly recognise N-galactosamine or mannose on the surface of various pathogenic microorganisms, which leads to the activation of MASP-1, MASP-2, C4, C2, and C3 [48]. *Panax* polysaccharides have immunomodulatory activities in the complement system, and can be used as potent regulators in the treatment of complement-related diseases. At low doses, all high-molecular-weight polysaccharide fractions of PN exhibit effective complement fixation activity in a dose-dependent manner. Zhu et al [49] showed that the complement-fixing activity of a water-soluble polysaccharide from PN was significantly greater than the activity of the polysaccharide isolated by Gao [50]. Compared with 1MD3-G1 and 1MD3-G3, 1MD3-G2 polysaccharide exhibits greater complement-fixing activity, suggesting that its fine structure is necessary for its complement-fixing activity [49]. A neutral polysaccharide, AGC1, predominantly composed of galactose, also exhibits complement fixation activity [26]. Therefore, the galactan cores of AG-II exhibit complement-fixing activity. Gao et al [51] reported that the anticomplement activity of PBGA12 polysaccharide is mediated through both the classical and alternative pathways.

4. Immunomodulatory activity of *Panax* polysaccharides *in vivo*

The immunomodulatory activity of *Panax* polysaccharides differs under physiological conditions, compared with *in vitro* conditions. The spleen and thymus play critical roles in the immune system, and lymphocytes are important immune cells that have been extensively studied in mouse models.

4.1. Effects of Panax polysaccharides on spleen and thymus indices

The spleen is the largest immune organ in the human body. It produces lymphocytes, especially B lymphocytes, which generate specific antibodies and participate in humoral immunity [35]. The thymus induces the differentiation and maturation of T cells and affects cellular immunity. The status of immune organs determines immune function. Spleen and thymus indices are expressed as their respective weights, relative to body weight. WGFPN polysaccharide treatment increases spleen and thymus indices in cyclophosphamide (CTX)-induced immunosuppression mice, enhances the immune function of immunosuppressed mice, and abolishes the inhibition effect of CTX [16]. Similarly, treatment with FGEF-CA, GFP1, PPQ, and PSPJ polysaccharides significantly elevate the weights of immune organs in a dose-dependent manner in mice. In addition to improving spleen and thymus indices, PJPS polysaccharide can enhance the carbon clearance index (K) in normal and immunosuppressed mice [52]. Overall, the results in mouse models indicate that *Panax* polysaccharides can restore damaged immune organs, increase the relative weights of immune organs, and enhance immune function in immunosuppressed mice.

4.2. Activation of T/B lymphocytes by Panax polysaccharides

Lymphocytes are key effector cells that comprise a critical part of the mammalian immune system. Lymphocyte proliferation is regarded as an indicator of the cellular and humoral immune response; T cells are involved in cellular immunity and B cells are involved in humoral immunity [19]. *Panax* polysaccharides significantly enhance concanavalin A- and lipopolysaccharide-induced T cell and B cell proliferation, respectively. Both with and without mitosis-promoting stimuli, WGPN polysaccharide stimulates lymphocyte proliferation following a bell-shaped dose-reaction curve; the maximum effect is reached at 50 mg/kg [53]. Wang et al [19] observed that GFP1 could promote concanavalin A- or lipopolysaccharide-induced cell proliferation in CTX-positive tumour-bearing mice, thereby enhancing cellular and humoral immunity, as well as antitumour activity.

CD4+ and CD8+ T cells are known as T helper lymphocytes (Th) and T cytotoxic lymphocytes, respectively. CD4+ T cells active the innate immune response and mediate non-adjuvant antiviral action, while CD8+ T cells are immune effector cells that directly clear target cells. CD4+ T cells are classically divided into Th1 and Th2 cells, according to their cytokine secretion profiles. IFN-γ and IL-2 are produced by Th1 cells, while IL-4 and IL-10 are secreted by Th2 cells. IL-2 is required for T cells to grow, proliferate, and differentiate, while IFN-γ regulates the immune system (e.g. by linking innate and adaptive immune reactions) [54]. IL-4 plays important roles in humoral immunity, particularly by regulating various cellular functions, including T and B cell proliferation and differentiation. IL-10 is an anti-inflammatory cytokine that helps to prevent excessive inflammatory responses [18]. Wang et al [19] reported that GFP1 treatment induced secretion of the Th1 cytokines IFN-γ and IL-2 to participate in immune-modulating activities in C57BL/6 mice carrying Lewis lung carcinoma (LLC). Ginseng leaf polysaccharide (GS-P) combined with ovalbumin (OVA) and Freund's incomplete adjuvant induced robust secretion of OVA-specific Th1 cytokines (i.e., IL-2, IFN-γ, and GM-CSF) and Th2 cytokines (i.e., IL-10) [18].

The synergistic effects of polysaccharides in combination with drugs are mediated by elevating the immune response to treatments. Zhang et al [52] found that PJPS polysaccharide from *P. japonicus* contributed to resistance against immunosuppression caused by cyclophosphamide by boosting the level of IgM in plasma. In specific immunity involving Th lymphocytes, Th1 and Th2 cells secrete IFN-γ and IL-4, respectively, to regulate the functions of B cells; they also convert responses from IgM to specific Ig classes. IL-4 stimulates B cells to induce IgG1 and IgE antibody production, while IFN-γ induces IgG2-type antibody production but inhibits IgE production [55]. One mouse study found that when the polysaccharide GS-P was admixed with OVA, antibody production was significantly greater than when OVA was administered alone. Using GS-P in combination with OVA and Freund's incomplete adjuvant induced greater levels of antigen-specific IgG1 and IgG2b antibodies, but dramatically reduced the production of IgE antibody [18]. Thus, *Panax* polysaccharides can enhance antibody levels and immune function in mice.

The molecular channels of polysaccharide-activated lymphocytes include B lymphocyte and T lymphocyte surface receptors. The B-cell receptor is a transmembrane receptor protein on the B cell membrane surface, which consists of IgM and CD79. The T-cell receptor (TCR) is expressed on T lymphocytes; it forms a complex with CD3 and binds MHC molecules to form the TCR co-receptor [56]. The TCR specifically recognises and binds to MHCI/II molecules, thereby mediating the activation of T lymphocytes. APG polysaccharide can promote the expression of the T cell transmembrane protein CD25, activating transcription factor Foxp3 to promote the production of immunosuppressive regulatory T cells [57]; this helps to maintain peripheral tolerance and actively suppress autoimmunity in a TCR-dependent manner.

4.3. *Activation of NK cells by Panax polysaccharides*

NK cells are non-specific lymphocytes that, unlike T and B lymphocytes, participate in antiviral and antitumor defence without prior sensitisation [19]. The cytotoxicity of NK cells can effectively reflect the immune function of the body. The polysaccharide WGPN markedly enhances the cytotoxicity of NK cells in a dose-dependent manner and can partially restore the activity of NK cells inhibited by 5-fluorouracil [53]. Various cytokines produced by NK cells (e.g., NO, IFN-γ, and Th1) are capable of mediating cytotoxic function to modulate immune responses. IL-2 can stimulate NK cell proliferation and lead to the secretion of various cytokines. The combination of RGAP polysaccharide and recombinant IFN-γ enhances NK cell cytotoxicity against tumour cells; however, RGAP alone has no effect [34]. In ovariectomized rats, RGAP affects the tumoricidal activity mediated by NK cells and is modulated by iNOS, while synergistically inducing NK cell activity [58]. GFP1 polysaccharide significantly enhances the cytotoxicity mediated by NK cells from splenocytes in LLC-bearing mice [19]; this effect was also observed in mice at 100 or 200 mg/kg doses of FGEP-CA polysaccharide [54]. Compared with the non-tumour control, transplantation of H22 cells significantly reduces the cytotoxic activity of NK cells and CD8+ T cells, which was reduced by PSPJ polysaccharide in a dose-dependent manner [59]. Through their cytotoxic activity and cytokine production, NK cells are involved in defence against virus infection and tumour formation, as well as immune regulation [56].

5. Immunomodulatory in intestine of *Panax* polysaccharides

The gut-associated lymphoid tissue (GALT) is a major component of intestinal immunity, including Peyer's patches located in the small intestinal wall. *Panax* polysaccharides can stimulate Peyer's patches to promote intestinal immunity. Kim et al [29] used α-amylase and amylase to enzymolyze KRG, and isolated immune-stimulating polysaccharides RG-CW-EZ-CP-4 and RG-CW-EZ-CP-8, which can regulate the intestinal immune system through Peyer's patches. Compared with non-enzyme-digested polysaccharides, enzyme-digested polysaccharides significantly improved intestinal immunomodulatory activity, which seemed to be due to the removal of amyloid polysaccharides.

6. Therapeutic potential of *Panax* polysaccharides

Basic studies have shown that *Panax* polysaccharides exhibit many beneficial therapeutic properties, including anti-tumour and chemoprotective effects [53,59], as well as anti-multiple sclerosis activity [57], immunomodulatory activity [16,54], anti-inflammatory activity [33], and anti-sepsis activity [60]. *Panax* polysaccharides have also shown promise for osteoporosis treatment [58], for use as immunoadjuvants [18,43], and for the induction of haematopoiesis [52]. *Panax* polysaccharides may also be used to enhance the efficacy of chemotherapy drugs and reduce their side effects [36,52].

APG polysaccharide can significantly reduce the symptoms and recurrence rate of EAE. It has beneficial effects on various types of EAE, which may lead to further insights regarding the immunosuppressive effects of EAE and multiple sclerosis [57]. Treatment with WGPN polysaccharide alone significantly increases relative spleen weight in S180-bearing mice, suggesting that it acts as an immunopotentiator. WGPN in combination with 5-fluorouracil has synergistic anti-tumour activity and can restore immune function following 5-fluorouracil injury; this indicates that WGPN has potential as an adjuvant for chemotherapy [53]. Ginsan polysaccharide upregulates phagocytosis, enhances bacterial clearance, downregulates inflammatory cytokine synthesis, and reduces inflammatory responses, ultimately rescuing animals from lethal sepsis [60]. Kim et al [58] showed that RGAP polysaccharide has potential as an immunotherapeutic agent for patients with osteoporosis. GS-P polysaccharide is a potent adjuvant that enhances OVA-specific humoral and cellular immune reactions, and may be useful as an adjuvant ingredient because it substantially diminishes IgE production [18]. In mice carrying LLC, GFP1 polysaccharide effectively inhibits tumour growth and lung metastasis by activating immune function [19]. PPQN polysaccharide may induce the production of cytokines to inhibit inflammation, which has therapeutic significance for the treatment of inflammation and inflammation-related diseases (e.g., tumours and atherosclerosis) [33]. PJPS polysaccharide and anticancer drugs are used in combination to inhibit the myelosuppressive and immunosuppressive effects of chemotherapy, which may decrease the risk of infection and the adverse effects of antibiotic use, thereby reducing psychological and economic stress and improving survival in patients with cancer [52].

7. Conclusion

This review summarized current information about different polysaccharide isolated from *Panax* species. However, the structure-activity relationships are yet not entirely understood. There was no report regarding to how to control the polysaccharide during the process. Moreover, the research on gut mucosal immunity was also limited. Determination of the structures and structural modification might be another interesting research area in future studies. We believe that our work will provide novel insights for future research and developments of the polysaccharides with clinical value.

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