Cytotechnology (2018) 70:1097–1110
https://doi.org/10.1007/s10616-018-0202-z

REVIEW

Plant-derived polysaccharides activate dendritic cell-based anti-cancer immunity

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Received: 16 May 2017 / Accepted: 5 February 2018 / Published online: 19 March 2018

Abstract Today, cancers pose a major public health burden. Although a myriad of cancer treatments are available, only a few have achieved clinical efficacy. This is partly attributed to cancers capability to evade host immunity by converting dendritic cells (DCs) from potent stimulators to negative modulators of immunity. Dendritic cell-based immunotherapy attempts to resolve this problem by manipulating the functional characteristics of DCs. Plant-derived polysaccharides (PDPs) can stimulate the maturation of DCs conferring on them the capacity to present internalised tumorigenic antigens to naïve T cells and subsequently priming T cells to eliminate tumours. PDPs have been used as immune modulators and later as anti-cancer agents by Traditional Chinese Medicine practitioners for centuries. They are abundant in nature and form a large group of heterogeneous though structurally related macromolecules that exhibit diverse immunological properties. They can induce antigen pulsed DCs to acquire functional characteristics in vitro which can subsequently be re-introduced into cancer patients. They can also be used as adjuvants in DC-based vaccines or independently for their intrinsic anti-tumour activities. Clinically, some in vitro generated DCs have been shown to be both safe and immunogenic although their clinical application is limited in part by unsatisfactory functional maturation as well as impaired migration to draining lymph nodes where T cells reside. We review the relative potencies of individual PDPs to induce both phenotypic and functional maturation in DCs, their relative abilities to activate anti-cancer immunity, the possible mechanisms by which they act and also the challenges surrounding their clinical application.

Keywords Anti-tumour immunity · Dendritic cell maturation · Polysaccharides · Toll-like receptors · T cells

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Introduction

At the heart of adaptive immunity are DCs. They are a diverse population of motile leukocytes of stellate morphology present in essentially all body tissues (Steinman 2007). DCs act as sentinel cells that efficiently identify and take up offending antigens which are then processed internally and later

presented to effector immune cells, subsequently activating both naive and memory immune responses and are thus commonly referred to as 'professional' Antigen Presenting Cells.

At resting state DCs exist in their immature state; they possess excellent antigen uptake capabilities in situ but poor T cell stimulatory qualities. The converse is true for mature DCs (Mellman and Steinman 2001). In DCs, maturity status can be distinguished by; expression of Major Histocompatibility Complex (MHC) and costimulatory molecules, production of specific cytokines and cell morphology, and determines immune activation or anergy (Reis e Sousa 2006; Steinman 2012). Therefore, following antigen uptake, the tightly controlled and complex process of maturation is critical for both migration of DCs into lymphoid tissue where effector T cells are resident. In lymphoid tissues DCs provide T cells with an antigenic signal mediated by a MHC-peptide complex, a co-stimulatory signal mediated by costimulatory molecules and a polarizing signal mediated by cytokines, like IL-12, all of which are required for the activation and expansion of antigen-specific cytotoxic $CD8^+$ T cells, and drives the development of immune responses toward different types of T helper cells (Kalinski 2009). In addition to its influence on $CD8^+$ T cells, IL-12 also promotes the polarization of $CD4^+$ T cells to interferon IFN-γ-producing type 1 T helper (Th1) cells. These cells play a role in orchestrating antibody production and activation and expansion of $CD8^+$ T cells. In summary, the efficiency of DC –based immunotherapy heavily relies on the activation and expansion of tumoricidal $CD8^+$ T cells (Carreno et al. 2013; Curtsinger et al. 2005; Okada et al. 2010).

Although DCs have been approved by the FDA as cellular biodrugs in cancer immunotherapy (Hovden and Appel 2010) several challenges have limited their clinical success. This include their low numbers and the impaired state of maturation at the tumour microenvironment. DCs generated in vitro do not fully resemble those resident in vivo at steady state and are impaired in their migration to lymph nodes (Sabado et al. 2017; Verdijk et al. 2008). Tumours also evade host immunity by secreting factors such as immunosuppressive cytokines, growth factors and microRNAs (Bennaceur et al. 2008; Kikete et al. 2016; Rabinovich et al. 2007).

Polysaccharides are extensively distributed in the cell membranes of animals, higher plants, algae, bacteria and fungi. Chemically, they are macromolecules composed of more than ten monosaccharide units linked by glycosidic bonds. The immunomodulatory properties of PDPs have been applied in Traditional Chinese Medicine (TCM) since ancient times. In those days most Chinese herbs were administered as aqueous extracts indicated either for the general well-being of the consumer or to treat specific diseases. The formulations tended to be composed of a plethora of bioactive compounds in addition to polysaccharides. Nevertheless, advances in science have enabled the isolation, purification and testing of herbal polysaccharides and as such many findings have validated the immunomodulatory claims attributed to PDPs from empirical knowledge (Jiang et al. 2010; Leung et al. 2006; Ma et al. 2013).

Since the turn of the century interest has been growing on the testing of PDPs as biological response modifiers partly because they are generally associated with low toxicity and few adverse events. Many seem to have excellent immune enhancing activities, and when used as maturation promoters or as adjuvants in DC-based immunotherapy they can effectively activate antigen specific immunity. These properties are dependent on structural characteristics namely; monosaccharide type and glycosidic-linkage composition, conformation, molecular weight, functional groups and branching characteristics (Ferreira et al. 2015). So far glucans, mannans and pectic polysaccharides are the most widely studied. The individual monosaccharaide units contained in them are many and varied but some of the common ones include; glucose, galactose, mannose, xylose, rhamnose and fructose. However, when used clinically, PDPs have several disadvantages; they are rapidly metabolized, have a short duration of action and lack specificity in their mechanisms of action.

DC ontogeny and characteristics

DCs are a heterogeneous population of specialized APCs that originate from bone marrow predecessors called monocyte and DC precursors (MDPs). Eventually two types of DCs emerge; plasmacytoid DCs (pDCs) and myeloid or conventional DCs (cDCs) (Reizis 2010). cDCs are distributed in virtually all

peripheral tissues while pDCs are mainly restricted to the T cell components of lymphoid organs.

Conventional DCs (cDCs) are MHC-II$^+$CD11c$^+$ and can be further classified into CD1c$^+$ DCs and CD141$^+$ DCs (Liu and Nussenzweig 2010). CD141$^+$ DCs are thought to be the human homologues of murine CD8$^+$ DCs, both of which have excellent ability to cross present both soluble and cell-associated antigens to CD8$^+$ T cells (Bachem et al. 2010). cDCs also differentially express TLRs. CD1c$^+$ DCs express TLRs 1-8 and can secrete IL-12, TNF-α, IL-8, and IL-10 upon stimulation. On the other hand, CD141$^+$DCs express TLR3 and 8 and secrete high levels of type I interferon upon stimulation with synthetic ds-RNA poly-ICLC (Meixlsperger et al. 2013). This DC subset is also known for producing high levels of IL-29 or type III interferon in response to TLR3 activation (Zhang et al. 2013).

pDCs are MHC-II$^+$CD303$^+$CD304$^+$Lin$^-$. They effectively respond to viral infections by secreting IFN-α and IFN-β and can rapidly cross-present influenza virus derived antigens to CD8$^+$ T cells. Their role in anti-viral immunity is aided in part by the high expression levels of TLR7 and TLR9, which enable them to recognize viral and self-nucleic acids (Lui et al. 2009; McKenna et al. 2005; O'Brien et al. 2016).

Maturation of DCs and induction of immunity

Mature DCs can be generated in vitro. First, the combination of GM-CSF and IL-4 (or IL-13) induces the initial differentiation of monocytes to acquire phenotypic features associated with dendritic cells and then development is completed following culture with maturation stimuli (Sallusto et al. 1995). On the contrary, in vivo studies reported that GM-CSF deficiency did not impair DC generation (Vremec et al. 1997). Later, several investigators discovered that administration of flt-3L in both humans and mice led to a remarkable expansion of DCs (Maraskovsky et al. 1996, 2000) and it was later established that DC precursors in bone marrow were responsive to flt-3L (D'Amico and Wu 2003). Upon stimulation, a complex and tightly controlled cascade of events directs the phenotypic, functional and morphological transformations in the course of maturation. Mature DCs are characterized by the upregulation of MHC I and II molecules, costimulatory molecules such as CD54, CD80, CD86, and more recently PD-L1/CD274 and PD-L2/CD273, chemokine receptors (e.g., CCR7), cytokine production (e.g., IL-12), adhesion molecules and immunoproteosomes. In the functional context, as DCs mature, there is a simultaneous reduction in antigen phagocytic capacity, enhancement in antigen processing and presentation, improved CCR7-dependent migration to and localization in T cell compartments in lymph nodes, induction of clonal expansion of antigen-specific naïve T-cells, enhanced capacity to release cytokines promoting the differentiation of naïve T cells and the activation of various immune cells (Gatto et al. 2013; Spörri and Reis e Sousa 2005). The nature of the maturation stimulus triggers the production of unique types of cytokines which direct the polarization and expansion of specific T cells in response to the nature of the threat. On the other hand, at steady state a fraction of DCs undergoes a maturation program that confers tolerogenic rather than immunogenic properties called homeostatic maturation (Lutz and Schuler 2002). These DCs induce the generation of regulatory T cells (Tregs) (Dalod et al. 2014). One of the mechanisms by which tumours evade host immunity is by directing the differentiation of DCs in favour of tolerogenic DCs.

Mature DCs undergo CCR7 dependent migration into T cell compartments of secondary lymphoid tissues in response to lymph node homing chemokines (CCL19 and CCL21). As regards tumour immunology, T cells, through the T cell receptors (TCRs) recognise tumour-specific antigens bound to MHC-I molecules on the DCs, a process called cross-presentation. This, together with other necessary cross-talk at the immunological synapse triggers signalling of molecules downstream of the TCR that results in the expansion and differentiation into memory and effector cells (Sabado et al. 2017). Mature DCs can also activate naïve and memory B cells primarily through their ability to stimulate CD4$^+$ T helper (Th) cells, which induce B cell proliferation and antibody production (Jego et al. 2004).

Exogenous maturation stimuli

Several exogenous stimuli can trigger the maturation of DCs. These may include: microbial products (Akira and Takeda 2004), endogenous inflammatory molecules (TNF-α, IL-1, IL-6 and IFN-α) (Škoberne et al. 2004), activation of intracellular sensors such as RIG-I (Kato et al. 2006) inflammasomes (Pedra et al. 2009), DC activating factors released by dead cells, such as heat-shock proteins, RNA and DNA (Gallo and Gallucci 2013). In clinic several TLRs have been used in combination based on the principle that concurrent activation of multiple TLRs can augment the effects of individual TLR stimulation (Boullart et al. 2008; Napolitani et al. 2005). As such, a cocktail of proinflammatory cytokines TNF-α, IL-1β, and IL-6 in combination with prostaglandin E_2 was initially established as the gold standard for DC maturation (Lee et al. 2002).

LPS is a potent TLR4 ligand and is widely used in the laboratory as a reference maturation stimulus. Clinical grade LPS has been used to mature DCs for vaccination in ovarian cancer patients (Chiang et al. 2013) but its clinical use in humans is limited due to concerns of its high toxicity to normal cells (Breckpot and Escors 2009). PDPs are structurally related to LPS and some were shown to be equipotent and thus could serve as potential alternatives. Polysaccharides generally induce TLR signalling, specifically TLRs 4, 2, 7 and 9. Moreover, *Ficus carica* polysaccharides were reported to act via Dectin-1 receptors (Tian et al. 2014a). They are believed to act on the named cell surface receptors because their large molecular size hinders them from crossing the cell membrane. In the majority of cases, downstream signalling appears to confluence at NF-κB and MAPK molecules. It is also known that DC maturation can be stimulated by engulfed antigens, including PDPs. Endocytosis of *Ganoderma lucidum* polysaccharides results in the upregulation of CD40, CD80, CD86 and MHCII. These maturation markers were significantly depressed when sodium azide (an endocytosis inhibitor) was co-cultured with polysaccharide treated DCs, indicating that endocytosis is critical for DC maturation (Zhu et al. 2016).

Activities and mechanisms by which PDPs promote maturation of DCs and subsequently induce T cell responses against tumours

Many plant-derived polysaccharide extracts have been tested for their influence on DC maturation and activation of anti-tumour immunity. Table 1. Summarises the findings.

Phenotypic maturation

Critical to the initiation of anti-tumour immunity is the stable and lasting interaction of DC and corresponding T cell surface molecules at the immune synapse. As mentioned earlier MHC molecules present peptides to the TCR while costimulatory molecules play a complementary role, with CD 80/86 binding CD28, and CD40 with CD40L on $CD8^+$ T cells, respectively. All PDPs tested and shown to promote DC maturation upregulated the expression of MHCs, CD80 and CD86, and in some cases CD40 albeit with variable potencies. For instance, *Plantago asiatica* and *Capparis spinosa* polysaccharides showed similar and superior phenotypic maturation capabilities, respectively, in comparison to LPS (Hamuti et al. 2017; Huang et al. 2014). As expected, non-polysaccharide plant extracts that suppress dendritic cell function similarly depress the expression of these maturation markers (Fu et al. 2014; Li et al. 2011, 2012a). Therefore, the evaluation of the relative influence on maturation marker expression is a useful predictor for potential DC based immunomodulatory properties of biological response modifiers before more elaborate studies are carried out.

Membrane receptors

The large molecular size of PDPs hinders them from crossing the cell membrane. They therefore exert their activity by surface binding to receptors at the cell surface. TLR4 was described as the signalling component of LPS and consequently triggered its cellular transduction, which led to DC maturation (Boele et al. 2009). Owing to their structural similarity with LPS, and reports from early studies that showed similar patterns of pharmacological activities, they were believed to share receptors. Indeed, of the eleven PDPs tested for mechanism in this review,

Table 1 Summary of activity and mechanisms mediated by plant-derived polysaccharides in DC-based anti-tumour immunity

Name	Phenotypic maturation	Functional maturation			Pathways		References
		Cytokine	T cell activation	Anti-tumour	Membrane target	Downstream signalling	
Achyranthes bidentata blume	↑MHC II, CD80, 86, CD40	↑IL-12	Not tested	Not tested	Not tested	Not tested	Zou et al. (2011)
Anelica gigas Nakai (Angelan)	↑MHC II, CD80, CD86	↑ IL-12,IFN-α, IFN-β, TNF-α, IL-1β but not IL-2, IL-4, IFN-γ	↑ T cell proliferation ↑IFN-γ, IL-2 in Th1 cells, and of IL-10 and IL-4 in Th2 cells.	In vivo; ┤ tumour growth ↑survival	TLR4	NF-κB/Rel, ↑nuclear translocation of NF-κB p50/p65, RelB, and c-Rel MAPK ↑phosphorylation of JNK, p38 and ERK,	Kim et al. (2011, 2007)
Astragalus mongholicus	↑CD-11c, MHC II	↑ IL-12p70	Not tested	In vitro: ↓viability of tumour cells, In vivo: ↓tumour growth	TLR4 ↑mRNA expression	↓ expression of IκB-α	Shao et al. (2006) and Tian et al. (2014b)
Caesalpinia spinosa (Galactomannan)	↑HLA-DR CD86, CD83, CD206.	↑ IL-12p70 < LPS, ↑IL-1β > LPS, ↑IL-6, IL-10, IL-12p70, TNF-α	Not tested	Not tested	mannan receptor	Not tested	Santander et al. (2011)
Capparis spinosa	Dose dependen↑CD40, CD80, CD86 > LPS	No Δ IL-12, TNF-α. n.s Δ in IL 10	↑proliferation of CD4 and CD8 T cells	Not tested	Not tested	Not tested	Hamuti et al. (2017)
Carthamus tinctorius	Dose dependent ↑CD86 < MHC-I = MHCII = CD80	In vitro: dose-dependent ↑TNF-α, IL-1β. Tumour lysate ↓IL-2. Dose dependent ↓ IL 10	In vivo: ↑ IL-2, IL-10 and IFN-γ, comparable to vaccine alone. IL-4 production ↓vaccine alone	↓JC tumour growth in vivo. ↑ CTL activity in vitro	Not tested	Not tested	Chang et al. (2011)
Dioscorea alata var. *purpurea*	combination with TCL, n.s. ↑ CD40, CD86. ↑CD80	dose-dependent ↑ IL-1, n.s ↑ IL-12, no Δ IL-10, ↓TGF-	Dose dependent ↑ T-cell proliferation. ↑ IL-17A, IFN-. N.s ↑ IL 4.	In vivo, dose-dependent ┤ tumour growth > LPS	Not tested	Not tested	Chang et al. (2013)
Ficus carica	↑ CD40, CD80, CD86, and MHCII	↑ mRNA expression of IL-12, IFN-γ, IL-6, and IL-23	↑ proliferation of $CD4^{+}CD25^{-}$ T cells	Not tested	Dectin-1 (not exclusive)	Syk	Tian et al. (2014a)

Table 1 continued

Name	Phenotypic maturation	Functional maturation			Pathways		References
		Cytokine	T cell activation	Anti-tumour	Membrane target	Downstream signalling	
Lycium barbarum	↑ I–A/I–E and CD11c	↑ IL–12 p40	↑ lymphocyte proliferation	Not tested	TLR2, TLR4	NF–κB, ↑ nuclear translocation of the NF–κB p65	Zhu et al. (2013)
Mori fructus	Dose dependent ↑ CD40, CD80, CD86, and MHC-I/II	↑ gene expression and production of IL-12 and IL-1β, TNF-α, and IFN-β	↑ T cell proliferation ↑ IL-2 and IFN-γ	Not tested	TLR4 (not exclusive)	MAPK (↑ phosphorylation of ERK, c-Jun, JNK, and p38 MAPKs) NFκB (↑ IκBα/β degradation,↑ nuclear translocation of NF-κB p65)	Shin et al. (2013)
Plantago asiatica	↑ MHC class II, CD80, CD86, CD40, = LPS	↑ IL-12p70 and simultaneously ↓ IL-10 ↑ TNF-α	↑ Ag-specific proliferation T cells.	Not tested	TLR4 > TLR2 > TLR7 > TLR9	Not tested	Huang et al. (2014)
Platycodon grandiflorum	Dose dependent ↑ CD40, CD80, CD86, and MHC-I/II	↑ IL-1β, IL-6, IL-10, IFN-β, and TNF-α. TNF-α > IL-12 and IL-1β	↑ Enhanced T cell proliferation ↑ IL-2 and IFN-γ production	Not tested	TLR4 (not exclusive)	MAPK (↑ phosphorylation of ERK, JNK, and p38 MAPKs) NFκB (↑ IκBα/β degradation, ↑ nuclear translocation of NF-κB p50 and p65)	Park et al. (2014)
Pleurotus ferulae	↑ CD40, CD80, CD86, MHC-II and CCR7	Dose dependent ↑ IL-12 and IL-6, TNF-α Synergism with CpG–ODN. ↓PGE_2	↑proliferation of $CD8^+$ T cells < CpG–ODN. ↑ IFN-γ. Synergism with CpG–ODN.	Not tested	TLR4 (not exclusive)	Not tested	Li et al. (2015)
Polyporus umbellatus	Dose-dependent ↑CD86	Dose dependent ↑IL-12 and IL-10	↑proliferation of T cells	Not tested	TLR4 ≫ TLR2	Not tested	Li et al. (2010)

Table 1 continued

Name	Phenotypic maturation	Functional maturation			Pathways		References
		Cytokine	T cell activation	Anti-tumour	Membrane target	Downstream signalling	
Pueraria lobata	Dose-dependent ↑CD40, CD86, and MHC-I	↑gene expression and production of IL-12, IL-1β, TNF-α, and IFN-β	↑T cell proliferation ↑ IL-2 and IFN-γ	Not tested	TLR4 (not exclusive)	MAPK (↑ phosphorylation of ERK, JNK, and p38 MAPK) NFκB (↑ IκBα/β degradation, ↑the nuclear translocation of NF-κB p65)	Kim et al. (2013)
Radix glycyrrhizae	↑CD80, CD86 and MHC I-A/I- E	↑ IL-12 p70. Time-dependent ↑IL-12 p40 mRNA expression	↑ proliferation T cells = LPS ↑IFN-γ	Not tested	TLR4 (not exclusive)	NF-κB. MAPK (p38 MAPK and JNK but not ERK)	Li et al. (2012b)

↑, increases; ↓, decreases; ┤, inhibits; Δ, change; ns, not significant

ten reported involvement of TLR4 signalling pathways in phenotypic and functional maturation of DCs. Most tested PDPs efficiently increased the maturation of TLR4 $^{+/+}$ DCs but C3H/HeJ mice with mutated TLR4 were hyporesponsive to angelan (a pure polysaccharide extract of *Angelica gigas* Nakai), *Mori fructus* polysaccharide, *Pueraria lobata* polysaccharide, *Platycodon grandiflorum* polysaccharide and LPS (Kim et al. 2007, 2013; Park et al. 2014; Shin et al. 2013). Nevertheless, introduction of TLR3 ligand poly(I:C) still enhanced DC maturation and function (Kim et al. 2013) indicating that although TLR4 appears to be the main pathway, other complementary pathways may be involved. In another study to investigate the relative importance of the various TLRs, Huang and colleagues reported that *P. asiatica* polysaccharides significantly up-regulated TLR2 and TLR4 mRNA expression and to a lesser extent TLR7 and TLR9. The up-regulation of TLR mRNA expression may be responsible for the overall responses to this polysaccharide which they showed to be a potent inducer of DC maturation. In relative terms, the order in decreasing importance was TLR4, TLR2, TLR7 and TLR9 (Huang et al. 2014). Treatment of immature DCs with *Astragalus mongholicus* also increases the mRNA expression of TLR4 (Shao et al. 2006).

Interestingly, galactomannan, a pure polysaccharide from *Caesalpinia spinosa* effectively induced the maturation of DCs in vitro, but decreased TLR4 expression by fivefold (Santander et al. 2011). It was earlier hypothesised that mannan could bind one of the C-type lectins mannose receptor family expressed by APCs (Sheng et al. 2006). The role of these receptors has not yet been fully understood but galactomannan could interact with DCs via the mannan receptor (CD206) which has high affinity for highly branched mannose structures. This is in light of the fact that galactomannan and mannan share a backbone composed of mannose and differ only in anomeric configuration.

Dectin-1 (CLEC 7A) a type-II C-type lectin receptor, is another transmembrane signalling receptor that mediates various cellular responses in myeloid cells, including antigen binding, uptake, and induction of cytokine production and chemokinesis (Brown 2006). Immature murine DCs treated with *F. carica* polysaccharides acquired maturation characteristics which positively correlated with an

increase in Dectin-1 expression. Upon introduction of anti-Dectin antibodies both the maturation characteristics and Dectin-1 expression were depressed (Tian et al. 2014a). Previous studies showed that the interaction of Dectin-1 and β-glucan can trigger intracellular signal transduction that can lead to responses including cell maturation, chemokine and cytokine production (Brown 2006; Reid et al. 2009).

TLR downstream signaling pathways

As stated earlier, TLR4 ligation is a major mechanism involved in the induction of DC maturation. This is mediated via two main downstream signalling pathways; MAPK and NF-κB (Banchereau et al. 2000; van de Laar et al. 2010). As expected, several of the PDPs tested mediated maturation via these pathways. Findings from several studies consistently reported that these polysaccharides increased the phosphorylation of MAPKs, specifically ERK, p38, and JNK and also induced the degradation of IκBα/β and nuclear translocation of p65 and p50 (Kim et al. 2013; Park et al. 2014; Shin et al. 2013). On further analysis, some of these downstream molecules play a bigger role in DC maturation. For instance, the activity of *Radix glycyrrhizae* polysaccharide was supressed when NF-κB and p38 MAPK were inhibited but not JNK (Li et al. 2012b). In another study that investigated the relative importance of the two downstream pathways, the investigators found that nuclear translocation of NF-κB p50/p65 and up-regulation of MHC-II expression attributed to angelan were markedly reduced with the introduction of TPCK (a direct inhibitor of both IKK activity and DNA binding of p65/RelA) but there was minimal effect when p38, ERK and JNK inhibitors were introduced in culture. Their findings appear to suggest that the angelan-induced DC maturation is largely depended on NF-kB signalling (Kim et al. 2011). Such reports probably influenced many follow up studies in which several investigators limited their investigation to the NF-κB pathway.

Later, Zhu and his group showed that TLR2 and TLR4 molecules have almost equal roles in directing DC maturation mediated by *Lycium barbarum* polysccharide. Interestingly, when the two TLRs are stimulated concurrently, there seems to be a confluence of the pathways at the level of NF–κB p65 since inhibition of this molecule significantly depresses maturation characteristics of DCs (Zhu et al. 2013). The above findings demonstrate that the intracellular events that mediate TLR signalling are not fully understood and more extensive studies are required in this area.

In addition, interaction of the transmembrane receptor Dectin-1 with *F. carica* polysaccharide resulted in increase in both acquisition of maturation characteristics by DCs concurrently with enhanced expression and phosphorylation of Syk. These results suggest that this polysaccharide activates DCs at least in part via the Dectin 1/Syk pathways (Tian et al. 2014a).

Functional maturation and anti-tumour activity

In general, the treatment of DCs with potent PDPs resulted in production of cytokines that were associated with the differentiation and proliferation of $CD8^+$ CTLs as well as polarizing the $CD4^+$ T cells towards the Th1 pathway. LPS is commonly used as the reference DC stimulus and several PDPs recorded appreciable activity even at low doses albeit with varied potencies. Some references reported a significant increase in IL-12 mRNA expression and production which was dose-dependent in some cases (Li et al. 2015; Li et al. 2010). Other cytokines whose production was generally enhanced in polysaccharide treated-DCs and which are associated with DC maturation and Th1 polarization include TNF-α, IL-1β and IL-10 (Wang et al. 2007). Subsequently, T cells were induced to proliferate and produce IFN-γ and IL-2 which indicated successful activation of immune responses.

Furthermore, some investigators went further and studied the anti-tumour immune responses. In vivo, CCR7 mediated DC migration is critical since the effector cells are resident in lymphoid organs. First, plant-derived polysaccharide-treated DCs can down-regulate expression of CCR1, CCR2, and CCR5 that normally direct DC trafficking into peripheral tissues and concurrently upregulate CCR7. Indeed, in vitro angelan enhanced expression of CCR7 and CXCR4. Similar results were obtained in vivo where angelan strongly enhanced migration to and localization of DCs in the T cell zones of lymph nodes (Kim et al. 2011).

The most rational use of PDPs in tumour immunotherapy is as vaccine adjuvants. Tumour

antigen loaded DCs show limited and sometimes depressed maturation characteristics which in turn hinder activation of immune responses. Treatment with PDPs can direct the maturation of tumour loaded DCs and this forms the basis of their use as adjuvants. There is no standardized protocol and different researchers used different methods but all involved the in vitro maturation of murine DCs in polysaccharide which ranged between 22 and 72 h. In all studies the antigen pulsed DCs were injected subcutaneously and in one study a booster vaccine was given after 10 days (Chang et al. 2013). In another study the polysaccharide was given intraperitoneally every alternate day (Kim et al. 2011). Despite the variations in protocols all findings for the tested PDPs confirmed that there was strong inhibition of tumour growth and prolonged lifespan of mice in the test groups (Chang et al. 2011, 2013; Kim et al. 2011). Finally, Dioscorea also strongly suppressed myeloid derived suppressor cells activity in tumour bearing mice. These cells are a major component of the immunosuppressive network present in the tumour microenvironment and are also known to prominently increase in tumour-bearing mice (Youn et al. 2008). Taken together, these findings indicate that indeed PDPs can be used as effective adjuvants in anti-cancer vaccines but more research is needed to determine the most potent ones and the ideal protocols for both in vitro maturation and administration.

Emerging trends and future approaches

Comparisons and combinations

Most of the studies that investigated the DC maturation and function properties of polysaccharide focused on polysaccharides extracted from one particular plant. The results thus limit one's ability to compare the immune response potency across plant and chemical species. Zhu and colleagues compared PDPs extracted from three herbs at the same concentration in vitro namely, *Astragalus membranaceus*, *G. lucidum* and *Radix ophiopogonis*, all of which were previously reported to positively influence DC maturation. For the parameters measured they concluded that *G. lucidum* exhibited the strongest activity while *R. ophiopogonis* exhibited the weakest one (Zhu et al. 2016). Previously, when comparing highly purified polysaccharide extracts from *A. membranaceus* and *Codonopsis pilosulae*, Chang et al. reported that in culture the former induced significantly a higher expression of CD40 while the latter induced higher expression of CD80 and CD86. This was probably due to the differences in the monosaccharide residues contained. Both had high glucose content but *C. pilosulae* had 15.59% galactose while *A. membranaceus* had none. Surprisingly, when tested for cytokine production they induced similar cytokine profile as well as similar anti-tumour activity when used as DC vaccine adjuvants. As expected, combination of the two provided the most efficacious result (Chang et al. 2015). It therefore appears that combinations of PDPs could potentially yield immunogenic synergy. Moreover, since combination strategies appear be the key to the success of DC vaccines, several permutations can be tested; for instance, PDPs in combination with checkpoint inhibitors, a class of drugs that has had a remarkable impact in immunotherapy in recent years. As such, further studies are required to ascertain the relative potencies as well as determine the combinations that provide the highest efficacies.

Structural modification

We know that the chemical structure and configuration of PDPs influence their DC maturation properties. However, we do not fully understand relationship between structure and function. In the continued quest to improve the induction of DC maturation and function by PDPs, protocols have been developed to guide the modification of these molecules in order to enhance activity (Upadhyaya et al. 2013; Wang and Zhang 2009). For instance, polysaccharides extracted from *Hericium erinaceus* were modified through selenylation to obtain nine derivatives. Among them, the most superior derivative, $sHEP_2$, significantly increased the expression of MHC-II and CD86, decreased DC endocytosis and enhanced IL-12 and IFN-γ production. In line with TLR4 activation, $sHEP_2$ induced NF-κB downstream signalling (Qin et al. 2017). Similarly, when polysaccharide extracts from *Radix Cyathulae officinalis* were structurally modified either by sulphation or phosphorylation, the derivatives exhibited significantly superior activities in terms of both expression

of cell surface markers and activation of T cell responses (Feng et al. 2015, 2017). Carboxymethylation of polysaccharides extracted from the seeds of *P. asiatica* L. too resulted in in vitro promotion of DC maturation characteristics such as maturation markers, cytokine production, induction of T cell proliferation, upregulation of chemokine receptor and so on (Jiang et al. 2014). LPS-matured DCs produce high quantities of IL-12p70 and induced tumour-specific T cell responses in melanoma patients. Due to its unfavourable toxicity profile a less toxic derivative, *O*-deacylated monophosphoryl lipid A (MPLA) was developed. Together with IFN-γ MPLA was demonstrated to produce high levels of IL-12 and induce $CD8^+$ T cell responses in vitro. It was approved for use in humans and is used in combination with Cervarix (Kolanowski et al. 2014). Lastly, small molecule synthetic TLR agonists can be effective adjuvants in anti-cancer vaccines. Nevertheless, their clinical application is restricted by undesirable pharmacokinetics and unacceptable side effects. Shinchi and colleagues conjugated a low molecular weight TLR7 ligand to various polysaccharides. They found that, in vitro the conjugates showed up to 1000-fold increase in potency in comparison to the unconjugated TLR7 ligand. Moreover, in vivo, the conjugates induced higher cytokine production and enhanced antigen specific immune responses (Shinchi et al. 2015). Taken together, these findings indicate that, in the context of DC-based immunotherapy, structural modification of natural polysaccharides can yield improved results.

Modification of delivery systems

A major limitation of PDPs in cancer immunotherapy is their rapid degradation in vivo. By providing controlled release of the payload at the site of action, encapsulation in liposomes can offer protection against degradation of PDPs in vivo, and thus resulting in a prolonged duration of action. Therefore, the liposomal carriers can effectively deliver the polysaccharide adjuvant and/or the antigen thus enhancing the activation of immune responses (Schwendener 2014). When *Rehmannia glutinosa* polysaccharides were encapsulated in a liposome and compared with the un-encapsulated one in a mouse model, the former posted superior results. First the encapsulated polysaccharide showed much higher stability for a longer period, it also induced significantly higher lymphocyte proliferation, cytokine production and upregulation MHCII and CD86 in lymph node DCs (Huang et al. 2016) In another study, PLGA polymer was used also for the co-encapsulation of paclitaxel and a TLR4 agonist (a phthalic acid derivative of LPS). This system effectively activated immune cells and facilitated the infiltration of T cells into the tumour microenvironment in a mouse model (Roy et al. 2013). These results show that in order to circumnavigate the challenges posed by the rapid degradation and metabolism of PDPs, it is necessary to develop better strategies to deliver the molecules to the intended site and maintain a longer duration of action. Thus the use of nanoparticles as vehicles for delivery of PDPs to their sites of action shows good promise.

Optimization of generation and injection of DCs

The technique used in the manipulation of DCs in vitro and subsequent injection into patients is critical to the induction of potent and long-lasting immune responses in vivo. Many current protocols typically mature DCs over 24 h. Emerging evidence suggests that DCs produce most cytokines in under 24 h and can only regain this capacity upon T cell interaction and CD40L ligation. Thus protocols that generate such DCs, also called Fast DCs may be more ideal especially because IL-12 production after injection is critical in vaccine efficacy. Fast DCs were shown to be as effective as standard-DCs in the activation of Ag-specific T-cell responses (Dohnal et al. 2009; Ramadan et al. 2004) and can help minimise costs as well as time spent. In addition, we are yet to conclusively determine, the optimum number of DCs to inject, the vaccination schedule, and the best route of injection. All these factors do influence the effectiveness of DC vaccines and warrant more extensive research. Lastly, immature DCs can be differentiated from human pluripotent stem cells (hPSCs) such as induced pluripotent stem cells and embryonic stem cells. This involves multi-step process that uses various growth factors such as BMP-4, VEGF, GM-CSF, SCF, Flt3L, and IL-4 at pre-determined intervals (Senju et al. 2010). Most importantly, such DCs can potentially be produced at a large scale using bioreactors (Li et al. 2014).

Conclusion

Dendritic cell-based immunotherapy holds much potential in targeting and eliminating cancers in vivo. Numerous techniques have been attempted to manipulate the functional maturation of DCs in vitro prior to re-introduction into cancer patients. Nevertheless, clinical outcomes have not been satisfactory partly because monocyte-derived DCs resemble more the cells that appear during inflammatory responses in vivo than DC subsets at steady state. Secondly, many in vitro generated dendritic cells have impaired capacity to migrate to lymph nodes and thirdly there is no ideal protocol for the maturation and subsequent injection of these DCs. However, findings from clinical trials show that better outcomes are achieved when a cocktail of maturation factors are used in combination.

Despite the plethora of maturation promoters available, PDPs remain prospective candidates, first owing to established effectiveness in inducing immunity against tumours and second due to their relative abundance in nature, appreciable safety profile and relative affordability. PDPs can condition the tumour environment to favour immune responses and can hence also be used as systemic adjuvants to vaccines. In addition to the above activities, some PDPs such as those from *Bupleurum chinense* and *Ascophyllum nodosum* (Abu et al. 2015; Tong et al. 2017) can exert their own intrinsic anti-tumour activity and thus may be investigated further with a view to examine the potential of exploiting multiple approaches in cancer therapy while using PDPs.

Being a diverse group of macromolecules that is abundant in nature, new and more potent ones could be discovered or existing ones could be combined to improve potency. In addition better protocols for DC maturation can be developed to optimize both maturation and systemic delivery of DC vaccines and adjuvants In addition, with the advancement of science they can be modified chemically to yield more effective and less toxic derivatives which may improve treatment outcomes in a number of cancers.

Acknowledgement This paper was supported by National Natural Science Foundation of China (NSFC No. 81573707) and The Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT, No. IRT_14R41).

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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