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REVIEW

Consumption of β-glucans to spice up T cell treatment of tumors: a review

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ABSTRACT

Introduction: Adoptive T-cell treatments of solid cancers have evolved into a robust therapy with objective response rates surpassing those of standardized treatments. Unfortunately, only a limited fraction of patients shows durable responses, which is considered to be due to a T cell-suppressive tumor microenvironment (TME). Here we argue that naturally occurring β-glucans can enable reversion of such T cell suppression by engaging innate immune cells and enhancing numbers and function of lymphocyte effectors.

Areas covered: This review summarizes timely reports with respect to absorption, trafficking and immune stimulatory effects of β-glucans, particularly in relation to innate immune cells. Furthermore, we list effects toward well-being and immune functions in healthy subjects as well as cancer patients treated with orally administered β-glucans, extended with effects of β-glucan treatments in mouse cancer models.

Expert opinion: Beta-glucans, when present in food and following uptake in the proximal gut, stimulate immune cells present in gut-associated lymphoid tissue and initiate highly conserved pro-inflammatory pathways. When tested in mouse cancer models, β-glucans result in better control of tumor growth and shift the TME toward a T cell-sensitive environment. Along these lines, we advocate that intake of β-glucans provides an accessible and immune-potentiating adjuvant when combined with adoptive T-cell treatments of cancer.

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1. Adoptive T cell therapy: a short introduction

Adoptive T cell therapy (AT) is a well-tested and promising approach to treat cancer and relies on the infusion of autologous tumor-specific T cells [1]. Besides the use of non-modified T cells, such as tumor-infiltrating lymphocytes (TILs) or peripheral T cell clones, one can also use T cells that are gene-engineered to express chimeric antigen receptors (CARs) or T cell receptors (TCRs). These CAR and TCR-engineered T cells recognize a chosen tumor antigen, and are redirected to selectively destroy cells expressing this antigen. While the use of CARs has shown impressive results in B cell leukemia's with response rates up to 94% (reviewed in [2]), which culminated in the recent FDA approval of *Kymriah* [3] and *Yescarta* [4], their current use in the treatment of solid tumors is lagging behind these successes in hematological tumors. TCR-engineered T cells have demonstrated clinical benefit in patients with multiple myeloma, metastatic melanoma and metastatic synovial sarcoma with response rates varying between 55% and 80% (reviewed in [5]). Notwithstanding these clinical results, particularly when treating solid tumors, AT is generally marked by a large fraction of patients with no or nondurable clinical responses. This suboptimal success coincides with limited accumulation and activation of T cells within tumors and poor persistence of these cells in the periphery [6,7]. To keep the positive momentum of AT and increase the durability of responses, local immune suppressive mechanisms need to be antagonized to ensure sufficient numbers and function of therapeutic T cells at the tumor site.

1.1. Tumor microenvironment and suppressive innate immune cells

The tumor microenvironment (TME) provides the tumor's architecture and nourishment, and consists of fibroblasts, endothelial cells and immune cells, and their products such as extracellular matrix components (EMC), cytokines and other mediators. There are several distinct immune cell types that actively contribute to an immunosuppressive TME, including (but not limited to) myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), tumor associated neutrophils (TANs), immature dendritic cells (imDCs) and regulatory T cells (Tregs). The TME counteracts the tumoricidal function of activated immune effector cells, such as CD8 T cells and Natural Killer (NK) cells, through various mechanisms, which are described in some detail below with emphasis on monocytic and granulocytic cell types that have been reported to be most responsive to β-glucans (see Section 2).

- *MDSCs*: these cells constitute a heterogeneous population of immature myeloid cells that arise from the bone marrow [8]. The expression of indoleamine 2,3-dioxygenase (IDO) by tumor cells is associated with MDSC infiltration [9]. MDSCs

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Article highlights

- Orally ingested β-glucans are taken up in the proximal gut via intestinal epithelial cells or M-cells in Peyer's patches, following which they are captured by subsets of CD103+ DCs and CXCR3+ macrophages within the GALT.
- Pro-inflammatory and T cell-enhancing effects such as diminishing T cell suppression by tumor-educated innate immune cells, and promoting T cell priming and Th1 differentiation have been attributed to β-glucans upon interaction with PRRs expressed by innate immune cells.
- Innate immune cells, following exposure to β-glucans, migrate to the bone marrow, spleen and lymph nodes where they support differentiation of effector lymphocytes, such as NK cells and CD8+ T cells.
- Amongst the β-glucans, those isolated from *L. edodes* and *S. cerevisiae* are the strongest biological and immunological modifiers according to clinical data with cancer patients and *in vivo* tumor mouse models.
- There is rationale to support adoptive T cell therapy with β-glucans derived from *L. edodes* and *S. cerevisiae*, not only because of T cell-potentiating abilities of these β-glucans but also because intake of β-glucans is safe, cheap, and enhances public health awareness.
- Chemistry, industrial production, and applications are currently not standardized and need to be better defined to select β-glucans as adjuvants.

This box summarizes key points contained in the article.

are generally divided into two major subsets: polymorphonuclear MDSC (PMN-MDSC) and monocytic MDSC (M-MDSC) that are morphologically and phenotypically similar to neutrophils and monocytes, respectively. Within the TME, M-MDSCs are the most prominent subset of MDSCs and can differentiate into TAMs (see below) [8]. MDSCs have the ability to disrupt mechanisms of immune control, such as antigen presentation by dendritic cells (DCs), natural killer (NK) cell cytotoxicity, and T cell activation [10].

For example, MDSCs have been reported to negatively regulate T cell responses by the production of galectin 9, a ligand for T cell immunoglobulin mucin-3 (Tim-3), the latter being an immune checkpoint that upon activation diminishes CD8 T cell responses [11]. Other examples by which MDSCs hamper T cell proliferation and effector functions include production of arginase-1, IDO and inducible nitric oxide synthase 2 (iNOS2) [12,13]. In particular, arginase-1 and IDO activity deplete local arginine and tryptophan, which promotes Treg development thereby blocking cytotoxic T cell function [11]. Interestingly, the frequency of MDSCs is correlated with disease progression in patients suffering from melanoma [14], non-small lung cancer (NSCLC) [15], breast cancer [16] and colorectal carcinoma [17], and was demonstrated to be inversely correlated with the presence of functional antigen-specific T cells in patients with advanced melanoma [18].

- *TAMs*: these cells constitute a population of myeloid cells that arise from tissue-resident macrophages of either embryonic or monocytic origin following their recruitment into the tumor tissue [19]. Tissue-resident macrophages or peripheral monocytes are recruited through colony-stimulating factor 1 (CSF-1), vascular endothelial growth factor (VEGF) and chemokines such as CCL2 and CCL5 produced by tumor and stromal cells [8,20]. TAMs represent a dominant myeloid cell population in many solid tumors and can display characteristics of both tumor-suppressive M1 as well as tumor-promoting M2 macrophages [21]. M1-like TAMs are induced by the T helper 1 (Th1)-type cytokine IFN-γ and the production of IL-12 and IL-23 by M1-like TAMs promotes or amplifies polarization of T cell toward a T helper 1 (Th1) phenotype [22]. However, tumors usually harbor TAMs with a M2-like phenotype, which can be locally induced by T helper 2 (Th2)-type cytokines, such as IL-4, IL-13 and IL-10 [23]. TAMs regulate tumor angiogenesis, metastasis, and immune suppression via different mechanisms. For example, TAMs acquire a more pro-angiogenic phenotype as a result from hypoxia, and become responsive toward cytokines, such as IL-10 and transforming growth factor (TGF-β), glucorticoids and immunoglobulin complexes [24]. Finally, M2-like TAMs suppress anti-tumor T cell responses through increased expression of programmed death-ligand 1 (PD-L1) and enhanced production of TGF-β and prostaglandin E2 (PGE2)[20]. In fact, TAMs take part in the progression of, for example, epithelial ovarian cancer with the frequency of TAMs being highest in high grade compared to low grade cancer tissues [25]. Moreover, the infiltration of TAMs has been shown to negatively associate with prognostic outcomes in several types of tumor such as lymphoma [26].
- *TANs*: these cells constitute a population of granulocytic cells that arise from peripheral neutrophils and PMN-MDSC [27]. Neutrophils are recruited to the tumor site via locally produced molecules such as CXCL8, CXCL5, CXCL6, and hydrogen peroxide. Two distinct subpopulations of TANs are described, either having immune suppressive (N2) or immune stimulatory (N1) functions [28], with type I interferons (IFNs) representing important inducers of N1 TANs and TGF-β of N2 TANs [29]. TANs with an N1-like phenotype show increased tumor cytotoxicity, expression of intercellular adhesion molecule 1 (ICAM1) and tumor necrosis factor alpha (TNF-α) [29], whereas TANs with an N2-like phenotype promote angiogenesis through the secretion of matrix metallopeptidase 9 (MMP-9), oncostatin M, CXCL8 and BV8/prokineticin-2 [30]. Interestingly, TANs can acquire antigen presenting cell (APC)-like functions and can stimulate intratumoral effector T cells, yet TANs were demonstrated to lose APC-like properties in later stages of tumor progression in patients with NSCLC. TANs are able to suppress T cell function via release of arginase-1 and iNOS (reviewed in [31]). In addition, infiltrated TANs were investigated in gastric cancer patients and were found to express high levels of PD-L1, which was associated with disease progression. This PD-L1 expression was induced by tumor-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) and actively contributed to T cell suppression [32].

1.2. Reversing immune *suppressive* innate immune cells

As already outlined above, IFNs as well as pro-inflammatory cytokines can counteract suppressive innate immune cells,

and are considered critical to obtain effective anti-tumor T cell responses. Interferons (IFNs) represent a large group of cytokines, typically divided among type I (generally IFN-α,β) II (IFN-γ) and III (IFN-λ) [33]. Pro-inflammatory cytokines, excreted from inflammation-promoting immune cells, such as macrophages and helper T cells, include interleukins-1 (IL-1), 6, 8, 12, 18, 21, GM-CSF, and TNF-α. Production of type I IFNs and pro-inflammatory cytokines generally occurs downstream of pattern recognition receptors (PRRs) following their ligation by pathogen or danger-associated molecular patterns (PAMPs or DAMPs). Type I IFNs inhibit the activity of MDSCs, resulting in the conversion of TAMs toward a more M1-like phenotype and promote activation, cross-presentation, and secretion of co-stimulatory molecules by DCs [34]. Also, type I IFNs can reverse N2-like TANs to those with enhanced production of TNF-α and anti-tumor activity [35]. It is noteworthy that during early stages of tumor development, a subset of DCs, expressing the basic leucine zipper transcription factor ATF-like 3 (Batf3) and the surface markers CD103 and CD8α, secrete IFN-β upon encounter with tumor cells [36]. This DC subset is specialized in priming and cross-presentation of antigens to CD8+ T cells, often contributing to a highly effective anti-tumor response [37]. In fact, the absence of gene signatures including type I IFNs and chemokines is associated with loss of accumulation and activation of intratumoral CD8+ T cells [38]. Anti-inflammatory cytokines such as TGF-β and IL-10, often produced by tumor cells and tumor-infiltrating immune cells, are involved in the impairment of DCs and suppression of effector T cells. Delivery of pro-inflammatory cytokines can shift the phenotypes of suppressive cell populations within the tumor. For example, IL-21 effectively converses TAMs into a more M1-like phenotype [39], which renders tumor cells more susceptible for AT. Also, administration of high doses of IL-12 leads to enhanced anti-tumor activity of NK cells in a B16 murine melanoma model [40]. Moreover, IL-12 was found to alter MDSCs and reprogram them to immune-stimulating myeloid cells [41]. Lastly, N2-like TANs were shown to re-express CCL3 and TNF-α following their inhibition, which led to enhanced attraction of innate (monocytes and granulocytes) as well as effector immune cells to the tumor site [29].

2. β-Glucans stimulate innate immune cells

Dietary fibers have been widely studied for their favorable effects on general health and well-being. Most studies have focused on non-starch poly- and oligosaccharides [42,43] and demonstrated beneficial effects in a diversity of diseases, such as Alzheimer, diabetes, inflammatory bowel disease, and cardiovascular disease [44–46]. Non-starch polysaccharides, particularly β-glucans have been demonstrated to become directly exposed to and potentiate innate immune cells in the small intestine through direct exposure to cells [47] or, alternatively, reduce unwanted colonic inflammation following microbiota-mediated fermentation into short-chain fatty acid [48,49]. These fatty acids could function as bioactive compounds and exert a beneficial action on specific intestinal bacteria linked to anti-inflammatory effects. Such immune effects may precede effects on general health (discussed in more detail in Section 3). Notably, humans have always encountered β-glucans either as part of their diet or as pathogens since β-glucans are cell wall components that are abundantly found in plants, yeast, fungi, and bacteria. These bioactive β-glucans consist of D-glucose monomers linked through β-glycosidic bonds with a β-(1→3) configuration and β-(1→6) or β- (1→4) linkages [50]. In Table 1, we have listed β-glucans that are most commonly used in studies of immune modulation, and in Figure 1 we have schematically exemplified the structural characteristics and variation in branching, which are considered critical toward the biological activities of β-glucans.

Immune modulation mediated by β-glucans requires: (1) intestinal uptake; (2) trafficking through the body; and (3) activation of immune cells at distant lymphoid organs. These different steps and necessary cellular interactions are shown in Figure 2. Below we have described these individual steps in more detail.

2.1. β-Glucan sampling from the gut and its biodistribution to lymphoid organs

Orally administered β-glucans arrive in an undigested form in the intestine as humans lack hydrolyzing enzymes. In the intestine, β-glucans are likely captured by intestinal epithelial cells (IECs), mucosal M cells, and/or subsets of CXCR3+ macrophages or CD103+ DCs (reviewed by Batbayer and colleagues [51]). To

Table 1. Source and chemical properties of most commonly reported β-glucans.[a]

	β-glucan	Organism/species	Glycosidic linkages	Mw (kDa)	Solubility in water	Refs.
Algae	Laminarin	*Laminaria digitata*	β-(1➞3, 1➞6)	3.5–7.7	Yes	[114,115]
Bacteria	Curdlan	*Alcaligenes faecali*	β-(1➞3)	53–2000	No	[116]
Fungi	Maitake	*Grifola frondosa*	β-(1➞3, 1➞6)	1000	Yes	[117]
	Lentinan	*Lentinula edodes*	β-(1➞3, 1➞6)	400–1000	Yes (slightly)	[118]
	Pachyman	*Poria cocos*	β-(1➞3, 1➞6)	N.D.	No	
	PGG	*Saccharomyces cerevisiae*	β-(1➞3, 1➞6)	120–205	Yes	[76]
	Pleuran	*Pleurotus ostreatus*	β-(1➞3, 1➞6)	N.D.	No	
	PSK	*Trametes versicolor*	β-(1➞3, 1➞4)	94–100	Yes	[119]
	Schizophyllan	*Schizophyllum commune*	β-(1➞3, 1➞6)	450	Yes	[120]
	Scleroglucan	*Sclerotium rolfsii*	β-(1➞3, 1➞6)	1000	Yes	[114]
	SSG	*Sclerotinia sclerotiorum*	β-(1 ➞ 3)	N.D.	Yes	
	WGP	*Saccharomyces cerevisiae*	β-(1➞3, 1➞6)	N.D.	No	
	Yeast	*Saccharomyces cerevisiae*	β-(1➞3, 1➞6)	35–5000	No	[121]
	Zymosan	*Saccharomyces cerevisiae*	β-(1➞3, 1➞6)	N.D.	No	
Higher plants	Barley	*Hordeum vulgare*	β-(1➞3, 1➞4)	23–137	Yes (slightly)	[116]
	Oat	*Avena sativa*	β-(1➞3, 1➞4)	40–359	Yes	[122]

[a]N.D.: Not determined; PGG: poly-glucopyranosyl-glucopyranose; PSK: polysaccharide-K; SSG: sclerotinan; WGP: whole glucan particles.

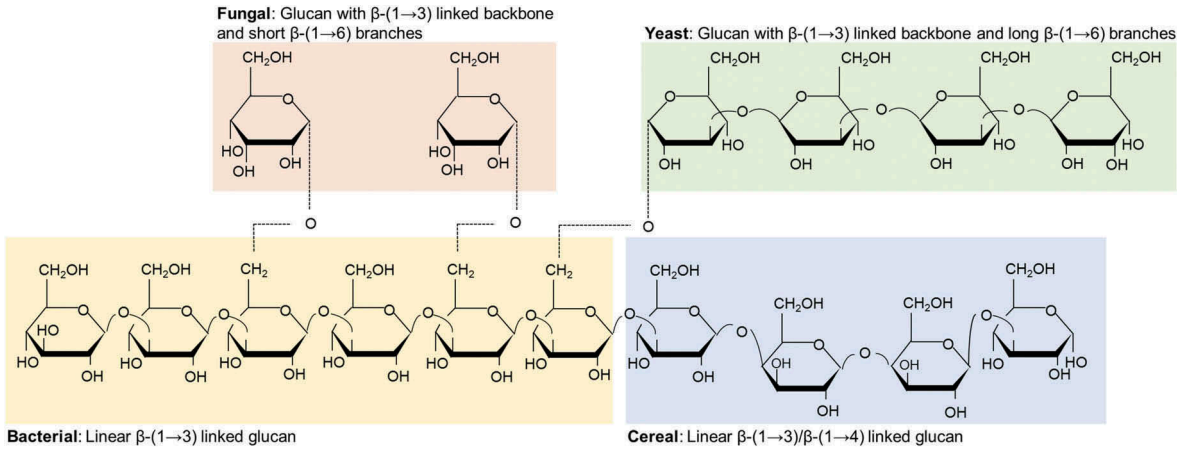

Figure 1. Glucans and their chemical structures. Examples and configurations of β-glucans derived from bacteria, fungi, yeast and cereal.

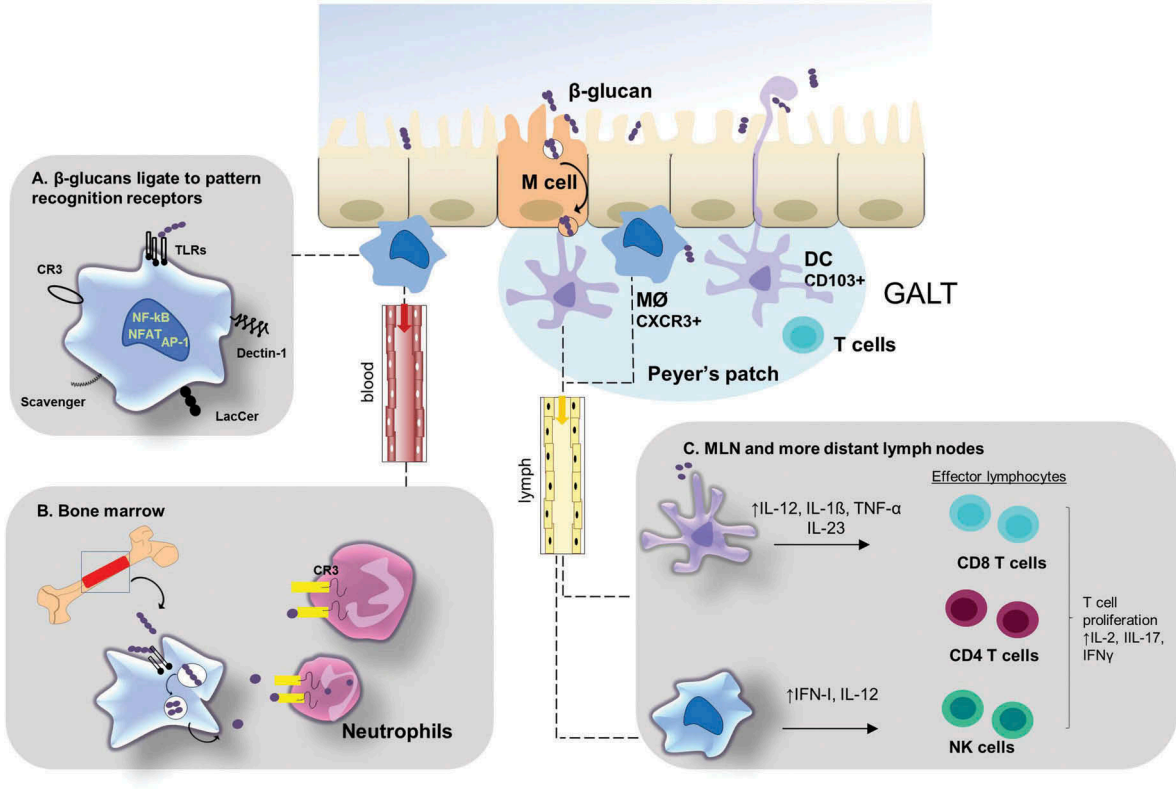

Figure 2. Uptake, trafficking and immune activation of β-glucans. Beta-glucans enter the proximal small intestine, via intestinal epithelial cells or M cells in Peyer's patches, cohere they are captured by CXCR3 macrophages or CD103 DCs. Exposure to β-glucans induces these cells to migrate via the bloodstream to the bone marrow or via the lymph system to mesenteric or more distant lymph nodes. (a) Beta-glucans bind to innate immune cells via PPRs generally resulting in signaling through conserved pathways, and yielding cellular activation. (b) Within bone marrow, degradation products of large β-glucans bind to CR3 on neutrophils resulting in their activation. (c) Within lymphoid organs, DCs and macrophages produce a variety of type-I IFNs and pro-inflammatory cytokines, ultimately culminating in enhanced differentiation of effector lymphocytes. Abbreviations: AP-1: Activator protein 1; CR3: Complement receptor 3; CXCR3: CXC chemokine receptor 3; DC: Dendritic cell; IFN-I: Type I interferons; IFN-γ: Interferon gamma; IL: Interleukin; LacCer: Lactosylceramide; MØ: Macrophage; NFAT: Nuclear factor of activated T-cells; NF-kB: Nuclear Factor Kappa Bèta; TNF-α: Tumor necrosis factor alpha; TLR: Toll-like receptor.

elucidate the exact route of uptake, Rice and colleagues fluorescently labelled and orally administered soluble β-glucans (scleroglucan, glucan phosphate and laminarin) to mice [52]. This study demonstrated that the first step in β-glucan uptake is its internalization by IECs and/or M cells in Peyer's patches. Uptake by IECs appeared to be independent of dectin-1 (a PRR that binds β-glucans, see below), whereas uptake by gut-associated lymphoid tissue (GALT) resulted in increased expression of dectin-1 as well as toll-like receptor 2 (TLR2), suggesting that these two receptors contribute to the uptake of β-glucans by M cells. Interestingly, uptake of β-glucans was accompanied by an increase in systemic IL-6 and IL-12 levels, but not IL-2, IFN-γ, or TNF-α [52]. In extension to this finding, there are multiple reports pointing out that β-glucans result in up-regulated expression of pro-inflammatory cytokines and other mediators, most likely derived from macrophages and DCs (see Table 2).

Table 2. Effects of β-glucans toward immune cells.[a]

Immune cell type[b]	β-glucan	Potentiation of:	Attenuation of:	Refs.
Neutrophils	β-(1→6)-glucan (from *Candida albicans*)[c] PGG[c]	• Gene expression of heat shock proteins • Production of ROS • Phagocytosis • Complement cascade and opsonization by C3b and further degradation in iC3b and C3dg		[123,124]
Macrophages	Barley, Oat, Lentinan[c] Wellmune Soluble, Lentinan[c]	• Gene expression of IL-1β, IL-8 and IL-10 • Gene expression of C-type lectin receptors • Skewing macrophages toward an alternative, M1-like gene expression profile		[125,126]
Dendritic cells	WGP[c] Curdlan[c]	• Gene expression of IL-6, IL-12, IL-2, TNF-α and IFN-γ • Surface expression of CD11c, HLA-DR, CD86 and CD40 • Production of IFN- γ, IL-2 and IL12p40 • Gene expression and protein secretion of IL-1β, IL-6, and IL-23		[127,128]
B lymphocytes	Curdlan, Zymosan[c]	• Production of TNF-α, IL-6 and IL-8	• IgM production and B-cell proliferation	[75]
Myeloid-derived suppressor cells	Curdlan[d]	• Gene expression of IL-12p35 • Surface expression of CD11c, CD40, CD86, MHCII and CD80	• Arginase activity and nitrites in spleen	[113]

[a]iC3b: inactivated C3b; IFN-γ: interferon gamma; IgM: immunoglobulin M; MHCII: major histocompatibility complex II; ROS: reactive oxygen species; TNF-α: tumor necrosis factor alpha.
[b]For immune cell types not listed (such as T lymphocytes), β-glucans were not (yet) reported to induce direct effects
[c]Human primary cells and cell lines
[d]Mouse study

In another study of β-glucan uptake, Hong and colleagues orally administered fluorescently labelled barley and yeast whole glucan particles (WGP) to mice [47]. In this study, β-glucan particles were detected 3 days after intake in splenic and lymph node macrophages, and 4 days after intake in bone marrow macrophages, which suggests migration of intestinal immune cells following exposure to β-glucans. Once captured, macrophages can breakdown β-glucans and secrete soluble fractions of β-glucans that bind and prime complement receptor 3 (CR3) present on macrophages and granulocytes. Fragmented and/or soluble β-glucans, however, might not unequivocally bind nor stimulate the same receptors as full length β-glucans. In fact, small β-glucan particles with a backbone length below seven glucose units cannot bind to dectin-1 [53], and soluble β-glucans cannot initiate clustering of dectin-1 in immunological synapses, and consequently cannot activate this receptor [54]. Another study employing oral administration of soluble β-glucans demonstrated the presence of significant serum levels of β-glucans after 14 days of administration, albeit a small fraction of the total amount of administered β-glucan [55]. This, in addition to the intestinal uptake and migration via innate immune cells, constitutes another route of β-glucan trafficking throughout the body in addition to the intestinal uptake and migration in β-glucan-activated innate immune cells. Collectively, the above studies argue that β-glucans, once having passed the intestinal epithelium, reach distant lymphoid organs via blood or lymph via two non-mutually exclusive routes either making use of subsets of innate immune cells or cell-free transport.

2.2. β-Glucan receptors

When β-glucans reach distant lymphoid structures, or solid tumors for that matter, they activate innate immune cells via ligation of β-glucan-specific PRRs. PPRs consist of two classes of intracellular receptors: RIG-I-like receptors and NOD-like receptors; as well as two classes of plasma membrane receptors: TLRs and C-type lectin-like receptors. Dectin-1 is one of the best characterized PRRs with respect to β-glucan binding which belongs to the class of C-type lectin-like receptors and is reported to bind to zymosan, scleroglucan, schizophyllan, lentinan, curdlan and WGP [53,54,56–59]. Dectin-1 is found on the surfaces of monocytes, macrophages, neutrophils, DCs, and T cells [60]. Studies using synthetic β-glucans revealed that binding to dectin-1 requires a configuration only consisting of β-(1→3) as β-glucans derived from barley with a mixed configuration consisting of β-(1→3, 1→4) are not recognized by dectin-1 [61]. Besides dectin-1 several other PPRs were demonstrated to bind β-glucans. In example, lactosylceramide receptor (LacCer), scavenger receptor, mannose receptor and CR3 were reported to bind extracts from *Pneumocystis carinii*, glucan phosphate, baker's yeast β-glucan, laminarin and zymosan [62–65]. Several studies reported collaborative signalling of dectin-1 in combination with TLRs [66]. In fact, dectin-1 was suggested to collaborate with TLR2 in its binding of curdlan and zymosan [67,68]. Expression patterns of these PRRs are not limited to immune cells, but also include epithelial cells, which suggests IECs can respond to β-glucan intake. It is noteworthy that β-glucan receptors are not limited to the binding of single β-glucans; and vice versa β-glucans are not limited to single receptors, making this ligand:receptor system highly redundant.

PRRs have evolved prior to other immune receptors and, when ligated by β-glucans, mediate signaling through highly conserved intracellular signaling pathways. These pathways consist of the activation of transcription factors, such as nuclear factor kappa-light-chain-enhancer (NF-κB) of activated B cells and interferon regulatory factors (IRFs), which results in production of type I IFNs and pro-inflammatory cytokines. For instance, a type I IFN gene signature was promoted in human DCs that were stimulated with WGP and curdlan [69] as well as in human and murine

macrophage cell lines stimulated with a β-glucan derived from *A. pullulan* [70]. More specifically, the intracellular receptors NOD1 and NOD2 can interact with the inflammasome NLRP3, which then leads to production of IL-1 cytokines [71]. Interestingly, NLRP3 was shown to be essential for curdlan-induced IL-1β secretion in human macrophages, which depended on both dectin-1 and spleen tyrosine kinase (SYK) signaling [72]. Ligation of dectin-1 and CD14 also triggers activation of the transcription factor nuclear factor of activated T-cells in macrophages and DCs (reviewed in [73]), often upstream of the production of pro-inflammatory cytokines. Other examples of β-glucans binding to PRRs and initiating the activation of transcription factors, include a β-glucan isolated from *G. lucidum*, which was reported to ligate TLR4, activate NF-κB, c-Jun N-terminal kinase (JNK) and extracellular-signal-regulated kinase (ERK), and result in expression of co-stimulatory receptors and production of pro-inflammatory cytokines by DC. These β-glucan-matured DCs were proved to be efficient T cell activators in an allogeneic *in vitro* setting [74]. Similarly, curdlan was shown to ligate dectin-1, signal through SYK, ERK, JNK, NF-κB and activator protein 1 (AP-1), and increase the production of TNF-α, IL-6 and IL-8, but not immunoglobulins by B cells [75]. Also, the high molecular weight β-(1→3), (1→6)-glucan from baker's yeast (PGG) was demonstrated to ligate the lactosylceramide receptor, activate NF-κB, and induce an oxidative burst in human neutrophils [76].

In extension to the above reports, other studies investigated whether β-glucan could revert phenotypes of immune suppressive innate immune cells. Along these lines, WGP was shown to inhibit M-MDSC activity by promoting the differentiation of this population into a more mature population through dectin-1 ligation and the activation of the major transcription pathway NF-κB [77]. In addition, zymosan and curdlan enabled conversion of immune-suppressive TAMs into M1-like TAMs with a potent T cell-stimulating activity ([78] and de Graaff *et al*., manuscript in preparation). For neutrophils is has been described that WGP primes these cells in a CR3-dependent manner. Complement activation within the tumor, which occurs when antibodies bind to tumor-associated antigens (TAA), leads to iC3b deposition and triggers phagocyte killing of iC3b-opsonized tumor cells by WGP-primed neutrophils [79].

3. Immune effects of β-glucans in healthy humans and cancer patients

During the last decade, numbers of clinical trials have investigated health benefits of β-glucans. These studies were performed with healthy volunteers, athletes or elderly and, in some cases, assessed immunological effects (see Table 3). Notably, β-glucans have also been tested as an oral adjuvant to cancer patients receiving standard of care therapy. These latter studies mostly focused on β-glucans' ability to reduce adverse effects of standard therapies and improve quality of life (see Table 4).

3.1. β-Glucans to improve well-being and immune activity in healthy subjects

The effects of β-glucans derived from *S. cerevisiae, L. edodus, P. ostreatus, S. uvarum, A. sativa, Agrobacterium spp*. and *H. vulgare* in healthy subjects have been tested in 15 different clinical studies from 2006 onwards. Importantly, no toxic or adverse effects were observed after oral administration of different doses of these β-glucans. Despite inconsistencies between studies regarding design, β-glucan dose (i.e. 50 mg to 10 g/day), duration of intervention (i.e. 4 to 90 days), and analyzed parameters, we have drawn the following general conclusions with respect to health, immunity, and microbiota.

First, health effects have been analyzed using various readouts with two studies reporting on upper respiratory tract infection (URTI) symptoms, one study on cold and flu symptoms, and another study on flow-mediated dilation of conduit artery (a measure used for nitric oxide-dependent endothelial function) [80–83]. These studies revealed a beneficial effect of β-glucan intervention on the mentioned parameters.

Second, immune effects have particularly been investigated with respect to immune effector cells, not innate immune cells, and included both humoral as well as cellular immunity. For example, a 6-week intervention with the β-glucan lentinan resulted in a significant increase in B cell numbers in blood [84]. This increase in B cell numbers matches reported increases in IgA levels in saliva in three other independent studies [80,85,86]. As low levels of IgA associate with increased risk of URTI, the β-glucan-mediated increase in IgA levels might protect against URTI [87]. As mentioned above, one of these studies indeed correlated the increase in IgA levels to a decreased frequency of cold/flu symptoms [80]. Another study reported that increased IgA levels were accompanied by reduced levels of C-reactive protein (CRP) in serum [86]. One may speculate that increased IgA levels might protect against and reduce inflammatory responses, which may be mirrored by reduced CRP levels.

In addition to effects toward humoral immunity, most clinical trials reported on effects toward NK cells, CD4 and CD8 T cells. Interestingly, two studies observed that Immunoglucan and *Agrobacterium* spp. R259 resulted in increased numbers of NK cells in blood and enhanced activity of blood-derived NK cells [83,88]. Furthermore, another study reported that Immunoglucan maintained NK cell activity during a recovery period (following a 20-min intensive exercise at the end of the supplementation period), whereas NK cell activity dropped in the placebo group [89]. Yeast β-glucan was also able to increase circulating fractions of monocytes after a period of exercise [90]. Two other studies described the intake of the 'active hexose correlated compound' (AHCC) in a non-exercise setting and reported increased numbers of CD11c-positive DCs [91] and increased numbers of IFN-γ and TNF-α producing CD4+ and CD8+ T cells in blood [92]. Notably, the latter effect was observed until 30 days after discontinuation of β-glucan intake.

Lastly, a study by Cosola and colleagues reported a decrease in p-cresyl sulfate levels in urine and an increase in short chain fatty acid levels in feces upon oral administration

Table 3. Orally administered ß-glucans in healthy subjects: health and immune effects[a].

ß-glucan (origin)	Study design	Dose; duration	Number of subjects	Health effects	Immune effects[b]	Refs.
SBG (*S. cerevisiae*)	Open-label	100 mg/day, 200 mg/day or 400 mg/day; 4 days	β-glucan: 18	N/A	↑ IgA levels in saliva = TNF-α, IL-6 levels in serum, IL-1β levels in saliva	[85]
Baker's yeast (*S. cerevisiae*)	Crossover, Double blinded, Placebo-controlled trial	250 mg/day; 10 days	β-glucan – Placebo: 30 Placebo – β-glucan: 30	= HR, perceived exertion = core body temperature	↑ Number of $CD14^+/CD16^+$ monocytes immediately post-exercise in blood	[90]
Non-specified β-glucan (*S. cerevisiae*)	Crossover, Randomized, Double-blinded, Placebo-controlled trial	250 mg/day; 10 days	Soluble β-glucan: 74 Insoluble β-glucan: 73 Placebo: 35 β-glucan – Placebo: 60 Placebo – β-glucan: 60	↓ number of cold/flu symptoms	↑ sIgA levels in saliva	[80]
Glucan #300 (*S. cerevisiae*)	Randomized, Open-label	1,000 mg/day; 7 days	β-glucan: 10 Placebo: 5	N/A	= TNF-α, IL-6, IL-10, IL-1β, IL-17, IL-22, IFN-γ production by *ex vivo* stimulated PBMCs	[129]
WGP (*S. cerevisiae*)	Randomized, Double-blinded, Placebo- controlled	250 mg/day; 90 days	β-glucan: 48 Placebo: 49	↓ number of days with upper respiratory tract infection symptoms	↓ MCP-1 levels in blood during upper respiratory tract infections = IL-1β, IL-8, MIP-1β, MIP-1α, G-CSF, MIG levels in plasma	[81]
Mixture of α- and β-glucan AHCC fermented product (*L.edodes*)	Open-label	3000 mg/day; 60 days	β-glucan: 30	N/A	↑ IFN-γ and TNF-α production by *ex vivo* stimulated blood $CD4^+$ and $CD8^+$ T cells	[92]
Lentinex® (*L. edodes*)	Crossover, Double blinded, Placebo- controlled	2.5 mg/day; 42 days	β-glucan – Placebo: 17 Placebo – β-glucan: 16	= diastolic or systolic blood pressure, HR	↑ number of B-cells in blood = number of NK cells in blood = C3, C4, IgG, IgA, IgM levels in blood = IL-8, IL-10, IL-12, CRP, TNF-α levels in blood	[84]
Non-specified β-glucan (*L. edodes*)	Randomized, Open-label	5000 mg or 10,000 mg/day; 4 weeks	β-glucan: 52	N/A	↑ sIgA levels in saliva ↓ CRP levels in serum	[86]
Mixture of α- and β-glucan AHCC fermented product (*L. edodes*)	Randomized, Double-blinded, Placebo-controlled	3000 mg/day; 4 weeks	β-glucan: 10 Placebo: 11	N/A	↑ number of $CD11c^+$ DCs in PBMCs = IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α production by *ex vivo* stimulated blood	[91]
Immunoglucan ® (*P. ostreatus*)	Randomized, Double blinded, Placebo-ontrolled	200 mg/day; 90 days	β-glucan: 25 Placebo: 25	↓ incidence of upper respiratory tract infection symptoms	↑ number of NK cells in blood	[83]
Immunoglucan ® (*P. ostreatus*)	Randomized, Double-blinded, Placebo-controlled	100 mg/day, 60 days	β-glucan: 9 Placebo: 11	N/A	↓ NK cell activity by *ex vivo* stimulated PBMCs in placebo group = number of monocytes, granulocytes in blood	[89]
CM-G (*S. uvarum*)	Randomized, Placebo-controlled	50 mg/day; 60 days	β-glucan: 26 Placebo: 26	= HDL, LDL, cholesterol levels in blood	= number of leukocytes, monocytes, lymphocytes in blood ↓ malondialdehyde levels in plasma	[130]
Non-specified β-glucan (*Agrobacterium* sp. R259)	Randomized, Double blinded, Placebo-controlled	700 mg/day; 56 days	β-glucan: 40 Placebo: 37	= cholesterol, glucose, calcium, creatine levels in blood = pulse rate	↑ NK activity by *ex vivo* stimulated PBMCs and IL-10 levels in serum = IFN-γ, IL-2, IL-4, IL-6, IL-12, TNF-α and IgG levels in serum	[88]
Oatvantage β-glucan (*A. sativa*)	Randomized, Double-blinded, Placebo-controlled	5600 mg/day; 18 days	β-glucan: 19 Placebo: 17	= upper respiratory tract infection symptoms	= NK cell activity by *ex vivo* stimulated PBMCs = IL-1ra, IL-6, IL-8 and IL-10 levels in plasma = IL-8, IL-1ra gene expression in blood	[131]
Non-specified β-glucan (*H. vulgare*)	Open-label	3000 mg/100g pasta; 60 days	β-glucan: 26	↑ brachial artery diameter (flow-mediated dilation) ↓ p-Cresyl sulfate levels in serum = Indoxyl sulfate levels in serum ↓ LDL and cholesterol levels in blood	N/A	[82]

[a] AHCC: active hexose correlated compound; CM-G: carboxymethyl-Glucan; CRP: C-reactive protein; DC: dendritic cell; G-CSF: granulocyte-colony stimulating factor; HDL: high-density lipoproteins; HR: heart rate; IgA: immunoglobulin A; LDL: low-density lipoproteins; MCP-1: monocyte chemoattractant protein-1; MIG: monokine induced by gamma interferon; MIP: macrophage inflammatory proteins; N/A: Not applicable; NK cell: natural killer cell; PBMCs: peripheral blood mononuclear cells; SIgA: secretory Immunoglobulin A; TNF-α: tumor necrosis factor alpha; WGP: whole glucan particle.

[b] Symbols: ↑: increase in β-glucan group; ↓: decrease in β-glucan group; = : no change in β-glucan group.

Table 4. Orally administered ß-glucans in cancer patients: health and immune effects[a].

ß-glucan (origin)	Type of cancer	Study design	Dose; duration	Number of subjects	Co-treatment	Health effects	Immune effects[b]	Refs.
Imuneks® (*S. cerevisiae*)	Invasive ductal and lobular breast carcinoma	Open-label	20 mg/day; 14 days	β-glucan: 23	No	N/A	↑ number of monocytes in blood ↑ frequency of $CD95^+$ within $CD14^+$ monocytes In blood ↑ frequency of $CD45RA^+$ within $CD14^+$ monocytes in blood	[101]
Imuneks® (*S. cerevisiae*)	Breast cancer	Randomized, Double-blinded, Placebo-controlled	20 mg/day; 21 days	β-glucan: 15 Placebo: 15	No	N/A	↓ IL-4 levels in serum ↑ IL-12 levels in serum = number of neutrophils, lymphocytes and monocytes in blood	[102]
Carboxymethyl-glucan (*S. cerevisiae*)	Prostate adenocarcinoma	Open-label	20 mg/day; 28 days	β-glucan: 30	Androgen deprivation therapy with goserelin acetate or leuprolide	↑ number of red blood cells and hematocrit ↑ hemoglobin levels and platelet counts in blood	↑ total number of leukocytes in blood	[100]
WGP (*S. cerevisiae*)	Non-small-cell lung carcinoma	Open-label	500 mg/day; 10–14 days	β-glucan: 43	No	N/A	↓ frequency of CD14−HLA-DR −$CD11b^+CD33^+$ MDSC in blood	[98]
LEM (*L. edodes*)	Breast cancer	Open-label	1,800 mg/day;21 days	β-glucan: 10	• 5-fluorouracil • epirubicin, • cyclophosphamide	= quality of life	= number of leukocytes and lymphocytes in blood	[95]
LEM (*L. edodes*)	Gastric and colorectal cancer	Open-label	1,800 mg/day; 28 days	β-glucan: 8	• 5-fluorouracil • Irinotecan • uracil and tegafur • levofolinate • mitomycin • taxol	↓ adverse effects of chemotherapy (nausea and abdominal pain)	↑ IFN-γ production by *ex vivo* stimulated blood-derived $CD4^+$, $CD8^+$ T and $CD56^+$ NK/NKT cells	[99]
LEM (*L. edodes*)	Breast cancer	Open-label	1,800 mg/day; 56 days	β-glucan: 20	• tamoxifen citrate • anastrozole • letrozole • toremifene citrate	↑ quality of life	= IFN-γ and IL-10 production by *ex vivo* stimulated blood	[96]
Superfine lentinan (*L. edodes*)	Colorectal cancer	Multicentre, Open-label	15 mg/day; 84 days	β-glucan: 71	• 5-fluorouracil • tegafur and uracil • tegafur • gimeracil and oteraci • doxifluoridine, • levofolinate, • irinotecan • gemcitabine • cisplatin	↑ quality of life	↑ binding ability of blood-derived $CD14^+$ monocytes to lentinan in improved status patients	[97]
Non-specified β-glucan	Colorectal cancer	Randomized, Double-blinded, Placebo-controlled	50 mg/day; 7 days	β-glucan; 31 Placebo: 31	• 5-fluorouracil	↓ adverse effects of chemotherapy (oral mucositis and diarrh ea)	= number of leukocytes, neutrophils in blood	[94]

[a]LEM: lentinula edodes mycelia extract; MDSC: myeloid-derived suppressor cells; N/A: Not applicable; NK: natural killer; NKT cells: natural killer T cells; WBM: white bottom mushroom; WGP: whole glucan particles.
[b] Symbols: ↑: increase in β-glucan group; ↓: decrease in β-glucan group; = : no change in β-glucan group.

of a β-glucan derived from *H. vulgare*, suggesting a saccharolytic shift in gut microbiota metabolism [82]. Zitvogel and colleagues demonstrated a dominance of distinct commensal species in patients who showed a clinical response toward PD-1 checkpoint inhibitors, making the observed effect of β-glucans on the microbiome highly relevant in the context of T cell therapies [93].

In general, these studies reported increased frequencies, but not activity, of various immune cell types which suggests enhanced immune-mediated alertness toward protrusion of homeostasis.

3.2. β-Glucans as adjuvants to anti-cancer therapy

β-glucans have been applied to cancer patients receiving standard treatments, such as chemotherapy, radiotherapy, monoclonal antibody or hormonal therapy. Of the 11 studies that have been performed so far, 10 studies have properly documented the name and origin of the β-glucan; in 6 studies cancer patients received *S. cerevisiae* (yeast) and in 4 studies patients received *L. edodes* (shiitake) (included in Table 4). In general terms, the use of β-glucans demonstrated a reduction of adverse effects of chemotherapy such as oral mucositis and diarrhea and an improvement of quality of life [94–97]. These effects appeared not related to a specific type of cancer or β glucan per se as observations included patients with colorectal, gynecological or breast cancer who received chemotherapy and were administered *L. edodes, S. cerevisiae* or *A. blazei*.

Numbers of studies report changes in immunological parameters. For instance, a study by Albeituni and colleagues reported on administration of WGP in NSCLC patients which resulted in decreased frequencies of MDSCs in blood (Table 4) [98]. Oral ingestion of *Lentinus edodes* mycelia extract (LEM) in patients with gastrointestinal cancer was accompanied by an increased frequency and activity of NK cells in blood [99]. The binding of lentinan, the β-glucan found in Lentinus edodes and in LEM, to CD14+ monocytes appeared to correlate with an improved quality of life as observed in patients with colorectal cancer [97]. Furthermore, an increase in total leukocyte count was found in patients with prostate adenocarcinoma who received carboxymethyl-glucan [100], and an increase in monocyte count was observed in breast cancer patients who received Imuneks β-glucan [101]. Besides changes in frequencies of immune cells, Imuneks also resulted in decreased levels of IL-4 and increased levels of IL-12 in serum from breast cancer patients, measured during two courses of chemotherapy [102].

Along these lines, it is noteworthy that a phase II clinical trial is currently being performed in which PGG is applied as adjuvant to Pembrolizumab, a humanized mAb against PD-1, in patients with advanced melanoma or triple negative breast cancer [103].

Taken together, it appears that when homeostasis becomes protruded (*only then*), β-glucans are expected to support a pro-inflammatory immune response, which according to *in vitro* and *in vivo* studies often culminates in a Th1-type T cell response. Although most studies focused on quality of life rather than tumor growth, we anticipate that the observed immune modulatory effects of β-glucans can support anti-tumor responses.

4. Supportive effects of β-glucans toward adoptive T cell therapy in mouse tumor models

Besides cancer patients, interventions with β-glucans have been analyzed in more detail for their effect on tumor growth

Open questions

- Do β-glucan-activated macrophages or DCs, or β-glucans themselves, migrate to tumour tissue to locally enhance T cell immunity; or does such T cell activation take place in tumour-draining lymph nodes?
- What is the receptor usage and initial signalling of different β-glucans?
- What attributes of innate immune cells, particularly in relation to T cell activation, are most effected by different β-glucans?
- Do β-glucans alter the composition of the gut microbiome and how does this affect adoptive T cell therapy?
- What treatment schedules are most optimal to combine β-glucans (and which ones) with adoptively T cell therapy?

in various mouse models. Oral administration of different β-glucans was tested in the following tumor models: mammary carcinoma; B cell lymphoma; lung carcinoma; breast adenocarcinoma; melanoma; and colon carcinoma, and in some of these models β-glucans were combined with chemotherapy, vaccination, proteins or antibody treatment; listed in detail in Table 5.

In mouse models β-glucans have been administered in curative and preventive settings. In one study, a direct comparison between a curative and preventive setting has been performed using a model of BALB/c mice with subcutaneous inoculated CT26 colon-carcinoma cells [104]. In this model, addition of LEM did not result in reduced growth of an already established tumor, but addition of this extract 1 week prior to tumor inoculation did significantly reduce tumor growth. In most studies, β-glucan interventions delayed both the onset of tumor growth [105] as well as the progression of already established tumors (all studies monitored tumor growth, except for one study that looked at cecum weight in colon carcinoma [106], and another study that looked at melanoma mass [107]). Notably, two studies documented in their materials and methods two transplanted cell lines but only reported the immune effects toward one of these cell line [108,109].

Various mice studies also investigated the effects of β-glucan intake on subsets of immune cells within TME, blood or other organs. There were 7 out of 16 studies that reported increased IFN-γ production following *ex-vivo* stimulation of either blood-derived T cells, splenocytes, cells from tumor or immune tissues, following uptake of β-glucans [106–108,110–113]. In the study where cells from GALT showed increased IFN-γ production following *ex-vivo* stimulation, authors argued that administered WGP β-glucans induced IL-12 and TNF-α production by DCs and stimulated DC migration into the tumor, which resulted in local expansion of T cells [108]. In another study, mice received T cells transgenic for an OVA-specific TCR (used as a model antigen), and demonstrated that the simultaneous addition of WGP resulted in an enhanced anti-tumor responses against established Lewis Lung Carcinoma (LLC) transfected with OVA [108]. Detailed analysis revealed that administration of WGP significantly increased the frequency of memory T cells in spleens (Table 5). Furthermore, two tumor-bearing mice models (lung carcinoma and breast adenocarcinoma) revealed that WGP administration led to conversion of M-MDSC to immune-potentiating APCs ($CD11c^+$ DC) [98], similar as observed in the human setting [98]. In these studies [98], APCs were shown to cross-present antigen and to prime CD8 T cells directed to OVA. Li and colleagues tested whether orally administered WGP to mice with established lung carcinoma and lymphoma would directly affect APCs [108], and observed significantly increased numbers of tumor-infiltrating DCs and macrophages upon WGP treatment. Moreover, expression of the co-stimulatory molecules CD80, CD86 and MHC class II was significantly upregulated by CD8α+ CD11c+ DCs in spleens that had captured apoptotic tumor cells in mice treated with WGP. These findings were linked to a stronger local expansion of antigen-specific CD4+ and CD8+ T cells, and likewise enhanced IFN-γ production by TILs. Despite these immunological analyses, it remains unclear how WGP affects the migration of DCs into the tumor tissue, and authors speculated that particulate WGP may in fact mobilize DC precursors from the bone marrow.

Ishikawa and colleagues investigated the effects of β-glucan on age-associated attenuation of immune competence, which paradoxically results from age-associated inflammation. Aged individuals demonstrated increased IL-6 and TNF-α levels in serum and early accumulation of MDSCs into tumor sites. The latter appeared to relate to the increased IL-6 levels which inhibited Th1 responses and increased numbers of MDSC. In this study an oral intervention with LEM retarded tumor growth of CT26 colon carcinoma in aged mice, which correlated with reduced IL-6 serum levels. Authors also demonstrated that neutralizing serum TNFα suppressed the induction of anti-tumor T cells, whereas neutralizing serum IL-6 augmented the induction of these cells. Collectively, these results suggest that LEM intervention specifically modulates the inflammatory immune responses from an anti-tumor effect toward a Th1-type T cell response [104].

5. Conclusion

There is an exponential growth in the number of AT studies for the treatment of different tumors. Despite clinical successes, there is however still a large number of patients that does not show durable response, which has been attributed in large part to T cell evasive mechanisms of tumors. To re-establish anti-tumor T cell responses, it is necessary to reverse immune-suppressive immune populations such as MDSCs, TAMs and TANs, that have co-evolved with tumors and helped sculpting an immune tolerant micro-environment, into more immune-potentiating APCs via induced expression of pro-inflammatory cytokines and type I IFNs. In this review, we argue that β-glucan fibers, acting as PAMPS or DAMPs and found in cell walls of cereals, plants, fungi and bacteria, activate innate immune cells, which can result in an immunologically favorable conversion of the TME, and sensitize tumors for enhanced T cell entry and activity. Along this hypothesis we have provided a comprehensive overview of the immune modulatory capacity of orally applied β-glucans in healthy humans and cancer patients and have delineated how these capacities can be exploited to support the safety and efficacy of AT. First, β-glucans reduce chemotherapy-related adverse effects and enhance quality of life. Second, β-glucans enhance blood frequencies and activities of APCs, such as monocytes and neutrophils, as well as effector lymphocytes, such as NK cells, which is mostly accompanied by reduced frequencies of MDSCs. In extension to these findings in humans, mice studies demonstrated that β-glucans delay outgrowth of the tumor, which again occurs hand in hand with effects toward APCs and enhanced numbers and activities of NK cells and CD4+ and CD8+ T cell within tumors. It is noteworthy that the clinical use of β-glucans is safe, can easily be implemented at low additional costs, and would support self-assertiveness and self-awareness amongst cancer patients.

6. Expert opinion

Development of AT during the past decades has resulted in clinical objective response rates up to 80%, with complete

Table 5. Orally administered β-glucans in cancer-bearing mice: anti-tumor and immune effects.[a]

ß-glucan (origin)	Tumor model	Co-treatment	Dose; start; duration	Anti-tumor effects	Immune effects[b]	Refs.
WGP (*S. cerevisiae*)	Mouse strain: Balb/c Transplanted cell lines: Ptas-64, mammary tumor (s.c.)	BAFF Receptor antibody [11C1]	Dose: 400 µg/day Start: day 9 after tumor inoculation Duration: 14 days	Stabilization	↑ IFN-γ levels in blood ↑ IL-4 levels in blood from tumor-bearing mice not receiving β-glucan-enhanced therapy	[110]
WGP (*S. cerevisiae*)	Mouse strain: C57Bl/6 Transplanted cell lines: LLC, lung tumor or EO771, breast tumor (s.c.)	No	Dose: 800 µg/day Start: day 8 after tumor inoculation Duration: 21 days	Delay	↑ gene expression of IL-12, TNF-α, IL-6 in TAMs (LLC and EO771) = gene expression of iNOS, IL-1β, IL-10 in TAMs (LLC and EO771) ↓ gene expression of Arginase in TAMs (LLC)	[78]
WGP (*S. cerevisiae*)	Mouse strain: OT-I and OT-II transgenic C57BL/6 mice Transplanted cell lines: LLC/OVA, lung tumor or RMA-S-MUC1, lymphoma (s.c.)	No	Dose: 100, 200, 400, 800 µg/day Start: day 11 after tumor inoculation or day −7 before tumor inoculation Duration: 21 days	Delay	↑ IFN-γ production by *ex vivo* stimulated splenic $CD8^+$ T cells (LLC) ↑ IFN-γ production by *ex vivo* stimulated $CD8^+$ and $CD4^+$ TILs (LLC) ↑ number of intratumoral DCs and macrophages (LLC) ↑ frequency of CD86 and MHC-II within $CD11c^+$ DCs in tumor (LLC)	[108]
WGP (*S. cerevisiae*)	Mouse strain: OT-I and OT-II transgenic RAG12/2 C57BL/6 mice and dectin-1 knock-out mice Transplanted cell lines: LLC, lung tumor or EO771, breast tumor (s.c.)	No	Dose: 800 µg/day Start: day 8 after tumor inoculation Duration: 24 or 27 days	Delay	↓ frequency of PMN-MDSC within spleens (EO771 and LLC) ↑ frequency of M-MDSC within tumors in EO771 model ↓ frequency of PMN-MDSC within tumors in EO771 model	[98]
WGP (*S. cerevisiae*)	Mouse strain: C57BL/6 Transplanted cell lines: LLC, lung tumor (s.c.)	No	Dose: 800 µg/day Start: day 8 after tumor inoculation Duration: 14 days	Delay	↓ frequency of $Gr\text{-}1^+CD11b^+$ MDSCs within spleen and tumor ↑ frequency of $F4/80^+$ macrophages within draining lymph nodes and tumor ↑ frequency of $CD11c^+$ DCs within draining lymph nodes and tumor	[77]
WGP (*S. cerevisiae*)	Mouse strain: C3-deficient, CR3/CD11b deficient mice and Dectin-1 knock-out C57BL/6 mice Transplanted cell lines: EO771/OVA, breast tumor (s.c.)	No	Dose: 800 µg/day Start: day 8 after tumor inoculation Duration:14 days	Delay	↑ frequency of macrophages and $CD8\alpha^+CD11c+$ DC within the tumor ↑ number of IFN-γ-producing $CD8^+$ TILs	[111]
WGP (*S. cerevisiae*)	Mouse strain: C57BL/6 Transplanted cell lines: LLC, lung tumor (s.c.)	Glucocorticoid-induced TNF receptor ligand	Dose: 800 µg every 2 days Start: day −7 before tumor inoculation Duration: 28 days	Delay	↑ frequency of $CD11c^+$ DCs within draining lymph node and tumor ↓ frequency of $CD4^+CD25^+Foxp3^+$ T cells in tumor ↑ Production of IFN-γ by *ex vivo* stimulation of $CD8^+$ T cells from tumors, draining lymph node and spleen	[132]
Soluble β-glucan (*S. cerevisiae*)	Mouse strain: Balb/c Transplanted cell lines: A20, B cell lymphoma (i.p.)	Vaccines: Survivin peptides Survivin protein	Dose:400 µg/day Start: day −7 before tumor inoculation Duration: 37 days	Delay	↑ Production of IFN-γ and IL-4 by *ex vivo* stimulated $CD8^+$ and $CD4^+$ splenic T cells, respectively ↑ number of macrophages and matured DCs in spleen	[133]
Soluble ß-glucan (*S. cerevisiae*)	Mouse strain: Balb/c Transplanted cell lines: A20, B cell lymphoma (s.c.)	Cyclophosphamide	Dose: 400 µg/day Start: day 9 after tumor inoculation Duration: 5 days	Delay	N/A	[105]
LEM (*L. edodes*)	Mouse strain: Balb/c and Balb/c nude Transplanted cell lines:C26, colon carcinoma (i.c.)	No	Dose: 1%, 2% extract (w/w; freely fed) Start: day 3 after tumor inoculation Duration: 11 days	N/A	↑ IFN-γ production by *ex vivo* stimulated T cells from mesenteric lymph node and spleen ↓ TGF-β production by *ex vivo* stimulated of $CD4^+$ and $CD8^+$ T cells from mesenteric lymph node ↓ TGF-β levels in plasma	[106]
LEM (*L. edodes*)	Mouse strain: C57BL/6 Transplanted cell lines: B16, melanoma (s.c.)	Vaccine: tyrosinase-related protein2$_{180–188}$ peptide	Dose: 2% extract (w/w; freely fed) Start: day 1 after tumor inoculation Duration: 20 days	Delay	↓ frequency of $Foxp3^+$ $CD4^+$ within tumor draining lymph nodes and spleen ↑ IFN-γ production by *ex vivo* stimulated T cells from lymph nodes and	[112]

(*Continued*)

Table 5. (Continued).

ß-glucan (origin)	Tumor model	Co-treatment	Dose; start; duration	Anti-tumor effects	Immune effects[b]	Refs.
LEM (*L. edodes*)	Mouse strain: Balb/c and BALB/c nude Transplanted cell lines: CT26, colon carcinoma (s.c.)	No	Dose: 50 mg or 75 mg/day Start: day −7 before tumor inoculation Duration: 14 days	Delay	↓ IL-6 levels in serum ↑ number of monocytic and granulocytic MDSCs intratumoral	[104]
LEM (*L. edodes*)	Mouse strain: C57BL/6 H-2^{b} and Balb/c nude Transplanted cell lines: B16, melanoma (s.c.)	Vaccine: Tyrosine-related protein $2_{181-188}$ peptide (C57BL/6)	Dose: 1%, 2% extract (w/w; freely fed) Start: day 1 after tumor inoculation Duration: 20 days	Delay	↓ number of Foxp3^{+} CD4^{+} T cells within draining lymph nodes and spleen ↓ TFG-β levels in plasma	[134]
Maitake D (MD)-Fraction (*G. frondosa*)	Mouse strain: BALB/c, Balb/c nude and C3H/HeJ Transplanted cell lines: C26, colon carcinoma or MM-46, mammary tumor (s.c.)	No	Dose: 5, 20, 80 mg/kg once every 19 days Start: 1 day after tumor inoculation Duration: 1 day	Delay	↑ gene expression of IL-12, IFN-γ in MLN, spleen and tumor (c26) ↑ number of IFN-γ expressing CD4^{+} and CD8^{+} T cells within spleen and tumor (c26) ↑ number of IFN-γ expressing CD4^{+} T cells in MLN and spleen (c26) ↑ gene expression of CXCL9 and CXCL10 in tumor (c26)	[109]
HMBG and LMBG (*Aureobasidium sp.*)	Mouse strain: C57BL6 Transplanted cell lines: B16, melanoma (s.c.)	No	Dose: 2.5 mg/day Start: day −7 before tumor inoculation Duration: 7 days	N/A	↑ IFN-γ and IL-2 production by *ex vivo* stimulated spleen cells ↑ number of CD4^{+} and CD8^{+} T cells in spleen	[107]
Curdlan (*A. faecalis*)	Mouse strain: C57/BL6 Transplanted cell lines: LLC, lung tumor (s.c.)	No	Dose: 800 µg every 2 days Start: day −7 before tumor inoculation Duration: 28 days	Delay	↑ frequency of CD11b^{+}Gr-1^{+} MDSCs within spleen ↓ arginase levels in serum ↑ frequency of IFN-γ expressing CD4^{+} and CD8^{+} T cells within spleen and dLN	[113]

[a]BAFF: B Cell Activating Factor; CXCL9: chemokine (C-X-C motif) ligand 9; Cy: cyclophosphamide; DC: dendritic cell; dLN: draining lymph node; DTX: docetaxel; HMBG: high molecular weight β-glucan; IFN-γ: interferon gamma; IL: Interleukin; iNOS: inducible nitric oxide synthase; i.p.: intraperitoneal; LEM: lentinus edodes mycelium; LLC: Lewis lung carcinoma; LMBG: low molecular weight β-glucan; MHC-II: major histocompatibility complex II; M-MDSC: monocytic myeloid-derived suppressor cell; Muc-1: mucin-1; OVA: ovalbumin; PMN-MDSC: polymorphonuclear myeloid-derived suppressor cell; s.c.: subcutaneous; TAM: tumor associated macrophages; TILs: tumor-infiltrating lymphocytes; TGF-β: transforming growth factor beta; TNF-α: tumor necrosis factor alpha; WGP: whole glucan particles.

[b] Symbols: ↑: increase in β-glucan group; ↓: decrease in β-glucan group; = : no change in β-glucan group.

response rates plateauing at 20%. One of the major current challenges of this therapy is to improve the durability of anti-tumor T cell responses. In the current contribution, we postulate that oral administration of β-glucans represents an adjuvant treatment to augment quantity and quality of intratumoral effector T cells, thereby supporting AT therapies.

Beta-glucans consist of polymeric D-glucose monomers with a backbone generally consisting of β-(1→3) bonds, and branched via β-(1→4) or β-(1→6) links. Beta-glucans are stable compounds found in cell walls of plants and micro-organisms that resist passage through the digestive tract. Figure 2 summarizes studies, often using fluorescently labelled β-glucans, that investigated intestinal uptake via epithelial cells and M cells, triggering of local CXCR3+ macrophages and CD103+ DCs, and activating effector NK and T cells in more distant lymphoid organs. Beta-glucans trigger innate immune cells via binding to PRRs, such as dectin-1, initiate type I IFNs and pro-inflammatory signaling cascades, and mediate the acquisition of T cell-recruiting and stimulating phenotypes.

The combination treatment of β-glucans and adoptive transfer of TCR-engineered T cells is illustrated in Figure 3. Along the lines of data put forward in Sections 2–4, we argue that β-glucans, such as WGP and LEM facilitate the change of an immune-tolerant tumor (Figure 3(a)) into one that is more immune-responsive. This conversion may be governed by increased numbers of antigen-presenting cells and an enhanced inflammatory state, thereby sensitizing the tumor for T cell entrance and activation (Figure 3(b)). Next, AT clearly increases the number of tumor-specific T cells that, because of the preceding β-glucan effects, are easily recruited into and activated within the tumor, where they can take part in an effective anti-tumor response (Figure 3(c)).

Preclinical studies with mice and clinical studies with healthy subjects and cancer patients performed to date clearly indicate that β-glucans potentiate innate immune cells and enhance accumulation and activity of intratumoral effector immune cells. Curdlan for example promotes the differentiation of MDSCs into a more mature state, which results in a reduced suppressive function [113]. In addition, orally administered WGP modulates DCs, leading to expansion and increased IFN-γ production of antigen-specific CD4+ and CD8+ T cells within tumors [108]. Skewing suppressive immune populations into APCs with enhanced production of type I IFNs and pro-inflammatory cytokines and chemo-

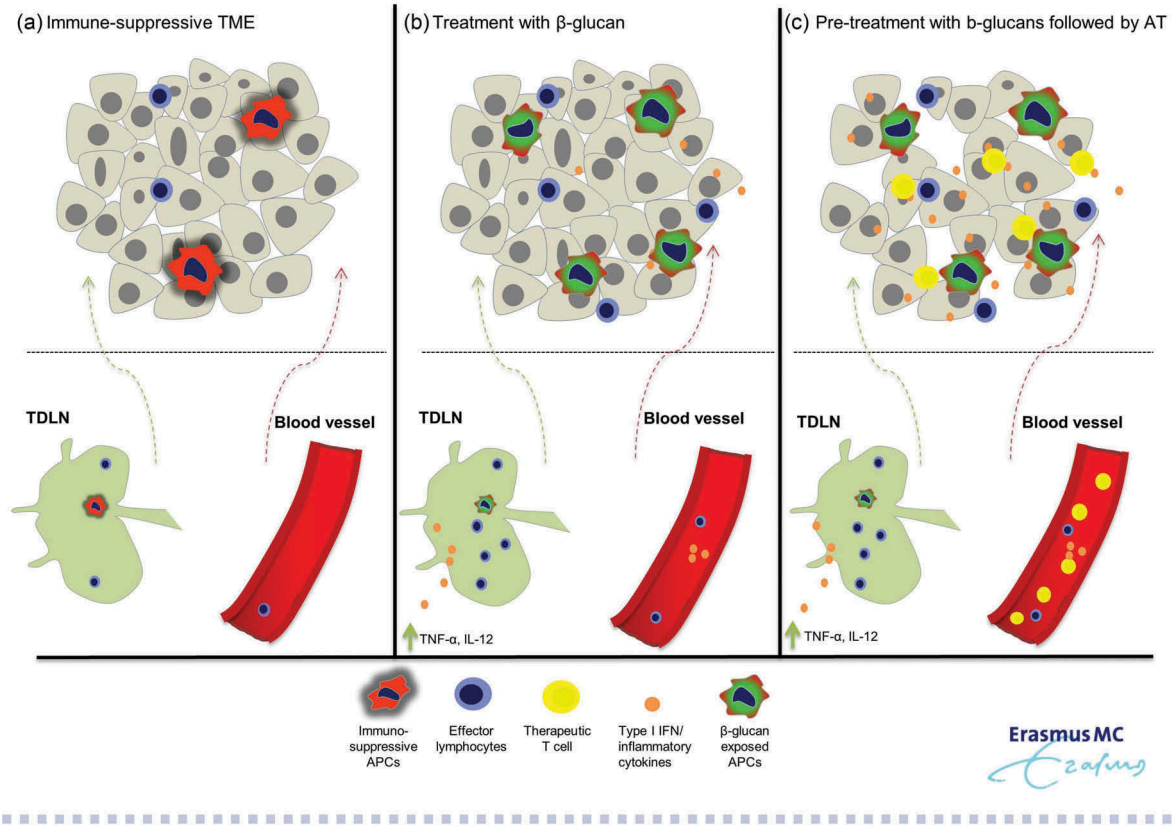

Figure 3. Beta-glucans support AT of solid tumors: proposed mechanisms of action. (a) Pre-treatment tumors harbor suppressive innate APCs like M2-TAMs, MDSCs and N2-TANs that actively suppress recruitment into the tumor and local activation of effector lymphocytes, such as NK cells and CD8+ T cells. (b) Oral administration of β-glucans results in tumors with increased numbers of immune-potentiating innate immune cells, as evidenced by their production of type I IFNs and inflammatory cytokines. These APCs are derived from TDLN or nearby blood vessels, toward which they migrated following exposure to β-glucans. Alternatively, these cells are converted from intratumoral suppressive innate immune cells due to a heightened inflammatory state of the TME or β-glucans that have reached the tumor tissue. Consequently, also the number and activation state of effector lymphocytes within the tumor increases. (c) Oral administration of β-glucans followed by AT results in an enhanced pool of therapeutic T cells (harboring a TCR transgene that recognizes a tumor antigen) in the bloodstream. These therapeutic T cells are recruited into the tumor, which has become sensitized by β-glucan treatment (as in panel B), and eradicate malignant cells. See text for more details. Abbreviations: APCs: antigen-presenting cells; AT: adoptive T cell therapy; IL: interleukin; TDLN: tumor-draining lymph node, TNF-α: tumor necrosis factor alpha.

attractants facilitates priming and differentiation of effector lymphocytes, such as NK and Th1 cells, thereby augmenting antitumor immune responses. Since tumors may escape recognition by CD8+ T cells via deficiencies in antigen processing and presentation, β-glucan-induced NK cell activity (besides effects toward T cells themselves) may further support AT.

Future studies are required to reveal whether β-glucans are transported to the TME and affect populations such as MDSCs, TANs and TAMs directly, or whether they end up in tumor-draining lymph nodes, where they trigger innate immune cells and induce immune cells to migrate toward the tumor site. Furthermore, it is worthwhile to assess whether immune-potentiating effects of β-glucans are mediated by the microbiome; not trivial since it has recently been demonstrated that distinct commensal bacterial species are related to clinical response toward PD-1 checkpoint inhibitors [93].

It is noteworthy that clinical trials are mainly performed with dietary insoluble particulate β-glucans derived from yeast (*S. cerevisiae*) and fungi (*L. edodus*) that consist of a β-(1→3) linked backbone with β-(1→6) linked side chains. As reviewed by Stier and colleagues, β-glucans derived from yeast and from fungi are known for their immune modulating effects [61] (Tables 3–5), making these β-glucans promising candidates to support AT. Also, in tumor mouse models, both WGP and LEM cause delayed tumor growth, which in some studies is accompanied by enhanced conversion of non-responsive TILs toward Th1 responses as well as anti-tumor T cell activity. When combining β-glucans with AT, this should be done in a manner which maximizes the persistency of CD8+ TCR T cells. To this end, when assessing the most optimal combination schedule, T cell numbers should be monitored in blood and tumor, and correlated with tumor growth as well as the immune composition of tumor tissues. Most studies observed stable tumor growth and ended their study after a fixed number of weeks; yet it is recommended to monitor immune parameters over longer time periods following administration of β-glucans.

One aspect that needs to be addressed to push the field forward is a lack of clear uniformity with respect to the annotation of these fibers as well as their source. In fact, currently there exists a large variation in primary chemical structures and molecular masses of β-glucans, which mostly depends on differences in extraction and preparation procedures, making comparisons between studies unnecessarily difficult. In addition, the relationship between configurations and bioactivity of β-glucans should be further studied to design or purify new β-glucans with higher bioactivities.

In short, β-glucans have demonstrated ability and impact with regards to reversion of immune suppressive innate cells to more pro-inflammatory APCs. Beta-glucans can be considered as oral adjuvants to AT, which is substantiated further due to ease of implementation, high safety, low additional costs, and support of self-assertiveness and self-awareness among cancer patients. Thus, the use of orally applied food adjuvants, such as β-glucans, would constitute a novel approach to rationally enhance endogenous immunity and support AT in treating cancer.

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