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Modulation of Toll-like receptor signaling in innate immunity by natural products

Luxi Chen [a], Jianhua Yu [b,c,*]

[a] Medical Scientist Training Program, The Ohio State University, Columbus, OH, USA
[b] Division of Hematology, Department of Internal Medicine, The Ohio State University, Columbus, OH, USA
[c] The Ohio State University Comprehensive Cancer Center, The James Cancer Hospital & Solove Research Center, Columbus, OH, USA

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ABSTRACT

For centuries, natural products and their derivatives have provided a rich source of compounds for the development of new immunotherapies in the treatment of human disease. Many of these compounds are currently undergoing clinical trials, particularly as anti-oxidative, anti-microbial, and anti-cancer agents. However, the function and mechanism of natural products in how they interact with our immune system has yet to be extensively explored. Natural immune modulators may provide the key to control and ultimately defeat disorders affecting the immune system. They can either up- or down-regulate the immune response with few undesired adverse effects. In this review, we summarize the recent advancements made in utilizing natural products for immunomodulation and their important molecular targets, members of the Toll-like receptor (TLR) family, in the innate immune system.

1. Introduction

Throughout history, compounds derived from natural sources have demonstrated their prowess as therapeutic agents in areas such as cardiovascular disease, metabolism, inflammation, and neurological disorders [1]. Recently, there has been a renewed interest in the scientific community to bring these natural products into clinical trials to provide safe and effective treatments for patients [2]. It is estimated that between 25 and 50% of marketed drugs today originate from natural sources [3]. Natural oils such as *Commiphora* (myrrh) and *Cupressus sempervirens* (Cypress) have been documented as medicinal therapies as early as ancient Mesopotamia and are still used today for treatment of the common cold, coughs, and inflammation [4]. Traditional Hindu Ayurveda as well as Traditional Chinese Medicine have been used for thousands of years and are gaining popularity in the field of Integrative Medicine among physicians [5, 6]. Examples of drugs derived from natural sources include morphine and codeine, which are isolated from the plant *Papaver somniferum* [7, 8]; anticancer agents taxol and halichondrin B, derived from the pacific yew tree [9] and marine sponges [10], respectively; and artemisinin, a Chinese Traditional Medicine used to treat malaria [11].

The human innate immune system provides the first line of defense against invading pathogens and is vital in early recognition of infection [12]. This sophisticated immune response relies on the recognition of microorganisms via a number of germline-encoded receptors known as pattern-recognition receptors or PRRs [13]. Distinctive PRRs react with specific evolutionarily conserved structures on pathogens called pathogen-associated molecular patterns (PAMPs), which are necessary for microorganism survival [14]. Perhaps the most extensively studied class of PRRs is the TLR family. To date, a total of 13 TLR members have been identified in mammals [15]. TLRs can further be divided into subfamilies based on the types of ligands they recognize. For instance, TLRs 1, 2, and 6 recognize lipopeptides and glycolipids, TLRs 7, 8, and 9 identify nucleic acids such as ssRNA and unmethylated CpG DNA, TLR3 distinguishes dsRNA associated with viral infection, TLR4 recognizes fibronectin, lipopolysacharrides (LPS), and heat shock proteins, TLR5 identifies bacterial flagellin, and TLRs 11 and 12 recognize profilin, an actin-binding protein [16].

A variety of immune cells, including dendritic cells (DCs), Natural Killer (NK) cells, T cells, and B cells, express TLRs. Recently, various groups showed that NK cells express high levels of TLRs 1, 3 and 6 [17, 18]. *In vivo*, DCs express TLRs 2, 4, 7, and 9 [19]. Human peripheral blood T cells express TLRs 1–5, 7, and 9 [20]. B cells also express TLRs, including TLRs 3, 4, and 9 [21]. TLR signaling affects myeloid and lymphoid progenitors in different ways. In culture, when stimulated with TLR ligands in the absence of growth and differentiation factors, myeloid progenitors become macrophages and/or monocytes while lymphoid progenitors give rise to DCs [22]. Additionally, TLR signaling plays different roles in myeloid and lymphoid cells. For example, LPS stimulation of mast cells does not result in IFN production while NK cells are activated to secrete IFN-γ [23, 24]. Furthermore, studies have shown that TRAF3, a downstream molecule of TLR signaling, constrains B cell TLR signaling yet activates myeloid cell TLR signaling [25, 26]. Activation of a TLR

* Corresponding author at: Division of Hematology, Internal Medicine, College of Medicine, The Ohio State University, 320 W 10th Ave, Columbus, Ohio, USA.
E-mail address: jianhua.yu@osumc.edu (J. Yu).

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with its ligand triggers signaling cascades that eventually result in the production of pro-inflammatory chemokines and cytokines. After pathogen recognition, TLR signal transduction is initiated via the Toll/Interleukin 1 Receptor (TIR) domain. Most TLRs use MyD88, a TIR-containing adaptor, to trigger a signaling pathway to activate NF-κB to express genes for inflammatory cytokines [27]. Therefore, innate immunity and its molecular targets play a critical role in the inflammatory response and protection against pathogens.

2. Natural health products and immune modulation

In the last century, several natural health products have been demonstrated to possess immunomodulatory actions, such as herbal medicines, probiotics, and fatty acids [28]. Probiotics, which are live bacteria capable of colonizing the gastrointestinal tract, have recently received a lot of attention in the scientific community. Not only do they assist in immune system maturation, but they also help manage inflammatory bowel disease [29] as well as atopic eczema and dermatitis in infants [30]. Strong evidence is also emerging to support the immunomodulatory effects of Vitamins E and C in the improvement of cognitive status in patients with Alzheimer's disease [31]. Vitamin D3 also possesses immunomodulatory properties, stimulating monocytes and macrophages to fight bacterial infections and regulating T cell development and migration [32]. Likewise, polyunsaturated fatty acids have successfully been employed in preventing allergic and inflammatory disorders [33, 34]. Furthermore, numerous experimental studies have demonstrated that green tea constituents and soy proteins can enhance the immune response and potentially lower the risk of certain cancers [35, 36]. Thus, current research has shown that compounds derived from natural sources offer new avenues for immune modulation and can be promising agents in preventing chronic diseases.

3. TLRs: linking natural products to innate immunity

TLRs are highly conserved PRRs that activate the innate immune system and participate in initiating the inflammatory response [37]. When TLR activity is dysregulated, there is an increased risk of developing chronic inflammatory and immune diseases [38]. Studies have shown that diets rich in fruits and vegetables are associated with lowered risk of cardiovascular disease and other inflammatory disorders [39, 40]. Many of the phytochemicals found in fruits and vegetables have beneficial anti-inflammatory actions in cells. Therefore, it is possible that these phytochemicals exert their mechanism of action by targeting TLRs and their signaling molecules downstream. Recently, Yi et al. reported that Ω-3 polyunsaturated fatty acids (PUFAs), abundant in nuts, oils, and fish, suppress the excessive inflammation in patients with severe trauma via a signaling pathway mediated by TLRs and NF-κB [41]. The group found that levels of COX-2, IL-2, and TNF-α substantially decreased in these patients. Additionally, Landmann et al. discovered that pretreating mice with chicoric acid found in the plant *Echinacea purpurea* attenuated the harmful effects of alcohol on the liver through suppression of mRNA expression of TNF-α and inducible nitric oxide synthase (iNOS) [42]. Currently, various natural compounds and their derivatives were found to act as agonists or antagonists for TLR family members and their downstream signaling molecules (Table 1, Fig. 1).

Recent reports have also shown the effect of natural products on the NK cell, an important regulator of innate immunity. Wu et al. published a study detailing how increased dietary intake of white button mushrooms promotes NK cell activity in C57BL/6 mice, enhancing the immune response against tumors and viruses [43]. The group discovered that production of IFN-γ and TNF-α, two key cytokines secreted by the NK cell, both increase upon intake of the mushroom. Another group in 2011 reported that daily consumption of blueberries for 6 weeks enhances NK cell count, decreases oxidative stress, and increases secretion of the anti-inflammatory cytokine IL-10 in athletes [44].

4. TLR dysregulation

Due to the importance of TLRs in maintaining innate immunity, dysregulation of TLR signaling pathways can lead to aging and immunosenescence [45] as well as a wide range of autoimmune diseases, including diabetes, hepatitis, rheumatoid arthritis, inflammatory bowel disease (IBD), and systemic lupus erythematosus [46]. For instance, a recent study showed that TLR3 gene polymorphisms may be associated with the pathogenesis of type 1 diabetes in a population of Black South Africans of Zulu descent [47]. Another study found that TLR2 expression increased in abdominal subcutaneous adipose tissue of patients with type 2 diabetes [48]. Furthermore, Kim et al. demonstrated the key role of the TLR4 signaling pathway in mediating insulin resistance and vascular inflammation in obesity induced by high-fat feeding [49]. Reports in the past decade have also clarified the importance of TLR signaling in the development of IBD. Compared to the healthy intestine, the IBD intestine has a distinct pattern of TLR upregulation and uncontrolled activation of TLR signaling [50]. Certain genetic defects, such as R753Q in TLR2 and S249P in TLR6, are associated with the progression of and susceptibility to IBD [51]. Given the significance of TLR dysregulation in the onset of disease, there is a need to understand compounds derived from natural sources that modulate TLR signaling pathways, especially in various disease settings. Below, we provide a summary of selected natural products acting on and affecting specific TLRs, namely TLRs 2, 4, and 9, as well as a combination of TLRs (i.e. both TLRs 2 and 4) and their downstream molecules.

4.1. TLR2

TLR2 binds to a wider range of ligands compared to any other member of the Toll-like Receptor family, recognizing bacterial, viral, fungal, and endogenous substances. Dimerization of TLR2 with TLR6 or TLR1 is crucial for identifying bacterial lipoproteins and lipopeptides [52]. Upon activation of TLR2, there is an increase in the NF-κB transcription factor via the MyD88/IRAK dependent pathway (Fig. 1), leading to the gene expression of cytokines such as IL-12 to enhance immunity. Therefore, TLR2 is a great target for immunotherapy, especially in malignant diseases to activate the innate immune response [53, 54]. Lu et al. recently investigated the immunomodulatory effects of polysaccharide krestin (PSK), an extract of the *Coriolus versicolor* mushroom [55]. PSK is commonly used as a treatment for cancer as a result of its potential immune potentiating effects. The group found that mechanistically, PSK acts as a selective TLR2 agonist to elicit a Type I inflammatory response, which is characterized by the recruitment of macrophages and

Table 1
Natural products and their TLR targets.

Compound name	TLR target	Type	Function	Potential therapeutic uses
PSK54	TLR2	Selective agonist	Elicit Type I inflammatory response	Breast cancer
*Astragalus mongholicus*61	TLR4	Agonist	Assist in dendritic cell maturation	Hypertension, stomach cancer
Oxymatrine69	TLR9	Agonist	Immuno-modulation	Chronic hepatitis B
PL-C72	TLR1/TLR6	Agonist	Activates NK cells to secrete IFN-γ	Cancer, viral infections
DHA81	TLR4, TLR9	Antagonist	Inhibits hepatic inflammation	liver fibrosis
Sparstolonin B78	TLR2, TLR4	Antagonist	Inhibit inflammatory cytokine expression in macrophages	Inflammatory diseases and cancer
Dioscin89	TLR4/MyD88	Antagonist	Up-regulation of HSP70	renal ischemia injury