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Astragali Radix (Huangqi): A promising edible immunomodulatory herbal medicine

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ABSTRACT

Ethnopharmacological relevance: Astragali Radix (AR, Huangqi in Chinese), the dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge., possesses diverse therapeutic effects against fatigue, dyspepsia, diarrhea, heart diseases, hepatitis, and anemia. In recent years, increasing evidence has indicated the multiple immunomodulatory activities of AR in preclinical and clinical studies.

Aim of the review: This review attempts to elaborate the immunomodulatory effects of AR and its potential application in the treatment of immune related diseases.

Materials and methods: A comprehensive literature search AR was carried out using multiple internationally recognized databases (including Web of Science, Google Scholar, PubMed, ScienceDirect, Wiley, ACS, Springer, Taylor & Francis, and CNKI).

Results: The immunomodulatory effects of AR are closely attributed to its active constituents such as polysaccharides, saponins, and flavonoids. We also demonstrate that AR can be used as a potential therapeutic intervention for immune related diseases through regulating immune organs, mucosal immune, and immune system (innate immunity and acquired immunity).

Conclusion: AR promotes the development of immune organs, enhances mucosal immune function, increases the quantity and phagocytic capacity of innate immunity, promotes the maturation and differentiation of acquired immunity cells, and improves the expression of antibodies in acquired immunity. We believe that AR has a broad research space in the adjuvant treatment of immune related diseases, which could be a breakthrough point to improve the application value of AR.

1. Introduction

In the past decade, immunotherapy has been shown to be a promising strategy to treat immune-related diseases, such as cancer, asthma, diabetes and inflammatory bowel disease (Asamoah et al., 2017; Bluestone et al., 2015; Catalan-Serra and Brenna, 2018; Ribas and Wolchok, 2018). An effective immune modulator is a key component for immunotherapy, and Chinese Herbal Medicine (CHM) has been shown to have promising immunomodulatory effects via multiple targets (Ma et al., 2013; Pan et al., 2019; Sharifi-Rad et al., 2018). Therefore, CHM has been widely used as a potential immunotherapy for immune-related diseases.

Astragali Radix (AR, Huangqi in Chinese, also known as astragalus), the dried root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge., is used as an ethnopharmacological herb in China, USA, Japan, Korea, Iran, Russia, and some other European countries (Li et al., 2019b). AR was first recorded in Shennong's Classic of Materia Medica of Qin or Han Dynasty (221 BC-220 AD) (Fu et al., 2014). As one of the most well-known Chinese medicinal herbs, AR possesses diverse therapeutic activities including

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anti-cancer, anti-viral and immunomodulatory effects (Ma and Zhao, 2000; Xu and Chen, 2005; Zhang et al., 2010). In the clinical practices of Traditional Chinese Medicine (TCM), AR is one of the essential and commonly used CHM to invigorate *Qi*, the substance that constitutes human body and maintains human life activities, and to promote *Yang*, the driving force of metabolism and physiological function of human body. These functions are considered as the primitive application of the immunoregulatory activity of this herb (Cho and Leung, 2007). It is also a well-known tonic that can be used by elderly adults who feel weak, patients who suffer from chronic diseases with low grade inflammation, and people whose work stress affects their health negatively (Wu et al., 2018).

Owing to its ability to regulate immunity, resist fatigue, and delay senescence, AR is widely used as a potential candidate for development of health products (Liu et al., 2017b). Remarkably, it is included in the edible herbal list created by the Ministry of Health of China, in which more than 100 herbs are identified as health products and medicinal drugs. Moreover, AR was categorized by the USA Dietary Supplement Health and Education Act of 1994 as a legal dietary supplement, therefore AR tea and capsules are being sold as over-the-counter (OTC) health products in the USA dietary supplement market (Zhang et al., 2011). In addition, AR has been produced in many different forms of supplements, including liquid extracts, capsules, powder, and tea, and it has also been widely used in animal husbandry due to its remarkable immunomodulatory effects, i.e., improving the immunity of animals and reducing the use of antibiotics (Farag and Alagawany, 2018).

The growth of AR is affected by many environmental factors. Its suitable habitats are mainly in mountainous areas with relatively less precipitation, such as Inner Mongolia, Heilongjiang, Gansu, and Ningxia Province, which cover an area of approximately 506,000 square kilometers (Peng and Guo, 2017). Due to the large-scale and long-term excavation, the amount of wild AR is dramatically decreased and in danger of extinction. As a result, the Chinese government has already categorized the wild AR as a third-class national protected plant, and now the clinically used AR is artificially manufactured.

Up to now, more than 100 compounds have been isolated and identified from AR, including saponins, flavonoids, polysaccharides, and amino acids (Auyeung et al., 2016; Fu et al., 2014; Liu et al., 2017b), in which saponins, flavonoids, and polysaccharides are deemed as the main bioactive constituents of AR that contributed to its immunomodulatory effects. Huang et al. revealed that the proteins from AR waste also exhibited potential immunomodulatory activities (Huang et al., 2019). Among them, astragalus polysaccharide (APS) is the most abundant and immunocompetent substance in AR (Ren et al., 2018). APS is mainly composed of dextran and heteropolysaccharides (Huang and Lu, 2003; Huang et al., 1982; Li et al., 2009a). Some of heteropolysaccharides in AR are composed of glucose, rhamnose, arabinose and galactose, and the other heteropolysaccharides consist of glucose and arabinose (Fu et al., 2014). In this review, we summarized the pharmacological effects of AR and focused on its immunomodulatory function, underlying mechanisms, and therapeutic applications for immune related diseases.

2. Ethnopharmacological immunomodulatory practices of AR

In the theoretical system of TCM, AR is sweet in flavor, warm in nature, and acts on the lung and spleen. Generally, AR is harvested in spring or autumn after growing for more than 3 years. After removing the aerial part and fibrous root, AR is washed, cut into thick slices, and dried. Then, it can be employed as herbal medicine in the clinic. Clinical practitioners of TCM consider that AR invigorates *Qi* and promotes *Yang*, thereby diffusing water, reducing swelling, fixing the surface, relieving sweat, promoting wound healing, and generating muscle (Auyeung et al., 2016). In the classic book titled “Compendium of Materia Medica”, it was declared that “AR is the leader of tonics among herb medicines.” Compared with equally well-known immunomodulatory medicinal herbs such as Ginseng Radix et Rhizoma, AR is more inclined to supplement the *Defense-Qi*, which has the physiological functions of defending exogenous pathogenic factors, warming and nourishing the whole body, and to replenish the *Middle-Qi*, which is the function motivity of spleen and stomach. Both of them are universally believed to be closely related to the immune system in TCM. In addition, its pharmacodynamic effects are more temperate and more suitable for weak and debilitated patients. Therefore, AR is a common and essential tonic herb medicine that has great developmental potential in food supplements.

AR, as a promising herbal medicine to regulate immunity, acts by promoting resistance to exogenous causative agents and strengthening general vitality. It is regarded as a significant tonic for up-regulating energy levels and regulating the immune system (Auyeung et al., 2016; Block and Mead, 2003). When used alone, it is generally soaked in water to drink, which is an effective method of AR utilization among folks (Jiao et al., 2015). It is also used in combination with other herbal medicines. For instance, some patients are susceptible to cold, which is called “exterior deficiency” according to TCM theory. AR can boost the *Defense-Qi* and avoid susceptibility to cold. It is generally used in combination with Ginseng Radix et Rhizoma, which is an appropriate combination for treating physical weakness caused by *Qi* deficiency, such as fatigue, inappetence, and spontaneous sweating (Wang et al., 2012a, 2018b). The compatibility of AR and Atractylodis Macrocephalae Rhizoma can enhance the function of replenishing the *Qi* and invigorating the spleen. The indications of this compatibility are fatigue, shortness of breath, and lassitude caused by spleen weakness and deficiency of vital energy (Zhou and Zhou, 2012). The combination of AR and Angelicae Sinensis Radix is suitable for improving internal injury, red muscular heat, thirst, deficiency of pulse, fatigue, sores, ulcers and fever due to *Blood* deficiency, and deficiency of the *Qi* and *Blood* (Wang et al., 2012b). On the basis of these compatibilities related to the regulation of immune system, numerous prescriptions for clinical and diet therapies have been developed in TCM.

3. Immunomodulatory constituents of AR

AR, like most botanical medicines, contains numerous natural products with different structural patterns. It is abundant with flavonoids, saponins, polysaccharides, amino acids, and other kinds of compounds, which show various bioactivities *in vivo* or *in vitro*. Nevertheless, not all these constituents from AR have immune regulatory function. After reviewing the relevant studies, we found that the main active constituents of AR for immune regulation are APS, astragalus saponins (AS), and astragalus flavonoids (AF) (Li et al., 2009a). Herein, we summarized the chemical constituents related to the immune regulation of AR. Their chemical structures are shown in Fig. 1.

3.1. Polysaccharides

Approximately 24 polysaccharides have been found in AR. Most of them are heteropolysaccharides. In terms of different species of raw materials or purification technology, different studies showed various results on the structural features of APS. Generally, the molecular weights of the heteropolysaccharides of APS are in the range of 8.7×10^3 Da to 4.8×10^6 Da with different ratios of monosaccharides, including glucose, galactose, rhamnose, arabinose, xylose, mannose, fructose, fucose, and ribose. Besides, glucuronic acid and galacturonic acid are also contained in APS (Jin et al., 2014). As early as in 1982, Huang et al. purified and isolated two kinds of heterosaccharides (AH-1 and AH-2) and two kinds of glacans (AG-1 and AG-2) from the water extract of AR. AG-1 was identified as an α-(1→4) (1→6) glucan with the ratio of α-(1→4) and α-(1→6) linkage approximately 5:2, while AG-2 was elucidated as an α-(1→4) glucan. Besides, AH-1 was an acidic polysaccharide that consists of monosaccharides, including galactose, arabinose, rhamnose, and glucose, with a ratio of 0.01:0.02:0.04:1.0.

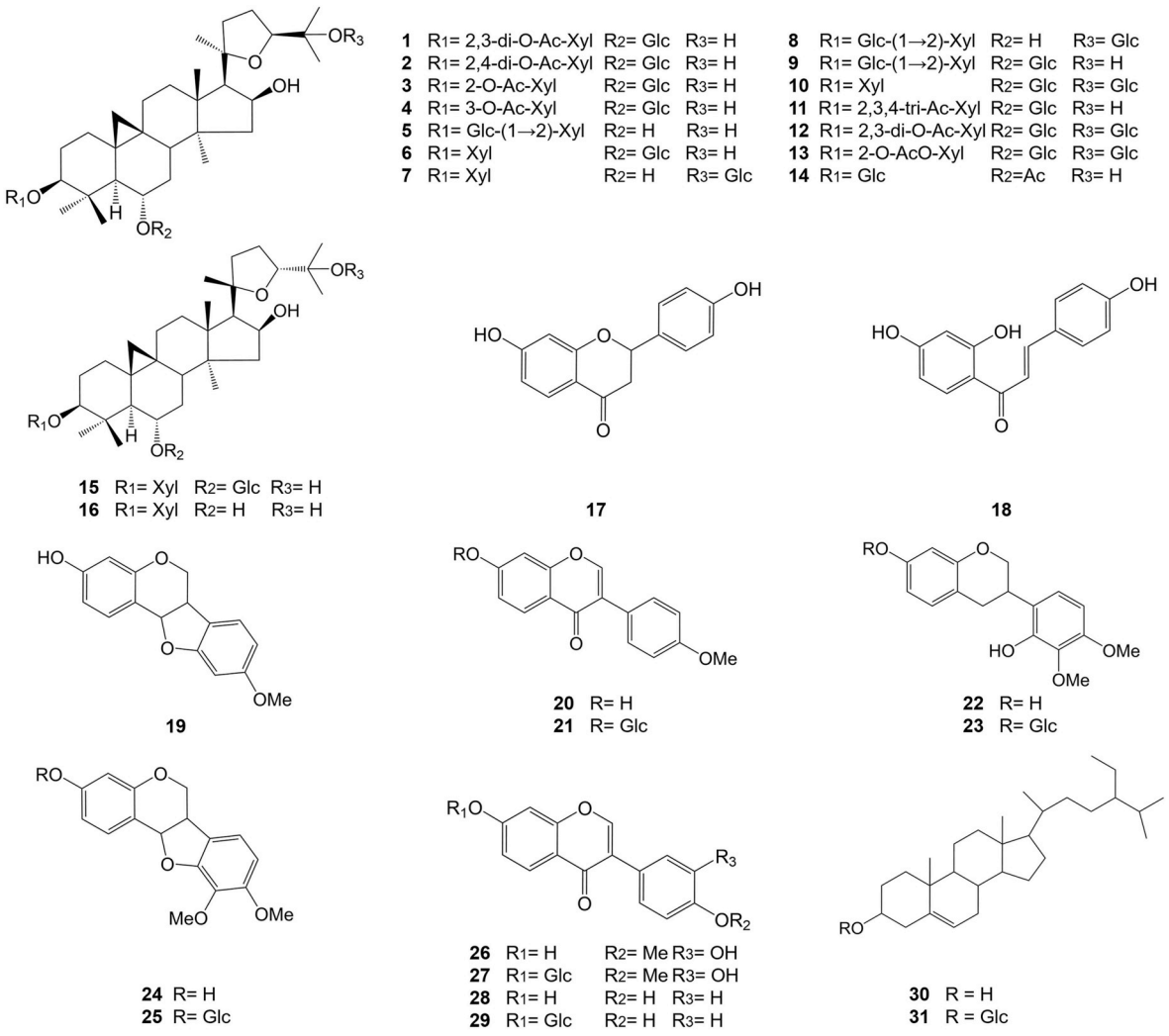

Fig. 1. Chemical structures of small molecular compounds associated with immunity in AR (**Ac:** acetyl; **Glc:** β-d-glucopyranosyl; **Xyl:** β-d-xylopyranosyl).

AH-2 consists of arabinose and glucose units at a ratio of 0.15:1. Notably, both polysaccharides have immune functions (Huang et al., 1982).

3.2. Saponins

Extensive phytochemical research on AR has documented that cyclolanostane-type saponins are the significant bioactive substances that are closely related to immunoregulation (Yin et al., 2004). Saponins in AR are main derivatives of the 20R, 24S form of cycloastragenol, called astragalosides (AST) **(1–14)**, which are expressed as [20(R), 24(S)-epoxy-9β, 19-cyclolanostane-β, 6α, 16β, 25-tetrol]. Some saponins, in the form of 20S,24R, are named astramembrainnins **(15, 16)**, which are expressed as [20(S), 24(R)-epoxy-9β, 19-cyclolanostane-β, 6α, 16β, 25-tetrol] (Rios and Waterman, 1997).

3.3. Flavonoids

Numerous flavones, which are main immunomodulatory components, have also been obtained from AR. They are mainly classified as flavonones (**17**), pterocarpans **(19, 24, 25),** isoflavans **(22, 23)**, and isoflavones **(20, 21, 26–29)** (Liu et al., 2017b). Among them, isoflavones are the most abundant class of substances. Notably, flavonoids containing glucoside account for a considerable proportion in AR (Fu et al., 2014; Lin et al., 2000).

4. Pharmacological activities on immune organs

Generally, the growth of immune organs in good situation is significant to promote the resistance to exogenous pathogens. Spleen, thymus, bursa of Fabricius (birds), and lymphatic tissue are the main immune organs (Harrison et al., 2011; Longenecker et al., 1966). AR and its derivatives play positive roles in promoting the development of immune organs in poultries. For instance, Wei et al. found that APS significantly increased liver, pancreas, spleen, and bursa of Fabricius indexes by adding 0.2%, 0.4%, and 0.6% of APS into the basic diet to one-day-old broilers for 7 days. However, no significant effect of APS on the organ index of 14-day-old broilers was observed, which indicated that APS efficiently promoted the development of immune organs in the early stage (Wei et al., 2011). Li et al. gave Astragali Radix extract (ARE) to 7-day-old chickens for 6 weeks by adding 3 mL of ARE (0.2 mg/L) to the water daily. After that, the immune organs, such as thymus, spleens, and bursa of Fabricius, were weighed, and the index of immune organs was calculated. The development of thymus and bursa of Fabricius of the drug-treated group was obviously more significant than that of the control group. In terms of the development of spleen, rapid growth occurred before they were 35 days old (Li et al., 2004). Li et al. also proved that chickens supplemented with 220 mg of APS and 4×10^{10} colony-forming units (CFU) probiotics to per kilogram of feed significantly improved the quality of the immune organs including thymus, spleen, and bursa of Fabricius. The combination of probiotics and APS in the feed displayed synergistic effects on the intestinal

Table 1
The effect of AR or AR derivatives on the immune system.

Forms of AR	Models	Dosages	Immunomodulatory actions	References
APS	1-day-old broilers	0.2%, 0.4%, and 0.6% of APS were added to the basic diet for 7 days	Promoted the development of immune organs in the early stage	Wei et al., (2011)
	Chickens	220 mg of APS and 4 × 10^{10} CFU probiotics were added to per kilogram of feed	Ameliorated the quality of immune organs such as spleen, thymus and bursa of Fabricius	Li et al., (2009b)
	Tumor-bearing BALB/c mice (H22 cells)	75,150, and 300 mg/kg were given by oral administration once daily for 15 days	Maintained the normal function of immune organs, up-regulated the percentage of lymphocyte subsets, and improved the pinocytosis of macrophages	Liu et al., (2017a)
	Four-week-old Yorkshire pigs	5, 10, and 20 mg/kg of APS were intramuscularly injected once a day over three successive days	Enhanced mRNA expressions of IFN-γ and IL-6, and up-regulated the titer of FMDV-specific antibody	Li et al., (2011)
	Weaned piglets	Basal diet supplemented with 800 mg/kg of APS for 28 days	Increased body weight. Exhibited higher level of serum IgM. Increased the expressions of NF-κB, TLR4 and MyD88	Yang et al., (2019)
	7-day-old chickens	20, 40, and 80 mg/kg of APS were given by oral administration for 7 days	Activated the chTLR4 pathway in bursa of Fabricius	Zhang et al., (2017b)
	Peritoneal macrophages of mice	1, 10, 100, and 200 μg/mL	Enhanced the killing effect of peritoneal macrophages on melanoma cells in mice	Yao et al., (2005)
	Nile tilapia (Oreochromis niloticus)	1500 mg of APS was added to per kg of diet, fed twice a day for 6 weeks	Ameliorated the phagocytic activity and respiratory burst of macrophages, and promoted activities of plasma lysozyme, the bactericidal, SOD, glutathione peroxidase (GPx), and amylase	Eman et al., (2014)
	Female B6C3F1 mice; RAW 264.7	10 and 30 mg/kg; 10, 50, and 100 μg/mL	Stimulated macrophages to express iNOS gene by activating NF-κB/Rel, and reduced expressions of NF-κB/Rel binding complexes and NO	Lee and Jeon, (2005)
	RAW264.7 macrophage	12.5, 25, 50, and 100 μg/mL	Increases expressions of NF-κB protein, and cytokines including TNF-α, GM-CSF, and NO	Zhao et al., (2011)
	Plasmacytoid DCs from acute myeloid leukemia patients	50, 100, and 200 μg/mL	Promoted differentiation and maturation of pDCs, and up-regulated secretions of IFN-α, TNF-α, and IL-6 in pDCs	Liu et al., (2010)
	BM-derived DCs of C57BL/6 mice	10, 50, 100, and 250 μg/mL	Promoted the co-expression of CD11c and MHC II molecules on DC surface	Shao et al., (2006)
	Gastric cancer model in wistar rats	100, 200, and 300 mg/kg of APS were given by oral administration once daily for 5 weeks	Up-regulated proliferation of spleen lymphocytes, and improved blood IL-2 levels and NK activities	Li et al., (2009a)
	25-day-old Haline white chickens	5 and 10 mg of APS were injected respectively for 6 consecutive days from the first day of infection	Promoted expressions of E-C3bRR, erythrocyte-C3b immune complex rosette rate (E-ICRR) and erythrocyte rosette forming enhancing rate (ERER)	Jiang et al., (2010)
	25-day-old chickens	5 and 10 mg/kg of APS were intramuscularly administered twice a day respectively for 31 days.	Ensured E-C3bRR at normal level, and increased E-ICRR. Improved the immune adherence by affecting the complement receptors on the membrane of erythrocytes	Li et al., (2007a)
	BV2 microglia cells	50, 100, and 200 μg/mL	Inhibited productions of NO and PGE2, gene expressions of iNOS and COX-2, and cytokine secretions of IL-1β and TNF-α. Related to inhibitions of NF-κB and PKB signaling pathway	Luo et al., (2015)
	CTX-induced immunosuppression mice	139 mg/kg of APS was orally administrated to mucosal immunosuppressed mice for 14 day	Increased the phagocytosis index, phagocytosis rate, and hemolysin level. Promoted secretions of sIgA in intestine and lung	Wu et al., (2011)
	Bacterial LPS-stimulates piglets	500 mg/kg of APS mixed with the feed	Reversed decreases of mast cells and intestinal histamine content caused by CTX to enhance the mucosal immune function of piglets	Ou et al., (2009)
	Sea cucumbers with an average initial weight of 49.3 ± 5.65 g	Basal diet was supplemented with ARE or APS at a rate of 3% body weight per day for 60 days	Decreased the cumulative symptom rates of *Vibrio splendidus*. Enhanced the immune responses of *Apostichopus japonicus* and resistance to infection by *Vibrio splendidus*	Wang et al., (2009)
	S180 Sarcoma-Bearing Mice; Intestinal intraepithelial γδT cells	150 and 300 mg/kg of APS were orally administrated daily; 40 and 80 μg/mL	Up-regulated the proliferation of γδT cells. Increased mRNA levels of IFN-γ, FasL and GrB, and secretions of TNF-α and IFN-γ. Decreased the level of IL-10 and TGF-β	Sun et al., (2014)
	Splenic DCs, $CD11c^{high}CD45R\text{-}B^{low}$ DCs, $CD11c^{low}CD45R\text{-}B^{high}$ DCs, splenic $CD4^+$ T cells of male BALB/c mice	50, 100, and 200 μg/mL	Promoted the differentiation of splenic DCs to $CD11c^{high}CD45RB^{low}$ DCs through shifting of Th2 to Th1 with improvement of T cells immune function *in vitro*	Liu et al., (2011)
	Dexamethasone induced Two-month-old immunosuppressive male Chinese Countryside Dogs	50, 100, and 200 mg/kg of APS were given by intravenous injection	Up-regulated expressions of $ANAE^+$ and $CD4^+$ T cells, and inhibited $CD8^+$ T cells. Improved contents of IL-2 and INF-γ, the cytophagic index, and the percentage of peritoneal macrophages	Qiu et al., (2010)

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Table 1 (*continued*)

Forms of AR	Models	Dosages	Immunomodulatory actions	References
	5 to 6-week-old male MF1-albino mice	250 mg/kg of APS was given for 4 consecutive weeks	Promoted the activity of neutrophils phagocytic and ROS production in intestinal tissues. Increased $CD4^+$ T cells, and decreased $CD8^+$ T cells in the spleens and thymus	Abuelsaad, (2014)
	Splenic T and B cells, mouse peritoneal macrophages form BALB/c and C3H/HeJ mice	30, 100, and 150 μg/mL	Ameliorated proliferations or cytokine productions of mouse B cells and macrophages, and activated B cells through membrane Ig in a TLR4-independent manner	Shao et al., (2004)
	Listeria infected mice	Intraperitoneally injected with 1 mL APS solution (1 g/L) for 6 days	Promoted the production of IgG against Listeria monocytogenes in mice	Xiang et al., (2007)
	PAM cell line 3D4/21; Ochratoxin A induced immune stress Kunming mice	20 μg/mL; 200 mg/kg	Decreased expressions of apoptosis, cytotoxicity and pro-inflammatory cytokine *in vitro*; alleviated spleen damages and decreased expressions of apoptosis-related proteins and pro-inflammatory cytokines. Enhanced expressions of AMPK/SIRT-1 and inhibited the level of NFκB *in vivo*	Liu et al., (2018a)
	Male BALB/c mice	1500 mg/kg gel once and 1000 mg/kg gel twice were administrated by subcutaneous injection (0.1 g/mL APS)	Increased values of immune organ indices, spleen lymphocyte proliferation, and serum IgM, IgG, IL-2 and IL-6	Yu et al., (2017)
AST IV	Peritoneal macrophages of mice	0.2, 0.6, 1.5, and 4 μg/μL	Up-regulated concentrations of IL-1 and IFN-γ and further enhanced the activation and phagocytosis of macrophages	Zhang et al., (2005)
	Inbred ICR mice; BALB/c mice; L929 cell	50 ~ 200 mg/kg of AST IV were orally administrated for 7 days	Enhanced T, B lymphocyte proliferations and the antibody production *in vivo* and *in vitro*. Inhibited the levels of TNF-α and IL-1 in macrophages *in vitro*	Wang et al., (2002)
	Lewis lung carcinoma cell-bearing C57BL/6 mice	40 mg/kg of AST IV was orally given daily; 80 and 160μg/mL	Suppressed the growth of tumor *in vivo*. Down-regulate Tregs and up-regulate CTLs both *in vivo* and *in vitro*	Zhang et al., (2014)
	$CD4^+CD25^+$ Tregs of male BALB/c mice	50 and 100 μg/mL	Rivaled the inhibitory effect of HMGB1 on immune function of Treg with a concentration-dependent manner *in vitro*	Huang et al., (2012)
	BALB/c mice	10 and 20 mg/kg of AST IV were administered by intraperitoneal injection	Boosted the Foxp3 expression, contents of IL-10 and TGF-β, and levels of IL-2 and IFN-γ. Promoted the proliferation of $CD4^+$ $CD25^-$ T cells	Li et al., (2016)
AST II	Female BALB/c and C57BL/6 mice; T cells of mice	50 mg/kg of AST II was orally administered once a day; 3, 10, 30, and 100 nmol/L	Improved mRNA levels of IFN-γ and T-bet in primary splenocytes, and expressions of CD25 and CD69 on primary $CD4^+$ T cells. Restored productions of IL-2 and IFN-γ and the splenic T cells proliferation	Wan et al., (2013)
AF	Chronic fatigue syndrome rats	20, 50 and 100 mg/kg of AF were given by intragastric administration once a day for 6 weeks	Produced more IL-2 but less IL-4. Promoted immunity by improving the reduced the spleen cell proliferation and balancing the abnormal cytokine level	Kuo et al., (2009)
	BALB/c mice; RAW 264.7	25, 50 and 100 mg/kg of AF were given by oral administration once per day for 7 days; 10, 25 and 100 μg/mL	Delayed type hypersensitivity, and up-regulated the level of serum hemolysin, the index of immune organ and the index of phagocytic *in vivo*. Enhanced expressions of NO, IL-1β, IL-6, IFN-γ and TNF-α *in vitro*	Guo et al., (2016)
	BALB/c mice	0.5 mg/d of AF were given by intramuscular injection	Enhanced the proliferation of lymphocytes induced by Con A, raised the total count of T cells, regulated disturbance of the T cell subset, and elevated the LAK activity induced by rIL-2	Jiao et al., (2001)
ARE	7-day-old chickens	0.2 mg/L of ARE was added to the water for 6 weeks	Promoted developments of thymus, spleens and bursa of Fabricius	Li et al., (2004)
	Mice	5 g, 25, and 50 g/kg of AER were administrated via intragastric administration for 30 days	Long-term administration of ARE contributed to the increase of the spleen index of mice	Liu et al. (2018)
	Ana-1 murine macrophages	10, 20, and 40 μL/mL	Increased mRNA levels and secretions of IL-1β and TNF-a in macrophages, and promoted the activity of HPA and cell migration	Qin et al., (2012)
	BM-derived DCs from C57BL/6 mice	15, 50, 150 and 500 mg/mL	Inhibited the proinflammatory response to *E. coli* and promoted *L. acidophilus*-induced production of IFN-β *in vitro*. The augmented level of IFN-β was associated with upregulations of TLR3 and IL-12, IL-6, IL-1β and TNF-α	Frokiaer et al., (2012)
	SD rats; NK92MI cells	3.1, 6.2, and 12.4 g/kg of ARE were given by gavage; Serum containing ARE was used for cell culture	Increased the expression of KLRK1 and the killing activity of NK cells	Liu et al., (2014a)
Proteins from AR	Splenic lymphocytes, peritoneal macrophages, and BM-derived cells isolated	0 ~ 180 μg/mL	Increased proliferations of splenic lymphocytes, peritoneal macrophages and BM-derived cells. Improved the secretion of phagocytosis and levels of IL6, TNF-α, NO, and H_2O_2 in macrophages. Promoted inflammatory cytokines in BMDCs	Huang et al., (2019)

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Table 1 (*continued*)

Forms of AR	Models	Dosages	Immunomodulatory actions	References
	from the spleen of ICR mice			
Powder of the stem and leaf of AR	Newcastle disease vaccine immunized One-day-old chickens	0.5%, 1%, 1.5% of ultrafine powder were added into the feedstuff for 49 days	Raised the antibody titers, IL-2 and IFN-γ contents	Xi et al., (2014)
	One-day-old chickens	0.5%, 1%, 2% of ultrafine powder were added into the feedstuff for 35 days	Increased the level of body weight and indexes of immune organs of chickens	Xue et al., (2014)
	One-day-old Japanese quail chickens	1%, 3%, or 5% of ultrafine powder were added into the feedstuff for 35 days	Promoted development of immune organs. Increased levels of C4 and C3, and IgA. Up-regulated activities of catalase and GPx	Guo et al., (2019)
AR Injection	Peripheral B lymphocytes of IgAN patients	200, 1000 mg/mL	Inhibited the expression of Cosmc, and up-regulated the secretion of IgA1 in peripheral B cells of IgAN patients	Ji et al., (2014)
	Apoptotic model of thymic lymphocyte induced by dexamethasone in mice	0.5, 1, 2 g/kg of AR Injection were administrated by intraperitoneal injection for 2 days	Up-regulated the expression of Bcl-2, which was related to anti-thymic atrophy and inhibition of thymic lymphocyte apoptosis	Yang et al., (2009)

microorganisms and the immune system, which was extremely significant for the exploration of new agents of immunity regulation (Li et al., 2009b). Besides, the ultrafine powder of the aerial part of AR showed a promotion in the development of immune organs of chickens (Guo et al., 2019; Xi et al., 2014; Xue et al., 2014). Liu et al. showed that APS decreased the expression of pro-inflammatory cytokines, cytotoxicity, and apoptosis induced by ochratoxin A via activating the 5′ AMP-activated protein kinase (AMPK)/Sirtuin 1 (SIRT-1) signaling pathway and protected spleen cells from immune stress (Liu et al., 2018a).

AR and ARE significantly affected the function and development of immune organs in mammal models. In order to observe the regulating effect of long-term administration of ARE, Li et al. treated mice with ARE (5, 25, and 50 g/kg) by intragastric administration for 30 days. As a consequence, the long-term administration of ARE contributed to strengthening the spleen index of mice (Liu et al., 2018b). In another study, APS group was subcutaneously injected with APS (250 mg/kg) once a day. APS-Gel group 1 was subcutaneously injected with APS gel (1500 mg/kg) on the 4th day. APS-Gel group 2 was subcutaneously administrated with APS gel (1000 mg/kg) on the 4th and 7th days. The compatibility of APS and gel effectively reduced the frequency of administration and improved the immune organ index of mice through subcutaneous injection (Yu et al., 2017). Notably, some studies have revealed that the immunoregulatory activity of AR on immune organs was obviously dose dependent. Liu et al. showed that APS induced a notable improvement in the thymus index and a reduction in the spleen index of tumor-bearing mice. Indeed, the spleen and thymus indexes of the mice in the high-dose group (300 mg/kg) were optimum (Liu et al., 2017a). Moreover, Kuo et al. showed that intragastric administration of AF (20, 50, and 100 mg/kg) consisted of demethylhomopterocarpin **(19)**, formononetin **(20)**, and ononin **(21)** to rats once a day for 6 weeks significantly enhanced immune function by ameliorating the reduced spleen cell proliferation and balancing the abnormal cytokine levels in rats (Kuo et al., 2009).

Chronically, the regulatory effects of AR and ARE on immune organs is comparatively prominent in TCM. However, the adequate elucidation of the relevant mechanism is still lacking and many researchers have attempted to explore. Zhang et al. found that APS administration activated toll-like receptor (TLR) 4 pathway in the bursa of Fabricius through myeloid differentiation primary response gene 88 (MyD88)-independent pathway (Zhang et al., 2017b). Moreover, AR injection has been proven to have a significant anti-thymic atrophy effect and inhibition of thymic lymphocyte apoptosis, which were related to the up-regulation of Bcl-2 expression (Yang et al., 2009).

5. Pharmacological activities on mucosal immune

Mucosal immune system refers to the lymphoid tissue that is widely distributed in the gastrointestinal tract, urogenital tract, respiratory tract submucosa, and some exocrine glands. They are the main sites for local nonspecific immune function. The mucosal immune system is mainly composed of mucosa-bound lymphoid tissues. It exerts the immunoregulatory activity by producing secretory immunoglobulin A (SIgA) and antigen-specific cell-mediated cytotoxicity, and secreting regulatory cytokines by regulatory cells located in the mucosa (McGhee et al., 1992). Wu et al. intravenously injected APS (139 mg/kg) with different molecular weights (157.7×10^3, 69.9×10^3, 22.4×10^3, 13.2×10^3, and 1.4×10^3 Da) into the mucosal immunosuppressed mice for 14 days. The levels of SIgA in the small intestine and lung lavage fluid, as well as the changes in the specific aggregate lymph nodes (Peyer's patches) of gastrointestinal mucosa-bound lymphoid tissues were detected to evaluate the immunoregulatory effects of APS on mucosal immunity. APS with large molecular weight significantly increased the phagocytic index and rate, and the secretion of SIgA in the intestine and lung (Wu et al., 2011). Another research proved that APS significantly promoted the proliferation of mice intestinal mucosa γδT cells, increased the mRNA levels of granzyme B (GrB), interferon (IFN)-γ, and Fas and Fas Ligand (FasL) in γδT cells, enhanced the viability and cytotoxicity of γδT cells in the intestinal mucosa of APS-treated mice, and increased the secretion of IFN-γ and tumor necrosis factor-alpha (TNF-α), whereas the transformed growth factor (TGF)-β and interleukin (IL)-10 levels were significantly decreased (Sun et al., 2014). In addition, Ou et al. investigated the activities of APS on the inflammatory mediators and mast cells induced by cyclophosphamide (CTX). APS (500 mg/kg) mixed with the feed obviously reversed the decrease in the number of mast cells and intestinal histamine content declining caused by CTX, thereby enhancing the mucosal immune function of piglets (Ou et al., 2009).

According to the above research, the regulation of AR on mucosal immune is mainly mediated by the increase of SIgA level. Besides, it is worth noting that AR can also up regulate the expression of γδT cells and mast cells in the mucosa, enhance the secretion of IFN-γ, TNF-α, and intestinal histamine, and decrease the levels of TGF-β and IL-10. These evidences suggest that the regulation of AR on mucosal immunity is a complex process.

6. Pharmacological activities on immune system

Recently, a growing number of academics and clinical practitioners have given importance to the research on the immunoregulatory activities of AR, because it is closely related to sterling clinical efficacy, such as anti-diabetic, anti-cancer and anti-virus, *etc.* (Fu et al., 2014). Literatures have documented that AR has promising immunomodulatory ability to regulate innate immunity (effects on macrophages, natural killer cells (NK cells), dendritic cells (DCs), erythrocyte immunity, and microglia), and acquired immunity (effects on T lymphocytes and B lymphocytes). The immune regulatory information of AR and its derivatives was showed in Table 1.

6.1. Innate immunity

6.1.1. Macrophages

Macrophage is one of the vital immune cells that can destroy and engulf dead cells and exogenous pathogens (Bratosin et al., 1998). After years of experimental researches, it has been demonstrated that APS has obvious superiorities in increasing macrophage number and activity. Ma and Zhao conducted a phagocytic test of macrophages in mice. In the research, each experimental group was intraperitoneally injected with different polysaccharides (100 mg/kg) separately once a day for 6 days. The result of phagocytic indexes showed that APS significantly increased the phagocytosis of macrophages (Ma and Zhao, 2000). Yao et al. found that APS (200, 100, 10, and 1 μg/mL) promoted nitric oxide (NO) synthesis in macrophages and enhanced the killing effect of peritoneal macrophages in melanoma cells *in vitro* and showed a certain concentration correlation (Yao et al., 2005). Eman et al. showed that a dietary supplement with APS (1500 mg/kg) improved the growth performances and immunological indexes of Nile tilapia, and up-regulated the phagocytic activity of macrophages (Eman et al., 2014). A clinical study by Jiang et al. found that the phagocytic capacity of macrophages in patients injected with AR significantly increased. Besides, the NO and TNF-α contents were significantly increased compared to those before the treatment (Jiang et al., 2005). Some studies have demonstrated that other compounds contained in AR also promoted the function of macrophages. Guo et al. showed that AF (25, 50, and 100 mg/kg) containing six main chemical components, including formononetin **(20)**, calycosin **(26)**, calycosin-7-O-β-D-glucopyranoside **(27)**, formononetin-7-O-β-D-glucopyranoside **(21)**, β-sitosterol **(30)**, and daucosterol **(31)**, enhanced the macrophage phagocytic index of mice *in vivo* (Guo et al., 2016).

Recently, many studies have clarified the underlying mechanisms. Lee and Jeon showed that APS stimulated macrophages to express inducible nitric oxide synthase (iNOS) gene by activating nuclear factor kappa-B (NF-κB)/Rel and reducing the expression of NF-κB/Rel binding complexes (Lee and Jeon, 2005). Zhao et al. considered that the effect of APS on RAW264.7 depended on the increasing levels of granulocyte-macrophage colony stimulating factor (GM-CSF), NO, and TNF-α. (Zhao et al., 2011). Qin et al. found that ARE activated macrophages by activating heparanase (HPA) to up-regulate cell migration and release immune response mediators. At the same time, ARE enhanced the secretion of macrophage release factor, promoted the expression of iNOS gene of macrophage, and increased the concentration of Ca^{2+} in macrophages, thereby affecting the phagocytosis of macrophages (Qin et al., 2012). In addition, Zhang et al. found that AST IV **(7)** increased the concentrations of IFN-γ and IL-1 and further enhanced the activation and phagocytosis of macrophages (Zhang et al., 2005).

6.1.2. Dendritic cells

As antigen-presenting cells, DCs play a momentous role in immune response (Banchereau and Steinman, 1998). Liu et al. found that APS promoted the maturation and prolonged the survival time of DCs, induced the directional differentiation of umbilical cord blood monocytes (precursor cells of DCs), enhanced the function of plasma cell-like dendritic cells in patients with acute myeloid leukemia, and promoted their differentiation and maturation (Liu et al., 2010). Shao et al. investigated the activity of APS on the maturation and function of murine bone marrow (BM)-derived DCs. APS increased the co-expression of major histocompatibility complex (MHC) II and CD11c on the surface of DCs. APS affected the immune regulation of BM in mice by regulating the function of BM-derived DCs (Shao et al., 2006). Du et al. showed that APS promoted the maturation of DCs, which was characterized by the increased levels of CD40, MHC I/II, CD86, and CD80, and down-regulating the frequency of the regulatory T cells (Treg) (Du et al., 2012). Li et al. demonstrated that AF, including daidzein 7-O-β-D-glucoside **(14)**, liquiritigenin **(17)**, isoliquiritigenin **(18)**, formononetin **(20)**, formononetin 7-O-β-D-glucoside **(21)**, isomucronulatol **(22)**, isomucronulatol 7-O-β-D-glucoside **(23)**, methylnissolin **(24)**, methylnissolin 3-O-β-D-glucoside **(25)**, calycosin **(26)**, calycosin 7-O-β-D-glucoside **(27)**, and daidzein **(28)**, down-regulated the production of proinflammatory cytokines, including IL-6 and IL-12 p40, in BM-derived DCs stimulated by lipopolysaccharide (LPS) (Li et al., 2014). Moreover, Frokiaer et al. revealed that 10, 50, 100, and 500 mg/mL of ARE marginally reduced the proinflammatory response to Escherichia coli and promoted the production of IFN-β induced by *Lactobacillus acidophilus in vitro*. The enhancement of IFN-β production was relevant for up-regulating the level of TLR 3, TNF-α, IL-1β, IL-6, and IL-12. Moreover, ARE obviously increased endocytosis in immature DCs (Frokiaer et al., 2012).

6.1.3. Natural killer cells

NK cells are immune cells with nonspecific killing effects on target cells, especially on a variety of tumor cells with rapid killing and lysis. This killing effect is independent of antibodies and complements (Vivier et al., 2008). AR can enhance the activity and killing effect of NK cells and promote their proliferation. For example, AR injection obviously promoted the activity of NK cells in patients with postoperative colorectal cancer and bronchial asthma with acute exacerbation to strengthen their immune function (Ji et al., 2011; Shi and Sun, 2012). Li et al. found that APS (100, 200, and 300 mg/kg) fed once daily for 5 weeks could significantly increase the activity of NK cells dose-dependently in rats with gastric cancer (Li et al., 2009a). The activity of APS on the immune reaction in pigs immunized with foot-and-mouth disease virus (FMDV) vaccine was investigated by Li and colleagues. In their study, three treated groups were injected with different doses of APS (5, 10, and 20 mg/kg). The effect of APS on the level of $CD3^-CD4^-CD8^+$ NKs among peripheral blood lymphocytes (PBL) in the three groups was remarkable compared to the vaccine group (Li et al., 2011). Cui et al. showed that AR had the strongest reversal effect on NK cell killing inhibition induced by Colon 26 among the anti-cancer herb medicines tested by targeting TGF-1, which was one of the vital mechanisms of the anti-cancer effect of AR (Cui et al., 2011). Another research suggested that the mechanism of AR in activating NK cells was related to its killer cell lectin-like receptor K1(KLRK1) activation (Liu et al., 2014a).

6.1.4. Erythrocyte immunity

The erythrocyte immune system was first proposed by Siegel and colleagues. At present, it has been described as a significant subsystem of immunity and one of the hotspots of immunology research (Siegel et al., 1981). Jiang et al. found that injection with 5 and 10 mg of APS to chickens once a day from the first day of infection bursa disease virus for 6 days significantly enhanced the immunocompetence of chicken erythrocytes (Jiang et al., 2010). Li et al. demonstrated that APS promoted chicken erythrocyte immunity by impacting the complement receptor 1 (CR1) on the membrane of erythrocytes (Li et al., 2007a). AR enhanced the immune function of chicken red blood cells by promoting the rate of erythrocyte-C3b receptor rosette rate (E-C3bRR) in the experimental group. The decline of the inhibition rate of E-C3bRR indicated that AR enhanced the immune activity of erythrocyte CR1,

thereby enhancing the erythrocyte immune function (Li et al., 2007c). Yang et al. showed that APS effectively participated in erythrocyte differentiation, regulated proto-oncogenes, including LIM domain only 2 (LMO2), Kruppel like factor (Klf) 1, Klf3, runt-related transcription factor 1 (Runx1), Ephrin type-B receptor 4 (EphB4) and specificity protein 1 (SP1), and increased the expression of K562 cell line protein and synthesis of fetal hemoglobin (Yang et al., 2010a).

6.1.5. Microglia

The microglia in central nervous system (CNS) play an important role in the immune and inflammatory responses (Aloisi, 2001). APS was reported to inhibit the production of prostaglandin E2 (PGE2) and NO, reduce the gene expression of cyclooxygenase-2 (COX-2) and iNOS, and decrease the secretion of TNF-α and IL-1β. The immunomodulatory effect of APS in microglia was related to the inhibition of protein kinase B (PKB) and NF-κB signaling pathway. APS possessed potential utilization in inflammatory and immune diseases, which was accompanied with microglia activation (Luo et al., 2015).

6.2. Acquired immunity

6.2.1. B lymphocytes

Fan et al. showed that 62.5–7.813 μg/mL of APS liposome significantly up-regulated the proliferation of B lymphocytes *in vitro* (Fan et al., 2012). Shao et al. reported that APS induced B cell proliferation by interacting with immunoglobulin (Ig) on the surface of B cells (Shao et al., 2004). Hong et al. showed that treating the mice with 200 mg/kg of an extract containing 26% of ARE significantly promoted the proliferation of B cells (Hong et al., 2018). In addition, Ji et al. found that 200 and 1000 mg/mL of AR injection reduced the level of IgA1 aberrant O-glycosylation and up-regulated the core I β3-Gal-T-specific molecular chaperone (Cosmc) expression in peripheral B lymphocytes *in vitro*, which was an important mechanism of AR injection in immunoglobulin A nephropathy (IgAN) treatment (Ji et al., 2014).

In addition, B lymphocytes produce antibodies to fight against exogenous pathogens, also known as humoral immunity. Hence, the level of antibody can reflect the extent of the action of somatic B cells to some degrees (Slifka et al., 1998). Xiang et al. found that the titer of serum against *Listeria monocytogenes* in mice intraperitoneally injected with APS was significantly higher than that in the saline control group. This finding demonstrated that APS promoted the production of IgG against *Listeria monocytogenes* in mice, thereby enhancing the humoral immunity to protect the host from intracellular bacterial infection (Xiang et al., 2007). Li et al. investigated the activity of APS in pigs administrated with vaccine for foot-and-mouth disease (FMD). APS significantly increased the titer of specific antibodies and promoted the mRNA expression of IL-6 and IFN-γ (Li et al., 2011). Wang et al. revealed that the phenoloxidase and lysozyme titers in humoral immunity and phagocytic ability of sea cucumber were significantly increased after APS administration. The dietary intake of APS significantly improved the immune response of sea cucumber (Wang et al., 2009). Furthermore, Guo et al. investigated the immune booster effect of APS-sulfated epimedium polysaccharide (sEPS) in immunosuppressed chicken model induced by CTX. The APS-sEPS surmounted the immunosuppression of chicken induced by CTX and significantly increased the titer of serum antibodies (Guo et al., 2012a). Chen et al. revealed that APS, combined with oxymatrine (OM) (mixed at a proportion of 2:3, dissolved, and diluted into 1, 2, and 4 mg/mL), synergistically increased the antibody levels in chickens vaccinated with Newcastle disease vaccine (Chen et al., 2010). Moreover, Nalbantsoy et al. demonstrated that AST VII **(10)** and macrophyllosaponin B generated significant specific cellular response and antibody against bovine serum albumin (BSA). Macrophyllosaponin B and AST VII remarkably improved the proliferation of splenocytes and levels of LPS and concanavalin A (Con A) in BSA-immunized mice, especially at 120 and 240 μg of AST VII. Besides, macrophyllosaponin B affected the stimulation of IFN-γ production (Nalbantsoy et al., 2011). In addition, considerable researches have documented that AR and ARE achieved anti-viral effects by increasing the number of antibodies (Guo et al., 2012b; Kallon et al., 2013; Londt et al., 2013).

6.2.2. T lymphocytes

T lymphocyte is the most vital and functional immune cell in acquired immunity. T lymphocyte has two subsets with different functions, $CD4^+$ and $CD8^+$ (Salgame et al., 1991). $CD4^+$ is a helper T cell that is important in assisting and inducing the immune process in the body, whereas $CD8^+$ has inhibitory effects. Therefore, preservation of the balance of $CD4^+$ and $CD8^+$ is vital to keep the immune system of the body normal (Jackute et al., 2015; Song et al., 2015). Liu et al. considered that APS regulated immunity by first inducing differentiation of T cells and then by activating T cells (Liu et al., 2011). Additionally, Li et al. showed that administration with 5–20 mg/kg of APS increased the number of $CD3^+CD4^+CD8^+$ memory T helper cells (Th) and $CD3^+CD4^-CD8^+$ cytotoxic T cells among PBL in four-week-old Yorkshire pigs (Li et al., 2011). By determining the percentages of peripheral blood $CD4^+$ cells, $CD8^+$ cells, $ANAE^+$ T lymphocytes, and $CD4^+/CD8^+$ ratio, a high dose of APS (200 mg/kg) up-regulated the cellular immunity in immunosuppressed dogs induced by dexamethasone (Qiu et al., 2010). Abuelsaad showed that APS enhanced $CD4^+/CD8^+$ T cell ratio, reduced reactive oxygen species (ROS) production, and down-modulated neutrophil activity in *Aeromonas hydrophila*-infected mice (Abuelsaad, 2014). A further study also proved that the effect of immune regulation of APS was parabolic. At APS concentration of 400 mg/L, the cell multiplication of static splenic lymphocytes of mice was the most pronounced (Guo et al., 2011).

Moreover, other monomer components of AR also have impacts on effective T lymphocyte regulation. Wang et al. demonstrated that AST IV **(7)** effectively improved the expression of antibody and increased the proliferation of T cells (Wang et al., 2002). Zhang et al. showed that 80 and 160 μg/mL of AST IV **(7)** enhanced the cytotoxic T lymphocyte (CTL) activity *in vitro*, whereas 40 mg/kg of AST IV down-regulated the activity of Treg in mice with lung cancer by oral administration and enhanced the direct killing effect in cellular immunity (Zhang et al., 2014). Huang et al. proved that 50 and 100 μg/mL of AST IV **(7)** decreased the activity of high mobility group box 1 protein (HMGB1) and down-regulated the shift of Th 2 to Th1 through increasing forkhead box protein 3 (Foxp3) expression in $CD4^+CD25^-$ Treg (Huang et al., 2012). AST I **(1)**, AST II **(3)**, AST III **(5)** and AST IV **(7)** increased the CD45-mediated hydrolysis with half maximal effective concentration (EC_{50}) values in the range of 3.33–10.42 μg/mL in a dose-dependent manner. AST II **(3)** (30 nmol/L) markedly promoted the expressions of CD25 and CD69 in primary $CD4^+$ T cells and up-regulated the mRNA expressions of T-bet and IFN-γ in primary splenocytes. AST II down-regulated the production of IL-2 and IFN-γ and the proliferation of splenic T cells in CTX-induced immunosuppressed mice (Wan et al., 2013). In addition, the flavonoids of AR stem and leaf have been corroborated to increase the T cell total count, elevate the lymphokine-activated killer cell (LAK) activity induced by recombinant interleukin-2 (rIL-2), increase the proliferation of lymphocytes induced by Con A, and regulate the disturbance of T cell subsets. These findings indicated that the flavonoids of AR stem and leaf also showed immune regulatory effects in immunosuppressive mice (Jiao et al., 2001).

6.3. Others

APS was also reported to alleviate LPS-induced immune stress in chickens mainly by reducing the gene transcription of TLR4 and NF-κB and improving energy and protein metabolism (Liu et al., 2015). Zheng et al. revealed that APS induced iNOS expression by regulating cytokines IL-6 and IL-1 (Zheng et al., 2013). Yuan et al. also found that APS regulated the expression of major immune genes in the kidney and head of carp (Yuan et al., 2008).

7. Pharmacological activities of AR in immunological diseases

7.1. Cancer

At present, few anti-cancer therapeutic agents can completely treat cancer. Some of them may even cause greater toxicity and side effects than curative effects. In order to achieve more acceptable and body-friendly treatment, contemporary medical research have begun to seek herbal medicines as adjuvants and alternative treatments for cancer. TCM has been demonstrated to exhibit great potential as an adjuvant because it reduces side effects and increases the sensitivity of a variety of first-line anti-cancer drugs. It also improves the survival probability and quality of life in patients (Konkimalla and Efferth, 2008). Xu and Chen showed that 125–625 mg/L of APS significantly inhibited the proliferation of Hepa cells in mice *in vitro*. This finding indicated that APS induced macrophages or splenocytes to produce TNF-α or INF-γ, so APS played an anti-cancer role *in vivo* by enhancing the immune function (Xu and Chen, 2005). Similarly, Yang et al. found that APS significantly improved the phagocytic function of macrophages in H22 tumor-bearing mice and increased the release of IFN-γ, TNF-α, and other cytokines. APS mainly improved the immune effect in tumor-carrying mice, thereby exhibiting anti-cancer effects (Yang et al., 2013a). Xiao et al. investigated the effect of APS on the cytokine level and tumor growth in tumor-bearing mice. APS increased the cytokine IL-2 level in the serum of tumor-carrying mice. Compared with the model group, APS obviously showed anti-cancer effects *in vivo* (Xiao et al., 2009). Li et al. revealed that APS down-regulated the growth and proliferation of Treg cells and reduced the secretion of TGF-β and IL-10 in the tumor microenvironment, which are the underlying mechanisms of its anti-cancer effect. APS also inhibited the growth and proliferation of $CD4^+$ $CD25^+$ Treg by inducing the expression of Foxp3 and re-building cytokine balance (Li et al., 2012). In addition, AR was found to enhance anti-cancer immunity, which was mainly related to TLR. For instance, Yin et al. found that APS possessed the effect on the treatment of bladder cancer. The main involved mechanisms were up-regulating the expression of TLR4 and enhancing the innate immune response in bladder epithelial cells, which were dominated by APS (Yin et al., 2010). Notably, Li et al. showed that although APS was failure to suppress the growth of MCF-7 cells, APS-activated RAW264.7 cells possessed a remarkable anti-cancer effect. The anti-cancer activity was evidenced by the regulation of apoptosis-related genes, including Bcl-2-associated X protein (Bax)/b-lymphocytoma-2 gene (Bcl-2), arrest of G1-phase cell cycle, and inhibition of cell proliferation (Li et al., 2019a), as shown in Fig. 2.

Therefore, the anti-cancer activity of AR is obviously related to the regulation of the levels of TLR, T cell subsets, TNF-α, IL-2, TGF-β, IFN-γ, and Foxp3. Cytokine IL-2 enhanced the proliferation of B, T, and NK cells and participated in the immune response and immune regulation. IL-2 improved the immunity of the body by promoting the killing activities of CTL, LAK, NK cells, and the generation of antibodies, and inducing the secretion of IFN-γ. It can be potentially used as a measure of the level of cellular immunity (Bream et al., 2003; Perussia et al., 1987). Li et al. also pointed out that the immunoregulatory effect of APS was mainly related to its structure α-9 (1→4) β-D-glucan. The existence of this structure significantly enhanced the immunoregulatory activity in cancer rats (Li et al., 2009a).

7.2. Virus

The anti-viral activity of AR is also an important bioactivity that has been comprehensively researched. Antiviral immunity has been confirmed to be closely related to CTL cells, $CD3^+CD4^+$, IL, IFN-γ, and NF-κB. Early studies suggested that AR induced a regulatory imbalance between T-helper cell and trihydrophobin 1 to up-regulate the production of IFN and enhance the efficacy against the sindbis, vesicular stomatitis, coxsackie, and influenza viruses (Song and Wu, 2001; You, 1993). Then, Zhang et al. showed that oral administration of APS significantly increased the humoral and cellular immune levels in FMDV-infected mice, including the enhancement of phagocytosis in peritoneal macrophages, promotion of the proliferation in splenic lymphocytes, and production of antibodies and cytokines, such as IL-4 and IL-10 (Zhang et al., 2010). In recent years, numerous studies were performed on the anti-viral activities of AR to discover new anti-viral mechanisms. Du et al. found that APS induced the proliferative activity of T cells, increased the level of hepatitis B surface antigen (HBsAg) antibody, improved the expression of IFN-γ in $CD8^+$ T cells, promoted $CD4^+$ T cells to produce IFN-γ, IL-2, and IL-4, and enhanced the activity of CTLs (Du et al., 2012). Xue et al. found that APS reduced porcine circovirus 2 (PCV2) replication by blocking NF-κB pathway and down-regulating oxidative stress. APS significantly reduced DNA replication, number of infected cells, malondialdehyde content, and ROS level of PCV2, thereby suggesting that APS has effect on preventing PCV2 infection (Xue et al., 2015). AR also had promising anti-avian influenza effects. For instance, Kallon et al. investigated the humoral immunization activity of APS against H9N2 avian influenza virus (H9N2 AIV) infection in chickens. Their results showed that the levels of IL-12, IL-10, IL-6, IL-4, lipopolysaccharide-induced tumor necrosis factor-alpha (LITAF), and antibody titers to H9N2 AIV increased after treatment with APS. APS significantly enhanced early humoral immune responses and reduced the replication of H9N2 AIV in the chicken model (Kallon et al., 2013). In addition, with the development of nanotechnology, AR derivatives have also been used to prepare nanoscale adjuvants. For example, Yakubogullari et al. combined AST VII and APS to prepare an adjuvant for the treatment of influenza A vaccine. The result showed that AST VII and APS combined as an adjuvant nanoparticle showed response towards Th1/Th2 balance and Th17 by producing IFN-c, IL-17A and IgG2a. Therefore, this nanoparticle is a good candidate as an adjuvants system to be utilized in treating influenza (Yakubogullari et al., 2019), as shown in Fig. 3.

7.3. Asthma

Asthma is a chronic inflammatory mucosal disease related to the overexpression of Th2 cytokines, eosinophilia, IgE, bronchial hyperresponsiveness, *etc.* (Lee et al., 2006). Chen et al. investigated the immunotherapeutic activity of AR in an airway murine asthma model induced by ovalbumin (OVA). After the oral administration of AR at 3 μg/kg, the IgE levels in serum, number of eosinophils, bronchoalveolar lavage fluid (BALF), and collagen deposition of lung sections were decreased. The RNA and protein expressions of Th2, as well as the ratio of the GATA3/T-bet mRNA and mRNA level of peroxisome proliferator-activated receptor γ (PPARγ) were declined. Those results indicated that AR possessed inhibitory effects on airway inflammation in the murine model of asthma by regulating the unbalance between Th2 and Th1 (Chen et al., 2014). Jin et al. reported that ARE (2.5, 5.0, and 10.0 g/kg) fed by oral administration evidently inhibited airway hyperresponsiveness to aerosolized methacholine in rats, thereby reducing the levels of IL-13, IL-5 and IL-4, inhibiting eosinophil counts, and increasing INF-γ levels in the BALF. AR also significantly down-regulated mucus secretion, collagen deposition and inflammatory infiltration in the lung tissues. AR enhanced the mRNA expression of $Foxp3^+$ and increased the population of $CD4^+CD25^+Foxp3^+$ Treg in the rat asthma model. The result indicated that the antiasthmatic activity of ARE was related to $CD4^+CD25^+Foxp3^+$ Treg (Jin et al., 2013). Yang et al. found that oral administration with ARE (0.5 g/kg) once a day for 28 days decreased the number of lymphocytes and eosinophils in BALF, goblet cell hyperplasia and attenuated the inflammation of lung, and airway hyperresponsiveness in OVA-induced asthmatic mice. ARE also reduced the expressions of major inducers of allergic Th2-associated cytokines, such as IL-4 and IL-5. Notably, ARE inhibited the expression of NF-κB and suppressed the translocation of NF-κB from the cytoplasm to the nucleus. Therefore, the mechanism of ARE in the treatment of asthma

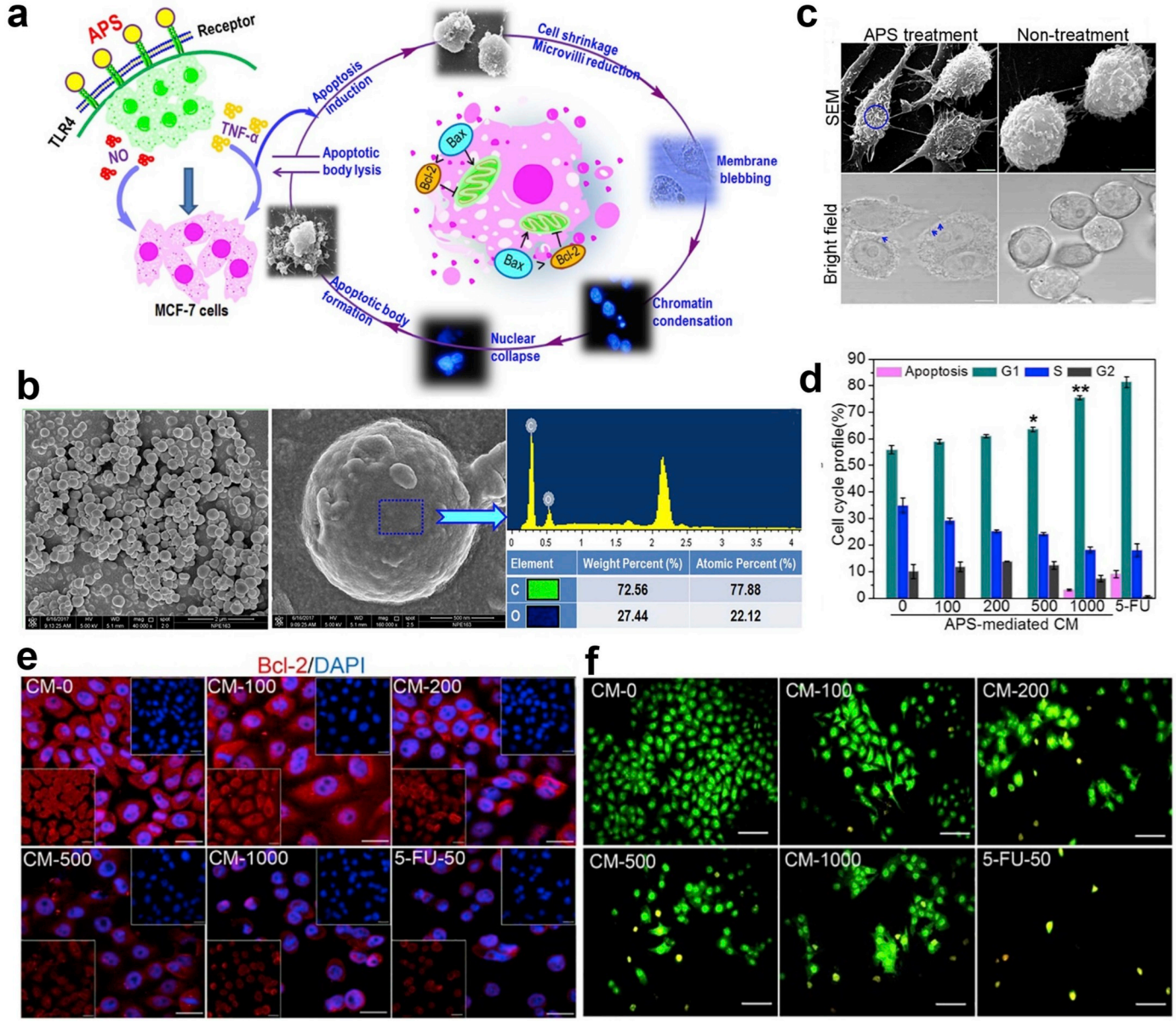

Fig. 2. APS showed a remarkable anti-cancer effect by activating macrophages. (a) Diagrammatic sketch of the apoptosis of MCF-7 cells caused by APS-activated macrophages. (b) Characterizations of morphology and chemical properties of APS. (c) Cellular morphology of RAW264.7 at P5 passage with or without the administration of APS under SEM and phase contrast microscopy. (d) Cell cycle profile of MCF-7 cells after exposure to conditioned medium for 48 h. The fraction of cells from apoptosis, G1, S and G2 phases were shown in as percentages. (e) Immunofluorescent pictures of Bcl-2/DAPI staining and their merge of MCF-7 cells after exposure to APS mediated conditioned medium and 5-FU, respectively. (f) APS mediated conditioned medium induced the apoptosis in MCF-7 cells. Figure reproduced with permission from (Li et al., 2019a). Copyright 2019, Elsevier.

was associated with the suppression of NF-κB pathway (Yang et al., 2013b). Du et al. showed that giving AST IV (7) (50 mg/kg) to BALB/c mice by gavage during each OVA challenge significantly reduced airway hyper-responsiveness, inflammation of eosinophilic airway, IL-13 and IL-4 levels, and the total IgE in serum. AST IV also significantly inhibited airway remodeling, including subepithelial fibrosis, smooth muscle hypertrophy, and goblet cell hyperplasia. AST IV also down-regulated the expression of TGF-β1 in lungs (Du et al., 2008). Clinical investigations also proved that AR had obvious anti-asthmatic effects. For instance, Wang et al. researched the influence of AR on Th1/Th2 equilibrium and T-box expressed in T cell expression. In their study, they found that the level of serum IL-4 was higher, but T-bet mRNA expression and IFN-γ level were lower in patients with asthma. After AR (60 μg/mL) intervention, the latter two parameters increased, whereas the IL-4 level decreased. After AR intervention, the abnormal increase of $CD4^+CCR3^+$ cells in patients was down-regulated, whereas the low level of $CD4^+CCR5^+$ cells was rectified. The abnormal change in the two indexes revealed a reversed trend of Th2 polarization. This finding indicated that AR increased the expression of T-bet mRNA and Th1 cytokines and reversed the predominant status of Th2 in patients (Wang et al., 2006).

AR also has been utilized by combining with other therapeutic agents to treat asthma. For instance, Qu et al. revealed that ARE combined with budesonide inhibited allergen-induced goblet cell hyperplasia and mucous gland hypertrophy, thereby increasing the thickness of bronchial airway and collagen deposition. Besides, TGF-β1 mRNA, TGF-β1, and drosophila mothers against decapentaplegic protein (Smad) 2/3 levels of lung tissue were obviously inhibited in mice treated with the drug combination. This finding revealed that ARE and budesonide inhibited the TGF-β1/Smads signaling pathway, which was

the main mechanism for the treatment of severe asthma airway (Qu et al., 2012). Yuan et al. found that the compatibility of AR and Angelicae Sinensis Radix inhibited the expression of IL-4 in nasal mucosa, lung tissue, and serum, and enhanced the expression of IFN-γ in lung tissue and serum. AR and Angelicae Sinensis Radix controlled airway allergic inflammation by keeping the balance between Th1 and Th2 (Yuan et al., 2012).

7.4. Radiation

Liu et al. investigated the protective activity of APS against ionizing radiation (IR) injury in liver. In their research, a radiation-induced oxidative stress mouse model was used. Before 60Co γ-irradiation, three groups of mice were orally administrated with 50, 100, and 200 mg/kg of APS once a day for 7 days separately. After irradiation, the APS-treated mice showed a significant reduction in lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase levels, as well as in the expression of NF-κB. IR induced the increase in thiobarbituric acid reactive substance, and the decrease in superoxide dismutase (SOD) were inhibited. Moreover, catalase and glutathione activities were down-regulated in APS-treated mice. The result demonstrated that administration of APS at 200 mg/kg/day significantly reduced the features of IR-induced injury of hepatic and pulmonary cells in mice. Therefore, APS effectively treated IR-induced damage of the liver in mice, and the mechanism was related to the inhibition of the radiation-induced oxidative stress (Liu et al., 2014b). Besides, after treating with ARE injection, the antibody response to the T-dependent antigen was promoted in radiation-treated mice. ARE injection improved the immunity and antibody of radiation-treated mice by activating Th cells (Zhao et al., 1990).

7.5. Toxoplasmosis

Approximately one-third of the people in the world are infected with *Toxoplasma gondii*. Although most infected people exhibit no or mild symptoms, people with low immune capacity, such as acquired immunodeficiency syndrome (AIDS) patients, pregnant women and others, infected with toxoplasma have serious consequences (Chahed Bel-Ochi et al., 2013). At present, no ideal drug exists to prevent and control toxoplasmosis. The effect of vaccine is not ideal, too. Yang et al. found that compared with the control group, ARE obviously inhibited intracellular replication of the parasite at 72, 96, and 120 h after administration. It demonstrated that ARE had remarkable activities against *T. gondii in vitro* (Yang et al., 2012). Yang et al. used APS (75 mg/kg/d) to treat toxoplasma mouse models and found that protective immunity significantly enhanced, survival time markedly increased, and liver histopathological score obviously decreased (Yang et al., 2010b).

7.6. Auxiliary treatment of autoimmune diseases

7.6.1. Diabetes mellitus type 1 (T1DM)

T1DM is mediated by effector T cells, which are activated by autoantigen. Such mediation resulted in the deficiency of insulin and destruction of pancreatic islets. Currently, the available therapeutic drugs for T1DM are insulin and various oral antidiabetic agents, including rosiglitazone, metformin, and sulfonylureas. However, the long-term treatment with conventional immunosuppressive agents can induce obvious side effects. Therefore, discovering some alternative therapeutic agents is urgently-needed (Aloisi, 2001). Zhou et al. investigated the immunopharmacological function of APS in multiple low dose streptozotocin (MLD-STZ)-induced T1DM and showed that galectin-1 expression was up-regulated in the serum of APS-treated experimental objects compared with the diabetic mouse model. The percentage of apoptotic $CD8^+$ T cells in spleens was correlated with the concentration of APS. Notably, the repression of galectin-1 by specific antibody inhibited the apoptosis of $CD8^+$ T cells *in vivo*. This finding suggested that APS enhanced the galectin-1 expression in the muscle of T1DM mice to accelerate the apoptosis of $CD8^+$ T cells (Zhou et al., 2011). Li et al. reported that administration with APS (100, 200, and 400 mg/kg) preserved β cells from apoptosis and attenuated insulitis in MLD-STZ-induced T1DM mice. Researches supposed that APS implemented immune regulation function by regulating the ratio of Th1/Th2 and was closely associated with the expression of PPARγ gene in the spleens (Li et al., 2007b). Moreover, Wang et al. found that the Chinese patent medicine named Shengqi granule (the main components are ARE and the extract of Ginseng Radix et Rhizoma) ameliorated T1DM symptoms in autoimmune non-obese diabetes (NOD) mice by raising the level of insulin and lowering blood glucose level. They demonstrated that Shengqi granule improved T1DM symptoms by up-regulating both $CD8^+CD122^+PD1^+$ and $CD4^+Foxp3^+$ Tregs. Moreover, Shengqi granule combined with cyclosporin A (CsA) inhibited T1DM by reversing the decrease of $CD4^+Foxp3^+$ Tregs that resulted from CsA administration (Wang et al., 2017).

7.6.2. Inflammatory bowel disease (IBD)

AR has obvious therapeutic potential in ameliorating antigen-induced colitis. Its specific effective components and activity mechanisms have been gradually investigated in recent years. Yang et al. revealed that APS markedly ameliorated 2,4,6-trinitrobenzenesulfonic acid solution (TNBS)-induced experimental colitis in rats by promoting the expressions of IL-1β and TNF-α, and nuclear factor of activated T-cells, cytoplasmic 4 (NFATc4) mRNA level. Therefore, APS was considered as the main effective component in AR to improve colitis (Yang et al., 2014). Lv et al. believed that APS treatment improved colitis histological scores and disease activity index, improved body weight and colon length, inhibited myeloperoxidase (MPO) activity and the levels of IL-17, IL-6, IL-1β, and TNF-α, and reduced NF-κB DNA phosphorylation activity (Lv et al., 2017). Liu et al. investigated the activity of APS on the expression of small intestinal mucosal lymphocyte factors in mice. APS suppressed the proliferation of induced cells and effector cells, reduced the expression of inflammatory factors, and increased the level of TGF-β *in vitro*. TGF-β, which was produced by T cells and macrophages, inhibited lymphocyte proliferation and promoted tissue repair. Therefore, APS promoted tissue repair by attenuating the lymphocyte activity and inflammatory factor expression in the mucosa to cure IBD (Liu et al., 2008). Similarly, Zhao et al. showed that after being treated with APS for 7 days, the levels of IL-23, IL-17, IL-6, IL-2, and related orphan receptor-γt (ROR-γt) in the colonic tissues decreased, whereas Treg in Peyer's patches, signal transducer and activator of transcription 5a (STAT5a) and TGF-β levels in the colonic tissues were increased (Zhao et al., 2016). Gao et al. found that both TNBS-induced histological colonic damage and macroscopic lesion were ameliorated by APS, which was accompanied by the reduced activity of MPO. TNBS enhanced the T-bet and GATA-3 levels. Nevertheless, the expression of GATA-3 was weaker than that of T-bet, thereby leading to an obvious decrease in the ratio of GATA-3/T-bet. APS increased the levels of GATA-3 and T-bet compared with the model group. APS up-regulated the expressions of Th1 and Th2 and favored a shift towards Th2 (Gao et al., 2016).

7.6.3. Multiple sclerosis (MS)

MS is a chronic autoimmune neuroinflammatory disease, and the young is the main susceptible population. Neuronal apoptosis induced by oxidative stress plays a major role in the pathogenesis of this disease. He et al. found that giving 20 mg/kg of AST IV (7) to mice daily obviously ameliorated the experimental autoimmune encephalomyelitis. They found that AST IV improved MS by alleviating oxidative stress, reducing the level of cellular ROS, enhancing antioxidant defense system, increasing anti-inflammatory and anti-apoptotic pathways, and modulating the differentiation of T cells and infiltration into the CNS (He et al., 2013). Another investigation demonstrated that the total

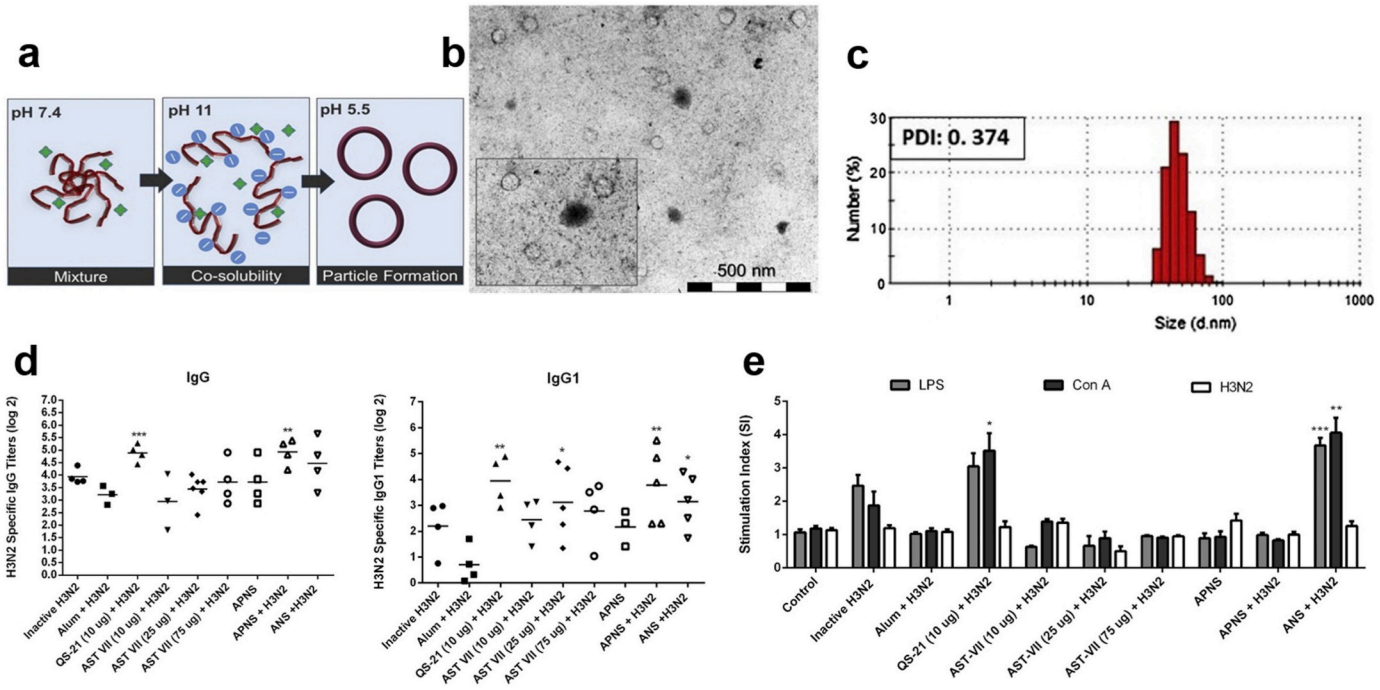

Fig. 3. (a) Diagrammatic sketch of AR nanocarrier system preparation through electrostatic interaction caused by pH change. (b) The TEM image of AR nanocarrier system. (c) The PDI value and particle size distribution of AR nanocarriers system. (d) The effects of adjuvanted or non-adjuvanted H3N2 vaccine on the production of H3N2 specific IgG and IgG1. (e) Stimulation indexes of Con A, LPS and H3N2 re-stimulated spleen cells obtained from mice immunized with formulated with adjuvants or H3N2 alone. Figure reproduced with permission from (Yakubogullari et al., 2019). Copyright 2019, Elsevier.

ASTs inhibited ROS production and lessened the infiltration of inflammatory cells. ASTs enhanced the mRNA expressions of Foxp3 and T-bet, and down-regulated RORγt to modulate T cell differentiation in peripheral immune systems. ASTs reduced neuroinflammation by reducing iNOS expression and other cytokine levels and inhibited ROS production by increasing the total superoxide dismutase (T-SOD) expression in the CNS. In addition, ASTs reduced p53 production and phosphorylation of tau by regulating the ratio of Bcl-2/Bax. ASTs arranged multiple means, such as anti-oxidative stress, anti-neuroinflammation, and immunoregulation, to prevent the aggravation of MS (He et al., 2014).

8. Safety

AR is an edible herb that can be used as medicine and food. It has been recorded in the dietary supplement catalogue list of China and the United States. The toxicity of this herb *in vivo* is extremely low, and no clinical side effects and serious adverse events have been reported. Allergic reactions of AR, including skin allergies, gastrointestinal reactions, *etc.*, have rarely been reported. In acute and subacute toxicity experiments, Sprague-Dawley rats (5.7–399.9 g/kg) and beagle dogs (2.855–39.9 g/kg) were orally administrated with ARE (main components were APS, AS, *etc.*) for three months. The result showed that ARE had no histopathological or toxicological effects on the body weight, organs, appetite, behavior, hematology, blood biochemistry and urine routine in rats and beagle dogs (Yu et al., 2007). Song et al. investigated the safety of HT042 which consisted of three standardized extracts from AR, *Phlomis umbrosa* root, and *Eleutherococcus senticosus* stem. The experiment result showed that the oral approximate lethal doses of HT042 and each of the herbs were > 5000 mg/kg (Song et al., 2017). Szabo assessed subchronic toxicity and genotoxic potential of cycloastragenol, which is a bioactive triterpene aglycone from AR and has obvious potential to be developed as a modern dietary ingredient. The result showed that the oral safe dose (no-observed-adverse-effect) for cycloastragenol was > 150 mg/kg/d in rats. Besides, ≤5000 μg/plate of cycloastragenol did not trigger transgenation in *Salmonella typhimurium* or *Escherichia coli* tester strains. Moreover, micronucleus assay showed that no clastogenicity appeared in peripheral erythrocytes administered 2000 mg/kg of cycloastragenol by intraperitoneal injection (Szabo, 2014). In addition, other researches also have proved that AR was a safe and effective herb in other therapeutic applications such as cancer (Zhang et al., 2017a), seasonal allergic rhinitis (Matkovic et al., 2010), and diabetic kidney disease (Zhang et al., 2019).

9. Product development

At present, the number of immunization-related patents of AR has reached 5,485 worldwide of which China has the largest number of patents, accounting for more than 3/4 of the total. The details of the patents related to the immune regulation of AR are shown in Fig. 4. At present, more than 100 kinds of prescription preparations with AR are considered as main medicines in China and Japan. Among them, two kinds of AR prescription preparations are recorded in Chinese Pharmacopoeia, namely AR Invigorating Stomach Ointment and AR Granules. Six AR patented medicines are recorded in the National Standard of Ministry of Health, including AR Granules, AR Injection, AR Essence, AR Shengmai Yin, AR Shengmai Granules, and AR Jianzhong Pills. In addition, six kinds of medicines are in the standards of China Food and Drug Administration (CFDA), including APS and APS injection. AR products are mainly composed of water extracts, polysaccharides, and other active fractions. The main dosage forms include granules, injections, pills, and oral liquids. The main effective components of AR are extracted by water or water extraction and alcohol precipitation method. After concentration, the finished products are prepared by adding excipients or by low temperature drying. Furthermore, AR is developed for the application in health care products and beverages. According to the CFDA database, 1,052 health foods contain AR or ARE in the Chinese market. They are mainly used for the daily health care in the elderly, sub-healthy people, or people with low immunity (CFDA, 2019).

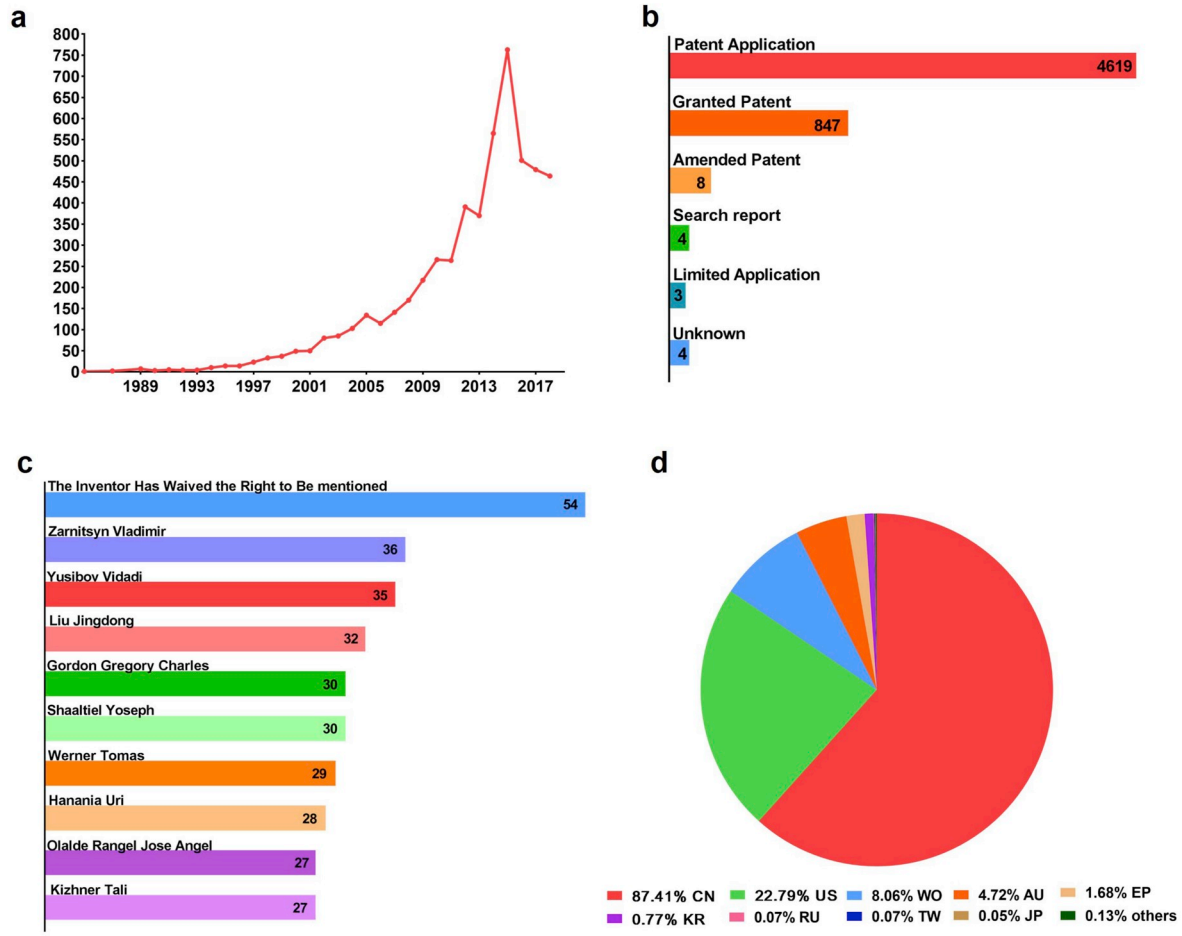

Fig. 4. Analysis of 5,485 patents browsed on LENS. ORG using search terms “Astragalus” and “immune”. (a) Patent applications per year; (b) Document type. (c) Inventors of the patents. (d) Jurisdictions (CN, China; US, United States; WO, World Intellectual property organization; AU, Australia; EP, European Patent Office; KR, South Korea; RU, Russia; TW, Taiwan; JP, Japan).

10. Conclusion and perspectives

As a promising edible immunomodulatory herb medicine, AR is widely used as a tonic dietary supplement. Generally, it is prepared as decoction for oral use in clinical applications with a general dosage ranging from 10 to 240 g/d. Different doses of AR are selected according to different medical purpose and diseases. The commonly used dosage for strengthening immunity is about 20 to 100 g/d (Wang et al., 2018a). In recent years, numerous developed patent medicines, including granules, capsules, injections, are used in clinic. The present review mainly summarized the ethnopharmacological immunomodulatory practices, immunomodulatory constituents, pharmacological activities on the immune system, and pharmacological activities on the immunological diseases of AR (Fig. 5). According to the research evidence that we have gathered, the immunomodulatory mechanisms of AR are briefly summarized below. i) AR can promote the development of immune organs in the growth and development stages to ensure that immune cells have an ideal location for expression, differentiation and maturation. ii) AR can increase the level of SIgA, thereby enhancing mucosal immune function. iii) AR can increase the quantity and phagocytic capacity of innate immunity cells to strengthen the ability in removing exogenous pathogens and endogenous aging, death, and cancer cells. iv) AR also promotes the maturation and differentiation of acquired immunity cells to exert a better specific immune effect. v) AR can effectively improve the expression of antibodies in acquired immunity, thus significantly eliminating antigens.

The immunomodulatory effects of AR are mainly attributed to its active constituents including APS, AF and AS, which showed distinct immunomodulatory features. Specifically, APS is a relatively systematic immunomodulator that involves immune organs, mucosal immunity, innate immunity and acquired immunity (Jin et al., 2014), while AF has a disposition to regulate innate immunity by enhancing the macrophage phagocytic index and affecting the immune activity of DCs (Guo et al., 2016; Li et al., 2014). Besides, AS tends to invigorate acquired immunity mainly by enhancing the direct killing effect of T lymphocytes and promoting the expression of CD25 and CD69 on primary $CD4^+$ T cells (Wan et al., 2013; Zhang et al., 2014). These distinctions indicate that in the clinical application and modern drug development of AR, the application of related components should be corresponding to different types of pathological changes. For instance, in the medical intervention of the systematic immune diseases, such as cancer, rheumatoid arthritis, systemic lupus erythematosus, *etc.*, APS and its developmental agents should be primarily utilized due to the complexity of these diseases. To improve immunity in the clinical or develop immunomodulation supplements, therefore it is reasonable to employ the application of AF due to innate immunity is the early defense of the immune system. After the body infected by pathogens, such as viruses or bacteria, and enter into the acquired immunity stage, the proportion of AS in the therapeutic agent should be increased. Expectedly, the rational use of different active ingredients of AR can effectively promote the clinical efficacy for the treatment of immune related pathological changes and improve the quality of developed drugs and dietary supplements of this plant.

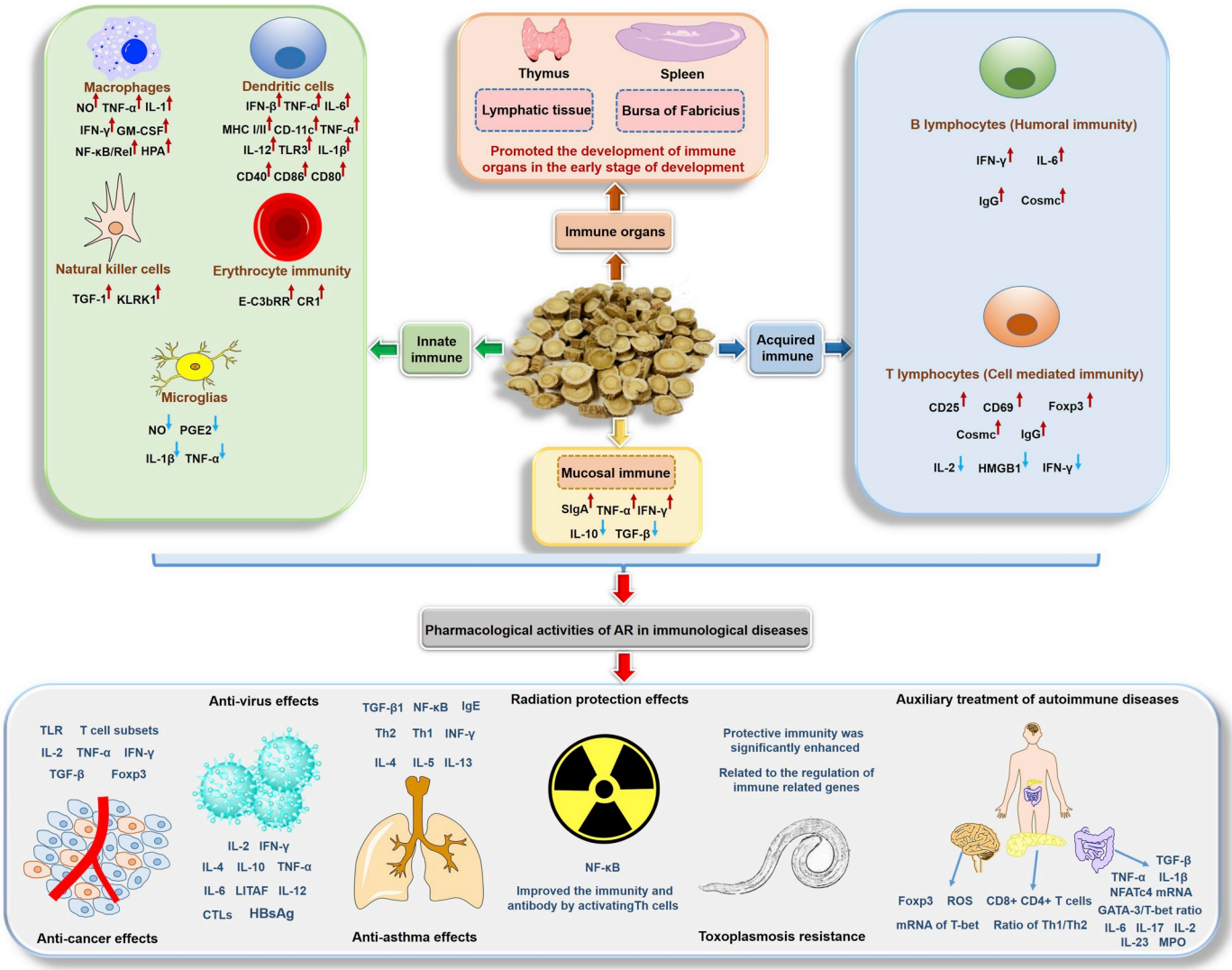

Fig. 5. AR modulates the immune system and immunological diseases.

Undoubtedly, AR exhibits various biological activities, such as anti-cancer, anti-virus, anti-asthma, radiation protection, toxoplasmosis resistance, and auxiliary treatment of autoimmune diseases activities. Growing evidence indicates that AR affects the immune system at organ, cellular, and even genetic levels. With the development of ethnic medicine in treating and preventing diseases, AR is regarded as one of the most prospective candidates for dietary supplements and modern drugs (Liu et al., 2017b). However, significant obstacles exist between fundamental research and extensive applications of AR in modern medicine. At present, some gaps still exist in terms of basic research on the immune regulation of AR. Several problems in the pharmacological study of AR remain unsolved. Macrophage is a momentous part of non-specific immunity. However, recent research of AR only focused on enhancing the number and phagocytic capacity of macrophage, without further exploring the mode of immunomodulatory action of AR in macrophage. Besides, NK cells play an important role in anti-cancer and anti-immunological diseases. Studies proved that AR can improve the production of IL-2 to promote the activity of NK cells. However, the researchers pay less attention to the direct effect of AR on NK cells. For the overall situation of the basic study of this plant, the main problem is the coexistence of the complexity of AR material base and the systematicness of immune system, which leads to the trend of fragmentation in the study of immune mechanisms of AR. Besides, the progress in systematic screening and evaluation of immune active ingredients is still limited. Most of the studies on the immunomodulatory effect of AR still focus on APS and single cytokine level, lacking systematic and in-depth exploration of the basis of immunoregulatory substances of AR. Moreover, the development and utilization of AR and its extracts are still insufficient. The effective substances extracted from herbal medicine can become more excellent after a certain preparation processing. For example, Yu et al. made a great attempt to combine APS with gels to achieve sustained release to regulate the immunity (Yu et al., 2017). Therefore, future research should be focused on the dosage forms by utilizing and developing polysaccharides, flavonoids, and saponins in AR, such as nano-preparations. For example, Yakubogullari et al. combined AST VII with APS to prepare nano-preparations as an adjuvant of influenza vaccine and achieved significant immunomodulatory and antiviral effects (Yakubogullari et al., 2019). Although AR and its extract have a positive effect on several autoimmune diseases, using AR alone is not recommended for the treatment of autoimmune diseases because of the complexity and uncertainty of the pathogenesis of these diseases. However, AR can be utilized as an adjuvant agent in combination with modern medical means to improve the quality of life of patients effectively. Other researchers can further investigate the combination of AR with biological or chemical drugs for the treatment of autoimmune diseases. Although AR has been proven to

be reliably safe, these safety evaluations were based on the experimental results of oral administration (Yu et al., 2007). At present, AR injectable products are certain to attract much attention and in the early development. We cannot ensure whether the effective substance of AR administered by injection is safe for patients, and injection safety is an important issue that must be investigated further by researchers. By solving the aforementioned deficiencies, we can better explore and utilize AR in the field of basic research, development of clinical drugs and health products systematically and scientifically in the near future.

Authors' contributions

Z.C., S.W. and Y.W. designed the study. Z.C. and L.L. collected the information and drafted the manuscript; C.G., W.C., C.T.V., P.Y., Y.Y., X.L., and X.T. revised the manuscript; S.W. and Y.W. supervised and revised the manuscript. All authors approved the final submitted version of the manuscript.

Declaration of competing interest

The authors have declared no conflict of interest.

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Abbreviations

AF	astragalus flavonoids
AIDS	acquired immunodeficiency syndrome
AMPK	5' AMP-activated protein kinase
APS	astragalus polysaccharide
AR	Astragali Radix
ARE	Astragali Radix extract
AS	astragalus saponins
AST	astragaloside
BALF	bronchoalveolar lavage fluid
Bax	Bcl-2-associated X protein
Bcl-2	b-lymphocytoma-2 gene
BM	bone marrow
BSA	bovine serum albumin
CFDA	China Food and Drug Administration
CFU	colony-forming units
CHM	Chinese Herbal Medicine
CNS	central nervous system
Con A	concanavalin A
Cosmc	core I β3-Gal-T-specific molecular chaperone
COX-2	cyclooxygenase-2
CR1	complement receptor 1
CsA	cyclosporin A
CTL	cytotoxic T lymphocyte
CTX	cyclophosphamide
DC	dendritic cell
EC50	half maximal effective concentration
E-C3bRR	erythrocyte-C3b receptor rosette rate
E-ICRR	erythrocyte-C3b immune complex rosette rate
EphB4	Ephrin type-B receptor 4
ERER	erythrocyte rosette forming enhancing rate
FasL	Fas and Fas ligand
FMD	foot-and-mouth disease
FMDV	foot-and-mouth disease virus
Foxp3	forkhead box protein 3
GM-CSF	granulocyte-macrophage colony stimulating factor
GPx	glutathione peroxidase
GrB	granzyme B
HBsAg	hepatitis B surface antigen
HMGB1	high mobility group box 1 protein
H9N2 AIV	H9N2 avian influenza virus
HPA	heparanase
IBD	inflammatory bowel diseases
IFN	interferon
Ig	immunoglobulin
IgAN	immunoglobulin A nephropathy
IL	interleukin
iNOS	inducible nitric oxide synthase
IR	ionizing radiation
Klf	Kruppel like factor
KLRK1	killer cell lectin-like receptor K1
LAK	lymphokine-activated killer cell
LITAF	lipopolysaccharide-induced tumor necrosis factor-alpha
LMO2	LIM domain only 2
LPS	lipopolysaccharide
MaxEnt	maximum entropy
MHC	major histocompatibility complex
MLD-STZ	multiple low dose streptozotocin
MPO	myeloperoxidase
MS	multiple sclerosis
MyD88	myeloid differentiation primary response gene 88
NFATc4	nuclear factor of activated T-cells, cytoplasmic 4
NF-κB	nuclear factor kappa-B
NK cells	natural killer cells
NO	nitric oxide
NOD	non-obese diabetes
OM	oxymatrine
OTC	over-the-counter drug
OVA	ovalbumin
PBL	peripheral blood lymphocytes
PCV2	porcine circovirus 2
PGE2	prostaglandin E2
PKB	protein kinase B
PPARγ	peroxisome proliferator-activated receptor γ
rIL-2	recombinant interleukin-2
ROR-γt	related orphan receptor-γt
ROS	reactive oxygen species
Runx1	runt-related transcription factor 1
sEPS	sulfated epimedium polysaccharide
SIgA	secretory immunoglobulin A
SIRT-1	Sirtuin 1
Smad	drosophila mothers against decapentaplegic protein
SOD	superoxide dismutase
SP1	specificity protein 1
STAT5a	signal transducer and activator of transcription 5a
T1DM	type 1 diabetes mellitus
TCM	Traditional Chinese Medicine
TGF	transformed growth factor
Th	helper T cell
TLR	toll-like receptor
TNBS	2,4,6-trinitrobenzenesulfonic acid solution
TNF-α	tumor necrosis factor-alpha
Treg	regulatory T cell
T-SOD	total superoxide dismutase

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