RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2022.051

ScienceAsia 48 (2022): **340–347**

Extraction and evaluation of biological activities of a polysaccharide from *Allium mongolicum* Regel

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Received 22 Jan 2021, Accepted 10 Dec 2021
Available online 28 Feb 2022

ABSTRACT: *Allium mongolicum* Regel polysaccharide (AMRP) acts as a main active substance of *A. mongolicum* Regel, but its structure and function are not fully understood. In this study, the AMRP fraction, AMRP-2 consisting of GalA, Rha, Glc, and Gal, was isolated and characterized. The antibacterial activity of AMRP-2 evaluated with the cup-plate method had a weak inhibitory effect against *Staphylococcus aureus*, *Staphylococcus albus*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*, but *Escherichia coli* and *Shigella dysenteriae* were relatively sensitive to AMRP-2. Furthermore, the immunoregulatory effects of AMRP-2 were evaluated both *in vitro* and *in vivo*. The results showed that after intragastric administration of AMRP-2, the proliferation of splenic T cells and the phagocytosis of peritoneal macrophages in mice were significantly enhanced, and the interleukin-2 (IL-2) and tumour necrosis factors α (TNF-α) levels in serum also increased sharply compared to the control group. These *in vivo* results were consistent with those of the *in vitro* study, i.e., splenic T cells proliferated dramatically after AMRP-2 treatment, and the secretion of IL-2 from mouse splenic T cells and TNF-α from peritoneal macrophages increased significantly. These findings indicated that AMRP-2 is an important immune modulator that has potential as an immunotherapy drug.

KEYWORDS: *Allium mongolicum* Regel, polysaccharide, antibacterial, immunomodulatory

INTRODUCTION

Allium mongolicum Regel (AMR) is a type of perennial xerophytic herb that is mainly distributed in desert regions, low mountains, droughty hillsides, and the grasslands of northwest China and Inner Mongolia [1]. In these regions, AMR plays a vital role in preventing wind and fastening sand. Additionally, this plant acts as a nutritious forage crop, a valuable medicine and a characteristic healthy vegetable with high palatability and a unique flavour [2]. AMR contains various active ingredients such as protein, fat, flavonoids, polysaccharides, and minerals [3–5]. It has been reported that dietary flavonoids extracted from AMR improve immunity, induce antioxidant effects, and promote growth in juvenile northern snakehead fish, and flavonoids could also promote growth as well as growth-related hormone expression in meat sheep [6]. Moreover, AMR extracts were confirmed to increase the average daily gain, feed intake capacity, and feed-in remuneration of broiler chickens [7].

AMR has been recorded to reduce blood pressure and blood lipids and to treat patients with renal disease. It has been reported that AMR ethanol extracts have immunological and antioxidant activities to animals [8]. Wang et al found that AMR aqueous extract scavenged free radicals and inhibited the activity of lipase and angiotensin-converting enzymes, which may be helpful for obesity and hypertension treatment. Xiao et al [9] found that ethanol extracts of AMR lowered blood lipid levels in hyperlipidaemic mice. However, most studies on AMR are concerned about the activity of ethanol extracts, which mainly include phenolics and flavonoids, whereas the specific structures of the active ingredients of AMR remain unknown. In the present study, polysaccharides, one of the most important components of AMR, were investigated [10]. First, the AMRP was extracted, separated, and purified, and then the structure was identified. Finally, the biological activities were explored both *in vitro* and *in vivo*. The possible mechanisms of action were also discussed.

MATERIALS AND METHODS

Materials and chemicals

Fresh AMR was collected from Erdos in Inner Mongolia, China, in June and July. Sephadex G-100 and dextran standards (T-10, T-40, T-70, T-110, and T-2000) were purchased from Pharmacia (Uppsala, Sweden). L-Rhamnose, D-glucose, D-xylose, L-arabinose, D-arabinose, D-mannose, D-galactose, D-galacturonic acid, D-glucuronic acid, and D-fructose were obtained from Shanghai Bioengineering Co. (Shanghai, China). Bovine serum albumin (BSA) and MTT were purchased from Sigma (MO, USA). RPMI 1640 medium and foetal bovine serum were obtained from Gibco (NY, USA). Lymphocyte separation medium was obtained from

TBD (Tianjin, China).

AMRP extraction

AMR was washed thoroughly and dried at 65 °C, and the polysaccharides were extracted with hot water. Briefly, the dried sample was ground into powder and treated with 98% aqueous ethanol under reflux for 12 h to remove the lipids and other hydrophobic compounds. After drying at 65 °C, the polysaccharides were extracted 3 times with distilled water (1:20 w/v) for 8 h each time at 80 °C. The supernatants were combined and concentrated to one-third of the original volume with a vacuum rotary evaporator (RE52CS, Shanghai YaRong Equipment company, China). The pigment was removed with 1% active carbon at 80 °C for 40 min. The free proteins present in the polysaccharides were precipitated by the Sevag [10] reagent (chloroform-n-butanol, v/v = 3:1) for 30 min, which was discarded after centrifugation. An equal volume of absolute alcohol was added to the supernatant to precipitate the polysaccharides of *A. mongolicum* Regel. After storage at 4 °C for 12 h, the crude polysaccharides were collected by centrifugation (4000 rpm, 30 min) and were obtained by vacuum drying and named AMRP.

Chromatography

The AMRP was dissolved in distilled water to a final concentration of 20 mg/ml. After centrifugation at 10 000 rpm for 5 min, the supernatant was loaded onto a Sephadex G-100 column (2.6 × 50 cm) for purification. The column was eluted with distilled water at a flow rate of 0.5 ml/min. The eluate was collected (3 ml per tube), and each fraction was assayed for the distribution of total sugars. The homogeneous fraction was collected, concentrated, lyophilized, and named AMRP-2.

General methods of chemical property analysis

Total carbohydrate content was evaluated by the phenol-sulphuric acid method using glucose as a standard [11]. Protein content was determined by the Bradford assay using BSA as a standard [12].

Homogeneity and molecular weight determination

The homogeneity and molecular weight of AMRP-2 were assessed by a Sephadex G-100 column (2.6 × 50 cm), and the column was eluted with distilled water at a flow rate of 0.5 ml/min. Different molecular weight dextrans were used (Dextran T-10, T-40, T-70, T-110, and T-2000). The molecular weight of AMRP-2 was calculated by the calibration curve constructed from the standard dextrans [13].

Sugar composition analysis

Ten milligrams of AMRP-2 were hydrolysed with 2 ml of HCl (6 mmol/ml) at 75 °C for 3 h, and the monosaccharide composition was determined by using high-performance liquid chromatography (HPLC) as described by Zhang et al [14].

Infrared spectral analysis

Ten milligrams of AMRP-2 were ground with 400 mg of KBr powder and pressed into pellets for Fourier Transform Infrared (FT-IR) measurement in the range of 1000 cm^{-1}– 400 cm^{-1} to detect functional groups.

NMR spectra analysis

For NMR analysis, AMRP-2 (20 mg) was dissolved in 0.5 ml D_2O (99.8%) and centrifuged to remove any undissolved AMRP-2. NMR spectra were recorded (15 000 scans) on a Bruker AVANCE III spectrometer (500-MHz, Bruker, Switzerland) at 20 °C. All date were processed and analysed using standard Bruker software.

Antimicrobial activity

The antibacterial potential of AMRP-2 was evaluated by the cup-plate method [15]. Amoxicillin was used as a standard. The microorganisms used in this trial were *E. coli* (ATCC44101), *S. aureus* (ATCC26003), *S. dysenteriae* (ATCC51285), *P. aeruginosa* (ATCC10102), *S. albus* (ATCC26101), and *P. vulgaris* (ATCC49001), which were purchased from the American Type Culture Collection (ATCC, USA). A sterile borer was used to make 3 cups in the agar medium cultured with microorganisms, and then AMRP-2 was added to each cup for a final quantity of 4 mg/cup with 5 μg/cup Amoxicillin as a control. All plates were kept at 4 °C for 1 h to enable the drug to fully diffuse into the medium, and then the plates were incubated at 37 °C for 24 h. The inhibition zone was evaluated as sensitive (S), intermediate (I), or resistant (R). The experiment was repeated 3 times, and the diameter of each inhibition zone was measured and recorded.

Animals

An equal number of healthy male and female Kunming mice, aged 6 weeks, weighing 20 ± 2 g, were provided by the Pharmacology Experimental Center of Inner Mongolia Medical University [approval number: SCXK (Inner Mongolia) 2002-0001, Hohhot, China]. The animal experiments have been approved by the Ethics Committee of Inner Mongolia Medical University. The mice were housed ad libitum with standard conditions at 25 ± 1 °C on a 12/12 h light/dark cycle. After 2 days of adaptive feed, mice were randomly divided into a control group (normal mice group) and test groups to receive treatment with different concentrations of AMRP-2. AMRP-2 was dissolved in distilled water and intragastrically administered daily (1.25 mg/kg/d, 12.5 mg/kg/d, and 125 mg/kg/d) for 35 consecutive days. The control group was given only distilled water. On the 36th day, the mice were

sacrificed. The spleens were extirpated, peritoneal macrophages were separated, and serum was isolated to determine the activities of the splenic lymphocytes and peritoneal macrophages and the levels of IL-2 and TNF-α in serum. For the *in vitro* assay, healthy mice were sacrificed. Splenic lymphocytes were isolated and treated with AMRP-2 (20 μg/ml, 50 μg/ml, or 100 μg/ml) for 72 h. T cell proliferation activity and IL-2 expression levels in lymphocyte cells were determined. Moreover, the level of TNF-α expressed in peritoneal macrophages was also studied.

Lymphocyte proliferation assay

Spleens aseptically extirpated from the mice were minced in D-Hanks solution and then filtered through a fine steel mesh. After centrifugation at 800 g for 10 min, the supernatant was discarded, and the sediment was resuspended in Lymphocyte Separation Medium to isolate the lymphocytes by centrifugation [16]. In a 96-well plate, the lymphocytes were seeded at a density of 5×10^6 per well and cultured in RPMI 1640 complete medium containing 5 μg/ml ConA [17, 18] for 44 h at 37 °C. MTT (100 μg/ml) was added to each well for an additional 4 h. After aspirating the supernatant, DMSO was added to dissolve the formazan crystals. The absorbance at 570 nm was measured using a Bio-Rad microplate reader (Benchmark Plus™, USA).

Macrophage phagocytosis assay

Peritoneal macrophage phagocytosis was evaluated as previously described [19]. Briefly, 24 h after the final administration of AMRP-2, mice were intraperitoneally injected with 1 ml of 0.5% chicken red blood cells (CRBCs). Thirty minutes later, the mice were sacrificed, and peritoneal macrophages were obtained. After staining with Wright-Giemsa stain, macrophages engulfing CRBCs were observed under a microscope, and the phagocytic rate (PR) was calculated as 100 × the number of macrophages containing CRBCs/the total number of macrophages.

Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-2 and TNF-α in serum were detected using an ELISA kit (Aditteram Diagnostic Laboratories, San Diego, USA). For the *in vitro* study, splenic lymphocytes were isolated from the spleen and co-cultured with 5 μg/ml ConA as well as different concentrations of AMRP-2 (50 μg/ml, 100 μg/ml, or 150 μg/ml) for 72 h. IL-2 secretion into the supernatant was analysed by ELISA [20]. Peritoneal macrophages were obtained and treated with 20 μg/ml LPS together with different concentrations of AMRP-2 (50, 100, or 150 μg/ml) for 72 h. The level of TNF-α in the supernatant was estimated by ELISA according to the manufacturer's instructions.

Statistical analysis

The values are expressed as the mean ± standard deviation (SD). Data were statistically analyzed using one-way analysis of variance (one-way ANOVA) or Student's *t*-test. The differences were considered significant when * $p < 0.05$ or ** $p < 0.01$. All experiments were performed at least 3 times, and representative results are presented.

RESULTS

Extraction and purification of polysaccharides

The crude polysaccharides AMRP were isolated by hot water extraction and ethanol precipitation. The pigment was removed by active carbon, and free protein was eliminated by the Sevag reagent. AMRP was fractionated by a Sephadex G-100 column and eluted with H_2O to give 2 fractions, AMRP-1 and AMRP-2 (Fig. 1A). The yield of AMRP-1 was low and is not discussed in this paper. The molecular weight distribution of the main component AMRP-2 was determined by using a Sephadex G-100 column, which was pre-calibrated by standard dextrans as described in materials and methods. The elution profile showed a single and symmetrical narrow peak, which suggested that AMRP-2 was homogeneous (Fig. 1B). The average molecular weight of AMRP-2 was 5.8 kDa. HPLC showed that AMRP-2 was primarily composed of GalA (86.5%), Glc (8.5%), Rha (2.6%), Gal (1.8%), and Man (0.6%).

Structural analysis of AMRP-2

The structural features of AMRP-2 were characterised by analysing the infrared and NMR spectra. The IR absorption spectrum exhibited a broad peak at 3414.04 cm^{-1} for —OH, while the weak peak at 2932.06 cm^{-1} indicated the axial deformation of CH in CH_2 groups (Fig. 2). The intense peaks at approximately 1738.40 cm^{-1} and 1601.38 cm^{-1} were attributed to the methoxycarbonyl group and the carboxyl group, respectively, which indicated the presence of uronic acid. The characteristic region between 1400 cm^{-1} and 1200 cm^{-1} was attributed to C—H variable angle vibrations. The large peak between 1200 cm^{-1} and 1000 cm^{-1} was the C—O stretching vibration peak of C—O—C and C—O—H. The region between 960 cm^{-1} and 930 cm^{-1} was attributed to a pyranoid ring, which was divided into different absorption peaks due to the difference in the three-dimensional structure (Fig. 2).

In the ^{13}C-NMR spectrum (Fig. 3), the signal at 173.2 ppm could be assigned to the C-6 resonance of α-GalA, while the peak at 100–99 ppm could be assigned to the C-1 resonance of GalA and methyl esterified GalA. The characteristic peaks of methyl ester groups were at 170.6 ppm, 52.8 ppm, and 20 ppm. The C-4, C-3 and C-2 resonances of α-GalA and methyl esterified GalA were at 78–77 ppm, 71–70 ppm, and 69–76 ppm,

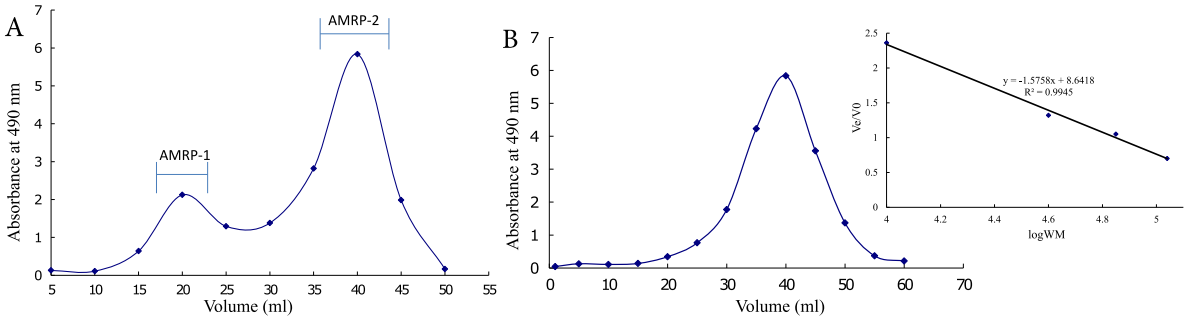

Fig. 1 Purification of AMRP-2. (A) Elution profiles of AMRP on a Sephadex G-100 column eluted with dH_2O. (B) Profile of AMRP-2 on Sephadex G-100 eluted with dH_2O. The subfigure is the standard curve of molecular weight.

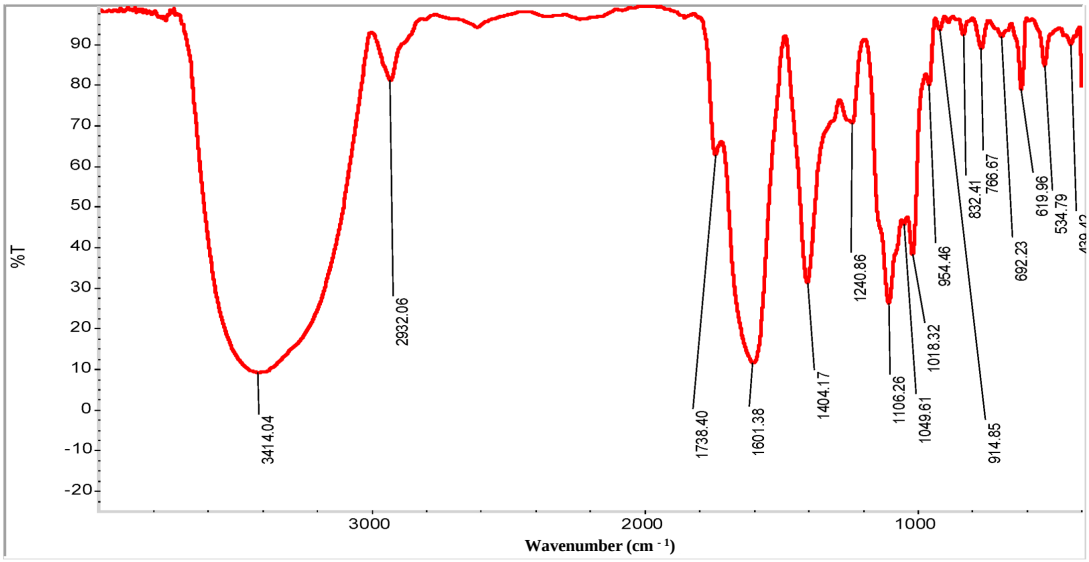

Fig. 2 Infrared spectrum of AMRP-2.

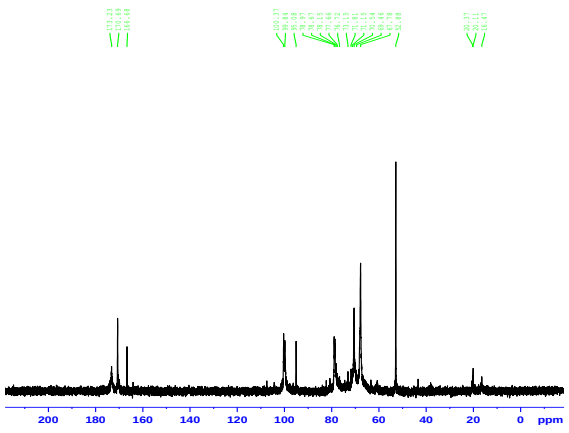

Fig. 3 ^{13}C-NMR spectrum of AMRP-2.

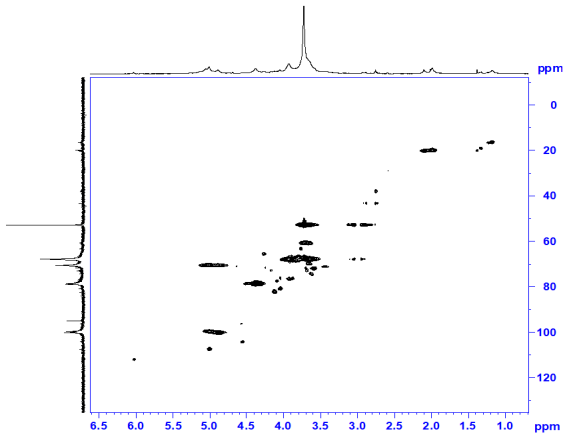

Fig. 4 ^{13}C—^{1}H COSY spectrum of AMRP-2.

Table 1 The antibacterial activity of AMRP-2.

Microorganism	Inhibitory zone (mm)	
	Amoxicillin	AMRP-2
E. coli	-	13.3 ± 0.3
S. dysenteriae	-	7.5 ± 0.3
S. albus	25.3 ± 0.4	7.1 ± 0.1
S. aureus	28.1 ± 0.1	9.9 ± 0.3
P. aeruginosa	27.5 ± 0.5	7.6 ± 0.4
P. vulgaris	17.2 ± 0.1	8.1 ± 0.7

(–) : No inhibition. The inhibitory zone (diameter) $p \leqslant 8$ mm, $\geqslant 13$ mm, and $\geqslant 16$ mm was recognized as resistant (R), intermediate (I), and sensitive (S), respectively.

respectively. These data indicated that AMRP-2 is a homogalacturonic (HG-type) polysaccharide with GalA as the main monosaccharide, in which a large amount of GalA is methyl esterified (Fig. 3). The results of 2D NMR were consistent with ^{13}C-NMR spectrum. As shown in Fig. 4, the peak δ100/4.80 ppm could be assigned to the C-1 resonance of GalA and methyl esterified GalA, which indicated that the structure of AMRP-2 belongs to pectin.

AMRP-2 exhibited weak antibacterial activity

As shown in Table 1, *S. albus*, *S. aureus*, *P. aeruginosa*, and *P. vulgaris* displayed sensitivity (S) responses towards amoxicillin, whereas the growth of *E. coli* and *S. dysenteriae* were not inhibited by amoxicillin. In the AMRP-2 treatment group, AMRP-2 exhibited antimicrobial activity against all the tested strains. For *S. albus*, *S. aureus*, *P. aeruginosa*, and *P. vulgaris*, AMRP-2 showed weaker inhibitory activity than that of amoxicillin. However, it is noteworthy that both *E. coli* and *S. dysenteriae* could be inhibited by AMRP-2. The results revealed that AMRP-2 has relatively weaker but broader spectrum antibacterial activity compared to amoxicillin.

AMRP-2 enhanced T cell proliferation and IL-2 secretion

The effects of AMRP-2 on ConA-induced splenic T lymphocyte proliferation and cytokine secretion were tested. As shown in Fig. 5A, in the presence of ConA, AMRP-2 promoted splenic T cell proliferation in a dose-dependent manner. While low dosages (1.25 mg/kg/d and 12.5 mg/kg/d) of AMRP-2 had little effect, the higher dose (125 mg/kg/d) significantly promoted T cell growth ($p < 0.05$). Moreover, AMRP-2 at the dosage of 125 mg/kg/d significantly stimulated IL-2 secretion into serum compared to the normal mice in the control group (Fig. 5B). Similar results were found in the *in vivo* analysis. Splenic lymphocytes were isolated from healthy mice and treated with AMRP-2; ConA was added at the same time as AMRP-2, and the mixture was co-incubated for 72 h. We found that AMRP-2 induced T cell proliferation (Fig. 5C) and IL-2 secretion (Fig. 5D) in a dose-dependent manner. When the working concentration of AMRP-2 was above 100 μg/ml, splenic T lymphocyte proliferation was significantly induced ($p < 0.05$), and IL-2 was significantly stimulated by T cells ($p < 0.05$). These results indicated that AMRP-2 might act as a potent T cell stimulator.

AMRP-2 promoted phagocytosis and TNF-α production in macrophages

The effects of AMRP-2 on murine macrophage function were also determined. Mice were administered AMRP-2 intragastrically for 35 days. CRBCs (chicken red blood cells) were injected into the peritoneum one day before the mice were sacrificed, and peritoneal macrophage phagocytosis was assessed by the Giemsa staining method. As shown in Fig. 6, in the presence of LPS, AMRP-2 significantly facilitated the phagocytosis of peritoneal macrophages in a dose-dependent manner. As shown in Fig. 5B, a dose-dependent increase in the production of TNF-α was observed in AMRP-2-treated mice. In addition, we found that AMRP-2 was able to induce the synthesis of TNF-α by macrophages (Fig. 5D).

DISCUSSION

In the present study, a purified fraction from the polysaccharides of AMR, AMRP-2, was obtained. AMRP-2 is a HG-type polysaccharide that harbours antibacterial and immunomodulatory effects.

The determination of antibacterial activity is of great significance for the screening of antimicrobial agents. Six strains of bacteria (*S. albus*, *S. aureus*, *P. aeruginosa*, *P. vulgaris*, and *E. coli*) were used to evaluate the antibacterial effects of AMRP-2. We found that *E. coli* and *S. aureus* were relatively sensitive to AMRP-2, indicating that AMRP-2 might be helpful to inhibit the growth of the intestinal flora. Yang et al [21] found similar results that sulfated polysaccharides from Codium fragile exhibited antibacterial activities against fish pathogenic bacteria including *Streptococcus parauberis*, *Lactococcus garvieae*, *Aeromonas salmonicida*, and *Edwardsiella tarda* at a minimum inhibitory concentration of 2 mg/ml, but not against *Streptococcus iniae* or *Vibrio anguillarum*. In addition, many literatures have reported that plant polysaccharides have antibacterial effects [22–24]. Therefore, plant polysaccharides can inhibit bacteria, and that may be related to the structure of polysaccharides.

Immune regulation is one of the most important biological activities of polysaccharides and has an immunoregulatory effect through both specific immunity and innate immunity [25–27]. It was reported that a basal diet supplemented with flavonoids or polysaccharides from AMR improved IgM concentrations in serum and affected cytokine TNF-a, IL-1b, and IL-8 transcription in *Channa argus* [3, 4, 28]. In ad-

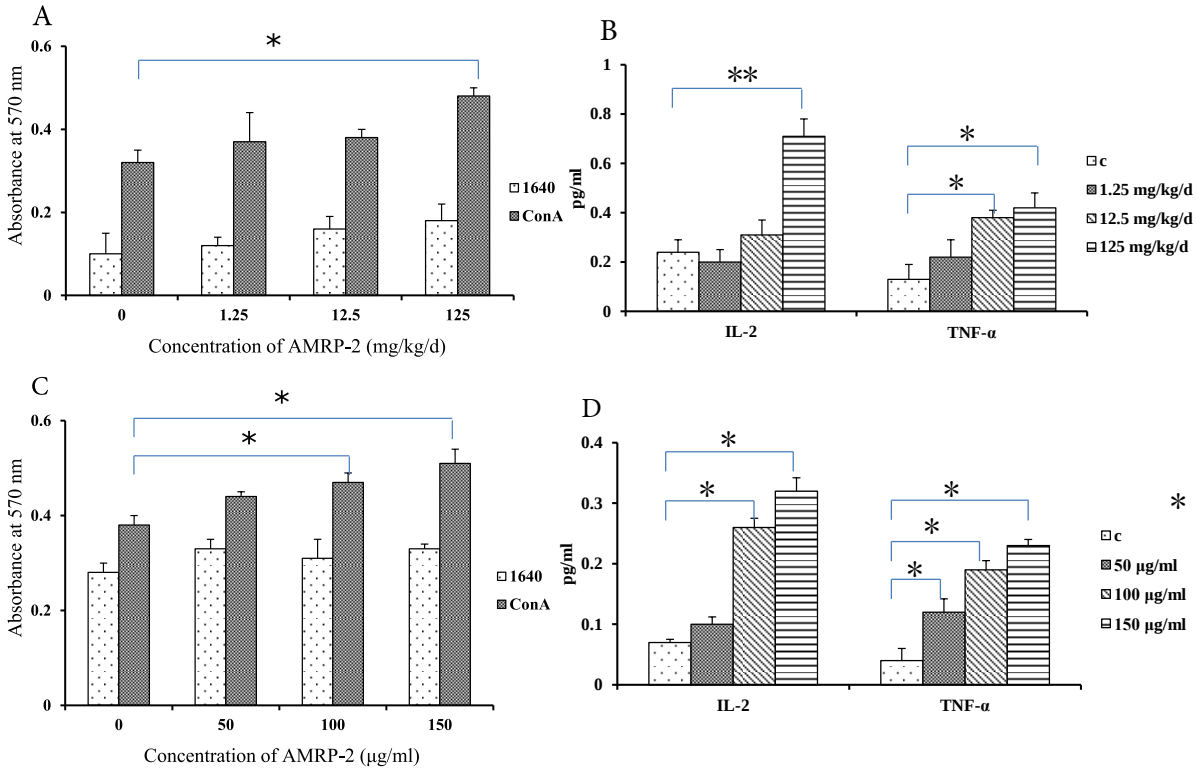

Fig. 5 Effects of AMRP-2 on T cell proliferation and IL-2 and TNF-α secretion. Splenic lymphocytes cultured with or without ConA. The cell number was measured by MTT assay (A), and the mouse serum levels of IL-2 and TNF-α were measured by ELISA (B). Splenic lymphocytes were co-cultured with different concentrations of AMRP-2 as well as ConA for 72 h (C, D). T cell proliferation was calculated by MTT assay (C). The secretion of IL-2 by T cells and the secretion of TNF-α by peritoneal macrophages were assessed by ELISA (D). Columns show the mean, and bars show the SD of 3 independent experiments; * $p < 0.05$, ** $p < 0.01$.

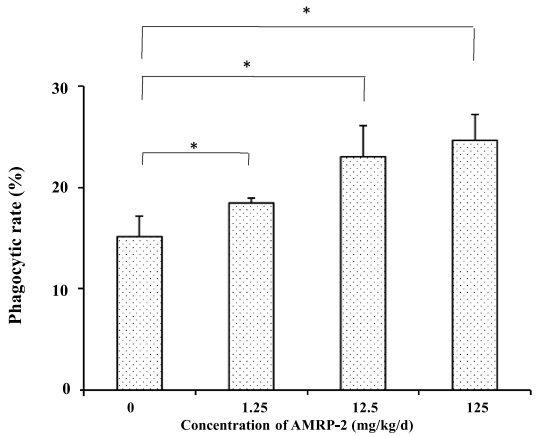

Fig. 6 Effects of AMRP-2 on macrophage phagocytosis. The percentage of phagocytic macrophages was calculated by counting the number of phagocytosed CRBCs present in the total macrophages. Columns show the mean, and bars show the SD of 3 independent experiments; * $p < 0.05$.

dition, Shang et al [29] found that polysaccharides extracted from Astragalus significantly increased $CD4^+$ levels. In the present study, we found that AMRP-2 significantly stimulated T cell proliferation and IL-2 secretion, which could be used as a T cell stimulator to exert a specific immune response. Moreover, AMRP-2 significantly promoted macrophage phagocytosis and TNF-α release from macrophages, which indicated that AMRP-2 could stimulate cellular innate immunity, as macrophage activation is one of the most important indicators to measure the innate immune functions of the body [26]. Thus, AMRP-2 might act as an important immune modulator.

Moreover, the effects of AMR juice and AMRP-2 on mice lymphocyte transformation and macrophage phagocytosis were compared. The results showed that AMRP-2 significantly promoted lymphocyte transformation and macrophage phagocytosis (compared to control group), whereas the effects of AMR juice (containing all the substances including phenolics and flavonoids) are not obvious (Table S1), which revealed the possibility that AMRP-2 is a major bioactive component in AMR.

CONCLUSION

We found that AMRP-2 showed inhibitory activity against a variety of bacteria, including *E. coli* and *S. dysenteriae*. Moreover, we also found that AMRP-2 can promote T cell proliferation and IL-2 secretion and can induce the synthesis of TNF-α in macrophages. Ongoing studies are focused on searching for the mechanism of action of AMRP-2 on immune regulation and detailed studies of the relationships of immunoregulatory activity to elucidate the molecular mechanisms of AMRP-2.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2022.051.

Acknowledgements: This work was supported by the Research Project of Inner Mongolia Pharmaceutical Collaborative Innovation Center (MYYXT201901), the Inner Mongolia Science and Technology Plan (2019GG160), Talent Term Project of Inner Mongolia Medical University (NYTD2018003), The 11th Grassland Talent Innovation Team of Inner Mongolia Autonomous Region, and the Inner Mongolia Natural Science Foundation Project (2019BS08013).

REFERENCES

1. Wang JK, Yang F, Zhao LH, Bao B (2013) Analysis and comparison of nutritional components in *Allium mongolicum* Regel and Chinese chive. *Acta Nutr Sin* **1**, 86–88.
2. Chen S, Man L, Jin S (2000) Mongolia plateau ethnobotany. *Adv Plant Sci* **3**, 245–251.
3. Li M, Guo W, Guo G, Zhu X, Niu X, Shan X, Tian J, Wang G, et al (2019) Effect of sub-chronic exposure to selenium and *Allium mongolicum* Regel flavonoids on *Channa argus*: Bioaccumulation, oxidative stress, immune responses and immune-related signaling molecules. *Fish Shellfish Immunol* **91**, 122–129.
4. Li M, Zhu X, Tian J, Liu M, Wang G (2019) Dietary flavonoids from *Allium mongolicum* Regel promotes growth, improves immune, antioxidant status, immune-related signaling molecules and disease resistance in juvenile northern snakehead fish (*Channa argus*). *Aquaculture* **501**, 473–481.
5. Wang W, Li J, Zhang H, Wang X, Fan J, Zhang X (2019) Phenolic compounds and bioactivity evaluation of aqueous and methanol extracts of *Allium mongolicum* Regel. *Food Sci Nutr* **7**, 779–787.
6. Qier MU, Changjin AO, Ruli SA, Wang T, Chen R, Muqile TE, Wang C (2016) Effects of flavonoids from *Allium mongolicum* Regel on antioxidant capacity of meat sheep. *Chin J Anima Nutr* **28**, 1823–1831.
7. Sqmg H (2008) Effects of *Allium mongolicum* Regel extract on antioxidation function activity and serum biochemical parameters in broilers. Master thesis, Inner Mongolia Agricultural University, China.
8. Li YL, Luo C, Fan YL, Jv N (2016) Study of *Allium mongolicum* Regel extracts on antioxidative effect of mutton sausage. *Food Res Dev* **37**, 6–10.
9. Xiao X, Zhao L, Zhang H, Zhao Q, Wu W, Bao B (2015) Inhibitory effect of ethanol extracts from *Allium mongolicum* Regel on hyperlipidemia mice. *J Food Saf Qual* **6**, 4147–4153.
10. Savag MG, Lackman DB, Smolens J (1938) The isolation of the components of *Streptococcal nucleoproteins* in serologically active form. *J Biol Chem* **124**, 425–436.
11. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* **28**, 350–356.
12. Sedmak JJ, Grossberg SE (1977) A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. *Anal Biochem* **79**, 544–552.
13. Wang GY, Liang ZY, Zhang LP, Miao CY, Zhang YS, Fei R (2001) Studies on the structure of JS1-the water-soluble polysaccharide isolated by alkaline from *Hippophae rhamnoides* L. *Chem J Chin Univ* **22**, 1688–1690.
14. Zhang X, Yu L, Bi H, Li X, Ni W, Han H, Li N, Wang B, et al (2009) Total fractionation and characterization of the water-soluble polysaccharides isolated from *Panax ginseng* CA Meyer. *Carbohydr Polym* **77**, 544–552.
15. David WW, Stout TR (1971) Disc plate method of microbiological antibiotic assay. *Microbiology* **22**, 659–665.
16. Xue H, Zhao Z, Lin Z, Geng J, Guan Y, Song C, Zhou Y, Tai G (2019) Selective effects of ginseng pectins on galectin-3-mediated T cell activation and apoptosis. *Carbohydr Polym* **219**, 121–129.
17. Harwell L, Skidmore B, Marrack P, Kappler J (1980) Concanavalin A-inducible, interleukin-2-producing T cell hybridoma. *J Exp Med* **152**, 893–904.
18. Stobo JD, Rosenthal AS, Paul WE (1972) Functional heterogeneity of murine lymphoid cells. I. Responsiveness to and surface binding of concanavalin A and phytohemagglutinin. *J Immunol* **108**, 1–17.
19. Yang F, Shi Y, Sheng J, Hu Q (2006) *In vivo* immunomodulatory activity of polysaccharides derived from *Chlorella pyrenoidosa*. *Eur Food Res Technol* **224**, 225–228.
20. Lequin RM (2005) Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). *Clin Chem* **51**, 2415–2418.
21. Yang Y, Lim J, Li C, Lee S, Hong S (2021) Effects of sulfated polysaccharides isolated from Codium fragile on inflammatory cytokine gene expression and *Edwardsiella tarda* infection in rockfish, *Sebastes schlegelii*. *Fish Shellfish Immunol* **112**, 125–134.
22. He F, Yang Y, Yang G, Yu L (2010) Studies on antibacterial activity and antibacterial mechanism of a novel polysaccharide from *Streptomyces virginia* H03. *Food Control* **21**, 1257–1262.
23. Yang B, Sa R, Zhang Y, Dalai B, Song Y, Song A, Chen W, Lu Y, et al (2016) Study on antioxidant and antibacterial activity *in vitro* of *Echinops latifolius* Tausch polysaccharides. *Acta Agric Bor Sin*, **31**, 233–238.
24. Singh A, Mittal A, Benjakul S (2021) Chitosan nanoparticles: Preparation, food applications and health benefits. *ScienceAsia* **47**, 1–10.
25. Rosenberg SA (2007) Interleukin 2 for patients with renal cancer. *Nat Clin Pract Oncol* **4**, ID 49722.
26. Mills CD, Lenz LL, Harris RA (2016) A breakthrough: macrophage-directed cancer immunotherapy. *Cancer Res* **76**, 513–516.
27. June CH, O'Connor RS, Kawalekar OU, Ghassemi S,

Milone MC (2018) CAR T cell immunotherapy for human cancer. *Science* **359**, 1361–1365.
28. Li M, Zhu X, Niu X, Chen X, Tian J, Kong Y, Zhang D, Zhao L, et al (2019) Effects of dietary *Allium mongolicum* Regel polysaccharide on growth, lipopolysaccharide-induced antioxidant responses and immune responses in *Channa argus*. *Mol Biol Rep* **46**, 2221–2230.
29. Shang L, Yang T, Yu H, Huang S, Zeng X, Qiao S (2019) Immunomodulation effect of antimicrobial peptide sublancin and Astragalus polysaccharides on immunosuppressed mice: a comparative study. *J Anim Husb Vet Med* **50**, 406–414.
30. Singh VK, Dwivedi P, Chaudhary BR, Singh R (2015) Immunomodulatory effect of *Gymnema sylvestre* (R. Br.) leaf extract: An *in vitro* study in rat model. *PLoS One* **10**, ID e0139631.

Appendix A. Supplementary data

Table S1 Effects of AMR and AMRP-2 on mice lymphocyte transformation and macrophage phagocytosis.

Group	Concentration	Lymphocyte transformation rate (%)	Macrophage phagocytic rate (%)
control	–	20.3 ± 3.6	19.2 ± 2.2
AMR juice	1 ml/d	22.5 ± 1.8	21.5 ± 2.3
AMRP-2	125 mg/ml/d	27.6 ± 3.4*	23.5 ± 3.7*

AMR of 500 g was squeezed into juice and intragastrically administered 1 ml/d for 35 consecutive days. The control group was given distilled water. On day 32 to day 35, PHA (2 mg/ml), a well-known T cell activator [30], was intramuscularly injected, 0.2 ml/d. The percentage of lymphocyte transformation in the peripheral blood was measured by Giemsa staining. The phagocytic rate of peritoneal macrophage was measured as described in Materials and Methods.