RESEARCH ARTICLE

Molecular Nutrition & Food Research
www.mnf-journal.com

Dietary Intake of Yeast-Derived β-Glucan and Rice-Derived Arabinoxylan Induces Dose-Dependent Innate Immune Priming in Mice

*Bart G. J. Moerings, Jurriaan J. Mes, Jeroen van Bergenhenegouwen, Coen Govers, Miriam van Dijk, Renger F. Witkamp, Klaske van Norren, and Suzanne Abbring**

Beta-glucans and arabinoxylans are known for their immunostimulatory properties. However, in vivo these have been documented almost exclusively following parenteral administration, underemphasizing oral intake. C57BL/6 mice are fed either a control diet or a diet supplemented with yeast-derived whole β-glucan particle (yWGP) or with rice-derived arabinoxylan (rice bran-1) at a concentration of 1%, 2.5%, or 5% weight/weight (w/w) for 2 weeks. Thereafter, cells from blood, bone marrow, and spleen are collected for ex vivo stimulation with various microbial stimuli. Dietary intake of yWGP for 2 weeks at concentrations of 1% and 2.5% w/w increases ex vivo cytokine production in mouse blood and bone marrow, whereas 5% w/w yWGP shows no effect. In the spleen, cytokine production remains unaffected by yWGP. At a concentration of 1% w/w, rice bran-1 increases ex vivo cytokine production by whole blood, but 2.5% and 5% w/w cause inhibitory effects in bone marrow and spleen.
This study demonstrates that dietary yWGP and rice bran-1 induce immune priming in mouse blood and bone marrow, with the strongest effects observed at 1% w/w. Future human trials should substantiate the efficacy of dietary β-glucans and arabinoxylans to bolster host immunity, focusing on dose optimization.

1. Introduction

Traditionally, innate immunity was believed to lack the ability to adapt after an initial encounter with a pathogen. However, recent observations have shown that upon stimulation various adaptations can occur in innate immune cells, resulting in either enhanced or diminished immune responses. Examples include cell differentiation, trained immunity, tolerance, and priming.[1–3] During priming, the initial stimulus alters the functional state of innate immune cells, and their immune status does not revert to baseline before the subsequent stimulation or infection occurs.[1] Consequently, the influence of a second challenge in primed cells typically exhibits an additive or synergistic effect with the initial stimulus. Unlike adaptive immunity, this altered innate immune response is not only specific to the initial trigger but also to unrelated secondary stimuli.[2]

B. G. J. Moerings, R. F. Witkamp, K. van Norren, S. Abbring
Division of Human Nutrition and Health
Wageningen University & Research
Wageningen 6708 WE, The Netherlands
E-mail: Suzanne.abbring@wur.nl
B. G. J. Moerings, J. J. Mes, S. Abbring
Wageningen Food and Biobased Research
Wageningen University & Research
Wageningen 6708 WG, The Netherlands

J. van Bergenhenegouwen, M. van Dijk
Danone Nutricia Research
Utrecht 3584 CT, The Netherlands
C. Govers
Cell Biology and Immunology Group
Wageningen University & Research
Wageningen 6708 WD, The Netherlands

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202300829

© 2024 The Authors. *Molecular Nutrition & Food Research* published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

DOI: 10.1002/mnfr.202300829

Although adaptations in innate immune compartments were initially identified in the context of vaccinations, subsequent research has demonstrated that specific nutritional components can also impact innate immune functioning.[4,5] This renders it one of the mechanisms through which dietary components contribute to shaping the immune system and the promotion of overall health.

Fungal-derived β-glucans have gained much attention as ligands for pattern recognition receptors (PRRs) which play key roles in immune modulation.[6] Beta-glucans are glucose polymers whose structure is determined by their source of origin.[7] For instance, β-glucans derived from fungi are mainly characterized by the presence of β-(1,6)-linked branches along the β-(1,3) linear backbone.[8] A substantial body of literature indicates that fungal-derived β-glucans are recognized as microbe-associated molecular patterns (MAMPs). Through activation of PRRs, they induce immune mechanisms and modulate the response to infection, inflammation, and cancer progression.[9,10] In addition, PRR activation by fungal β-glucans has also been shown to induce trained innate immunity, as demonstrated through extensive in vitro studies using various cellular models[11–13] and, to a lesser extent, in vivo experiments.[14] As an example, when human monocytes are pre-exposed to β-glucans, their immune response is enhanced upon a subsequent secondary stimulation with microbial ligands in vitro.[12] Furthermore, animal studies have demonstrated that systemic administration of β-glucan provides macrophage-mediated protection against subsequent pathogenic challenges such as *Candida albicans* and *Staphylococcus aureus*.[12,15]

Apart from β-glucans, arabinoxylans have also gained attention as potent immunomodulators.[16] Arabinoxylans, found in cereals, constitute a major part of human dietary fiber intake and consist of a β-(1,4)-linked xylose backbone with arabinose branches substituted at the xylose O-2 and/or O-3 positions.[17] In vitro studies have demonstrated that arabinoxylans have a wide variety of immunostimulatory effects on innate immune cells.[18–21] For example, we recently reported that rice bran-derived arabinoxylan preparations were able to enhance immune activity in primary human macrophages.[5] In vivo studies have revealed that orally administrated arabinoxylans can activate the innate immune system, leading to increased NK cell activity against neuroblastoma and tumor formation, as well as elevated numbers of myeloid-derived dendritic cells in the peripheral blood of multiple myeloma patients.[22–24] Furthermore, a dietary intervention study in healthy older individuals revealed that oral administration of an arabinoxylan preparation tended to increase antibody titers and seroconversion rates following influenza vaccination.[25]

Despite recent progress in understanding immune responses following parenteral administration of β-glucans, the effectiveness of orally administered β-glucans and arabinoxylans is not well understood.[26,27] Particularly in human clinical studies, there remains uncertainty about the extent of gastrointestinal absorption and the subsequent peripheral availability of β-glucans. For instance, Leentjens et al. reported minimal detectable serum concentrations of β-glucan, along with a lack of effect on leukocyte cytokine production and microbicidal activity.[28] Although established systemic presence in humans is lacking, there is evidence suggesting favorable outcomes for upper respiratory infections (URTIs) following oral ingestion.[29,30] Beta-glucans and arabinoxylans, as part of a healthy diet or supplements, offer promising candidates that would support host immunity in the defense against pathogens.[31] However, additional research is needed to determine the efficacy of oral administration of these fibers, including the identification of optimal sources and dosages.

The current study aims to investigate whether dietary intake of β-glucan and arabinoxylan enhances innate immune responsiveness in mice. In addition, we aimed to identify the most effective oral dose. To this end, mice were fed either a diet supplemented with yeast-derived whole glucan particle (yWGP), or a diet supplemented with an arabinoxylan preparation derived from rice bran (rice bran-1) at a concentration of 1%, 2.5%, or 5% weight/weight (w/w) for 2 weeks. Cells from different immunological organs were subsequently isolated and ex vivo challenged to assess a potential priming effect.

2. Experimental Section

2.1. Diets

Yeast-derived whole β-glucan particle (yWGP; Invivogen, Toulouse, France) or a preparation of arabinoxylans isolated from rice bran (rice bran-1 (RiFiber); RiceBran Technologies, The Woodlands, TX, USA) was added to the AIN-93 M diet at a concentration of 1%, 2.5%, or 5% w/w (with a correction for fiber purity) at the expense of cellulose (Table S1, Supporting Information; Ssniff Spezialdiäten GmbH, Soest, Germany). The AIN-93 M diet without β-glucan or arabinoxylan supplementation was used as control diet. The characteristics of yWGP and rice bran-1, such as solubility, protein content, molecular weight distribution, branching and linkages, monosaccharide composition, total saccharide content (i.e., purity), and LPS/LTA contamination levels can be found in Table S2, Supporting Information.

2.2. Animals

Male C57BL/6J mice, aged 4 weeks (Envigo, Horst, The Netherlands), were housed in conventional type II cages (n = 3/cage) with wood–wool bedding, a play tunnel, and a gnawing stick on a 12 h light/dark cycle in humidity- (66 ± 2.5%, mean ± SD) and temperature (21 ± 0.5 °C)-controlled conditions with unlimited access to food and water. Mice arrived at the animal facilities in groups of three which prevented re-randomization and aggression. Upon arrival, cages were randomly assigned to the control and experimental groups and mice were habituated to the laboratory conditions for 12 days prior to the start of the study. This study was conducted under an ethical license of the national competent authority (CCD, Centrale Commissie Dierproeven) including a positive advice from the local animal ethics committee (Wageningen University, The Netherlands), and all animal procedures were approved by the Animal Welfare Body, following principles of good laboratory animal care—securing full compliance

© 2024 The Authors. *Molecular Nutrition & Food Research* published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

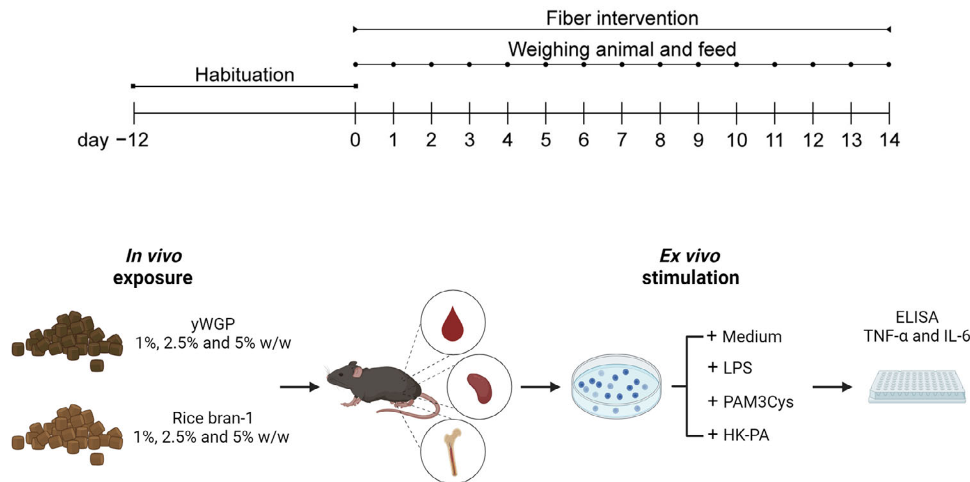

Figure 1. Schematic overview of the study design. Male C57BL/6J mice were acclimated on AIN-93 M control feed to the laboratory conditions for 12 days before the start of the study. Following this habituation period, experimental groups (n = 12 per group) received a specific fiber-supplemented diet (1% yWGP, 2.5% yWGP, 5% yWGP, 1% rice bran-1, 2.5% rice bran-1, or 5% rice bran-1 w/w) for a duration of 2 weeks. The control group (n = 12) remained on the AIN-93 M control diet. Body weight and food intake were assessed daily. At the end of the 2-week exposure period, mice were anesthetized and euthanized by cervical dislocation. Blood and immunological organs were collected for ex vivo analysis.

to the European Directive 2010/63/EU for the use of animals for scientific purposes.

2.3. Experimental Design

After a habituation period of 12 days on the AIN-93 M control diet, the experimental groups (n = 12 per group) were switched to either a yWGP- or rice bran-1-supplemented diet for 2 weeks. For both diets, three different fiber concentrations were tested, namely 1%, 2.5%, and 5% w/w. The control group (n = 12) remained on the AIN-93 M control diet. During the study period, body weight and food intake were assessed daily. Food intake was determined by weighing back the food left in the cages. After 2 weeks of exposure to the different fiber-specific diets, mice were anesthetized with isoflurane followed by blood collection via orbital bleeding. Immediately thereafter, mice were euthanized by cervical dislocation, and immunological organs were collected for the ex vivo assessment of a potential priming effect. A schematic representation of the experimental design was shown in **Figure 1**.

2.4. Whole Blood Stimulation

Lithium heparin-anticoagulated blood was collected to assess immune responsiveness of whole blood. Whole blood samples were stimulated ex vivo for 6 and 24 h with 1 µg mL^{-1} lipopolysaccharide (LPS; derived from *Escherichia coli* serotype O111:B4; Sigma-Aldrich, Zwijndrecht, The Netherlands), after which the samples were centrifuged for 10 min at 2000 × g. Plasma was aliquoted and stored at −80 °C until further cytokine analysis.

2.5. Isolation and Stimulation of Bone Marrow Cells

Following euthanasia of the mice, hind legs were removed from the hip joint and bone marrow was thoroughly flushed from both femurs and tibiae using a needle and syringe. The resulting bone marrow cell suspension was passed through a 70 µm cell strainer (Falcon, Corning Incorporated, Corning, NY, USA), centrifuged (1400 × g, 7 min), and incubated with lysis buffer (1.5 M NH_4Cl, 0.1 M $NaHCO_3$, and 10 mM EDTA [all from Merck, Darmstadt, Germany] in demineralized water, filter-sterilized) to remove red blood cells. Cells were then resuspended in RPMI 1640—Glutamax—HEPES culture medium supplemented with 10% FBS, 1% MEM non-essential amino acids, 1% sodium pyruvate, and 1% penicillin/streptomycin (all from Gibco, Bleiswijk, The Netherlands) and added to 24-well tissue culture-treated plates (5×10^5 cells/well; Greiner Bio-One, Alphen a/d Rijn, The Netherlands). To ex vivo assess a potential priming effect, whole bone marrow cells were treated with 1 µg mL^{-1} LPS, 10 µg mL^{-1} PAM3Cys (EMC Microcollections, Tübingen, Germany), or 1×10^7 cells mL^{-1} heat-killed *Pseudomonas aeruginosa* (HK-PA; InvivoGen, San Diego, CA, USA) for 24 h at 37 °C, 5% CO_2. To examine a priming effect in adherent bone marrow cells, non-adherent cells were removed after overnight incubation by replacing the culture medium, and adherent cells were stimulated with LPS, PAM3Cys, or HK-PA for 6 and 24 h. The resulting supernatants were collected and stored at −20 °C until further analysis.

2.6. Isolation and Stimulation of Splenocytes

To isolate splenocytes, spleens were collected and homogenized using a syringe and a 70 µm cell strainer. The resulting single cell suspensions were incubated with lysis buffer to eliminate red blood cells. Splenocytes (5×10^5 cells well^{-1}) were then cultured in flat-bottomed 96-well tissue culture plates (Corning Costar, New York, NY, USA). To ex vivo investigate a potential priming effect, splenocytes were stimulated with 1 µg mL^{-1} LPS, 10 µg mL^{-1} PAM3Cys, or 1×10^7 cells mL^{-1} HK-PA for 4 and 24 h at 37 °C, 5% CO_2. A potential priming effect was also assessed in spleen adherent cells. To this end, splenocytes were cultured overnight

© 2024 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

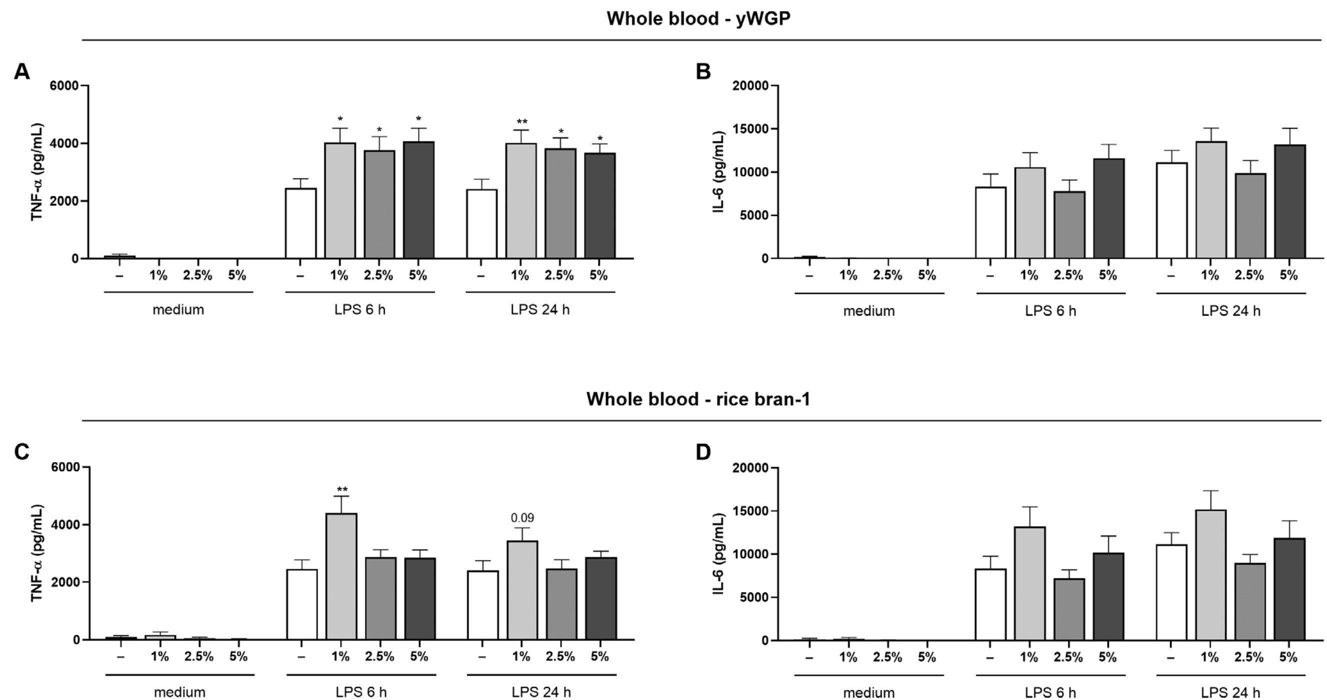

Figure 2. Exposure to yWGP and rice bran-1 increased pro-inflammatory cytokine levels after ex vivo LPS challenge in whole blood cultures. TNF-α (A,C) and IL-6 (B,D) release by whole blood cultures from both rice bran-1- and yWGP-exposed animals measured in plasma samples collected 6 h after LPS stimulation and 24 h after medium or LPS stimulation. Data are presented as mean ± SEM, n = 12 for control animals and n = 12 for yWGP- or rice bran-1-exposed animals. *$p < 0.05$, **$p < 0.01$, as analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test.

at 37 °C, 5% CO_2. The following day, nonadherent splenocytes were removed by replacing the culture medium, and adherent cells were stimulated with LPS, PAM3Cys, or HK-PA for 6 and 24 h. Supernatants were collected and stored at −20 °C for further analysis.

2.7. Cytokine Measurements

The production of tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and IL-10 in the obtained supernatants was determined by means of ELISA (BioLegend, San Diego, CA, USA) according to manufacturer's protocol.

2.8. Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM) and differences compared to the control group were statistically determined using one-way ANOVA followed by Dunnett's multiple comparisons test. When data were not normally distributed, log transformation was applied. If log transformation did not result in normality, the non-parametric Kruskal–Wallis test followed by Dunn's multiple comparisons test was used. For the results on cytokine production after ex vivo stimulation of bone marrow cells and splenocytes, a sample size of $n = 6$ (instead of $n = 12$) was used for the control group as values between the two cohorts used showed significant differences. Food intake and body weight were analyzed with two-way repeated measures ANOVA and mixed-effects model for repeated measures, respectively, followed by Dunnett's multiple comparisons test. All statistical analyses were performed using GraphPad Prism software (version 9.3.1; GraphPad Software, San Diego, CA, USA) and results were considered statistically significant when $p < 0.05$.

3. Results

3.1. Both yWGP and Rice Bran-1 Induce Innate Immune Priming in Circulating Immune Cells

All experimental diets were consumed equally and no differences in body weight were observed between the control and experimental groups (Figure S1, Supporting Information).

To assess whether dietary intake of different concentrations of yWGP or rice bran-1 induces immune priming in innate immune cells in the circulation, TNF-α and IL-6 production was measured after ex vivo stimulation of whole blood with LPS. TNF-α and IL-6 are prototypical pro-inflammatory cytokines and are considered direct measures of innate immune functioning. Animals exposed to all three yWGP-supplemented diets showed increased TNF-α levels in whole blood cultures compared to control animals for both 6 and 24 h LPS stimulation (**Figure 2**A). In addition, exposure to 1% and 5% yWGP resulted in a higher release of IL-6. However, this difference did not reach statistical significance (Figure 2B). Production of the anti-inflammatory cytokine IL-10 was not affected by yWGP exposure (Figure S2A, Supporting Information).

For rice bran-1, exposure to the 1% concentration also increased TNF-α levels compared to control animals (Figure 2C). This effect was observed for both 6 and 24 h LPS stimulation

© 2024 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

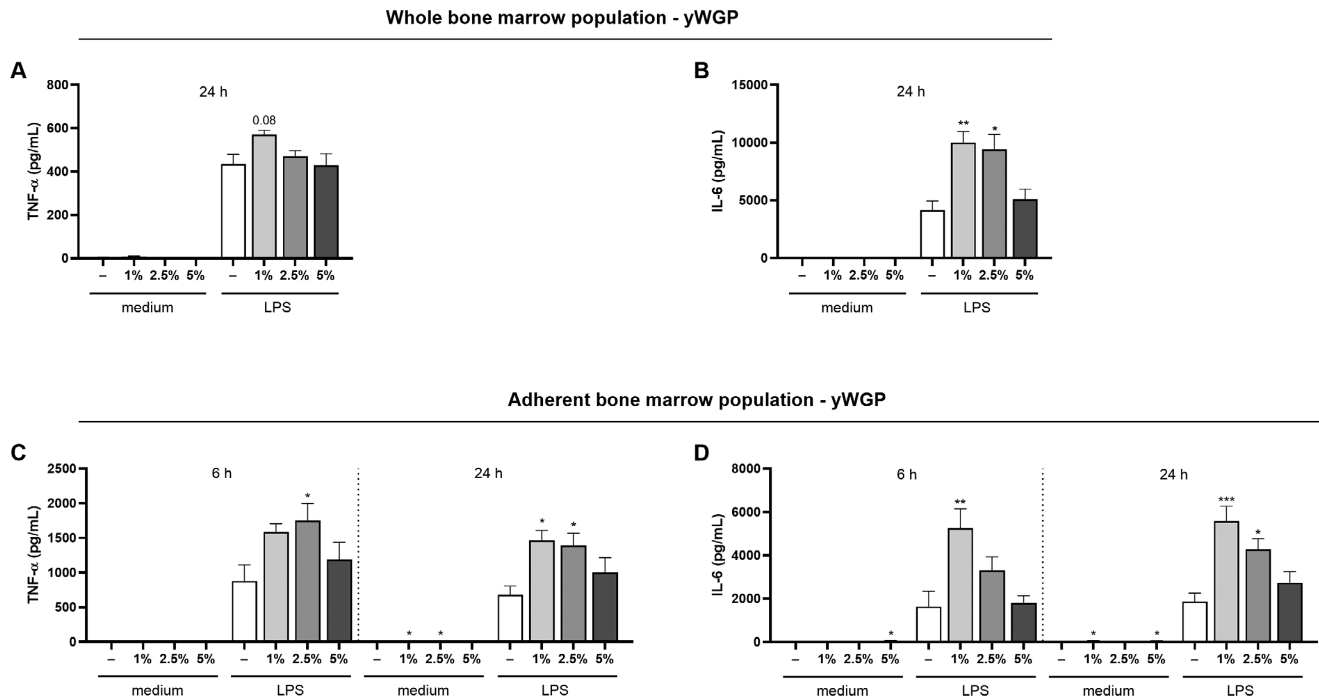

Figure 3. yWGP exposure enhanced immune responsiveness in bone marrow-derived cells. TNF-α and IL-6 concentrations measured in supernatant after ex vivo stimulation of whole (A,B) and adherent (C,D) bone marrow-derived cell populations with medium or LPS for 6 and 24 h. Data are presented as mean ± SEM, $n = 6$ for control animals and $n = 12$ for yWGP-exposed animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, as analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test.

(Figure 2C). IL-10 levels also tended to increase in these animals (Figure S2B, Supporting Information). No effect of rice bran-1 on IL-6 production was observed (Figure 2D).

3.2. yWGP Exposure Increases Ex Vivo TNF-α and IL-6 Release Upon Stimulation of Bone Marrow Cells

Modulation of myeloid progenitor cells in the bone marrow is an integral component of the long-lasting protective effects of trained immunity.[32] Whole and adherent bone marrow cell populations from animals orally exposed to yWGP were therefore stimulated ex vivo with different microbial stimuli (i.e., LPS, PAM3Cys, and HK-PA) to assess effects on pro-inflammatory cytokine responses. Exposure to yWGP clearly induced innate immune priming effects in both the whole bone marrow population as well as in the adherent cells (**Figure 3A–D**, **Tables 1** and **S3**, Supporting Information). One percent yWGP-exposed animals showed an enhanced TNF-α production in most conditions tested (with the exception of the whole bone marrow population stimulated with PAM3Cys for 24 h and the adherent bone marrow cells stimulated with LPS for 6 h; Figure 3A,C, and Table 1). IL-6 production in these animals was elevated in all conditions tested (Figure 3B,D, and Table S3, Supporting Information). The priming effects of 2.5% yWGP were most clearly observed in the adherent bone marrow population, particularly for TNF-α (Figure 3C and Table 1). IL-6 production in these animals was mainly increased upon LPS stimulation (Figure 3B,D, and Table S3, Supporting Information). Interestingly, in contrast to what was observed in the whole blood compartment, animals orally exposed to 5% yWGP showed no change in the secretion of TNF-α and IL-6 (Figure 3A–D, Tables 1 and S3, Supporting Information).

3.3. Differential Effects of Rice Bran-1 on Immune Responsiveness of Bone Marrow Cells

Like for yWGP, immune priming effects were also assessed in bone marrow cells from rice bran-1-exposed animals after an ex vivo challenge. Upon 24 h LPS stimulation, the whole bone marrow cell population of 1% rice bran-1-exposed animals released higher amounts of TNF-α compared to the whole bone marrow cell population of control animals (**Figure 4A**), whereas no effect on IL-6 release was observed (Figure 4B). In contrast, exposure to 2.5% and 5% rice bran-1 did not affect TNF-α production upon LPS or PAM3Cys stimulation, and even caused a decrease after HK-PA stimulation (Table 1). This decrease in TNF-α production was also observed in the adherent bone marrow cell population. After 6 h of stimulation with LPS, PAM3Cys, or HK-PA, TNF-α levels were decreased in all rice bran-1-exposed animals (Figure 4C and Table 1). For IL-6, the reduction was only observed in animals exposed to the 2.5% and 5% concentration (Figure 4D and Table S3, Supporting Information). Upon ex vivo stimulation for 24 h with LPS (Figure 4C,D), PAM3Cys, or HK-PA (Tables 1 and S3, Supporting Information), adherent bone marrow cells did not exhibit any changes in TNF-α and IL-6 production.

© 2024 The Authors. *Molecular Nutrition & Food Research* published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1. TNF-α levels of bone marrow cell populations stimulated with LPS, PAM3Cys, and HK-PA.

Cell population	Length of stimulation	Stimulation	Diet							
			yWGP				Rice bran-1			
			–	1%	2.5%	5%	–	1%	2.5%	5%
Whole bone marrow-derived population	**24 h**	**Medium**	4 ± 2	8 ± 2	2 ± 1	0 ± 0	3 ± 1	4 ± 1	1 ± 1	2 ± 1
		LPS	435 ± 44	571 ± 20^{+}	472 ± 24	428 ± 54	431 ± 11	511 ± 18**	420 ± 11	445 ± 17
		PAM3Cys	166 ± 21	164 ± 17	141 ± 12	121 ± 14	132 ± 15	137 ± 13	152 ± 11	165 ± 8
		HK-PA	212 ± 28	373 ± 27*	321 ± 23	247 ± 47	415 ± 23	391 ± 35	303 ± 17$^{\#}$	319 ± 17$^{\$}$
Adherent cells bone marrow	**6 h**	**Medium**	0 ± 0	3 ± 1	3 ± 1	5 ± 3	0 ± 0	1 ± 0	3 ± 1	6 ± 1
		LPS	881 ± 230	1591 ± 115	1749 ± 251*	1188 ± 248	1483 ± 160	838 ± 61$^{\#\#}$	694 ± 109$^{\#\#\#\#}$	787 ± 104$^{\#\#\#}$
		PAM3Cys	348 ± 122	729 ± 88*	578 ± 96	372 ± 91	712 ± 66	545 ± 46	373 ± 59$^{\#\#}$	374 ± 43$^{\#\#}$
		HK-PA	476 ± 198	1271 ± 160^{+}	1266 ± 255^{+}	775 ± 227	2252 ± 449	1037 ± 120$^{\#\#}$	972 ± 222$^{\#\#}$	822 ± 159$^{\#\#\#}$
	24 h	**Medium**	17 ± 0	16 ± 0$^{\#}$	14 ± 1$^{\#}$	17 ± 0	17 ± 2	16 ± 0	13 ± 1	19 ± 1^{+}
		LPS	682 ± 124	1461 ± 147*	1395 ± 172*	1000 ± 216	646 ± 52	618 ± 37	496 ± 52	580 ± 49
		PAM3Cys	466 ± 70	716 ± 45^{+}	700 ± 47^{+}	507 ± 87	681 ± 34	749 ± 23	651 ± 24	617 ± 19
		HK-PA	627 ± 188	1390 ± 156*	1342 ± 190^{+}	867 ± 211	1170 ± 105	1291 ± 139	935 ± 107	896 ± 136
####	$p < 0.0001$	Decrease								
###	$p < 0.001$									
##	$p < 0.01$									
#	$p < 0.05$									
$	$p < 0.1$									
	$p > 0.1$	–								
+	$p < 0.1$	Increase								
*	$p < 0.05$									
**	$p < 0.01$									
***	$p < 0.001$									
****	$p < 0.0001$									

yWGP, yeast-derived whole β-glucan particle; 1%, 2.5%, 5%, dietary fiber concentration expressed as w/w; HK-PA, heat-killed *Pseudomonas aeruginosa*. $^{*}/^{\#}p < 0.05$, $^{**}/^{\#\#}p < 0.01$, $^{***}/^{\#\#\#}p < 0.001$, $^{****}/^{\#\#\#\#}p < 0.0001$, as analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test. $^{+}/^{\$}p < 0.1$ was considered a trend.

© 2024 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

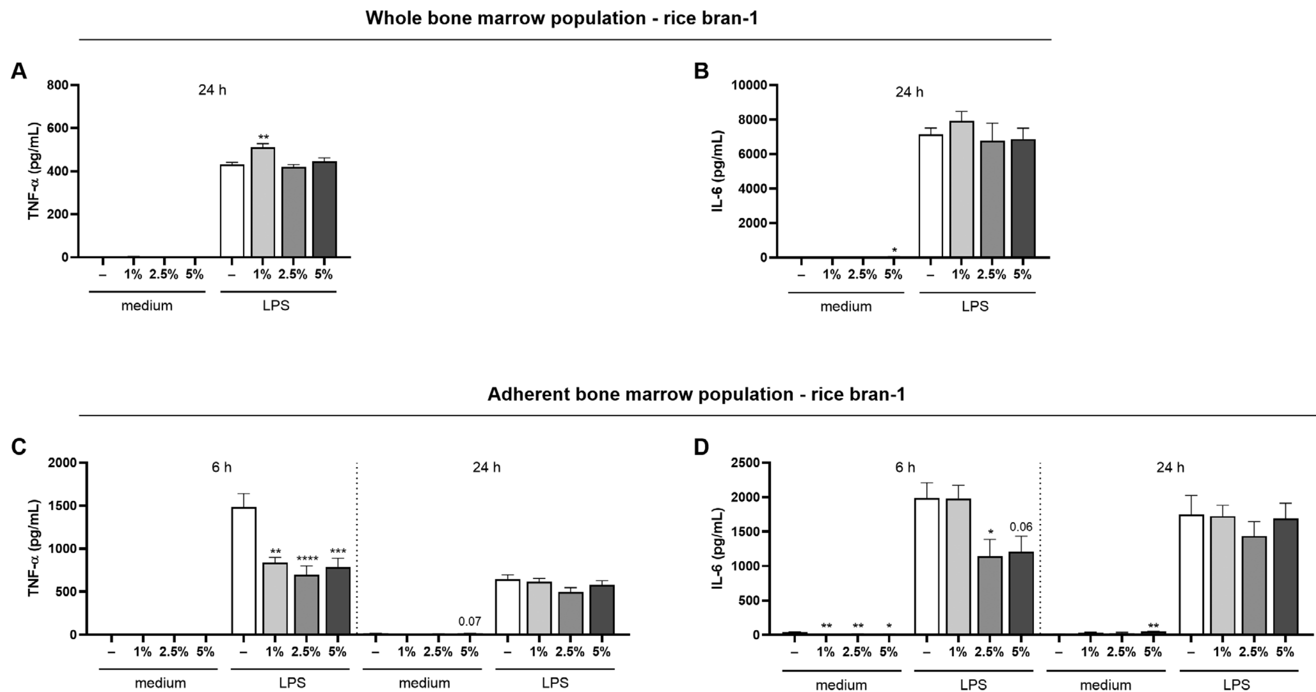

Figure 4. Opposing effects of rice bran-1 on bone marrow-derived cell responses. The release of TNF-α and IL-6 by whole (A,B) and adherent (C,D) bone marrow-derived cells from rice bran-1-exposed and control animals was measured in supernatant collected 6 and 24 h after ex vivo medium or LPS stimulation. Data are presented as mean ± SEM, $n = 6$ for control animals and $n = 12$ for yWGP-exposed animals. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, as analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test.

3.4. Exposure to yWGP Does Not Affect Splenocyte Responsiveness

The spleen functions as a reservoir of undifferentiated monocytes that can be mobilized to the circulation in response to inflammatory stimuli.[33] Splenic immune cell populations of animals orally exposed to yWGP were therefore stimulated ex vivo to investigate whether priming effects were also observed in the spleen. Results showed no significant difference in TNF-α and IL-6 levels in whole and adherent splenocyte populations of animals exposed to 1%, 2.5%, or 5% yWGP compared with control animals after 4 h (whole splenocyte population) or 6 h (adherent splenocyte population) LPS stimulation ex vivo (**Figure** 5A–D). After 24 h stimulation with LPS, the whole splenocyte population of animals exposed to 5% yWGP showed reduced IL-6 levels compared to the control group (Figure 5B), while TNF-α levels were not affected (Figure 5A). Adherent splenocyte populations of yWGP-exposed animals showed no altered TNF-α or IL-6 production after ex vivo stimulation for 24 h with LPS compared to control animals (Figure 5C,D). Comparable to LPS stimulation, ex vivo stimulation with PAM3Cys or HK-PA did not alter TNF-α responses by both splenocyte populations of yWGP-exposed animals (**Table** 2). PAM3Cys stimulation did reduce IL-6 secretion by the whole splenocyte population of animals exposed to 5% yWGP (Table S4, Supporting Information), which is in line with the effects observed after LPS stimulation. IL-6 production by adherent splenocytes was not affected after PAM3Cys stimulation in these animals. The same applied to IL-6 production after HK-PA stimulation (Table S4, Supporting Information).

3.5. Splenic Immune Cell Populations of 2.5% and 5% Rice Bran-1-Exposed Animals Show Reduced Pro-Inflammatory Cytokine Production after Ex Vivo Challenge

To determine whether innate immune priming was induced in the spleen after dietary intake of rice bran-derived arabinoxylan preparations, cytokine production by splenocyte populations was measured after ex vivo stimulation. Stimulation with LPS (**Figure** 6A,B), PAM3Cys, and HK-PA (Tables 2 and S4, Supporting Information) for 4 h reduced TNF-α and IL-6 production from the whole splenocyte population of animals exposed to 5% rice bran-1 compared to control animals. This reduction in cytokine production was also observed in splenocytes harvested from animals exposed to 2.5% rice bran-1 after PAM3Cys and HK-PA stimulation (Tables 2 and S4, Supporting Information). The 1% rice bran-1 group only showed a reduction in IL-6 production after PAM3Cys stimulation (Table S4, Supporting Information). After 24 h of stimulation, none of these effects were observed anymore (Figure 6A,B, Tables 2 and S4, Supporting Information). For the adherent splenocyte population, inhibition of cytokine production by rice bran-1 was mainly observed after 24 h stimulation. Exposure to both 5% and 2.5% rice bran-1 reduced the TNF-α production after LPS and PAM3Cys stimulation (Figure 6C and Table 2). A trend towards a decrease in the production of TNF-α was observed after HK-PA stimulation (Table 2). For IL-6, effects were less pronounced. Only a tendency towards a reduction was observed in animals exposed to 5% rice bran-1 after LPS and HK-PA stimulation (Figure 6D and Table S4, Supporting Information). A reduction in cytokine production was also observed after

© 2024 The Authors. *Molecular Nutrition & Food Research* published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

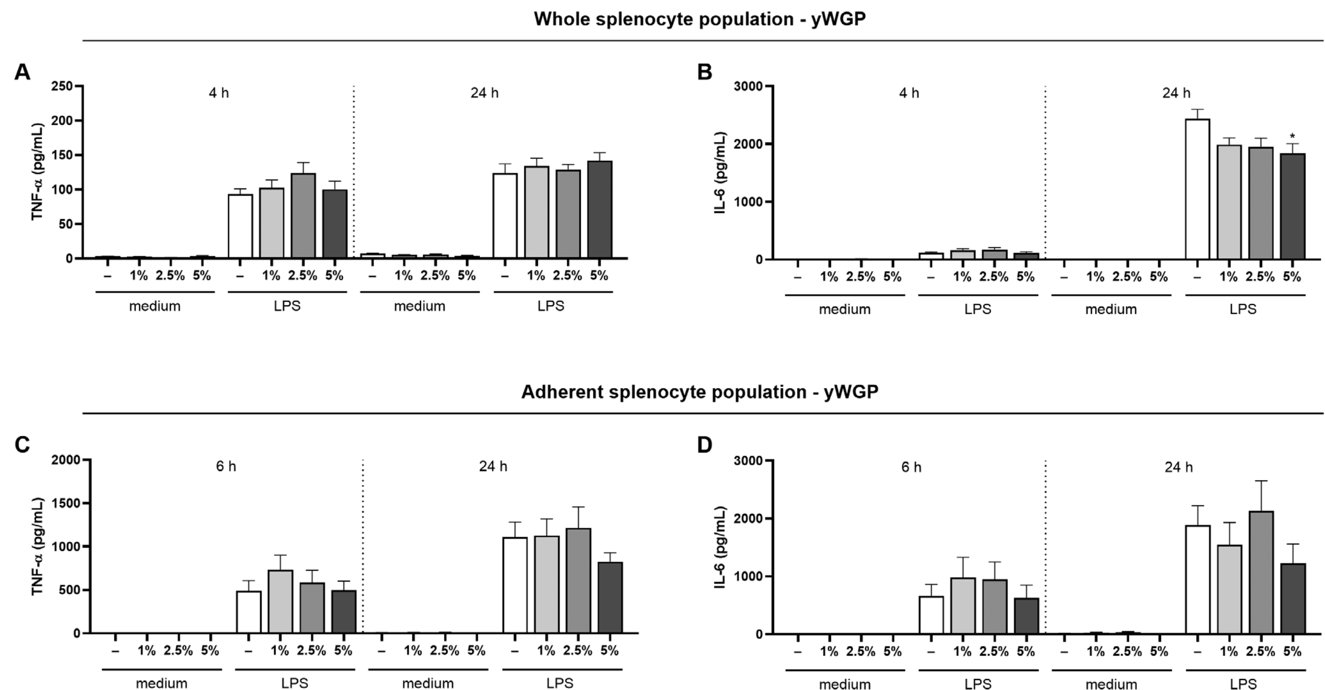

Figure 5. yWGP did not induce innate immune priming in whole and adherent splenocyte populations. After 2 weeks on a yWGP-supplemented (1%, 2.5%, and 5% w/w) diet, the whole (A,B) and adherent (C,D) splenocyte populations were collected and ex vivo stimulated with medium or LPS. TNF-α and IL-6 release by splenocytes from yWGP-exposed and control animals was measured in supernatants collected 4, 6, and 24 h after medium or LPS stimulation. Data are presented as mean ± SEM, $n = 6$ for control animals and $n = 12$ for yWGP-exposed animals. $^{*}p < 0.05$, as analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test.

6 h stimulation of adherent splenocytes, but only for PAM3Cys stimulation (Tables 2 and S4, Supporting Information).

4. Discussion

The stimulation and maintenance of host defence mechanisms against pathogens are of high relevance for immunocompromised individuals, including the elderly. Beta-glucans and arabinoxylans hold significant promise as immune activators, as evidenced by numerous in vitro and in vivo studies involving both animals and human subjects.[2,12,16,25,29] However, the in vivo evidence has been predominantly acquired following parenteral administration (either peritoneal or intravenous).[34–37] By contrast, evidence supporting their immune-enhancing effects after oral intake, naturally the most practical method for regular consumption, is only limited.[22,25,30] The present study demonstrates the dose-dependent impact of dietary exposure to yeast-derived β-glucan (yWGP) or arabinoxylan (rice bran-1) on immune cell responsiveness in mice. Both yWGP and rice bran-1 increased TNF-α and IL-6 levels in whole blood and bone marrow cells after ex vivo stimulation. The highest immune reactivity was observed at the lower concentrations tested (1% and 2.5% w/w for yWGP and 1% w/w for rice bran-1). Interestingly, no priming effects were observed in the spleen.

Beta-glucans and arabinoxylans, found in various foods and used in dietary supplements, are non-digestible dietary fibers. The yWGP utilized in this study has been extensively investigated for its immune-enhancing properties.[29,38] When administered intravenously, intraperitoneally, or subcutaneously, β-glucans increased immune responsiveness.[39,40] However, human clinical trials involving oral consumption have reported less convincing effects thus far.[41–43] Although numerous reports suggest enhanced resistance to URTIs among immunocompromised populations following oral administrations of β-glucans, these primarily rely on self-reported symptoms. In the vast majority of cases, minimal or no significant alterations were detected in immune parameters.[41,42] Studies exploring the effects of oral β-glucan administration on immune function have often been limited to assessing its impact on the levels of circulating cytokines and chemokines.[44–46] Additionally, these studies often focus on investigating only one specific source and dose of β-glucan. In the current study, we not only explored a dose range, but assessed the effects of oral yWGP on the functionality of innate immune cells isolated from various compartments.

Our findings in mice that oral administration of yWGP increases the ex vivo-stimulated cytokine release in whole blood cultures are in line with the few human studies that also involved assessing cytokine production upon ex vivo stimulation.[29,47,48] Similar to β-glucans, we previously reported that rice bran-derived arabinoxylan preparations have the ability to enhance innate immune responsiveness in vitro.[5] Among the tested arabinoxylan sources, rice bran-1 exhibited the strongest effects in vitro. Consequently, this specific arabinoxylan preparation was selected for the present study. In line with our previous in vitro findings and the current results obtained with yWGP, animals exposed to rice bran-1 showed increased TNF-α levels in ex vivo-stimulated whole blood cultures. For rice bran-1, the results were dependent on the fiber dose. Only the whole blood cultures from

© 2024 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2. TNF-α levels of splenic immune cell populations stimulated with LPS, PAM3Cys, and HK-PA.

Cell population	Length of stimulation	Stimulation	Diet							
			yWGP				Rice bran-1			
			–	1%	2.5%	5%	–	1%	2.5%	5%
Whole splenocyte population	**4 h**	**Medium**	3 ± 0	3 ± 0	1 ± 0	4 ± 1	2 ± 1	4 ± 1	0 ± 0	2 ± 0
		LPS	93 ± 8	103 ± 11	124 ± 16	101 ± 12	124 ± 13	148 ± 13	102 ± 7	83 ± 8#
		PAM3Cys	13 ± 1	11 ± 1	13 ± 2	11 ± 1	17 ± 2	13 ± 2	14 ± 2	10 ± 1#
		HK-PA	13 ± 2	13 ± 3	15 ± 4	14 ± 3	107 ± 24	117 ± 30	33 ± 4##	23 ± 4###
	24 h	**Medium**	7 ± 1	6 ± 0	6 ± 1	4 ± 1	16 ± 6	9 ± 1	7 ± 2	6 ± 3
		LPS	124 ± 14	134 ± 11	129 ± 8	142 ± 11	192 ± 17	214 ± 17	158 ± 10	201 ± 12
		PAM3Cys	149 ± 37	170 ± 20	125 ± 19	157 ± 17	228 ± 32	329 ± 49	206 ± 19	240 ± 28
		HK-PA	236 ± 35	244 ± 26	248 ± 30	212 ± 35	319 ± 21	340 ± 22	254 ± 30	288 ± 15
Adherent splenocyte population	**6 h**	**Medium**	0 ± 0	0 ± 0	4 ± 1	4 ± 0	5 ± 2	3 ± 0	2 ± 1	3 ± 1
		LPS	491 ± 118	731 ± 169	587 ± 141	496 ± 107	584 ± 118	467 ± 127	356 ± 89	390 ± 85
		PAM3Cys	158 ± 34	186 ± 53	208 ± 60	136 ± 35	1066 ± 217	436 ± 140	277 ± 88##	305 ± 78#
		HK-PA	44 ± 16	88 ± 50	109 ± 47	43 ± 13	71 ± 22	84 ± 25	56 ± 27	94 ± 31
	24 h	**Medium**	9 ± 2	10 ± 2	10 ± 5	2 ± 1	12 ± 3	19 ± 2	3 ± 3	5 ± 3
		LPS	1112 ± 170	1126 ± 194	1215 ± 243	824 ± 105	950 ± 145	809 ± 152	544 ± 142#	442 ± 71#
		PAM3Cys	719 ± 98	709 ± 123	787 ± 166	513 ± 65	1495 ± 296	1192 ± 213	760 ± 138#	705 ± 115#
		HK-PA	354 ± 56	422 ± 94	459 ± 120	254 ± 42	542 ± 119	346 ± 91	261 ± 81$	237 ± 55$

Color key	p-value	
####	$p < 0.0001$	Decrease
###	$p < 0.001$	
##	$p < 0.01$	
#	$p < 0.05$	
$	$p < 0.1$	
	$p > 0.1$	–
+	$p < 0.1$	Increase
*	$p < 0.05$	
**	$p < 0.01$	
***	$p < 0.001$	
****	$p < 0.0001$	

yWGP, yeast-derived whole β-glucan particle; 1%, 2.5%, 5%, dietary fiber concentration expressed as w/w; HK-PA, heat-killed *Pseudomonas aeruginosa*. $*/^{\#}p < 0.05$, $**/^{\#\#}p < 0.01$, $***/^{\#\#\#}p < 0.001$, $****/^{\#\#\#\#}p < 0.0001$, as analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test. $^{+}/^{\$}p < 0.1$ was considered a trend.

© 2024 The Authors. *Molecular Nutrition & Food Research* published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

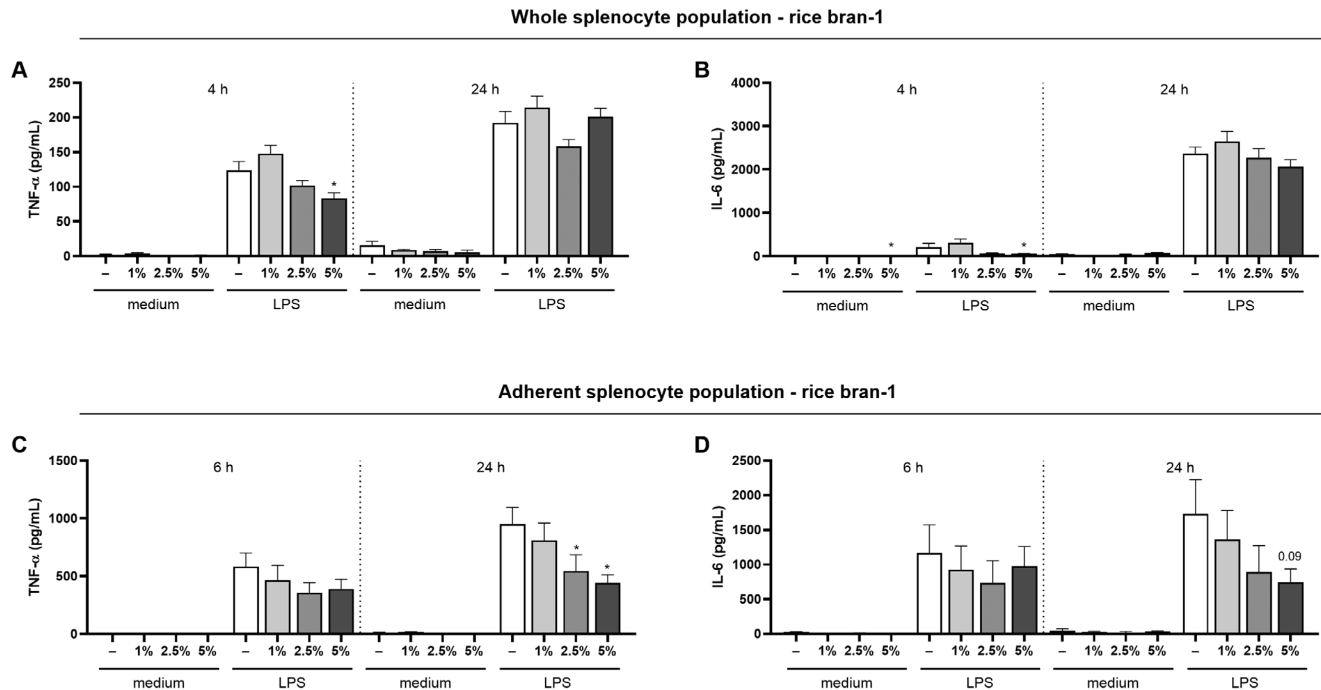

Figure 6. Splenocytes from rice bran-1-exposed animals show reduced pro-inflammatory cytokine production following ex vivo LPS challenge. After a 2-week period of receiving a rice bran-1-supplemented diet (1%, 2.5%, or 5% w/w), the whole (A,B) and adherent (C,D) splenocyte populations were isolated and ex vivo stimulated with medium or LPS. Release of TNF-α and IL-6 by splenocytes from rice bran-1-exposed and control animals was measured in the collected supernatant at 4, 6, and 24 h after medium or LPS stimulation. Data are presented as mean ± SEM, $n = 6$ for control animals and $n = 12$ for yWGP exposed animals. $^*p < 0.05$, as analyzed with one-way ANOVA followed by Dunnett's multiple comparisons test.

animals that had consumed the 1% fiber diet displayed an increased ex vivo cytokine response.

The discovery of memory function in hematopoietic stem cells[32] provides a possible explanation for the phenomenon of short-lived immune cells in blood acquiring memory. It is noteworthy that monocytes and dendritic cells have a relatively short lifespan in both mice and humans, typically around 5–7 days.[49] Consequently, reprogramming of myeloid progenitors within the bone marrow contributes to the generation of trained immunity within the myeloid cell compartment comprising blood monocytes and tissue macrophages.[35] For this reason, we investigated whether dietary exposure to yWGP or rice bran-1 for 2 weeks also induces innate immune priming in bone marrow-derived cells. We observed, for the first time, that whole bone marrow cultures as well as the adherend cell fractions displayed an enhanced cytokine secretion upon re-stimulation with heterologous stimuli. Animals fed with 1% and 2.5% yWGP showed enhanced release of TNF-α and IL-6. Interestingly, no effect was observed in animals fed 5% yWGP, indicating the dose-dependency of the yWGP-induced priming effect (which was not observed in whole blood cultures). In contrast to yWGP, rice bran-1 showed differential effects in restimulated whole bone marrow cultures and adherend cell cultures. While only the 1% rice bran-1 induced enhanced TNF-α release in whole bone marrow cultures, an inverse effect was observed in adherend cell cultures where TNF-α and IL-6 levels were reduced following LPS stimulation. This effect was particularly noticeable at concentrations of 2.5% and 5%. These observations align with recent in vitro research indicating that low-dose priming with PRR ligands induces training effects in primary macrophages, while high-dose priming leads to prolonged inhibition of inflammatory cytokine release.[50,51] Furthermore, the difference in cytokine release between whole bone marrow cells and adherent cells may be attributed to differences in cell composition. Previous studies indicate that non-adherent bone marrow cells consist mainly of immature/nuclear red blood cells, lymphocytes, monocytes, and granulocytes.[52,53] With selective adhesion, there is a decrease in lymphocytes and immature/nuclear red blood cells, while the populations of monocytes and granulocytes remain stable.[53] As a result, adherent bone marrow cells consist mainly of monocytes/macrophages and granulocytes, with TNF-α production mediated mainly by these cells. This production is less influenced by lymphocytes, among others. This shift in cell composition likely contributes to the increased production observed in adherent bone marrow cell cultures compared with whole bone marrow cells.

The spleen is a site of extramedullary hematopoiesis and a reservoir of undifferentiated monocytes.[54] However, our findings revealed no evidence of increased cytokine production upon ex vivo challenge in splenic immune cell populations derived from animals orally exposed to varying concentrations of yWGP/rice bran-1. These results are in line with the study of Ferreira et al. demonstrating that removal of the spleen does not modulate the ability of animals exposed intraperitoneally to β-glucan to elicit a proinflammatory cytokine response against a secondary stimulation.[55]

The increased ex vivo-stimulated release of pro-inflammatory cytokines that we observed following oral administration of yWGP or rice bran-1 could be attributed to immune priming, in

© 2024 The Authors. Molecular Nutrition & Food Research published by Wiley-VCH GmbH
16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

which the initial fiber stimulus modifies the functional state of innate immune cells, causing an additive response upon the secondary stimulation (the ex vivo stimulation).[1] Since epigenetic alterations are known to underlie this phenomenon, further research in this area is of great interest. In addition, it would be interesting for future research to investigate training regimes involving a wash-out period between fiber exposure and ex vivo challenge. Our findings demonstrate that the effects of yWGP and rice bran-1 on immune responsiveness are dose-dependent. The strongest priming effects were consistently observed at doses corresponding to a content of 1% w/w for both fiber types. Consumption of feed with concentrations of 2.5% w/w showed effects only in the case of yWGP, while an even higher concentration of 5% w/w failed to induce innate immune priming (except in whole blood cultures from yWGP animals). Intriguingly, consuming feed containing 5% w/w rice bran-1 for 2 weeks even diminished cytokine responses in splenocytes and adherent bone marrow cultures of the mice. These findings suggest that, particularly for rice bran-1, the doses used might be somewhat high for effectively inducing innate immune priming. For yWGP, an optimal response might be attainable with a concentration lower than those currently studied. Consequently, additional experiments are warranted to more accurately determine the optimal dosage for dietary supplementation. In addition to priming, immune modulation by dietary fibers could also be caused by an indirect prebiotic effect. Future exploration of alterations in microbiota composition and its metabolites therefore also holds considerable intrigue.

To the best of our knowledge, this is the first demonstration of oral exposure to yWGP and rice bran-1 leading to innate immune priming in the blood and bone marrow compartments of mice. The effects were found to be dose-dependent, with most substantial outcomes observed at the lowest dose given, corresponding to 1% w/w. The capacity to induce immune priming appears to also depend on the fiber source, with β-glucan showing greater potency compared to arabinoxylan at the dosages used. While the precise underlying mechanisms are yet to be determined, our results indicate that oral administration of yWGP and rice bran-1 enhances immune responses in mice. This aligns with in vitro training studies on human monocytes and in vivo studies using parenteral administration. The efficacy of β-glucans and arabinoxylans as dietary ingredients or supplements for bolstering host immunity warrants further substantiation in future human trials, with an emphasis on dose optimization.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This research is funded by the partnership between NWO domain Applied and Engineered Sciences and Danone Nutricia Research and with additional financial support from Topsector Agri and Food.

Conflict of Interest

J.v.B. and M.v.D. are employed by Danone Nutricia Research. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors Contributions

B.G.J.M., J.J.M., J.v.B., C.G., M.v.D., K.v.N., and S.A. conceived of and designed the study. B.G.J.M. and S.A. performed the experiments. B.G.J.M. and S.A. analyzed the data and wrote the manuscript, which was reviewed by all co-authors.

Keywords

β-glucan, arabinoxylan, dietary intake, dose optimization, innate immune priming

Received: November 22, 2023
Revised: March 26, 2024
Published online: April 29, 2024

[1] M. Divangahi, P. Aaby, S. A. Khader, L. B. Barreiro, S. Bekkering, T. Chavakis, R. van Crevel, N. Curtis, A. R. DiNardo, J. Dominguez-Andres, R. Duivenvoorden, S. Fanucchi, Z. Fayad, E. Fuchs, M. Hamon, K. L. Jeffrey, N. Khan, L. A. B. Joosten, E. Kaufmann, E. Latz, G. Matarese, J. W. M. van der Meer, M. Mhlanga, S. Moorlag, W. J. M. Mulder, S. Naik, B. Novakovic, L. O'Neill, J. Ochando, K. Ozato, et al., *Nat. Immunol.* **2021**, *22*, 2.
[2] M. G. Netea, L. A. Joosten, E. Latz, K. H. Mills, G. Natoli, H. G. Stunnenberg, L. A. O'Neill, R. J. Xavier, *Science* **2016**, *352*, aaf1098.
[3] M. G. Netea, J. Quintin, J. W. van der Meer, *Cell Host Microbe* **2011**, *9*, 355.
[4] A. Christ, P. Gunther, M. A. R. Lauterbach, P. Duewell, D. Biswas, K. Pelka, C. J. Scholz, M. Oosting, K. Haendler, K. Bassler, K. Klee, J. Schulte-Schrepping, T. Ulas, S. Moorlag, V. Kumar, M. H. Park, L. A. B. Joosten, L. A. Groh, N. P. Riksen, T. Espevik, A. Schlitzer, Y. Li, M. L. Fitzgerald, M. G. Netea, J. L. Schultze, E. Latz, *Cell* **2018**, *172*, 162.
[5] B. G. J. Moerings, J. van Bergenhenegouwen, M. Furber, S. Abbring, H. A. Schols, R. F. Witkamp, C. Govers, J. J. Mes, *Int. J. Biol. Macromol.* **2022**, *209*, 942.
[6] E. J. Murphy, E. Rezoagli, I. Major, N. J. Rowan, J. G. Laffey, J. Fungi, *(Basel)* **2020**, *6*, 356.
[7] E. A. Murphy, J. M. Davis, M. D. Carmichael, *Curr. Opin. Clin. Nutr. Metab. Care* **2010**, *13*, 656.
[8] A. Nakashima, K. Yamada, O. Iwata, R. Sugimoto, K. Atsuji, T. Ogawa, N. Ishibashi-Ohgo, K. Suzuki, *J. Nutr. Sci. Vitaminol. (Tokyo)* **2018**, *64*, 8.
[9] J. P. Angeli, L. R. Ribeiro, M. F. Bellini, M. S. Mantovani, *Arch. Toxicol.* **2009**, *83*, 81.
[10] H. P. van Steenwijk, A. Bast, A. de Boer, *Nutrients* **2021**, *13*, 1333.
[11] B. G. J. Moerings, P. de Graaff, M. Furber, R. F. Witkamp, R. Debets, J. J. Mes, J. van Bergenhenegouwen, C. Govers, *Front. Immunol.* **2021**, *12*, 672796.
[12] J. Quintin, S. Saeed, J. H. A. Martens, E. J. Giamarellos-Bourboulis, D. C. Ifrim, C. Logie, L. Jacobs, T. Jansen, B. J. Kullberg, C. Wijmenga, L. A. B. Joosten, R. J. Xavier, J. W. M. van der Meer, H. G. Stunnenberg, M. G. Netea, *Cell Host Microbe* **2012**, *12*, 223.
[13] S. Saeed, J. Quintin, H. H. Kerstens, N. A. Rao, A. Aghajanirefah, F. Matarese, S. C. Cheng, J. Ratter, K. Berentsen, M. A. van der Ent, N. Sharifi, E. M. Janssen-Megens, M. Ter Huurne, A. Mandoli, T. van Schaik, A. Ng, F. Burden, K. Downes, M. Frontini, V. Kumar, E. J. Giamarellos-Bourboulis, W. H. Ouwehand, J. W. van der Meer, L. A. Joosten, C. Wijmenga, J. H. Martens, R. J. Xavier, C. Logie, M. G. Netea, H. G. Stunnenberg, *Science* **2014**, *345*, 1251086.

 © 2024 The Authors. *Molecular Nutrition & Food Research* published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

[14] J. C. Dos Santos, A. M. Barroso de Figueiredo, M. V. Teodoro Silva, B. Cirovic, L. C. J. de Bree, M. Damen, S. Moorlag, R. S. Gomes, M. M. Helsen, M. Oosting, S. T. Keating, A. Schlitzer, M. G. Netea, F. Ribeiro-Dias, L. A. B. Joosten, *Cell Rep.* **2019**, *28*, 2659.
[15] F. Bistoni, A. Vecchiarelli, E. Cenci, P. Puccetti, P. Marconi, A. Cassone, *Infect. Immun.* **1986**, *51*, 668.
[16] S. Y. Zhang, W. Li, C. J. Smith, H. Musa, *Crit. Rev. Food Sci. Nutr.* **2015**, *55*, 1035.
[17] B. Siurek, J. Rosicka-Kaczmarek, E. Nebesny, *Food Sci. Technol. Int.* **2012**, *18*, 559.
[18] M. Bermudez-Brito, N. M. Sahasrabudhe, C. Rosch, H. A. Schols, M. M. Faas, P. de Vos, *Mol. Nutr. Food Res.* **2015**, *59*, 698.
[19] M. Ghoneum, M. Matsuura, *Int. J. Immunopathol. Pharmacol.* **2004**, *17*, 283.
[20] C. Govers, Y. F. Tang, E. H. Stolte, H. J. Wichers, J. J. Mes, *Food Funct.* **2020**, *11*, 7073.
[21] A. Perez-Martinez, J. Valentin, L. Fernandez, E. Hernandez-Jimenez, E. Lopez-Collazo, P. Zerbes, E. Schworer, F. Nunez, I. G. Martin, H. Sallis, M. A. Diaz, R. Handgretinger, M. M. Pfeiffer, *Cytotherapy* **2015**, *17*, 601.
[22] L. Cao, X. Liu, T. Qian, G. Sun, Y. Guo, F. Chang, S. Zhou, X. Sun, *Int. J. Biol. Macromol.* **2011**, *48*, 160.
[23] D. Cholujova, J. Jakubikova, B. Czako, M. Martisova, L. Hunakova, J. Duraj, M. Mistrik, J. Sedlak, *Cancer Immunol. Immunother.* **2013**, *62*, 437.
[24] S. M. Zhou, X. Z. Liu, Y. Guo, Q. A. Wang, D. Y. Peng, L. Cao, *Carbohydr. Polym.* **2010**, *81*, 784.
[25] C. Laue, Y. Stevens, M. van Erp, E. Papazova, E. Soeth, A. Pannenbeckers, E. Stolte, R. Bohm, S. L. Gall, X. Falourd, S. Ballance, S. H. Knutsen, I. Pinheiro, S. Possemiers, P. M. Ryan, R. P. Ross, C. Stanton, J. M. Wells, S. V. Werf, J. J. Mes, J. Schrezenmeir, *Nutrients* **2021**, *13*, 2683.
[26] P. J. Rice, E. L. Adams, T. Ozment-Skelton, A. J. Gonzalez, M. P. Goldman, B. E. Lockhart, L. A. Barker, K. F. Breuel, W. K. Deponti, J. H. Kalbfleisch, H. E. Ensley, G. D. Brown, S. Gordon, D. L. Williams, *J. Pharmacol. Exp. Ther.* **2005**, *314*, 1079.
[27] A. B. C. Samuelsen, J. Schrezenmeir, S. H. Knutsen, *Mol. Nutr. Food Res.* **2014**, *58*, 183.
[28] J. Leentjens, J. Quintin, J. Gerretsen, M. Kox, P. Pickkers, M. G. Netea, *PLoS One* **2014**, *9*, e108794.
[29] R. Fuller, M. V. Moore, G. Lewith, B. L. Stuart, R. V. Ormiston, H. L. Fisk, P. S. Noakes, P. C. Calder, *Nutrition* **2017**, *39–40*, 30.
[30] S. Talbott, J. Talbott, *J. Sport Sci. Med.* **2009**, *8*, 509.
[31] J. J. Volman, J. D. Ramakers, J. Plat, *Physiol. Behav.* **2008**, *94*, 276.
[32] I. Mitroulis, K. Ruppova, B. M. Wang, L. S. Chen, M. Grzybek, T. Grinenko, A. Eugster, M. Troullinaki, A. Palladini, I. Kourtzelis, A. Chatzigeorgiou, A. Schlitzer, M. Beyer, L. A. B. Joosten, B. Isermann, M. Lesche, A. Petzold, K. Simons, I. Henry, A. Dahl, J. L. Schultze, B. Wielockx, N. Zamboni, P. Mirtschink, U. Coskun, G. Hajishengallis, M. G. Netea, T. Chavakis, *Cell* **2018**, *172*, 147.
[33] F. K. Swirski, M. Nahrendorf, M. Etzrodt, M. Wildgruber, V. Cortez-Retamozo, P. Panizzi, J. L. Figueiredo, R. H. Kohler, A. Chudnovskiy, P. Waterman, E. Aikawa, T. R. Mempel, P. Libby, R. Weissleder, M. J. Pittet, *Science* **2009**, *325*, 612.
[34] B. El-Din, N. K. E. Noaman, M. Ghoneum, *Nutr. Cancer* **2008**, *60*, 235.
[35] T. Chavakis, I. Mitroulis, G. Hajishengallis, *Nat. Immunol.* **2019**, *20*, 802.
[36] P. Garcia-Valtanen, R. M. Guzman-Genuino, D. L. Williams, J. D. Hayball, K. R. Diener, *Immunol. Cell Biol.* **2017**, *95*, 601.
[37] C. L. Stothers, K. R. Burelbach, A. M. Owen, N. K. Patil, M. A. McBride, J. K. Bohannon, L. M. Luan, A. Hernandez, T. K. Patil, D. L. Williams, E. R. Sherwood, *J. Immunol.* **2021**, *207*, 2785.
[38] E. Mah, V. N. Kaden, K. M. Kelley, D. J. Liska, *J. Med. Food* **2020**, *23*, 416.
[39] M. L. Patchen, T. J. MacVittie, *Int. J. Immunopharmacol.* **1985**, *7*, 923.
[40] V. Vetvicka, L. Vannucci, P. Sima, *Scand. J. Immunol.* **2020**, 91.
[41] A. Auinger, L. Riede, G. Bothe, R. Busch, J. Gruenwald, *Eur. J. Nutr.* **2013**, *52*, 1913.
[42] G. Lehne, B. Haneberg, P. Gaustad, P. W. Johansen, H. Preus, T. G. Abrahamsen, *Clin. Exp. Immunol.* **2006**, *143*, 65.
[43] H. Stier, V. Ebbeskotte, J. Gruenwald, *Nutr. J.* **2014**, *13*, 38.
[44] R. Fuller, H. Butt, P. S. Noakes, J. Kenyon, T. S. Yam, P. C. Calder, *Nutrition* **2012**, *28*, 665.
[45] J. M. Gaullier, J. Sleboda, E. S. Ofjord, E. Ulvestad, M. Nurminiemi, C. Moe, A. Tor, O. Gudmundsen, *Int. J. Med. Mushrooms* **2011**, *13*, 319.
[46] H. A. Zabriskie, J. C. Blumkaitis, J. M. Moon, B. S. Currier, R. Stefan, K. Ratliff, P. S. Harty, R. A. Stecker, K. Rudnicka, R. Jager, M. D. Roberts, K. Young, A. R. Jagim, C. M. Kerksick, *Nutrients* **2020**, *12*, 1144.
[47] K. C. Carpenter, W. L. Breslin, T. Davidson, A. Adams, B. K. McFarlin, *Br. J. Nutr.* **2013**, *109*, 478.
[48] B. K. McFarlin, A. S. Venable, K. C. Carpenter, A. L. Henning, S. Ogenstad, *Front. Physiol.* **2017**, *8*, 786.
[49] A. A. Patel, Y. Zhang, J. N. Fullerton, L. Boelen, A. Rongvaux, A. A. Maini, V. Bigley, R. A. Flavell, D. W. Gilroy, B. Asquith, D. Macallan, S. Yona, *J. Exp. Med.* **2017**, *214*, 1913.
[50] M. Bauer, S. Weis, M. G. Netea, R. Wetzker, *Trends Immunol.* **2018**, *39*, 438.
[51] D. C. Ifrim, J. Quintin, L. A. Joosten, C. Jacobs, T. Jansen, L. Jacobs, N. A. Gow, D. L. Williams, J. W. van der Meer, M. G. Netea, *Clin. Vaccine Immunol.* **2014**, *21*, 534.
[52] S. Fricke, C. Fricke, C. Oelkrug, N. Hilger, U. Schonfelder, M. Kamprad, J. Lehmann, J. Boltze, F. Emmrich, U. Sack, *Cell Mol. Life Sci.* **2010**, *67*, 4095.
[53] A. Hinze, A. Stolzing, *J. Inflamm. (Lond.)* **2012**, *9*, 12.
[54] V. Bronte, M. J. Pittet, *Immunity* **2013**, *39*, 806.
[55] A. V. Ferreira, R. F. Uijen, O. Bulut, M. I. de Jonge, J. Dominguez-Andres, M. G. Netea, *J. Leukocyte Biol.* **2022**, *111*, 9.

© 2024 The Authors. *Molecular Nutrition & Food Research* published by Wiley-VCH GmbH

16134133, 2024, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202300829 by Wageningen University And Research Facilitair Bedrijf, Wiley Online Library on [17/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License