Journal of Functional Foods 94 (2022) 105121

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Combination of different probiotics and berry-derived (poly)phenols can modulate immune response in dendritic cells

Valentina Taverniti [a,*], Cristian Del Bo' [b], Walter Fiore [a,c], Giorgio Gargari [a], Stefania Arioli [a], Patrizia Riso [b], Simone Guglielmetti [a], Hanne Frøkiær [d]

[a] Division of Food Microbiology and Bioprocesses, Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Italy
[b] Division of Human Nutrition, Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Italy
[c] Sofar S.r.l., Trezzano Rosa, Milano, Italy
[d] Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg, Denmark

ARTICLE INFO

Keywords:
L. paracasei
B. bifidum
Cranberry
Blueberry
IL-10
Synergism

ABSTRACT

The immunomodulatory potential of probiotics and (poly)phenols (PP) is recognized; however, studies regarding microorganisms-PP synergisms are yet to be explored. Here, we investigated the cooperation between probiotics and berry-derived PP extracts in modulating the cytokine responses in dendritic cells. Bacteria elicited immune responses in a strain-dependent manner. PP extracts showed different modulation of cytokine triggered by bacteria. Also with LPS, used as pro-inflammatory stimulus, PP from blueberry (BB) and cranberry (CB) most efficiently reduced IL12 production. *L. paracasei* LPC-S01 and *B. bifidum* MIMBb23sg resulted the best bacterial association in abrogating IL12 and increasing IL10. The use of PP fraction from BB50f and CB1 with the LPC-S01 + MIMBb23sg association resulted the most efficient combinations in terms of anti-inflammatory activity. These results provide bases for further investigation *in vivo*, in the perspective to develop food supplements that might conceivably deliver the single and combined benefits of probiotics and berry (poly)phenols.

1. Introduction

Consumption of fruits and vegetables have been associated with reduced blood inflammatory and oxidative markers (Root et al., 2012) and public health recommendations world-wide encourage a higher consumption of such foods (Guilbert, 2003), which positively correlate with an improvement of disease-related parameters (Van Duyn and Pivonka, 2000). Particularly, the (poly)phenolic (PP) fraction of fruits, especially berries, has been acknowledged and demonstrated to be a component with significant health-promoting properties, through its anti-inflammatory and immune-modulating activities (Fraga et al., 2019; Shama, 2016; Folmer et al., 2014). The anti-inflammatory effect of berry PP have been demonstrated on the postprandial response to meals as well as to exercise-induced inflammation in humans, *e.g.* by reducing the circulatory postprandial level of C-Reactive protein, Plasminogen activator inhibitor-1, interleukin (IL)1β and IL-6 (Edirisinghe et al., 2011; Ellis et al., 2011; McAnulty et al., 2011). More studies yet have investigated the effect of berry extracts on chronic low-grade inflammation in groups of individuals suffering from overweight or metabolic syndrome (Chiva-Blanch, 2017) and, although not all studies demonstrated significant anti-inflammatory effects of the berry PP, the overall trend is an attitude towards mild anti-inflammatory effects. Numerous animal studies support the findings of anti-inflammatory effects of berry PP (Land Lail et al., 2021). The effects of PP are often immune-mediated through anti-oxidant activities and modification of the expression of genes, including pro-inflammatory cytokines and enzymes (Yahfoufi et al., 2018). In recent years, much has been learned regarding the mechanism of action of the PP. Depending on their specific structure, the different PP may bind to one or more proteins involved in cell signaling. In particular, enzymes involved in the down-stream signaling of pattern recognition receptors (PRRs) expressed by immune cells and epithelial cells can become inhibited by polyphenols (Zhao et al., 2011). In addition, the specific structure of the PP molecule may be critical for how easily the molecule will pass through the cell membrane, an event required for the PP to exert the inhibitory effects on cell proteins. Hence, the different polyphenols found in berries may have highly diverse potentials as anti-inflammatory agents. Related to bioactives, another important and continuously increasing segment is that

* Corresponding author.
E-mail address: valentina.taverniti@unimi.it (V. Taverniti).

https://doi.org/10.1016/j.jff.2022.105121
Received 24 January 2022; Received in revised form 17 May 2022; Accepted 17 May 2022
Available online 25 May 2022

of probiotics, which are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). Besides their effects on pathogen inhibition, modulation of microbiota composition and enhancement of epithelial barrier (Azad et al., 2018; Markowiak and Ślizewska, 2017; Kechagia et al., 2013; Bermudez-Brito et al., 2012), probiotics have been widely demonstrated to possess immunomodulatory activity (Azad et al., 2018; Liu et al., 2018). Some of the most convincing effects found in human interventions have been prevention of upper respiratory airway infections (Hao et al., 2015), atopic dermatitis (Panduru et al., 2015; Pelucchi et al., 2012) and inflammatory bowel diseases (Jia et al., 2018). In addition, numerous studies using animal disease models support these findings and reveal different mechanism of actions (Patel et al., 2015). In contrast to (poly)phenols, probiotics act by stimulating specific receptors on myeloid cells and epithelial cells, initiating signaling cascades such as those leading to the activation of the nuclear factor (NF)-κB and the mitogen-activated protein kinase (MAPK) pathways, all leading to the production of cytokines, involved in recruitment of immune cells and priming of lymphocytes towards specific phenotypes, thus resulting in modulation of host physiology (Carneiro et al., 2019). Probiotic bacteria have been demonstrated to stimulate different signaling pathways, in turn giving rise to the production of distinct cytokine profile (Christensen et al., 2002). Accordingly, with respect to stimulation of cytokine production, probiotics and (poly)phenols may interact, thus giving rise to different and unpredictable effects. (de Souza et al., 2019; Banerjee and Dhar, 2019; dos Santos et al., 2019). Some studies have investigated the combined effects of PP and probiotics. In this regard, probiotics have been shown to perform biotransformation of (poly)phenolic compounds through the activity of different enzymes, such as glycosyl-hydrolases, causing the release of aglycones from glycol-conjugated phenolic compounds, also resulting in improvement of absorption and bioavailability of such molecules. From the other side, PP at gut level may exert a prebiotic function and favor the population of beneficial bacteria, including probiotics (de Souza et al., 2019; Banerjee and Dhar, 2019; dos Santos et al., 2019; Taverniti et al., 2017). All these data demonstrate that, in the gastrointestinal tract, the presence of probiotics may influence the absorption of PP and PP may affect the growth and survival of probiotics. Both probiotics and PP may, however, transverse the gut epithelium, where the PP and probiotics can affect immune cells, in particular macrophages and dendritic cells, thus affecting the cytokines produced, in turn impacting on the physiological state. Dendritic cells, in particular, are antigen-presenting cells which form the bridge between the innate and the adaptive immunity and direct both types of immune responses by the cytokines that are being produced. Therefore, we decided to employ DCs as *in vitro* model to study the combinatory effects of bacteria and polyphenols, by screening several combinations of probiotic strains (among lactic acid bacteria and bifidobacteria) and PP fractions extracted from different berries, in order to investigate their modulatory actions on cytokines production, in order to identify specific combinations that could be worthy for further *in vivo* studies. Our results evidenced that a specific coupling of a *Lacticaseibacillus paracasei* and a *Bifidobacterium bifidum* strain employed together with two different PP extracts of blueberry and cranberry enhanced the immunomodulatory potential of the berry extracts, also in presence of LPS, used in our model as pro-inflammatory stimulus. On the basis of these observations, the *in vitro* screening performed in this study provides a rationale that encourages further *in vivo* studies, finally targeted to the full characterization and potential development of a food supplement that might be helpful to prevent or help in dampening inflammatory conditions.

2. Materials and methods

2.1. Bacterial strains, preparation, and growth conditions

All the strains used for this study were grown in de Man-Rogosa-Sharpe (MRS) broth (Difco Laboratories Inc., Detroit, MI, USA) supplemented with 0.05% L-cysteine hydrochloride (Sigma-Aldrich) (cMRS), in order to standardize bacterial preparation conditions between lactic acid bacteria (LAB) and bifidobacteria. Bacteria were inoculated from frozen glycerol stocks and sub-cultured twice in cMRS using a 1:100 inoculum; LAB *Lactobacillus* and *Lacticaseibacillus* were incubated at 37 °C in static conditions, whereas bifidobacteria were incubated at 37 °C in anaerobic conditions (Anaerocult A System; Merck, Darmstadt, Germany). Bacterial cells from an overnight culture were collected and washed twice with sterile PBS (Sigma-Aldrich) supplemented with pre-reduced 0.05% L-cysteine (cPBS). After that, a viable cell count of bacterial suspensions was performed by using an Accuri C6 flow cytometer (BD Bio-sciences, Milan, Italy) equipped with a 488 nm laser beam excitation. The following threshold settings were applied: FSC-H 3000 and SSC-H 1000, 50 μl collected at a medium flow rate. Cell suspensions were stained with SYBR Green I (Sigma-Aldrich) cells staining at 37 °C for 15 min in the dark. Fluorescence of SYBR Green I was recovered in the FL1 channel (excitation 488 nm, emission filter 530/30 nm). The rate of events was generally lower than 2000 events/s. All parameters were collected as logarithmic signals. The data obtained were analysed using the BD ACCURI C6 software version 1.0 (BD Biosciences, Milan, Italy) (Arioli et al., 2018). On the basis of the obtained counts, each bacterial strain was resuspended at a known concentration in cPBS added with sterile glycerol (1:6 v/v) and stored at −80 °C in aliquots.

2.2. Preparation of berry (poly)phenolic fractions

Wild blueberry powder 3% (w/w) polyphenol (BB3; Ref OK705055, lot K347/002/A15), Wild blueberry powder 50% (w/w) fibers (BB50f; Ref OK705001, lot C056/003/D16), Strawberry (SB) powder 100% (w/w) fruit (SB; Ref ON200003) and Cranberry 1% (w/w) proanthocyanidins (CB1; Nutricran 90S_06155, Ref EK036155, batch number K066/001/A13, lot numer pH 142288) were produced in an industrial process at Naturex Inc, USA. Elderberry (EB) spray dried fruit (EB; Code 70120053, Batch L 1601300421, lot pH 160422) was produced by Iprona AG-S.p.A., Italy. Cranberry 15% (w/w) proanthocyanidins (CB15; product name Urophenol Extract: Pur, code number URO-std-Pur 15% PAC BL-DMAC, lot number 636 or pH 160169) was produced by Nutra Canada Inc., Canada. All the berry powders have been analyzed per the norms in vigor for microorganism content, pesticides (European pharmacopoeia) and heavy metals. The berry powders were used to extract two different fractions: the water-soluble fraction (containing mainly simple sugars and organic acids), the methanol soluble fraction (containing mainly (poly)phenols). Extraction was performed following the method described by Wrolstad et al. (Wrolstad, 2005) with some modifications. Briefly, 500 mg of each berry powder was suspended in 40 ml of deionized water, vortexed for 3 min, sonicated for 15 min, and centrifuged at 3000 *g* for 10 min at 20 °C (step 1). Supernatant was collected and 6 ml of aqueous supernatant was loaded through solid-phase extraction (SPE) cartridges (Strata-X 300 mg/3 ml, Phenomenex, Torrence, CA, USA), while the pellet was used for a second extraction in 10 ml of MetOH, sonicated, vortexed and centrifugated as previously reported (step 2). The elution of water- soluble (WS) compounds from the cartridges was carried out with 0.01 N HCl (5 ml). The WS fraction was discarded, whereas the PP extract was obtained by loading methanol (5 ml) containing 0.1% HCl and the methanolic supernatant obtained from step 2. A third and final elution was performed with acetone (3 ml). Fractions obtained from methanol and acetone elution were combined and dried under vacuum with a rotavapor (RC Jouan 10, Jouan, Winchester, VA, USA) at 20° C. The dried PP extracts were dissolved in 1 ml of methanol acidified with HCl (0.05 mM) and stored at −20° C until analysis for their total (poly)phenol content.

2.3. Total (poly)phenol, anthocyanin and proanthocyanidin content

Total (poly)phenol content (TPC) of the PP extract was determined by Folin-Ciocalteu method (Wrolstad, 2001). Briefly, 5 μl of the berry PP extracts solution was diluted in methanol/acetone/water solution (495 μl; 30:30:30 v/v, acidified with 0.1% acetic acid). Next, the diluted sample (200 μl) was put in a tube containing 1 ml of Folin-Ciocalteu's reagent and 800 μl of a saturated solution of sodium carbonate. Samples were mixed and incubated for 60 min at room temperature in the dark. Finally, the absorbance was measured spectrophotometrically at 765 nm. The results were ex-pressed as gallic acid equivalents (GAE) using a calibration curve obtained with gallic acid standard.

Anthocyanin content of the PP extracts was determined as previously reported (Del Bò et al., 2010). Briefly, 5 μl of the berry PP extracts solution was diluted in methanol acidified with 0.1% TFA. The chromatographic system consisted of an Alliance model 2695 (Water, Milford, MA, USA) equipped with a model 2998 photodiode array detector (Waters). The separation was carried out through a C18 Kinetex column (150 × 4.6 mm, 2.6 μm, Phenomenex) at 45 °C with a 1.7 ml min^{-1} flow rate. The eluents were (A) 1% H3PO4 and (B) acetonitrile/water (35:65, v/v) while the mobile was linear as follow: 0–15 min, 14% B; 15–25 min, from 14 to 20% B; 25–35 min, from 20 to 32% B; 35–45 min, from 32 to 50% B; 45–48 min, from 50 to 90% B; 90% for 3 min. The calibration curve (from 2 to 50 μg ml^{-1}) for each ACN (Cy-, Dp-, Pt-, Pe-, and Mv-3-O-glc, Cy-and Pt-3-O-gal, and Pt-3-O-ara) was obtained by diluting the stock solution with acidified methanol (0.1% TFA). Each analysis was carried out in duplicate. Chromatographic data were acquired in the range of 200–700 nm and integrated at 520 nm. The single ACNs were identified by LC coupled to electrospray ionization − mass spectrometry (ESI-MS) operated in positive full-scan mode in the range of 200–800 Da. The capillary voltage was set to 3.5 kV, the cone voltage to 20 V, the source temperature to 130 °C, and the desolvating temperature to 350 °C. Data were acquired by Masslinx 4.0 software (Micromass, Beverly, MA, USA).

Total proanthocyanidin (PAC) content of the PP fractions/extracts was determined according to Prior et al. (Prior et al., 2010), with slight modification, as previously reported (Gardana and Simonetti, 2019). Briefly, an amount of 50 mg of 4-dimethylamino-cinnamaldehyde (DMAC) reagent was dissolved in 50 ml of acidified ethanol prepared by mixing 75 ml of ethanol with 12.5 ml of 37% HCl and 12.5 ml of deionized water. Then, 70 μl of sample or standard was added to 2.1 ml of DMAC solution. The reaction was monitored every minute for 60 min at 640 nm by a Lambda 20 spectrophotometer (PerkinElmer, Waltham, MA, USA). The blanks were reagents and samples diluted in acidified ethanol. The assay was performed in duplicate, and the total percentages of the PACs, ex-pressed as PAC-A2 equivalents, were calculated as previously reported (Gardana and Simonetti, 2019).

2.4. Generation of bone marrow-derived dendritic cells

Bone marrow-derived DCs were prepared as described previously (Christensen et al., 2002). Briefly, bone marrow from C57BL/6 mice (Tactonic, Lille Skensved, Denmark) was flushed out from the femur and tibia and washed. 3×10^5 ml^{-1} bone marrow cells were seeded into 10 cm Petri dishes in 10 ml RPMI 1640 (Sigma-Aldrich, St. Louis, MO, USA) containing 10% (v/v) heat inactivated fetal calf serum supplemented with penicillin (100 U ml^{-1}), streptomycin (100 mg ml^{-1}), glutamine (4 mM), 50 mm 2-mercaptoethanol (all purchased from Cambrex Bio Whittaker) and 15 ng ml^{-1} murine GM-CSF (harvested from a GM-CSF transfected Ag8.653 myeloma cell line). The cells were incubated for 8 days at 37 °C in 5% CO_2 humidified atmosphere. On day 3, 10 ml of complete medium containing 15 ng ml-1 GM-CSF was added. On day 6, 10 ml were removed and replaced by fresh medium. Non-adherent, immature DC were harvested on day 8.

2.5. Stimulation of DCs with bacterial cells and berries

Immature DCs were resuspended in fresh medium (2×10^6 cells ml^{-1}) supplemented with 10 ng ml^{-1} GM-CSF, and 500 ml $well^{-1}$ were seeded in 48-well tissue culture plates (Nunc, Roskilde, Denmark). All bacterial strains were used at a MOI of 5, both when used alone and in combination with berry extracts. When bacteria were used in association of two or three strains together, the final MOI was kept at 5; therefore, for the combination of two bacteria, 2.5 MOI was used for each strain, whereas 1.6 MOI was used for each strain in the combination of three bacteria. (Poly)phenolic extracts obtained from berry powder were preliminary tested on DCs at a polyphenols concentration of 15 μg ml^{-1}, based on the total polyphenol content (TPC) analyzed in the PP extracts (paragraph 2.3) and showed in the last column of Table 1; however, as with this concentration we could not see any modulation, we afterwards used in all the experiments a final PP concentration of 50 μg ml^{-1}, calculated as above described. Piceatannol (Sigma-Aldrich) was used as reference in the *in vitro* model at a concentration of 25 μM (corresponding around to 5 μg ml^{-1}; Supplementary Fig. 3). Control conditions included DCs stimulated only with media, DCs stimulated with the vehicle used for bacteria preparation (cPBS + 10% glycerol, v/v), DCs stimulated with berry extracts solvent, which was MetOH + 0.05 mM HCl (MetOH/HCl, final concentration 0.3% (v/v)) and DCs stimulated with piceatannol solvent (DMSO, final concentration 0.1% (v/v)). MetOH/HCl concentration of 0.3% (v/v) corresponded to the volume needed to obtain a PP concentration of 50 μg ml^{-1} of the extract with the lowest PP content, as based on the PP quantifications (Table 1). In order to standardize all the experimental conditions and make results comparable, the volume of MetOH/HCl was adjusted so that the final concentration was 0.3% (v/v) in all the samples and conditions. All the conditions (bacteria and berries alone and in combinations) were tested also in presence of LPS from *E. coli* O26:B6 (Sigma-Aldrich) at a concentration of 1 μg ml^{-1}. Upon stimulation, the DCs were incubated for 20 h at 37 °C in 5% CO_2 humidified atmosphere, prior to the collection of supernatants for ELISA assay. Bacterial strains and polyphenolic extracts of berry powders were used in combinations to test the capability of berry PP to condition DCs to either prevent a pro-inflammatory condition, mimicked by LPS and/or to modulate the immune response elicited by bacteria. To this aim, for these experiments DCs were first pre-stimulated with berry extracts for 1 h, to allow berry PP to condition the DCs, followed by 1 h stimulation with bacteria, before the addition of LPS. All the experiments have been conducted at least twice, and with two technical replicates for each experiment. Figures show one representative experiment and error bars refer to technical replicates.

2.6. Cytokine quantification in DCs supernatant

The protein production of IL12(p70), IL10 and TNF-α was analyzed using commercially available ELISA kits (R&D systems, Minneapolis, MN, USA).

2.7. Statistical analysis

Statistical calculations for the comparison between bacterial strains and bacterial combinations have been performed by ANOVA and the Tukey's post hoc test. Value with different suffix letters significantly differ at a $P < 0.05$. Statistical analysis for the comparison of bacterial associations with berry PP extracts were analyzed by unpaired heteroscedastic Student's *t* test with two-tailed distribution. Differences of $P < 0.05$ were considered to be significant.

2.8. Ethics statement

All animals used as a source of bone marrow cells were housed under conditions approved by the Danish Animal Experiments Inspectorate (Forsøgdyrstilsynet), Ministry of Justice, Denmark, and experiments

Table 1
Description of the six commercial berry powders and Total (poly)phenols content (TPC) in the six berry polyphenolic (PP) extracts employed in the study.

Berry extract (Dry powders)	Polyphenol content (Dry powders)	Description (Dry powders)	Other components (Dry powders)	Supplier	TPC in PP extract (mg ml^{-1})*
Elderberry (EB)	8.85% anthocyanins 10% polyphenols	No information	Maltodextrin	Iprona	60.59 ± 1.11
Cranberry 15% proanthocyanidins (CB15)	>15% proanthocyanins	No information	No information	Canada Nutra	78.99 ± 0.89
Cranberry 1% proanthocyanidins (CB1)	>1% proanthocyanins	No information	No information	Naturex	64.04 ± 1.88
Strawberry (SB)	No information	1 kg of product from 15 kg fresh fruit	None	Naturex	57.80 ± 1.21
Blueberry 3% polyphenols (BB3)	>3% polyphenols	1 kg of product from 6 kg fresh fruit	Maltodextrin, silicon dioxide	Naturex	54.40 ± 1.19
Blueberry 50% fiber (BB50f)	>3% polyphenols	1 kg of product from 5 kg fresh fruit	Guar gum, sunflower lecithin	Naturex	50.12 ± 1.32

Data are reported as mean ± standard deviation.
* Equivalent of gallic acid.

were carried out in accordance with the guidelines 'The Council of Europe Convention European Treaty Series 123 for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes'. Since the animals were employed as sources of cells, and no live animals were used in experiments, no specific approval was required for this study. Hence, the animals used for this study are included in the general facility approval for the faculty of Health and Medical Sciences, University of Copenhagen.

3. Results

3.1. Total polyphenols content in the PP extracts

Table 1 reports the total polyphenol content (TPC) of the different PP extracts obtained from the six commercial berry powders, as specified in paragraph 2.3 of Materials and Methods. Cranberry (CB15 and CB1) showed the highest TPC content, followed by elderberry, strawberry, and blueberry (BB3 and BB50f). The Polyphenol content, Description and Other components columns of Table 1 show information as reported on technical sheets of berry powders, provided from the different Suppliers. TPC obtained from our analyses on PP extracts seems to reflect the powder extraction yield from fresh fruit, as specified by Suppliers for the powders of strawberry, BB50f and BB3.

3.2. Selected probiotic strains induce different cytokine profile in DCs

The probiotic strains under study were compared between each other in their attitude to induce cytokines IL12, IL10 and TNF-α upon contact with DCs, also with the LPS stimulus (Supplementary Fig. 1). Statistic differences between bacteria are evidenced by different suffix letters above histograms ($P < 0.05$; Fig. 1and Supplementary Fig. 1).

In the stimulatory activity of bacteria on DCs, LAB strains *L. paracasei* DG and CF3 displayed comparable induction of all the three cytokines analyzed (Fig. 1). In contrast, *L. paracasei* strain LPC-S01 induced a significantly lower amount of IL12 compared to the other two *L. paracasei* strains (around 50% less). Conversely, the production of IL10 was about 4 times higher with LPC-S01 (4029 pg ml^{-1}) compared to DG (1002 pg ml^{-1}) and CF3 (1156 pg ml^{-1}). Concerning TNF-α, *L. rhamnosus* GG displayed the highest induction among all LAB tested, with a significant difference compared with other LAB (Fig. 1). Regarding bifidobacteria, both with *B. bifidum* MIMBb23sg and *B. animalis* subsp. *lactis* BB-12 the induction of IL12 was negligible (Fig. 1). In the production of IL10, the levels induced by MIMBb23sg were around 2.5 times more compared to BB-12 (respectively, 2154 and 834 pg ml^{-1}; Fig. 1). Even though all five LAB strains induced an IL12 response comparable to or higher than the IL12 production induced by LPS, the combined stimulation of DCs by LPS and LAB led to lower IL12 levels, compared to bacteria or LPS used separately (Fig. 1 and Supplementary Fig. 1). The two *Bifidobacterium* strains decreased the LPS-induced level of IL12 to < 50% (Supplementary Fig. 1). Among all bacteria, MIMBb23sg was the strain that reduced the most IL12 levels, compared to DCs stimulated only with LPS (from 1810 to 277 pg ml^{-1}; Supplementary Fig. 1). Both LAB and bifidobacteria in combination with LPS induced IL-10 levels corresponding to, often, more than the sum of the IL-10 levels elicited by the bacteria and the LPS alone (Supplementary Fig. 1). LAB strains used alone induced more than the double of the TNF-α production compared to the levels induced by LPS; however, in combination with LPS, in most of the cases (excluding LPC-S01) the

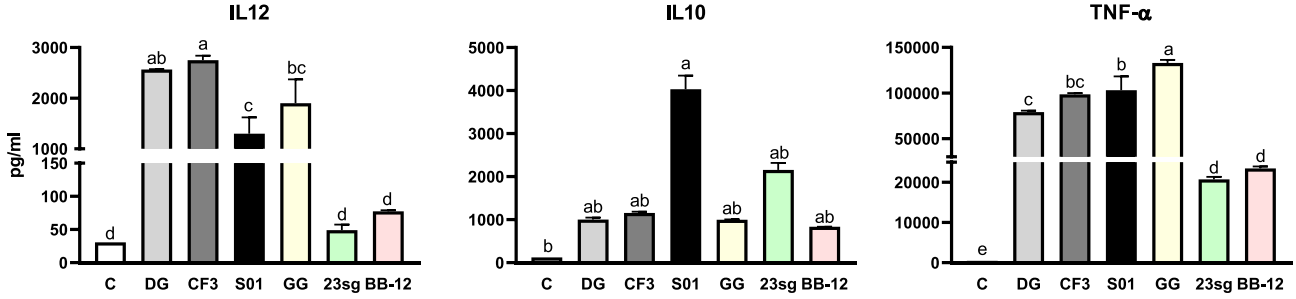

Fig. 1. Cytokine production in DCs upon stimulation with lactic acid bacteria and bifidobacteria. Protein levels of the cytokines IL12p70, IL10 and TNF-α were measured in the supernatants of DCs by ELISA after 20 h. Bacteria were used at multiplicity of infection (MOI) of 5. C: Control condition, DCs without bacterial cells. DG: *L. paracasei* DG, CF3: *L. paracasei* CF3; S01: *L. paracasei* LPC-S01; GG: *L. rhamnosus* GG; 23sg: *B. bifidum* MIMBb23sg; BB-12: *B. animalis* subsp. *lactis* BB-12. Data were analysed by ANOVA and the Tukey's post hoc test. Value with different suffix letters significantly differed at $P < 0.05$.

TNF-α levels were reduced compared to bacteria used alone (Fig. 1 and Supplementary Fig. 1). When LPS and bifidobacteria were tested together on DCs, the levels of TNF-α almost corresponded to the sum of the cytokine levels induced by LPS and bifidobacterial strains separately (Fig. 1 and Supplementary Fig. 1).

To summarize, *L. paracasei* LPC-S01 and *B. bifidum* MIMBb23sg were the most potent IL10 inducers, while eliciting the lowest levels of IL12. Regarding TNF-α, DG induced a lower production compared to LPC-S01 and GG. With the addition of LPS, MIMBb23sg decreased the most, among all bacteria, the IL12 levels compared to LPS-stimulated DCs, with a trend in a higher production of IL10 (5898 pg ml^{-1}) compared to the other *Bifidobacterium* strain BB-12 (3913 pg ml^{-1}), used as reference. Analogously, *L. paracasei* LPC-S01 had the most significant trend among LAB in reducing IL12 levels triggered by LPS (from 1810 to 724 pg ml^{-1}) and, overall, induced the highest levels of IL10 (from 1319 of DCs + LPS to 14353 pg ml^{-1}) which were significantly different from levels induced by the other bacterial strains. Strain DG induced the lowest levels, among the other LAB, of TNF-α in presence of LPS, with statistic difference compared to GG and LPC-S01.

3.3. Berry extracts modulate in DCs the immune response induced by bacteria also in presence of LPS.

3.3.1. Berry extracts differently modulate LPS effects.

Cytokine levels in dendritic cells treated with berry PP extracts were the same as for the control samples (DCs media + MetOH/HCl; Fig. 2). Interestingly, when used in presence of LPS, berry extracts showed different ability to modulate the pro-inflammatory response in DCs (Supplementary Fig. 2). Specifically, BB3, BB50f, and CB1 significantly decreased IL12 levels induced by LPS respectively by 47% ($P < 0.01$), 70% ($P < 0.01$) and 29% ($P < 0.05$), whereas CB15, SB and EB had no significant impact (Supplementary Fig. 2). Conversely, no significant effect was evidenced by any berry PP extracts on TNF-α and IL10 production induced by LPS, except for an increase in TNF-α induced by CB15 and SB compared to LPS (Supplementary Fig. 2, $P < 0.01$).

3.3.2. Berry extracts modify the cytokine profile induced by probiotic bacteria.

As for the ability to modify the effect of LPS, depending on the origin of the berries we observed different effects of PP extracts on bacterial-induced response in DCs. In addition, the modulation exerted by each

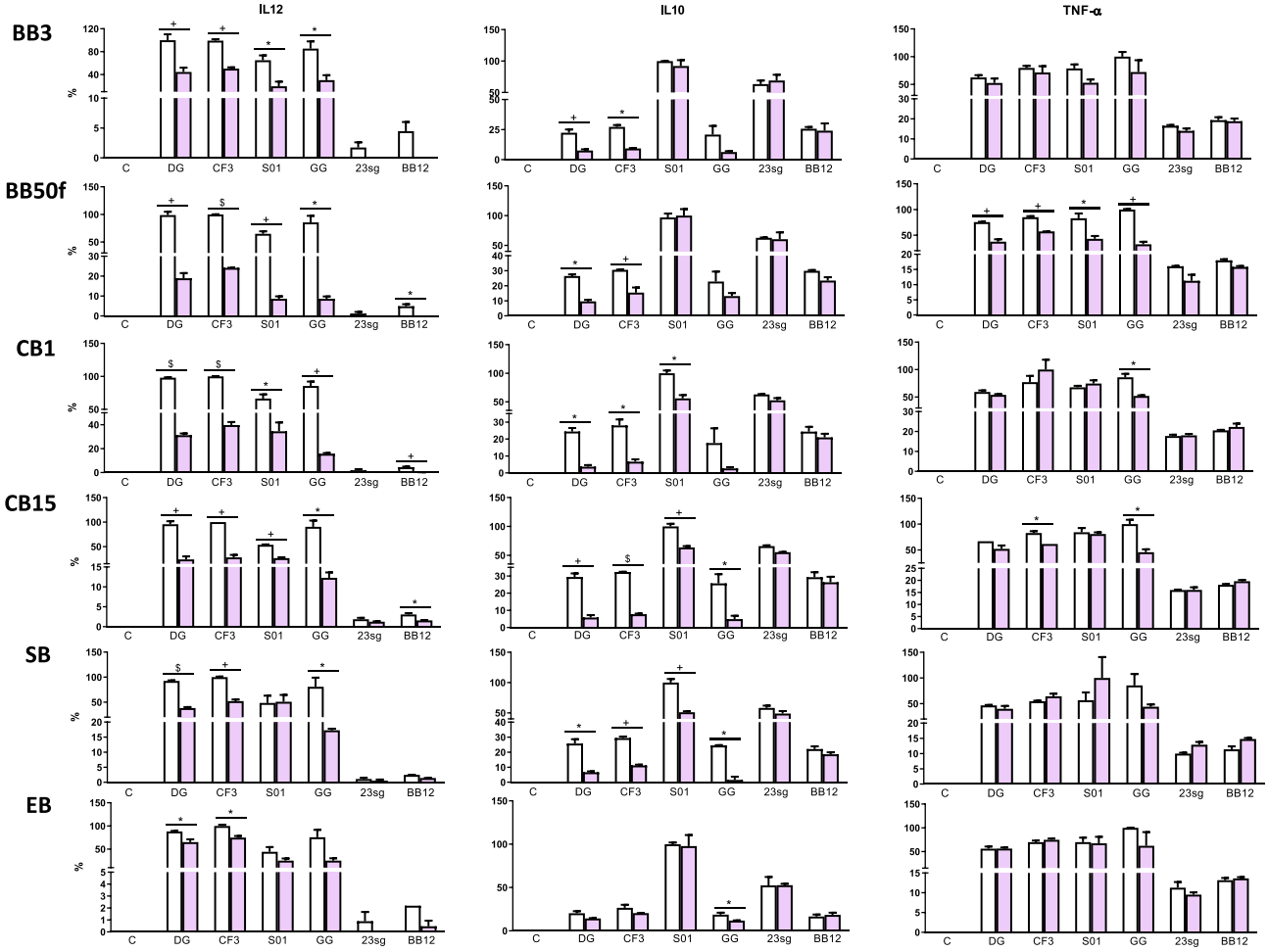

Fig. 2. Cytokines profiles derived from all the combinations of six berry PP extracts and six bacterial strains. Protein levels of the cytokines IL12p70, IL10 and TNF-α were measured in the supernatants of DCs by ELISA after 20 h. (Poly)phenolic extracts from berries were used at a concentration of 50 μg ml^{-1}. Bacteria were used at multiplicity of infection (MOI) of 5. C: Control condition, DCs without bacterial cells in media + MetOH/HCl as control for berry PP extracts. White histograms indicate conditions without berry PP extracts, whereas lilac histograms indicate conditions with berry PP extracts treatment. DG: *L. paracasei* DG, CF3: *L. paracasei* CF3; S01: *L. paracasei* LPC-S01; GG: *L. rhamnosus* GG; 23sg: *B. bifidum* MIMBb23sg; BB-12: *B. animalis* subsp. *lactis* BB-12. BB3: Blueberry powder 3% polyphenols; BB50f: Blueberry powder 50% fibers; CB1: Cranberry 1% proanthocyanidins; CB15: Cranberry 15% proanthocyanidins; SB: Strawberry; EB: Elderberry. Data are reported as the percentage of cytokine level in each condition compared to the control sample (*i.e.* pg ml^{-1} deriving from DCs added with media + MetOH/HCl was set as 0). Asterisks and symbols indicate statistically significant differences ($: $P < 0.001$; +: $P < 0.01$; *: $P < 0.05$) of samples with berry PP extracts (either control samples and samples with bacteria) compared to corresponding conditions without PP extracts, according to unpaired *t*-test.

berry extract varied depending on the bacterial strain combined. Specifically, BB3 reduced the IL12 production induced by *L. rhamnosus* GG and by the *L. paracasei* strains DG, CF3 and LPC-S01, leaving the levels of TNF-α unaffected (Fig. 2). A reducing effect by BB3 was displayed also on IL10 production, but only in the conditions with *L. paracasei* DG and CF3. The other blueberry extract, BB50f, exerted a more pronounced effect compared to BB3. Specifically, the IL12 production by strains *L. paracasei* DG and CF3 together with BB50f was 5 times less, compared to 2 times less with BB3; in presence of *L. paracasei* LPC-S01, IL12 production was 7 times less with BB50f compared to 3 time less with BB3 + LPC-S01, whereas it was 9 times less vs 2.75 when BB50f and BB3, respectively, were used in co-stimulation with *L. rhamnosus* GG. Moreover, BB50f caused also a reduction in TNF-α production when used together with all the LAB strains (Fig. 2). Regarding IL10, like for BB3, the reduction induced by addition of BB50f occurred only with strains DG and CF3 (Fig. 2).

The addition of the cranberry extract CB1 induced a decrease in IL12 with all LAB strains and with *B. animalis* subsp. *lactis* BB-12. CB1 reduced also the IL10 levels in combination with *L. paracasei* DG, CF3 and S01 and the *L. rhamnosus* GG-dependent production of TNF-α (Fig. 2). CB15, similarly to CB1, induced a reduction on IL12 production in presence of all LAB and *B. animalis* subsp. *lactis* BB-12. However, compared to CB1, the reducing ability on TNF-α of CB15 was observed not only with *L. rhamnosus* GG, but also in presence of *L. paracasei* CF3. CB15 also decreased the level of IL-10 when added in combination with all LAB strains (Fig. 2).

Addition of strawberry extract SB to DCs decreased IL12 for the *L. paracasei* strains DG and CF3 and for *L. rhamnosus* GG; no effect was observed with any of the six bacterial strains for TNF-α. IL10 induction was lowered in combination with all LAB strains (Fig. 2).

EB affected IL12 production only for DG and CF3 and reduced IL10 production induced by GG (Fig. 2).

3.3.3. Specific berry extracts modulate the bacteria-induced cytokine profile also in presence of LPS.

The modulating effect by berry extracts on bacteria-dependent cytokine production was tested also by challenging DCs with the addition of LPS. With the addition of the blueberry extracts, the reduction yield of IL12 in presence of LPS, in most of the cases, was similar or even more pronounced than in absence of LPS, except for SB (Supplementary Fig. 2). In presence of LPS, BB50f was not able to reduce TNF-α, differently from what observed when added to *L. rhamnosus* GG, *L. paracasei* DG and LPC-S01 without LPS; the only TNF-α reduction ability was retained with strain CF3 and appeared withstrain MIMBb23sg (Supplementary Fig. 2). When the two cranberry extracts CB1 and CB15 have been employed, the ability to decrease the bacteria-dependent IL12 levels was still evident also in presence of LPS and, in addition, also for MIMBb23sg in the case of CB1 (Supplementary Fig. 2). For what it concerns the effect on IL10, interestingly, the reduction induced both by blueberry and cranberry extracts on LAB without LPS addition (especially on *L. paracasei* strains) was not observed anymore in presence of LPS, except for CB1 with strain GG and BB-12 and for CB15 for strain GG (Supplementary Fig. 2).

With SB, the IL12 decreasing effect observed for *L. paracasei* DG, CF3 and *L. rhamnosus* GG was retained only for GG when tested together with LPS. With SB in presence of bacteria + LPS the TNF-α levels were increased for DG, CF3 and BB-12. Regarding IL10, SB reduced the production elicited by DG, CF3 and BB-12 in presence of LPS (Supplementary Fig. 2).

With EB, the reduction activity on IL12 without LPS was confirmed also in presence of LPS with *L. paracasei* DG, CF3 and in addition with *L. rhamnosus* GG and *B. bifidum* MIMBb23sg; for TNF-α, a significant decrease was displayed in combination with DG, CF3, LPC-S01, GG and BB-12 (Supplementary Fig. 2), that was not present in absence of LPS (Fig. 2). The reduction of IL10 production by GG was confirmed also in presence of LPS (Supplementary Fig. 2). Regarding the association of bifidobacteria and berry PP extracts on IL12 modulation, in presence of LPS with BB3, BB50f, CB1 and EB emerged a reduction, that was not estimable at baseline (in absence of LPS) because of the inability of *Bifidobacterium* strains to trigger IL12 *per se* (Fig. 2 and Supplementary Fig. 2). The addition of BB50f to MIMBb23sg in presence of LPS induced also a reduction of TNF-α, that was not evident in the condition of BB50f added to the strain in absence of LPS (Fig. 2 and Supplementary Fig. 2). Based on these results, BB3, BB50f, CB1 and CB15 were selected to be further tested with bacterial combinations, as these PP extracts provided the more pronounced extents of IL12 and TNF-α reduction for the largest number of strains, both with and without LPS.

3.4. Addition of selected berry extracts to selected bacterial combinations enhance the immunomodulatory ability

3.4.1. Combination of selected probiotic strains promotes synergism in the immuno-modulatory effect on DCs

In the perspective to select the probiotic bacteria displaying the most effective immunomodulatory potential, strains *L. paracasei* DG and LPC-S01, for LAB, and *B. bifidum* MIMBb23sg, for bifidobacteria, have been chosen based on their higher ability to induce IL10 and to trigger the lowest levels of IL12 and TNF-α (Fig. 1). Associations of either two and three strains have been subsequently tested, in order to find a combination suitable to be therefore employed with the most effective berry PP extracts, based on previous results (Fig. 2 and Supplementary Fig. 2). In Fig. 3 are shown the comparisons between single strains and all the strain combinations for IL12, IL10 and TNF-α upon stimulation with DCs (statistic differences between bacteria are evidenced by different suffix letters above histograms ($P < 0.05$)).

Regarding IL12, when *B. bifidum* MIMBb23sg was used in combination with *L. paracasei* DG or *L. paracasei* LPC-S01 or with both strains, it was able to abrogate the LAB-dependent production of IL12 (down to 41.7 pg ml^{-1} compared to DG; down to 53.8 pg ml^{-1}compared to LPC-S01; Fig. 3). *L. paracasei* LPC-S01 was also able, to a lesser extent than MIMBb23sg, to reduce the IL12 production when used together with DG (from 2361 pg ml^{-1} to 1742 pg ml^{-1}). However, the use of three strains together (the two *L. paracasei* strains + MIMBb23sg) did not have an additional advantage compared to the combination of DG + MIMBb23sg or LPC-S01 + MIMBb23sg (Fig. 3). Also for what it concerns TNF-α, the addition of *B. bifidum* MIMBb23sg strongly reduced the cytokine level induced by DG (from 94,253 to 13050 pg ml^{-1}) and by LPC-S01 (from 81,110 to 18189 pg ml^{-1}, Fig. 3). The combination of DG and LPC-S01 did not differ from the LAB strains used separately and the use of the two LAB + *Bifidobacterium* was not different from the effect induced by MIMBb23sg when added to the solely DG or LPC-S01 (Fig. 3).

Regarding the anti-inflammatory cytokine IL10, the combination of *B. bifidum* MIMBb23sg with *L. paracasei* LPC-S01, which was the strain showing the highest induction of the anti-inflammatory cytokine IL10 among all bacteria (Fig. 1, Fig. 2 and Fig. 3), resulted in a more elevated production of IL10, compared to LPC-S01 used alone (from 2428 to 3857 pg ml^{-1}: Fig. 3). Also the addition of MIMBb23sg to DG increased the IL10 production compared to DG (from 531 to 1596 pg ml^{-1}; Fig. 3), however not reaching the level obtained by the association LPC-S01 + MIMBb23sg. LPC-S01 increased the IL10 level of DG when employed together (up to 1675 pg ml^{-1}; Fig. 3) that was however significantly lower compared to the level induced by LPC-S01 alone (Fig. 3). The use of three strains induced a lower production of IL10 (3020 pg ml^{-1}) compared to LPC-S01 + MIMBb23sg(Fig. 3).

3.4.2. Addition of berry extracts to bacterial combinations enhances the immunomodulatory effect

The previously selected combinations of two and three bacteria (2 LAB and one *Bifidobacterium*) were tested together with selected berries (BB3, BB50f, CB1, CB15), both in presence and absence of LPS. Concerning IL12 production, BB3 significantly dampened IL12 production in all the bacterial combinations, both in absence and in presence of LPS

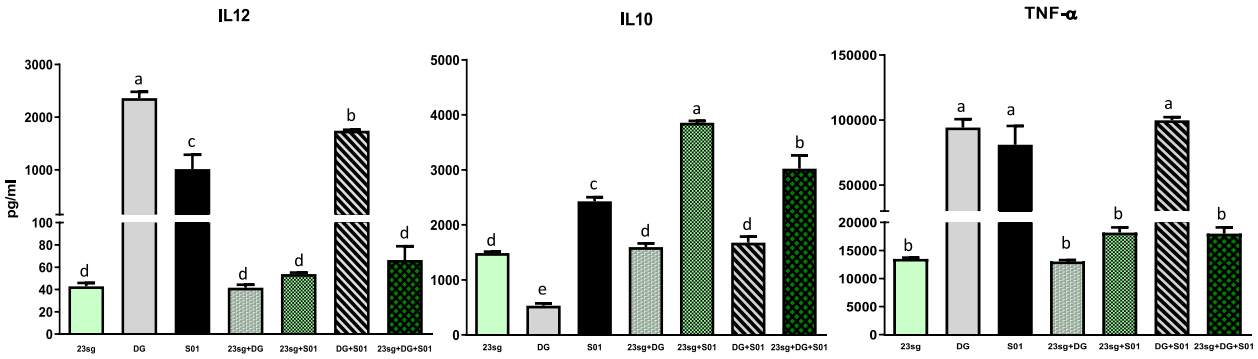

Fig. 3. Anti-inflammatory potential of combinations of bacterial strains. Protein levels of the cytokines IL12p70, IL10 and TNF-α were measured in the supernatants of DCs by ELISA after 20 h. Bacteria alone and in combination were used at a final multiplicity of infection (MOI) of 5. DG: *L. paracasei* DG; S01: *L. paracasei* LPC-S01; 23sg: *B. bifidum* MIMBb23sg. Data are presented as pg ml^{-1}. Data were analysed by ANOVA and the Tukey's post hoc test. Value with different suffix letters significantly differed at $P < 0.05$.

(Fig. 4 and Supplementary Fig. 4). BB50f behave analogously, except for the condition LPC-S01 + MIMBb23sg and LPC-S01 + DG + MIMBb23sg, for which we did not see a significant reduction without LPS (Fig. 4), even though it appeared in presence of LPS(Supplementary Fig. 4). Both CB1 and CB2 reduced significantly IL12 production with all bacterial association, with and without LPS, with the only exception of CB15 added to LPC-S01 + MIMBb23sg in absence of LPS (Fig. 4). Regarding IL10, of interest in a perspective of anti-inflammatory boost, we found with BB3 a trend in the increase of the IL10 induced by LPC-S01 and MIMBb23sg, even though not significant (Fig. 4). More clearly, instead, CB1 enhanced the increase of IL10 levels observed with the association of *L. paracasei* LPC-S01 and *B. bifidum* MIMBb23sg, as evidenced by the comparison between LPC-S01 + MIMBb23sg and CB1 + LPC-S01 + MIMBb23sg ($P < 0.05$, Fig. 4). Moreover, CB1 addition to LPC-S01 + MIMBb23sg, in presence of LPS, provoked also a reduction of TNF-α levels, compared to the solely bacterial association ($P < 0.05$, Supplementary Fig. 4) and also for the association of DG + S01 and DG + MIMBb23sg (Supplementary Fig. 4). BB50f decreased TNF-α levels in the association LPC-S01 + MIMBb23sg, both in absence ($P < 0.05$, Fig. 4) and presence of LPS ($p < 0.01$, Supplementary Fig. 4), where the

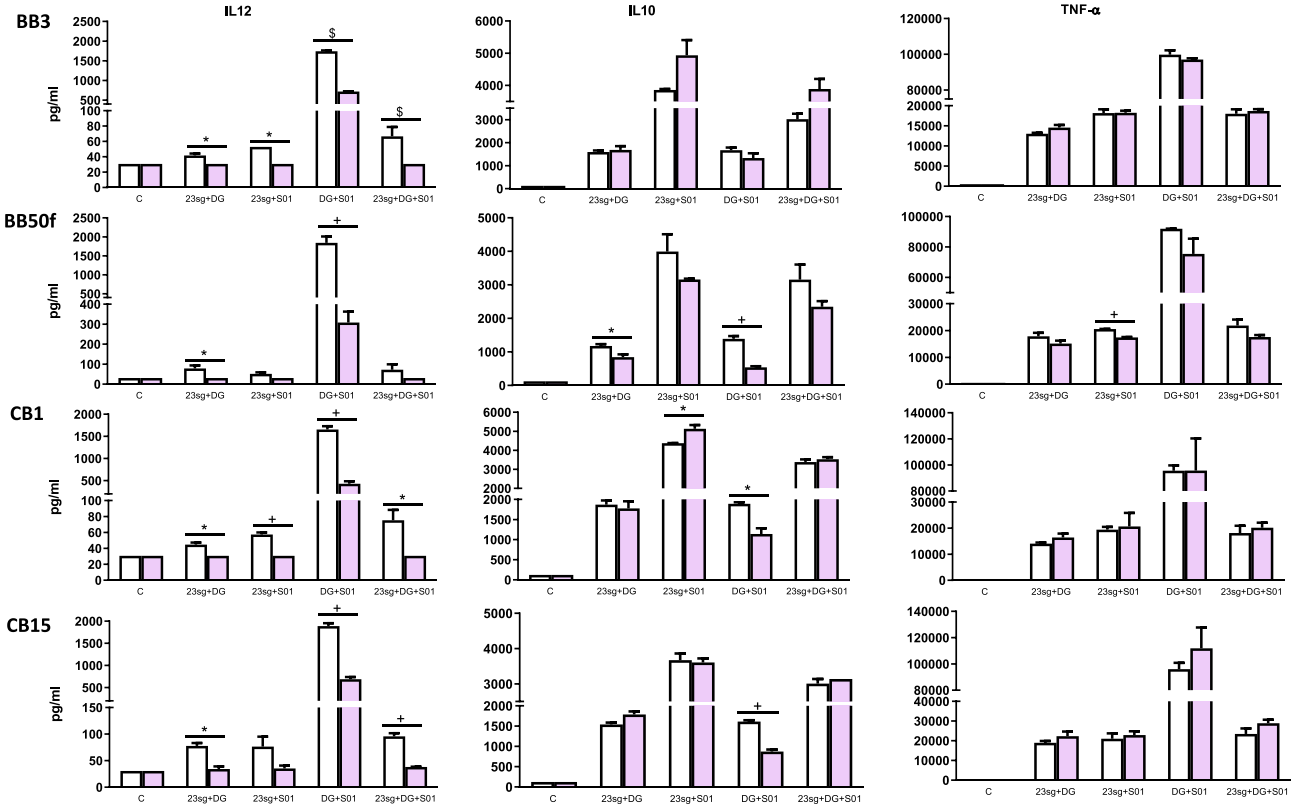

Fig. 4. Anti-inflammatory potential of combinations of bacterial strains together with berries. Protein levels of the cytokines IL12p70, IL10 and TNF-α were measured in the supernatants of DCs by ELISA after 20 h. (Poly)phenolic extracts from berries were used at a concentration of 50 μg ml^{-1}. Bacterial combinations were used at a final multiplicity of infection (MOI) of 5. C: Control condition, DCs without bacterial cells in media + MetOH/HCl. White histograms indicate conditions without berry PP extracts, whereas lilac histograms indicate conditions with berry PP extracts treatment. DG: *L. paracasei* DG; S01: *L. paracasei* LPC-S01; 23sg: *B. bifidum* MIMBb23sg. BB3: Blueberry powder 3% polyphenols; BB50f: Blueberry powder 50% fibers; CB1: Cranberry 1% proanthocyanidins; CB15: Cranberry 15% proanthocyanidins. Data are presented as pg ml^{-1}. Asterisks and symbols indicate statistically significant differences ($: $P < 0.001$; +: $P < 0.01$; *: $P < 0.05$) between conditions with and without berry PP extracts according to unpaired *t*-test.

reduction was evident for all the bacterial combinations.

From these results, the PP extracts blueberry BB50f and, in particular, the cranberry CB1 emerged as the most effective in the immunomodulatory potential on the bacterial association of *L. paracasei* LPC-S01 and *B. bifidum* MIMBb23sg in DCs, even challenged with LPS. In specific, BB50f reduced IL12 production without LPS, while decreasing the TNF-α levels in both conditions tested (with and without LPS); CB1 decreased IL12 levels either in absence and presence of LPS, reduced TNF-α in presence of LPS and, in addition, increased IL10 in absence of LPS.

3.5. Total anthocyanins and proanthocyanidins content in the BB and CB extracts, ACNs and PAC profile

Based on the *in vitro* results, BB and CB extracts were further characterized for their anthocyanins and procyanidins content. In general, all the major ACN compounds were higher in concentration in BB extract compared to CB extracts. The main compound in the BB extracts (Table 2) was Mv-glc (30.4 vs 30.5% for BB3 and BB50f, respectively) followed by Mv-gal (15.3 vs 14.0% for BB3 and BB50f, respectively) and Pet-glc (11.4 vs 8.9% for BB3 and BB50f, respectively). Regarding CB extracts, only three ACNs were detected in our analysis: Cy, Peo and Mv, linked to different sugars such as glucose, galactose and arabinose (Table 2). In detail, Cy-gal was quantified in CB1 (20.9%) but it was absent in CB15, and Peo-gal was 34.8% in CB1 compared to 3% in CB15. Conversely, Cy-ara and Mv-gal were higher in CB15 (respectively, 41.8 and 47.5%) than in CB1 (19.1% and 16.7%, respectively). Peo-glc was comparable in the two cranberry extracts (8.5 and 7.7% in CB1 and CB15) (Table 2).

No proanthocyanidins (PAC, expressed as total procyanidin A2) were detectable in the BB and CB extracts, apart from CB15 showing, however, an amount<0.1%.

4. Discussion

Maintenance of a balanced immune response is essential for the general homeostasis, and for the prevention of the numerous pathologies that may derive from a misregulated immune system, such as metabolic syndrome and some cancers (Eberl, 2010; Nicholson, 2016). In this perspective, functional foods, food supplements and nutraceuticals can constitute a valid tool in the regulation of physiological processes for the promotion of host's health (Chanda et al., 2019; Cory et al., 2018; Pan et al., 2017; Cencic and Chingwaru, 2010). Probiotic microorganisms, through different mechanisms, have been shown to positively impact on immune system (Maldonado Galdeano et al., 2019) and systemic inflammation (Liu et al., 2018). Similarly, (poly)phenols (PP) are bioactive compounds, mainly present in berry fruit, that are regarded as therapeutic molecules for their widely-described antioxidant and immunomodulatory properties towards an anti-inflammatory action (Silva and Pogačnik, 2020). PP have been shown to modulate immune processes by inactivating cellular pathway, thus inhibiting mechanisms that are involved in activation and differentiation of immune cells (Yahfoufi et al., 2018; Taverniti et al., 2014; Cuevas et al., 2013). While PP display a more pronounced anti-inflammatory attitude, probiotics may modulate immune system towards stimulatory or regulatory outcomes, depending on the strain (Maldonado Galdeano et al., 2019). Certain probiotic microorganisms induce Th1 responses, with production of pro-inflammatory cytokines, active against infections and cancer cells ("immunostimulatory probiotics"), whereas other bacterial strains are more prone to activate Treg cells, with the release of anti-inflammatory cytokines ("immunoregulatory probiotics"), which induce tolerance and resolution of inflammation (Azad et al., 2018). Based on this evidence, in the study here described, we aimed at performing a preliminary screening to select a combination of probiotic bacteria and berry-derived PP with immunomodulatory potential, to be further studied *in vivo* for a possible exploitation as a food supplement. Bacterial strains selected for this study were already investigated for their probiotic features, particularly *L. paracasei* DG (Cremon et al., 2018; Ferrario et al., 2014); *L. paracasei* LPC-S01 (Koirala et al., 2020; Balzaretti et al., 2015) and *B. bifidum* MIMBb23sg (Taverniti et al., 2021). For *L. paracasei* CF3, which derives from the intestine of a healthy adult, probiotic properties have been investigated only *in vitro* (unpublished). *L. rhamnosus* GG and *B. animalis* subsp. *lactis* BB-12 have been used as reference probiotic strains for lactic acid bacteria and for bifidobacteria, respectively, based on the body of literature and evidences of their beneficial properties (Segers and Lebeer, 2014; Jungersen et al., 2014). Regarding the berry extracts employed, we obtained the fraction containing (poly)phenols starting from the commercial powder of Blueberry with 3% polyphenols, Blueberry with 50% fibers content, Cranberry with 1% and 15% pro-anthocyanidins, Strawberry and Elderberry. Besides blueberries and cranberries, which have been widely described in literature (Silva et al., 2020; Colletti et al., 2021), there are emerging studies regarding the health promoting properties of other berry fruit, like strawberry (Chen et al., 2019; Miller et al., 2019) and elderberry (Carneiro et al., 2019; Ho et al., 2017). As reference for bioactive molecules, we used piceatannol, a derivative from resveratrol with known anti-inflammatory activity (Piotrowska et al., 2012). To evaluate the potential of probiotics and berries on immune system, murine bone marrow-derived DCs have been employed as *in vitro* model, to test the induction of pro-inflammatory (IL12, TNF-α) and anti-inflammatory (IL10) cytokines. We, in addition, employed LPS, as

Table 2
Percentage of anthocyanin (ACN) compounds detected and quantified in the blueberry (BB) and cranberry (CB) PP extracts used in the study.

ACN compounds	Blueberry 3% PP (% ACNs)	Blueberry 50% FB (%ACNs)	Cranberry 1% PA (%ACNs)	Cranberry 15% PA (%ACNs)
D-gal	3.6	3.8	/	/
D-glc	8.9	6.1	/	/
Cy-gal	2.3	3.0	20.9	/
D-ara	1.5	2.3	/	/
Cy-glc	9.9	8.7	/	/
Cy-ara	1.8	2.9	19.1	41.8
Pet-gal	2.3	3.4	/	/
Pet-glc	11.4	8.9	/	/
Peo-gal	0.7	0.8	34.8	3.0
Pet-ara	0.9	2.0	/	/
Peo-glc	6.5	6.5	8.5	7.7
Mv-gal	15.3	14.0	16.7	47.5
Mv-glc	30.4	30.5	/	/
Mv-ara	4.4	6.8	/	/
unidentified	/	0.5	/	/
Total	100	100	100	100

List of abbreviation for sugar moieties of ACNs: glc = glucose; gal = galactose; ara = arabinose. List of abbreviation for ACN compounds: D = delphinidin; Cy = cyanidin; Peo = peonidin; Pet = petunidin; Mv = malvidin.

endotoxins are among the stimuli that mainly trigger inflammatory response (Monguió-Tortajada et al., 2018). In our experiments, DCs were first pre-stimulated with berry extracts for 1 h, followed by 1 h stimulation with bacteria, before the addition of LPS. This procedure was chosen in order to test a potential protective effect of berry extracts and probiotics towards LPS effect. The choice to defer the addition of bacteria was applied to allow the activity of berry extracts on DCs, avoiding the concurrent presence of other stimuli. We acknowledge that, to verify a plausible mutual effect between probiotics and berry extracts on bacterial viability, as well as on PP and derived molecules bioavailability, *in vivo* investigations would be required, that were however beyond this preliminary *in vitro* screening. As first evaluation, we observed that, when used alone, berry-derived PP had no activity in the modulation of cytokines in DCs, as expected (Taverniti et al., 2017; Frøkiær et al., 2012). Nonetheless, BB3, BB50f and CB1 were able to significantly modulate IL12 production triggered by LPS, as similarly reported in previous *in vitro* studies performed in DCs (del Cornò et al., 2016). Regarding the behavior of the different probiotic strains, we found a significant difference between LAB and bifidobacteria in the ability to induce IL12, which was already shown (Weiss et al., 2011). By screening all bacteria, *L. paracasei* LPC-S01 and *B. bifidum* MIMBb23sg displayed the most pronounced anti-inflammatory profile, also in presence of LPS, in terms of lowest production of stimulatory cytokines IL12 and TNF-α, and the highest induction of the anti-inflammatory IL10. The species *B. bifidum* has been already shown to have the most evident anti-inflammatory properties compared to other species of the genus (Khokhlova et al., 2012; Preising et al., 2010; Riedel et al., 2006). However, the immunomodulatory properties of microorganisms are strictly strain-dependent (Hill et al., 2014). Regarding this aspect, we have confirmed what already reported for the species *L. paracasei* (reclassified as *Lacticaseibacillus paracasei* (Zheng et al., 2020) for which it was already observed a high heterogeneity in the immune response when 40 strains were tested on different population of immune cells (Cassard et al., 2016). When *B. bifidum* MIMBb23sg was used in combination with *L. paracasei* LPC-S01 and *L. paracasei* DG, it abrogated the production of IL12, and significantly decreased the levels of TNF-α. Similar switching mechanisms exerted by *B. bifidum* species on LAB immune activity have been already observed in the same model (Weiss et al., 2010). In addition, the combination of *L. paracasei* LPC-S01 and *B. bifidum* MIMBb23sg resulted also in a higher production of IL10, compared to strains used alone, resulting the best association of probiotic strains in terms of immunomodulatory effect. Interestingly, this result suggests that the increased induction of the anti-inflammatory IL10 could be attributable to a mechanism of synergism between the two bacteria, rather than an additive effect since, when employed together, each strain was added to DCs at MOI of 2.5 in order to reach the final MOI of 5, which was the concentration chosen to test bacterial activity individually (Taverniti et al., 2012). When the Blueberry (BB3 and BB50f) and the Cranberry (CB1 and CB15) extracts, selected especially on the basis of their degree of dampening activity on inflammatory cytokines, were added to the LPC-S01 + MIMBb23sg bacterial combination, BB50f and CB1 resulted the most effective in the immunomodulatory effect. In fact, also in presence of LPS, they could further reduce the production of IL12 and TNF-α induced by bacteria. CB1, in addition, also increased IL10 levels induced by LPC-S01 + MIMBb23sg (in absence of LPS). These results confirm some other preliminary data on beneficial combination of probiotics and PP (Banerjee and Dhar, 2019; dos Santos et al., 2019; Taverniti et al., 2017). However, based on data collected in this study, we cannot precisely extrapolate which are the actual bioactives and activated mechanisms responsible for the more efficacious activity of the two combinations identified (*i.e.* the bacterial combination LPC-S01 and MIMBB23sg + berry extracts BB50f and CB1). Regarding the content in specific PP, there are differences between the berries selected and the extracts obtained for this study. It is well known that blueberry fruits are generally rich in anthocyanidins (*i.e.* malvidin, which was also confirmed in our analysis), procyanidins B and chlorogenic acid (Stevenson and Scalzo, 2012). In the present study, we found that BB extracts contained much higher amounts of total ACNs compared to CB, while no procyanidins were detected. Anthocyanins are widely documented to exhibit anti-inflammatory activity (Vendrame and Klimis-Zacas, 2015) thus, we cannot exclude that the modulation observed could be attributable to these compounds or due to a synergistic effect with other PP (*i.e.* chlorogenic acids and other phenolics) and bioactives not analyzed, and potentially responsible of the different effects observed (BB3 vs. BB50f). Regarding cranberry, its profile generally stands out by its richness of the type A procyanidins (Blumberg et al., 2013). In our extracts, the content of ACNs was 10 times lower compared to BB and limited mainly at cyanidin and peonidin, phenolic acids and flavanols (Neto, 2007). Furthermore, no PACs were detectable in the two CB extracts after extraction from the powder, apart from a small amount of procyanidin A in the CB15 sample. The apparent absence of PACs in the extracts was in part surprising. We cannot exclude limitations related to the method of extraction (even if widely used) and/or quantification. In fact, the quantification of PACs was performed spectrophotometrically as total procyanidins instead of single compounds, thus we cannot exclude a minor sensibility of detection. Also for the CB extracts, the analysis of the single phenolic acids was not performed and thus we cannot exclude their possible contribution in the modulation observed, as well as for the presence of other classes of bioactive molecules, as stilbenes (Neto, 2007). Moreover, as also reported in literature, the lack of PAC quantification might be related to the presence of insoluble PACs, whose quantification might be an issue as they remain associated with proteins and fibers after extraction, requiring specific procedures and standards, as butanol-HCl extraction (Gullickson et al., 2020). The other berry extracts, which showed milder effect in this study are characterized by a different composition, *i.e.* ellagitannins and glycosides of pelargonidin prevailing in strawberry (Nowicka et al., 2019) and monoglycosides of cyanidin, constituting around 10% of elderberry (Silva et al., 2017). However, besides the quantification of the total content of polyphenols in extracts from a berry matrix, looking at the results for elderberry and strawberry obtained in this study, which showed a good yield of total PP, it emerges the importance to investigate in detail the composition at the level of the different classes of PP compounds, that can give rise to different extent of biological effects. It is also important to underline that the number of hydroxyl groups on the PPs and their putative methylation can influence their immune modulating activity and these variations in PP profile within the berry extracts may represent a factor affecting the resulting cytokine profiles.

Summarizing, the study here described reports a screening performed on six probiotic strains and six berry-derived PP extracts for their immunomodulatory potential. Results collected evidenced different behavior between lactic acid bacteria and bifidobacteria in the immune response induced on dendritic cells. When used together, some combinations of bacteria resulted in a synergism in the immunomodulatory activity. In specific, a combination of a *Lacticaseibacillus paracasei* and a *Bifidobacterium bifidum* strain together with the PP fractions from a blueberry and from a cranberry extract, resulted to be the most effective association in reducing pro-inflammatory cytokines IL12 and TNF-α and increasing anti-inflammatory IL10, also in presence of LPS. In the perspective of a combined use of berry and bacteria, it is possible to hypothesize that, besides the immunomodulatory potential deriving from a synergism in the probiotics and berry-derived PP association, other beneficial outcomes are expected to be exerted as well on the host, *e.g.* the anti-oxidant effects of berry-PP and the modulatory effects on microbiota and host physiology, already demonstrated for the bacterial strains used in the present study (Koirala et al., 2020; Balzaretti et al., 2015; Taverniti et al., 2021). This suggests that some beneficial/protective food components, as berry (poly)phenols, may be also exploited to quench the stimulatory activity of some bacteria, plausibly not affecting other probiotic features and, therefore, making their application more versatile. The presented results can be relevant for future

studies aimed at addressing the specific molecules involved in the anti-inflammatory activity of the berry extracts used in this study, by testing the single compounds separately. This kind of investigation would, as first, require the evaluation of PP molecules impact on probiotic viability. Several PP compounds have been shown to have antimicrobial effects; even though this kind of activity has mainly been tested with the purpose to antagonize pathogens (Suriyaprom et al., 2022), inhibitory effects have been described also towards probiotic microorganisms (Gil-Sánchez et al., 2018). Nonetheless, there are also several evidence that LAB can benefit from certain PP compounds for their growth (Hervert-Hernández et al., 2009; Bravo et al., 2007), even though the outcome strictly depends on the type of PP molecules and bacterial strains, as shown in a study performed on different bifidobacterial species (Gwiazdowska, 2015). This implies also that the metabolic activity of microbes, which can exploit PP to grow, exerts a transformation of such compounds into derivatives, which might eventually differ in their bioactivity (Stevens and Maier, 2016). As an example, combination of grape pomace PP and a strain of *L. plantarum* was shown to improve not only the probiotic growth, but also allowed a synergistic effect between PP and the bacterium in counteracting pathogenic strains growth (Caponio et al., 2022). However, synergistic effects to benefit host health due to (poly)phenols and probiotics association, as also the ones described in this paper, should be proven *in vivo*, as *in vitro* investigations are useful particularly to perform screening, but cannot be predictive of the complexity of the *in vivo* environment. Often the fate of administered PP molecules in the organism, upon digestion and absorption, is not known, as well as the effect exerted from the metabolic activity of the microbiota and on microbiota (van Duynhoven et al., 2011; Makarewicz et al., 2021).. Effects of PP administration on microbiota composition are reported (Guglielmetti et al., 2013; Xu et al., 2022). Investigations in animal models would allow to address if the synergistic effect found *in vitro* is retained and whether a combined use of PP and probiotics might improve the modulation of gut microbiota, as previously suggested (Westfall et al., 2018; Dueñas et al., 2015). Therefore, studies devoted in observing the *in vivo* outcome of the selected PP-probiotics combinations are warranted. All this evidence could be finally targeted to the possible development of food supplements that can be useful in the maintenance and/or restoration of immune homeostasis.

Declaration of Competing Interest.

W.F. is an employee of the company that commercializes some of the probiotic strains investigated in this study. V.T. and S.G. receive royalties from the selling of *B. bifidum* MIMBb23sg. The authors declare no conflict of interest.

CRediT authorship contribution statement

Valentina Taverniti: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Cristian Del Bo':** Investigation, Writing – review & editing. **Walter Fiore:** Conceptualization. **Giorgio Gargari:** Formal analysis, Visualization. **Stefania Arioli:** Investigation, Writing – review & editing. **Patrizia Riso:** Conceptualization, Writing – review & editing, Supervision. **Simone Guglielmetti:** Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision, Project administration. **Hanne Frøkiær:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Antonella Brusamolino, Claudio Gardana and Mirko Marino for their contribution in the preparation and analysis of the PP extracts, and Giacomo Mantegazza for manuscript revision. We acknowledge the European Joint Programming Initiative "A Healthy Diet for a Healthy Life" (JPI-HDHL—http://www.healthydietforhealthylife.eu/) and Mipaaft (Italy; D.M. 8245/7303/2016) for contributing to the grant awarded to G.G.

Funding

This research was funded by Sofar S.p.A.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2022.105121.

References

Root, M. M., Mcginn, M. C., Nieman, D. C., Henson, D. A., Heinz, S. A., Andrew Shanely, R., et al. (2012). Combined fruit and vegetable intake is correlated with improved inflammatory and oxidant status from a cross-sectional study in a community setting. *Nutrients, 4*, 29–41. https://doi.org/10.3390/nu4010029

Guilbert, J. J. (2003). The world health report 2002 - Reducing risks, promoting healthy life [2]. *Educ Heal, 16*, 230. https://doi.org/10.1080/1357628031000116808

Van Duyn, M. A. S., & Pivonka, E. (2000). Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: Selected literature. *Journal of the American Dietetic Association, 100*, 1511–1521. https://doi.org/10.1016/S0002-8223(00)00420-X

Fraga, C. G., Croft, K. D., Kennedy, D. O., & Tomás-Barberán, F. A. (2019). The effects of polyphenols and other bioactives on human health. *Food & Function, 10*, 514–528. https://doi.org/10.1039/c8fo01997e

Shama. Fruit Polyphenols: A Review of Anti-inflammatory Effects in Humans, Critical Reviews in Food Science and Nutrition Crit Rev Food Sci Nutr 2016;56:419-44. https://doi: 10.1080/10408398.2013.767221.

Folmer, F., Basavaraju, U., Jaspars, M., Hold, G., El-Omar, E., Dicato, M., et al. (2014). Anticancer effects of bioactive berry compounds. *Phytochemistry Reviews, 13*, 295–322. https://doi.org/10.1007/s11101-013-9319-z

Edirisinghe, I., Banaszewski, K., Cappozzo, J., Sandhya, K., Ellis, C. L., Tadapaneni, R., et al. (2011). Strawberry anthocyanin and its association with postprandial inflammation and insulin. *British Journal of Nutrition, 106*, 913–922. https://doi.org/10.1017/S0007114511001176

Ellis, L. Z., Liu, W., Luo, Y., Okamoto, M., Qu, D., Dunn, J. H., et al. (2011). Green tea polyphenol epigallocatechin-3-gallate suppresses melanoma growth by inhibiting inflammasome and IL-1β secretion. *Biochemical and Biophysical Research Communications, 414*, 551–556. https://doi.org/10.1016/j.bbrc.2011.09.115

McAnulty, L. S., Nieman, D. C., Dumke, C. L., Shooter, L. A., Henson, D. A., Utter, A. C., et al. (2011). Effect of blueberry ingestion on natural killer cell counts, oxidative stress, and inflammation prior to and after 2.5 h of running. *Applied Physiology, Nutrition and Metabolism, 36*, 976–984. https://doi.org/10.1139/h11-120

Chiva-Blanch, G.; Badimon L. Effects of Polyphenol Intake on Metabolic Syndrome: Current Evidences from Human Trials. Oxid Med Cell Longev 2017:5812401.

Land Lail, H., Feresin, R. G., Hicks, D., Stone, B., Price, E., & Wanders, D. (2021). Berries as a Treatment for Obesity-Induced Inflammation: Evidence from Preclinical Models. *Nutrients, 13*, 334. https://doi.org/10.3390/nu13020334

Yahfoufi, N., Alsadi, N., Jambi, M., & Matar, C. (2018). The immunomodulatory and anti-inflammatory role of polyphenols. *Nutrients, 10*. https://doi.org/10.3390/nu10111618

Zhao, L., Lee, J. Y., & Hwang, D. H. (2011). Inhibition of pattern recognition receptor-mediated inflammation by bioactive phytochemicals. *Nutrition Reviews, 69*, 310–320. https://doi.org/10.1111/j.1753-4887.2011.00394.x

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., et al. (2014). Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*, 506–514. https://doi.org/10.1038/nrgastro.2014.66

Azad, M. A. K., Sarker, M., Li, T., & Yin, J. (2018). Probiotic Species in the Modulation of Gut Microbiota: An Overview. *Biomed Research International, 2018*. https://doi.org/10.1155/2018/9478630

Markowiak, P., & Ślizewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients, 9*. https://doi.org/10.3390/nu9091021

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., et al. (2013). Health Benefits of Probiotics: A Review. *ISRN Nutr, 2013*, 1–7. https://doi.org/10.5402/2013/481651

Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic Mechanisms of Action. *Annals of Nutrition & Metabolism, 61*, 160–174. https://doi.org/10.1159/000342079

Liu, Y., Alookaran, J. J., & Rhoads, J. M. (2018). Probiotics in autoimmune and inflammatory disorders. *Nutrients, 10*. https://doi.org/10.3390/nu10101537
Hao, Q., Dong, B. R., & Wu, T. (2015). Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Systematic Review*. https://doi.org/10.1002/14651858.CD006895.pub3
Panduru, M., Panduru, N. M., Sălăvăstru, C. M., & Tiplica, G.-S. (2015). Probiotics and primary prevention of atopic dermatitis: A meta-analysis of randomized controlled studies. *The Journal of the European Academy of Dermatology and Venereology, 29*, 232–242. https://doi.org/10.1111/jdv.12496
Pelucchi, C., Chatenoud, L., Turati, F., Galeone, C., Moja, L., Bach, J.-F., et al. (2012). Probiotics Supplementation During Pregnancy or Infancy for the Prevention of Atopic Dermatitis. *Epidemiology, 23*, 402–414. https://doi.org/10.1097/EDE.0b013e31824d5da2
Jia, K., Tong, X., Wang, R., & Song, X. (2018). The clinical effects of probiotics for inflammatory bowel disease. *Medicine (Baltimore), 97*, Article e13792. https://doi.org/10.1097/MD.0000000000013792
Patel, S., Shukla, R., & Goyal, A. (2015). Probiotics in valorization of innate immunity across various animal models. *Journal of Functional Foods, 14*, 549–561. https://doi.org/10.1016/j.jff.2015.02.022
Carneiro NVQ, Da SILVA HBF, Da Silva RR, Carneiro TCB, Costa RS, Pires AO, et al. Sambucus australis modulates inflammatory response via inhibition of nuclear factor kappa b (NF-kB) in vitro. An Acad Bras Cienc 2019;91. https://doi.org/10.1590/0001-3765201920170831.
Christensen, H. R., Frøkiær, H., & Pestka, J. J. (2002). Lactobacilli Differentially Modulate Expression of Cytokines and Maturation Surface Markers in Murine Dendritic Cells. *Journal of Immunology, 168*, 171–178. https://doi.org/10.4049/jimmunol.168.1.171
de Souza, E. L., de Albuquerque, T. M. R., dos Santos, A. S., Massa, N. M. L., & de Brito Alves, J. L. (2019). Potential interactions among phenolic compounds and probiotics for mutual boosting of their health-promoting properties and food functionalities – A review. *Critical Reviews in Food Science and Nutrition, 59*, 1645–1659. https://doi.org/10.1080/10408398.2018.1425285
Banerjee, A., & Dhar, P. (2019). Amalgamation of polyphenols and probiotics induce health promotion. *Critical Reviews in Food Science and Nutrition, 59*, 2903–2926. https://doi.org/10.1080/10408398.2018.1478795
dos Santos, A. S., de Albuquerque, T. M. R., de Brito Alves, J. L., & de Souza, E. L. (2019). Effects of Quercetin and Resveratrol on in vitro Properties Related to the Functionality of Potentially Probiotic *Lactobacillus* Strains. *Frontiers in Microbiology, 10*. https://doi.org/10.3389/fmicb.2019.02229
Taverniti V, Dalla Via A, Minuzzo M, Del Bo' C, Riso P, Frøkiær H, et al. In vitro assessment of the ability of probiotics, blueberry and food carbohydrates to prevent S. pyogenes adhesion on pharyngeal epithelium and modulate immune responses. Food Funct 2017;8:3601–9. https://doi.org/10.1039/C7FO00829E.
Arioli, S., Eraclio, G., Della Scala, G., Neri, E., Colombo, S., Scaloni, A., et al. (2018). Role of Temperate Bacteriophage ϕ20617 on Streptococcus thermophilus DSM 20617T Autolysis and Biology. *Frontiers in Microbiology, 9*. https://doi.org/10.3389/fmicb.2018.02719
Wrolstad RE. Handbook of food analytical chemistry. Pigments, colorants, flavors, texture, and bioactive food components, vol 2. Wiley, New Jersey 2005; 473–5.
Wrolstad. Current Protocols in Food Analytical Chemistry. Curr Protoc Food Anal Chem 2001. https://doi.org/10.1002/0471142913.
Del Bò, C., Ciappellano, S., Klimis-Zacas, D., Martini, D., Gardana, C., Riso, P., et al. (2010). Anthocyanin Absorption, Metabolism, and Distribution from a Wild Blueberry-Enriched Diet (Vaccinium angustifolium) Is Affected by Diet Duration in the Sprague−Dawley Rat. *Journal of Agriculture and Food Chemistry, 58*, 2491–2497. https://doi.org/10.1021/jf903472x
Prior, R. L., Fan, E., Ji, H., Howell, A., Nio, C., Payne, M. J., et al. (2010). Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *Journal of the Science of Food and Agriculture, 90*, 1473–1478. https://doi.org/10.1002/jsfa.3966
Gardana, C., & Simonetti, P. (2019). Evaluation of the Degree of Polymerization of the Proanthocyanidins in Cranberry by Molecular Sieving and Characterization of the Low Molecular Weight Fractions by UHPLC-Orbitrap Mass Spectrometry. *Molecules, 24*, 1504. https://doi.org/10.3390/molecules24081504
Eberl, G. (2010). A new vision of immunity: Homeostasis of the superorganism. *Mucosal Immunology, 3*, 450–460. https://doi.org/10.1038/mi.2010.20
Nicholson, L. B. (2016). The immune system. *Essays in Biochemistry, 60*, 275–301. https://doi.org/10.1042/EBC20160017
Chanda, S., Tiwari, R. K., Kumar, A., & Singh, K. (2019). Nutraceuticals inspiring the current therapy for lifestyle diseases. *Advances in Pharmacological Sciences, 2019*. https://doi.org/10.1155/2019/6908716
Cory H, Passarelli S, Szeto J, Tamez M, Mattei J. The Role of Polyphenols in Human Health and Food Systems: A Mini-Review. Front Nutr 2018;5. https://doi.org/10.3389/fnut.2018.00087.
Pan L-L, Li J, Shamoon M, Bhatia M, Sun J. Recent Advances on Nutrition in Treatment of Acute Pancreatitis. Front Immunol 2017;8. https://doi.org/10.3389/fimmu.2017.00762.
Cencic, A., & Chingwaru, W. (2010). The role of functional foods, nutraceuticals, and food supplements in intestinal health. *Nutrients, 2*, 611–625. https://doi.org/10.3390/nu2060611
Maldonado Galdeano, C., Cazorla, S. I., Lemme Dumit, J. M., Vélez, E., & Perdigón, G. (2019). Beneficial effects of probiotic consumption on the immune system. *Annals of Nutrition & Metabolism, 74*, 115–124. https://doi.org/10.1159/000496426
Silva, R. F. M., & Pogačnik, L. (2020). Polyphenols from food and natural products: Neuroprotection and safety. *Antioxidants, 9*. https://doi.org/10.3390/antiox9010061
Taverniti V, Fracassetti D, Del Bo' C, Lanti C, Minuzzo M, Klimis-Zacas D, et al. Immunomodulatory Effect of a Wild Blueberry Anthocyanin-Rich Extract in Human Caco-2 Intestinal Cells. J Agric Food Chem 2014;62:8346–51. https://doi.org/10.1021/jf502180j.
Cuevas, A., Saavedra, N., Salazar, L. A., & Abdalla, D. S. P. (2013). Modulation of immune function by polyphenols: Possible contribution of epigenetic factors. *Nutrients, 5*, 2314–2332. https://doi.org/10.3390/nu5072314
Cremon, C., Guglielmetti, S., Gargari, G., Taverniti, V., Castellazzi, A. M., Valsecchi, C., et al. (2018). Effect of Lactobacillus paracasei CNCM I-1572 on symptoms, gut microbiota, short chain fatty acids, and immune activation in patients with irritable bowel syndrome: A pilot randomized clinical trial. *United Eur Gastroenterol J, 6*, 604–613. https://doi.org/10.1177/2050640617736478
Ferrario, C., Taverniti, V., Milani, C., Fiore, W., Laureati, M., De Noni, I., et al. (2014). Modulation of fecal clostridiales bacteria and butyrate by probiotic intervention with Lactobacillus paracasei DG varies among healthy adults. *Journal of Nutrition, 144*, 1787–1796. https://doi.org/10.3945/jn.114.197723
Koirala, R., Gargari, G., Arioli, S., Taverniti, V., Fiore, W., Grossi, E., et al. (2020). Effect of oral consumption of capsules containing Lactobacillus paracasei LPC-S01 on the vaginal microbiota of healthy adult women: A randomized, placebo-controlled, double-blind crossover study. *FEMS Microbiology Ecology, 96*, 1–12. https://doi.org/10.1093/femsec/fiaa084
Balzaretti, S., Taverniti, V., Rondini, G., Marcolegio, G., Minuzzo, M., Remagni, M. C., et al. (2015). The vaginal isolate Lactobacillus paracasei LPC-S01 (DSM 26760) is suitable for oral administration. *Frontiers in Microbiology, 6*. https://doi.org/10.3389/fmicb.2015.00952
Taverniti, V., Cesari, V., Gargari, G., Rossi, U., Biddau, C., Lecchi, C., et al. (2021). Probiotics Modulate Mouse Gut Microbiota and Influence Intestinal Immune and Serotonergic Gene Expression in a Site-Specific Fashion. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.706135
Segers, M. E., & Lebeer, S. (2014). Towards a better understanding of Lactobacillus rhamnosus GG - host interactions. *Microbial Cell Factories, 13*. https://doi.org/10.1186/1475-2859-13-S1-S7
Jungersen, M., Wind, A., Johansen, E., Christensen, J., Stuer-Lauridsen, B., & Eskesen, D. (2014). The Science behind the Probiotic Strain Bifidobacterium animalis subsp. lactis BB-12®. *Microorganisms, 2*, 92–110. https://doi.org/10.3390/microorganisms2020092
Silva, S., Costa, E. M., Veiga, M., Morais, R. M., Calhau, C., & Pintado, M. (2020). Health promoting properties of blueberries: A review. *Critical Reviews in Food Science and Nutrition, 60*, 181–200. https://doi.org/10.1080/10408398.2018.1518895
Colletti A, Sangiorgio L, Martelli A, Testai L, Cicero AFG, Cravotto G. Highly Active Cranberry's Polyphenolic Fraction: New Advances in Processing and Clinical Applications. Nutrients 2021;13:2546. https://doi.org/10.3390/nu13082546.
Chen, T., Shi, N., & Afzali, A. (2019). Chemopreventive effects of strawberry and black raspberry on colorectal cancer in inflammatory bowel disease. *Nutrients, 11*. https://doi.org/10.3390/nu11061261
Miller, K., Feucht, W., & Schmid, M. (2019). Bioactive compounds of strawberry and blueberry and their potential health effects based on human intervention studies: A brief overview. *Nutrients, 11*. https://doi.org/10.3390/nu11071510
Ho, G. T. T., Wangensteen, H., & Barsett, H. (2017). Elderberry and elderflower extracts, phenolic compounds, and metabolites and their effect on complement, RAW 264.7 macrophages and dendritic cells. *International Journal of Molecular Sciences, 18*. https://doi.org/10.3390/ijms18030584
Piotrowska, H., Kucinska, M., & Murias, M. (2012). Biological activity of piceatannol: Leaving the shadow of resveratrol. *Mutat Res - Rev Mutat Res, 750*, 60–82. https://doi.org/10.1016/j.mrrev.2011.11.001
Monguió-Tortajada, M., Franquesa, M., Sarrias, M. R., & Borràs, F. E. (2018). Low doses of LPS exacerbate the inflammatory response and trigger death on TLR3-primed human monocytes article. *Cell Death and Disease, 9*. https://doi.org/10.1038/s41419-018-0520-2
Frøkiær, H., Henningsen, L., Metzdorff, S. B., Weiss, G., Roller, M., Flanagan, J., et al. (2012). Astragalus Root and Elderberry Fruit Extracts Enhance the IFN-β Stimulatory Effects of Lactobacillus acidophilus in Murine-Derived Dendritic Cells. *PLoS ONE, 7*. https://doi.org/10.1371/journal.pone.0047878
del Cornò, M., Scazzocchio, B., Masella, R., & Gessani, S. (2016). Regulation of Dendritic Cell Function by Dietary Polyphenols. *Critical Reviews in Food Science and Nutrition, 56*, 737–747. https://doi.org/10.1080/10408398.2012.713046
Weiss, G., Christensen, H. R., Zeuthen, L. H., Vogensen, F. K., Jakobsen, M., & Frøkiær, H. (2011). Lactobacilli and bifidobacteria induce differential interferon-β profiles in dendritic cells. *Cytokine, 56*, 520–530. https://doi.org/10.1016/j.cyto.2011.07.024
Khokhlova, E. V., Smeianov, V. V., Efimov, B. A., Kafarskaia, L. I., Pavlova, S. I., & Shkoporov, A. N. (2012). Anti-inflammatory properties of intestinal Bifidobacterium strains isolated from healthy infants. *Microbiology and Immunology, 56*, 27–39. https://doi.org/10.1111/j.1348-0421.2011.00398.x
Preising, J., Philippe, D., Gleinser, M., Wei, H., Blum, S., Eikmanns, B. J., et al. (2010). Selection of bifidobacteria based on adhesion and anti-inflammatory capacity in vitro for amelioration of murine colitis. *Applied and Environment Microbiology, 76*, 3048–3051. https://doi.org/10.1128/AEM.03127-09
Riedel, C. U., Foata, F., Philippe, D., Adolfsson, O., Eikmanns, B. J., & Blum, S. (2006). Anti-inflammatory effects of bifidobacteria by inhibition of LPS-induced NF-κB activation. *World Journal of Gastroenterology, 12*, 3729–3735. https://doi.org/10.3748/wjg.v12.i23.3729
Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., et al. (2020). A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. *International Journal of Systematic and Evolutionary Microbiology, 70*, 2782–2858. https://doi.org/10.1099/ijsem.0.004107

Cassard, L., Lalanne, A. I., Garault, P., Cotillard, A., Chervaux, C., Wels, M., et al. (2016). Individual strains of Lactobacillus paracasei differentially inhibit human basophil and mouse mast cell activation. *Immunity, Inflammation and Disease, 4*, 289–299. https://doi.org/10.1002/iid3.113

Weiss, G., Rasmussen, S., Fink, L. N., Jarmer, H., Nielsen, B. N., & Frøkiær, H. (2010). Bifidobacterium bifidum actively changes the gene expression profile induced by Lactobacillus acidophilus in murine dendritic cells. *PLoS ONE, 5*. https://doi.org/10.1371/journal.pone.0011065

Taverniti, V., Minuzzo, M., Arioli, S., Junttila, I., Hämäläinen, S., Turpeinen, H., et al. (2012). In Vitro Functional and Immunomodulatory Properties of the Lactobacillus helveticus MIMLh5-Streptococcus salivarius ST3 Association That Are Relevant to the Development of a Pharyngeal Probiotic Product. *Applied and Environment Microbiology, 78*, 4209–4216. https://doi.org/10.1128/AEM.00325-12

Stevenson, D., & Scalzo, J. (2012). Anthocyanin composition and content of blueberries from around the world. *Journal of Berry Research, 2*, 179–189. https://doi.org/10.3233/JBR-2012-038

Vendrame, S., & Klimis-Zacas, D. (2015). Anti-inflammatory effect of anthocyanins via modulation of nuclear factor- B and mitogen-activated protein kinase signaling cascades. *Nutrition Reviews, 73*, 348–358. https://doi.org/10.1093/nutrit/nuu066

Blumberg, J. B., Camesano, T. A., Cassidy, A., Kris-Etherton, P., Howell, A., Manach, C., et al. (2013). Cranberries and their bioactive constituents in human health. *Adv Nutr, 4*, 618–632. https://doi.org/10.3945/an.113.004473

Neto, C. C. (2007). Cranberry and blueberry: Evidence for protective effects against cancer and vascular diseases. *Molecular Nutrition & Food Research, 51*, 652–664. https://doi.org/10.1002/mnfr.200600279

Gullickson, E. R., Krueger, C. G., Birmingham, A., Maranan, M., & Reed, J. D. (2020). Development of a Cranberry Standard for Quantification of Insoluble Cranberry (Vaccinium macrocarpon Ait.) Proanthocyanidins. *Journal of Agriculture and Food Chemistry, 68*, 2900–2905. https://doi.org/10.1021/acs.jafc.9b03696

Nowicka, A., Kucharska, A. Z., Sokół-Łętowska, A., & Fecka, I. (2019). Comparison of polyphenol content and antioxidant capacity of strawberry fruit from 90 cultivars of Fragaria × ananassa Duch. *Food Chemistry, 270*, 32–46. https://doi.org/10.1016/j.foodchem.2018.07.015

Silva, P., Ferreira, S., & Nunes, F. M. (2017). Elderberry (Sambucus nigra L.) by-products a source of anthocyanins and antioxidant polyphenols. *Industrial Crops and Products, 95*, 227–234. https://doi.org/10.1016/j.indcrop.2016.10.018

Suriyaprom S, Mosoni P, Leroy S, Kaewkod T, Desvaux M, Tragoolpua Y. Antioxidants of fruit extracts as antimicrobial agents against pathogenic bacteria. Antioxidants (Basel) 2022;11:602. https://doi: 10.3390/antiox11030602.

Gil-Sánchez, I., Cueva, C., Sanz-Buenhombre, M., Guadarrama, A., Moreno-Arribas, M. V., & Bartolomé, B. (2018). Dynamic gastrointestinal digestion of grape pomace extracts: Bioaccessible phenolic metabolites and impact on human gut microbiota. *Journal of Food Composition and Analysis, 68*, 41–52. https://doi.org/10.1016/j.jfca.2017.05.005

Hervert-Hernández, D., Pintado, C., Rotger, R., & Goñi, I. (2009). Stimulatory role of grape pomace polyphenols on *Lactobacillus acidophilus* growth. *International Journal of Food Microbiology, 136*, 119–122. https://doi.org/10.1016/j.ijfoodmicro.2009.09.016

Bravo, K. S., López, F. A., García, P. G., Quintana, M. D., & Fernández, A. G. (2007). Treatment of green table olive solutions with ozone. Effect on their polyphenol content and on *Lactobacillus pentosus* and *Saccharomyces cerevisiae* growth. *International Journal of Food Microbiology, 114*(60–68). https://doi.org/10.1016/j.ijfoodmicro.2006.09.032

Gwiazdowska, D.; Jús, K.; Jasnowska-Małecka, J.; Kluczý nska, K. The impact of polyphenols on *Bifidobacterium* growth. Acta Biochim. Pol 2015; 62: 895–901. https://doi. 10.18388/abp.2015_1154.

Stevens JF, Maier CS. The chemistry of gut microbial metabolism of polyphenols. Phytochem Rev 2016;15:425-444. https://doi: 10.1007/s11101-016-9459-z.

Caponio GR, Noviello M, Calabrese FM, Gambacorta G, Giannelli G, De Angelis M. Effects of grape pomace polyphenols and in vitro gastrointestinal digestion on antimicrobial activity: recovery of bioactive compounds. Antioxidants (Basel) 2022;11:567. https:// doi: 10.3390/antiox11030567.

van Duynhoven J, Vaughan EE, Jacobs DM, Kemperman RA, van Velzen EJ, Gross G, Roger LC, Possemiers S, Smilde AK, Doré J, Westerhuis JA, Van de Wiele T. Metabolic fate of polyphenols in the human superorganism. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4531-8. https://doi: 10.1073/pnas.1000098107.

Makarewicz M, Drożdż I, Tarko T, Duda-Chodak A. The interactions between polyphenols and microorganisms, especially gut microbiota. Antioxidants (Basel) 2021;10:188. https://doi: 10.3390/antiox10020188.

Guglielmetti S, Fracassetti D, Taverniti V, Del Bo' C, Vendrame S, Klimis-Zacas D, Arioli S, Riso P, Porrini M. Differential modulation of human intestinal bifidobacterium populations after consumption of a wild blueberry (*Vaccinium angustifolium*) drink. J Agric Food Chem 2013; 61:8134-40. https://doi: 10.1021/jf402495k.

Xu X, Guo Y, Chen S, Ma W, Xu X, Hu S, Jin L, Sun J, Mao J, Shen C. The positive influence of polyphenols extracted from pueraria lobata root on the gut microbiota and its antioxidant capability. Front Nutr 2022;9:868188. https://doi: 10.3389/fnut.2022.868188.

Westfall, S., Lomis, N., & Prakash, S. (2018). A novel polyphenolic prebiotic and probiotic formulation have synergistic effects on the gut microbiota influencing *Drosophila melanogaster* physiology. *Artificial Cells, Nanomedicine, and Biotechnology*, (46), 441–455. https://doi.org/10.1080/21691401.2018.1458731

Dueñas M, Muñoz-González I, Cueva C, Jiménez-Girón A, Sánchez-Patán F, Santos-Buelga C, Moreno-Arribas MV, Bartolomé B. A survey of modulation of gut microbiota by dietary polyphenols. Biomed Res Int 2015;2015:850902. https://doi: 10.1155/2015/850902.