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Review

Probiotic Molecules That Inhibit Inflammatory Diseases

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Featured Application: Exopolysaccharide from *Bacillus subtilis* induces an anti-inflammatory response that protects mice from several inflammatory diseases, including enteric and blood-borne pathogens, allergic eosinophilia, and graft versus host disease. This EPS, designated EPS^{Bs}, has potential as a therapeutic for inflammatory diseases in humans.

Abstract: Consumption of probiotics for health purposes has increased vastly in the past few decades, and yet the scientific evidence to support health benefits from probiotics is only beginning to emerge. As more probiotics are studied, we are beginning to understand the mechanisms of action by which they benefit human health, as well as to identify the bacterial molecules responsible for these benefits. A new era of therapeutics is on the horizon in which purified molecules from probiotics will be used to prevent and treat diseases. In this review, we summarize the active molecules from probiotic bacteria that have been shown to affect innate and adaptive immunity and have health benefits in experimental settings. We focus particularly on the cellular and molecular mechanisms of the probiotic *Bacillus subtilis* and its active molecule, exopolysaccharide (ESP^{Bs}).

Keywords: probiotic; *Bacillus subtilis*; exopolysaccharide; anti-inflammatory

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1. Introduction Mod

The World Health Organization defines probiotics as "live microorganisms that when administered in adequate amounts, confer a health benefit on the host." (FAO/WHO 2002). Consumption of live organisms for maintaining good health is a concept accepted by the general population, and in fact, The National Health Interview Survey showed that probiotics were the third most used non-vitamin, non-mineral dietary supplement in 2012 [1]. Despite their popular use, probiotics have yet to be approved for any medical indication by the US Food Drug Administration [2], in part because results from clinical trials on the efficacy of probiotics in the treatment or prevention of disease have been equivocal [3]. The lack of clarity emanating from the clinical trials has created a state of confusion regarding the clinical use of probiotics. Some of the confusion may arise from the use of different probiotic strains, or combinations thereof, batch preparation, and the outcomes readout. Furthermore, humans vary widely in their diets, genetic backgrounds, and gut microbiome composition, all of which likely influence the efficacy of probiotics.

The probiotic bacteria most widely studied belong to the *Lactobacillus* sp., *Bacterioidetes* sp., *Bifidobacteria* sp., and *Bacillus* sp. These bacteria have been used to treat diarrhea, irritable bowel syndrome [4], and atopic dermatitis [5], among other diseases. Probiotic bacteria can be beneficial to human health, for example, by inhibiting growth of pathogenic bacteria in the intestine and by secreting bioactive metabolites, such as short-chain fatty acids [6]. The use of intact bacteria for clinical interventions can be challenging, however, in part because the administration of bacteria may alter the endogenous microbiome,

causing dysbiosis, and also affect intestinal immunity in a manner not yet fully understood. An alternative approach to administering live bacteria is to isolate and administer the active bacterial molecule responsible for the health benefits. As discussed below, we argue that future scientific research into probiotics should focus not only on specific probiotic organisms but more importantly, on identifying the active molecules of these organisms and determining the mechanism of action by which they provide protection from disease. Such a process will lead to probiotic molecules or probiotic-treated host cells as therapeutics. Here we focus on probiotic molecules that affect the innate and/or adaptive immune system.

Bioactive probiotic molecules with anti-inflammatory properties include cell envelope molecules, secreted proteins, and exopolysaccharides, many of which are shown in Table 1. Although bacterial metabolites, such as short-chain fatty acids, lactate, and bacteriocins, can also be active probiotic molecules, we do not discuss them in this review. Instead, we briefly describe cell envelope-associated probiotic molecules, as well as secreted protein and carbohydrate probiotic molecules, and discuss in-depth the anti-inflammatory probiotic exopolysaccharide (EPS) produced by *Bacillus subtilis*. This EPS designated EPS^{Bs}, ameliorates disease due to enteric or blood-borne pathogens, allergic eosinophilia, and graft vs. host disease [7–13].

Table 1. Probiotic bacterial molecules with anti-inflammatory activity.

Organism	Molecule	Results	Reference
Bacillus subtilis	Lipoteichoic acid (LTA)	LTA activates a TLR2-dependent inflammatory response and concomitantly induces activation of MerTK signaling to counteract the inflammation *in vitro*.	[14]
	EPS^{Bs}	EPS^{Bs} reduces *Citrobacter rodentium* infection and generates peritoneal anti-inflammatory macrophages.	[9]
		EPS^{Bs} prevents allergic eosinophilia.	[10,12]
		EPS^{Bs} ameliorates GvHD and can generate tolerogenic DCs *in vitro*.	[8]
		EPS^{Bs} protects against systemic infection of *Staphylococcus aureus*.	[7]
			[11,13]
Bacteroides fragilis	Polysaccharide A (PSA)	PSA is protective and therapeutic in murine models of colitis and multiple sclerosis via the induction of IL-10 secreting Tregs.	[15–19]
		PSA activates colonic DCs and produces IFN-β that enhances resistance to viral infection in murine models. This protection is dependent on TLR4.	[20]
	Glycosphingolipids	Glycosphingolipids decrease the number of invariant natural killer T cells in the colonic lamina propria leading to improved outcomes in a murine colitis model.	[21,22]
Bifidobacterium adolescentis	EPS^{Ba}	EPS^{Ba} induces IL-10 production, protects from colitis by activation of DCs and macrophages, and increases the Treg/Th17 cell ratio in mice.	[23]
Bifidobacterium breve UCC2003	EPS^{Bb}	EPS^{Bb} reduces *Citrobacter rodentium* infection in mice.	[24]
		EPS^{Bb} prevents the maturation of DCs and activation of antigen-specific CD4+ T cells.	[25]
		EPS^{Bb} reduces the rate of small epithelial cell shedding in a mouse model of pathological cell shedding.	[26]
Bifidobacterium breve WBBR04	EPS^{Bb}	EPS^{Bb} enhances the intestinal barrier integrity to prevent allergen infiltration and food allergy in mice.	[27]
Bifidobacterium longum	Serpin	Serpin inhibits eukaryotic elastase-like serine proteases, which are dysregulated in inflammatory disorders.	[28,29]

Table 1. *Cont.*

Organism	Molecule	Results	Reference
Bifidobacterium Longum 35624	EPS^{Bl}	EPS^{Bl} dampens pro-inflammatory cytokines and reduces inflammatory symptoms in a T cell transfer colitis model.	[30]
		EPS^{Bl} stimulates the release of IL-10 in a TLR2-dependent manner and reduces eosinophil recruitment in the lungs in a respiratory inflammation mouse model.	[31]
Bifidobacterium Longum YS108R	EPS^{Bl}	EPS^{Bl} reduces the pro-inflammatory cytokines IL-6 and IL-17A, alleviating inflammation in a colitis murine model.	[32]
Bifidobacterium sp.	Fimbriae	Fimbriae facilitate gut colonization and stimulation of macrophage cytokine production, TNF-α, IL-6, and Il-10.	[33–35]
Escherichia coli Nissle 1917	Flagellin	Flagellin induces the release of β-defensin-2 in epithelial cells through NF-κB- and AP-1-dependent pathways *in vitro.*	[36]
Lactobacillus acidophilus NCFM	Surface layer protein A (SlpA)	SlpA binds the lectin-receptor DC-SIGN and increases IL-10 and reduces IL-12p70 production from DCs.	[37]
Lactobacillus casei	Lactoceptin	Lactoceptin can selectively hydrolyze pro-inflammatory chemokine IP-10 leading to reduced lymphocyte recruitment in an ileitis murine model.	[38]
Lactobacillus casei BL23	p40 and p75	Secreted proteins p40 and p75 stimulate Akt activation, display anti-apoptotic activity, and prevent epithelial barrier damage in colitis murine models.	[39–41]
Lactobacillus casei Shirota	High molecular components of cell wall	High molecular weight cell wall components of *Lactobacillus casei Shirota* decrease LPS-induced IL-6 production in macrophages.	[42]
	Polysaccharide peptidoglycan complex	Polysaccharide peptidoglycan complex improves ileitis and inhibits IL6/STAT3 signaling in a murine colitis model.	[43]
Lactobacillus helveticus KLD1.8701	EPS^{Lh}	EPS^{Lh} reduces intestinal inflammation and improves mucosal barrier function in a murine colitis model.	[44]
Lactobacillus kefiranofaciens	EPS^{Lk}	EPS^{Lk} increases the number of IgA^{+} cells in the small and large intestines and increases the levels of IL-4 and IL-12 in the intestinal fluid and serum.	[45]
Lactobacillus plantarum	Serine-threonine peptide (STp)	STp changes the phenotype of DC from ulcerative colitis patients by reducing TLR expression, increasing activation markers, and restoring stimulatory capacity.	[46]
Lactobacillus plantarum	LTA	LTA from *L. plantarum* and *Staphylococcus aureus* alleviates atopic dermatitis by regulating the complement regulatory proteins CD55 and CD59 and reducing activation of the complement system.	[47]
		LTA inhibits the release of TNF-α and IL-10 from stimulated THP-1 cells by dephosphorylating c-Jun N-terminal kinase (JNK) and p38, respectively.	[48]
Lactobacillus plantarum K8	LTA	LTA suppresses inflammatory cytokine-mediated complement activation through the inhibition of C3 synthesis.	[49]
Lactobacillus plantarum L-14	EPS^{Lp}	EPS^{Lp} suppresses the pro-inflammatory cytokine mediators, COX-2, IL-6, TNF-α, and IL-1β induced by LPS.	[50]
Lactobacillus plantarum N-14	EPS^{Lp}	EPS^{Lp} activates RP105/MD1 on intestinal epithelial cells to reduce inflammatory pathways.	[51]
Lactobacillus rhamnosus GG	Pili	Pili helps the adhesion of *Lactobacillus rhamnosus GG* to epithelial and the release of anti-inflammatory IL-10, IL-8, and IL-6 from epithelial cells.	[34,52]
Lactobacillus rhamnosus GG	Lipoteichoic acid (LTA) Supernatants	LTA improves colitis in a murine model. Administration of culture supernatants reduces eosinophil numbers, goblet cells, and lung inflammation in murine allergy model.	[53,54]

Table 1. *Cont.*

Organism	Molecule	Results	Reference
Lactobacillus sakei	LTA	LTA inhibits the secretion of TNF-α from UVA-exposed derma fibroblasts.	[55]
Lactobacillus salivarius Ls33	Peptidoglycan	Peptidoglycan protects mice from chemically induced colitis in a NOD2-IL-10-dependent manner.	[56]
Lactobacillus Sp	Teichonic acids	Teichonic acid induces IL-10 in a TLR2-dependent manner in macrophages.	[57]
Lactobacillus paraplantarum BGCG11	EPS^{Lp}	EPS^{Lp} reduces pro-inflammatory cytokines in a hyperalgesia rat model that results in anti-hyperalgesic and anti-edematous outcomes.	[58]
Lactobacillus plantarum NCU116	EPS^{Lp}	EPS^{Lp} regulates the tight junction proteins occluding and ZO-1 by activating STAT3.	[59]
Lactobacillus rhamnosus GG	EPS^{Lr}	EPS^{Lr} reduces hydrogen peroxide-induced intestinal oxidative damage and apoptosis by Keap1/Nrf2 and Bax/Bcl-2 pathways *in vitro*.	[60]
	LTA	LTA protects intestinal epithelial cells from radiation injury through the activation of pericryptal macrophages. These macrophages release CXCL12 that binds to CXCR4 on COX-2 expressing mesenchymal stem cells and stimulate the release of PGE, which protects epithelial stem cells from radiation.	[61]
Lactobacillus rhamnosus KL37	EPS^{Lr}	EPS^{Lr} inhibits T cell-dependent immune response reducing the arthritogenic antibodies in an arthritis murine model.	[62]
Propionibacterium freudenreichii	Guanidine surface protein extract	Treatment of human peripheral blood with guanidine surface protein extract releases IL-10 and IL-6, while having no effect on IL-12, TNF-α, and IFNγ.	[63]

2. Cell Envelope Molecules

Probiotics' cell envelope molecules have immunomodulatory properties that can reduce pro-inflammatory cytokines, increase production of anti-inflammatory IL-10, generate T regulatory cells (Tregs), and help protect from radiation damage. One of the best characterized anti-inflammatory probiotic molecules is the capsular polysaccharide A (PSA) from *Bacteroides fragilis*. Oral administration of PSA, a zwitterionic polysaccharide, is protective and therapeutic in murine models of colitis and multiple sclerosis by inducing IL-10-secreting Tregs. Protection by PSA is TLR2 and MHCII-dependent, likely due to TLR2 signaling in plasmacytoid dendritic cells to induce Tregs and IL-10 production [15–19,64]. Furthermore, *B. fragilis* produces glycosphingolipids that decrease the number of invariant natural killer T cells in the colonic lamina propria, leading to improved outcomes in a murine colitis model [21,22]. Recently, *B. fragilis* PSA was shown to activate colonic DCs and produce IFN-β in a TLR4-dependent manner that enhances resistance to viral infection in murine models [20].

A polysaccharide peptidoglycan complex on *L. casei* Shirota was shown to improve ileitis and inhibit IL-6/STAT3 signaling in a murine colitis model using bacterial mutants *in vivo* and purified peptidoglycan *in vitro* [65]. The oral administration of probiotic *L. rhamnosus* GG mutant with modified lipoteichoic acid (LTA) molecules improved colitis in a murine model, correlating with decreased TLR expression and pro-inflammatory cytokine secretion [53]. Further, oral gavage with WT *L. rhamnosus* GG or intraperitoneal (i.p.) injection with *L. rhamnosus* LTA protected intestinal epithelial cells from radiation injury through the activation of pericryptal macrophages. These macrophages release CXCL12 that binds to CXCR4 on COX-2 expressing mesenchymal stem cells and stimulates the release of PGE, which protects epithelial stem cells from radiation [61]. Supernatants from *L. rhamnosus* GG cultures also reduced eosinophil numbers, goblet cell numbers, and lung inflammation in an allergy inflammation murine model [54]. Peritoneal administration of

isolated peptidoglycan from *L. salivarius* Ls33 protects mice from chemically induced colitis in a NOD2-IL-10-dependent manner [56]. Other cell wall components from probiotics have been identified as listed in Table 1. As suggested above, these examples illustrate the potential for using purified active components of probiotics to treat human diseases prophylactically and therapeutically.

3. Secreted Molecules

A. Proteins. Numerous extracellular and secreted bacterial proteins are known to diminish inflammation. These include serpin from *Bifidobacterium longum*, which inhibits eukaryotic elastase-like serine proteases that are dysregulated in inflammatory disorders, such as celiac disease [28,29], and p40 and p75, molecules from *Lactobacillus casei* BL23 that *in vitro* protect from disruption of epithelial cell tight junctions induced by hydrogen peroxide in a PKC and MAP kinase-dependent manner [39]. A p40 secreted molecule from *L. rhamnosus GG* activated EGFR *in vitro* and, after oral administration, prevented DSS-induced intestinal epithelial damage in mice [40,41]. Moreover, *L. casei* secretes lactoceptin, which hydrolyzes the pro-inflammatory chemokine IP-10, and its i.p. administration leads to reduced lymphocyte recruitment in an ileitis murine model [38]. Another extracellular molecule that moderates the immune response is flagellin from *Escherichia coli* Nissle 1917, which induces the release of the antimicrobial peptide β-defensin 2 in epithelial cells *in vitro* [36]. Other secreted probiotic proteins that can modulate immune responses are summarized in Table 1. These examples illustrate the potential of using extracellular and secreted peptides from probiotics for therapeutic treatments of immune-mediated diseases.

B. Exopolysaccharides. Many probiotics secrete polysaccharides that have anti-inflammatory properties, which can reduce the production of pro-inflammatory cytokines, increase anti-inflammatory cytokines, enhance the intestinal epithelial barrier, and inhibit T cell-dependent immune responses. These polysaccharides are usually obtained by alcohol precipitation from culture supernatants, and the solubilized precipitate is designated as exopolysaccharide (EPS). As discussed below, EPS molecules of different origins vary widely in the anti-inflammatory effects and the mechanism of protection. Although the term "EPS" is used for all of these samples, the composition and structure of them are generally not known. Likely, EPS from different microorganisms have different compositions and structures, leading to diverse functions and mechanisms of immune regulation. A defined classification of EPS from different organisms will require the structure and structure/function relationship of these polysaccharides. In this review, we distinguish the EPS preparations by using superscripts to symbolize the bacterium from which a specific EPS is isolated, e.g., EPS^{Bs} from *Bacillus subtilis*.

Using bacterial mutants, EPS^{Bb} from *Bifidobacterium breve* has been implicated in reducing colitis during *Citrobacter rodentium* infection in mice [24] and in reducing rates of epithelial cell shedding [26]. Oral administration of EPS^{Bb} from *B. breve* has also been shown to enhance the intestinal barrier integrity, thereby preventing allergen infiltration and food allergy [27]. Another species of *Bifidobacterium*, the non-aggregating strain IF1-03 *B. adolescentis*, protects from colitis by inducing IL-10 production, activating dendritic cells (DCs) and macrophages, and increasing the ratio of Treg/Th17 cells in mice [23]. Based on data from a bacterial mutant, EPS^{Bl} from still another *Bifidobacterium* species, *B. Longum* 35624, dampens pro-inflammatory cytokines and reduces inflammatory symptoms in the T cell transfer colitis model [30], and in an allergy model, EPS^{Bl} stimulates the release of IL-10 in a TLR2-dependent manner that reduces recruitment of eosinophils to the lungs [31].

For *Lactobacillus*, numerous immunomodulatory effects of EPS^{Lh} have been identified and are reviewed in Laiño et al. [66]. Oral administration of EPS^{Lh} isolated from *L. helveticus* KLDS1.8701 reduces intestinal inflammation and improves mucosal barrier function in a colitis model [44], whereas EPS^{Lr} from *L. rhamnosus* KL37 inhibits T cell-dependent immune responses and reduces the arthritogenic antibodies in an arthritis murine model [62]. Several other examples of the anti-inflammatory effects of EPS from probiotics are listed in Table 1. While the molecular mechanisms by which EPS induces anti-inflammatory

responses are not well understood, it is noteworthy that EPS^{Lp} from *L. plantarum* N-14 reduces TLR4-mediated pro-inflammatory cytokine production by porcine intestinal epithelial cells. This anti-inflammatory response by EPS^{Lp} is due to the induction of negative regulators of TLR signaling, especially RP105, a type I transmembrane molecule, considered part of the TLR family [51]. RP105 complexes with the accessory protein, MD1, and together inhibit LPS signaling through the TLR4/MD2 complex [67]. These data suggest that EPS from some probiotic species may dampen an inflammatory response or induce an anti-inflammatory response by binding to receptors that negatively regulate TLR4 signaling. EPS is easily extractable from bacteria, and as the structures and functions become better defined, EPS from numerous microorganisms may become widely used for therapeutic purposes.

4. EPS^{Bs} as Probiotic

The EPS (EPS^{Bs}) that we study is derived from *Bacillus subtilis* and induces anti-inflammatory responses that protect mice from several inflammatory diseases. Below, we discuss these diseases, the cells and molecules that promote protection, and the potential of EPS^{Bs} as a therapeutic for humans.

A. *C. rodentium*-induced colitis. Oral administration of a single dose of *B. subtilis* spores was first shown to reduce colitis in mice after infection with the enteric pathogen, *Citrobacter rodentium* [9]. In this model, *B. subtilis* does not function by reducing the colonization of *C. rodentium*, but instead alters the inflammatory disease process, as indicated by reduced epithelial hyperplasia, diarrhea, and goblet cell loss (Figure 1). Analysis of *B. subtilis* mutants revealed that a mutation in *epsH*, which regulates biofilm synthesis [68], did not protect from disease caused by *C. rodentium*, suggesting that biofilm-associated carbohydrate exopolysaccharide (EPS^{Bs}) was required for protection. EPS^{Bs} was isolated and purified by treatment with DNase, RNase, proteinase K, and gel filtration [10,12], and indeed, it protected from disease. In fact, a single intraperitoneal injection of EPS^{Bs} (2.5 mg/kg) administered one day prior to or as much as 3 days after infection with *C. rodentium,* was sufficient to reduce epithelial hyperplasia, diarrhea, and goblet cell loss. Protection by EPS^{Bs} is mediated by anti-inflammatory macrophages, sometimes designated as M2 macrophages. Intraperitoneal administration of EPS^{Bs} results in the accumulation of macrophages with M2 macrophage markers, IL4Ra, CD206, arginase, and PD-L1 in the peritoneum, and adoptive transfer of these cells to untreated mice protects them from colitis after infection with *C. rodentium* [10,12]. These findings demonstrate the anti-inflammatory potential of EPS^{Bs}, and of the anti-inflammatory macrophages induced by EPS^{Bs}.

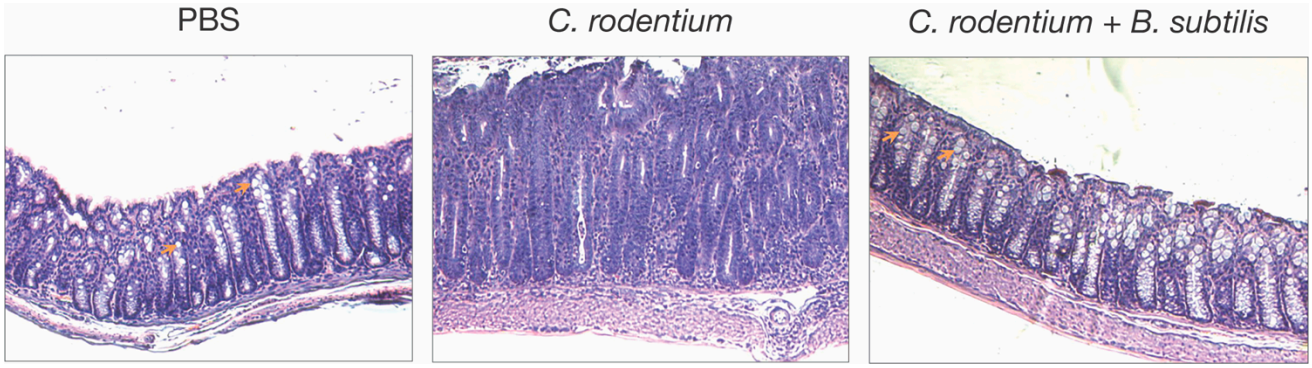

Figure 1. Colonic histological analysis of the effect of *B. subtilis* spores in *C. rodentium* disease 10 days post infection. Note goblet cells present in normal tissue (arrows), but reduced in colon infected with *C. rodentium*. Reprinted from [9].

B. Systemic infection with *Staphylococcus aureus*. Similar to infection with the enteric pathogen, *C. rodentium*, EPS^{Bs} also moderates disease caused by infection with blood-borne *S. aureus* [11]. In this case, EPS^{Bs} increases survival by reducing weight loss and systemic inflammation, as evidenced by decreased levels of inflammatory cytokines and chemokines in blood and bacterial burden [11]. EPS^{Bs} induced hybrid-like M1/M2 macrophages, which not only inhibited T cell activation, characteristic of M2 macrophages but also inhibited *S. aureus* growth through reactive oxygen species (ROS), characteristic of M1 macrophages [69]. Together, data from infection by *C. rodentium* and *S. aureus* show that EPS^{Bs} from *B. subtilis* induces an anti-inflammatory environment with decreased inflammatory cytokines and increased anti-inflammatory macrophages that limit T cell activation, as well as macrophages that restrict the growth of bacteria.

C. Allergic eosinophilia. The association of changes in microbiota to allergic disease is well known, not only because of the hygiene hypothesis [70] but also because of a landmark study by Stein et al., who showed that children who grow up in a farm environment with close proximity to farm animals develop considerably fewer allergies than children that grow up without much interaction with farm animals [71]. This "farm effect" is likely explained by the interaction of children with microbes of the farm animals [72]. Swartzendruber et al. orally administered *B. subtilis* spores to mice and showed that they prevented the development of allergic eosinophilia in response to intranasal administration of house dust mite (HDM) antigen [8]. The infiltration of eosinophils is due in part to cytokines secreted by T cells [73]. Because DCs are also crucial for the activation of T cells and the development of eosinophilia, Swartzendruber et al. hypothesized that EPS^{Bs}-treated DCs could mitigate the allergic eosinophilia caused by an allergy to HDM. Intranasal adoptive transfer of EPS^{Bs}-treated bone marrow-derived DCs (BMDCs) prevented eosinophilia induced by HDM-pulsed DCs, indicating that EPS^{Bs} induces anti-inflammatory DCs, which can prevent an allergic response, as might be predicted by previous studies [70,72].

D. Graft versus host disease (GvHD). Another T cell-mediated disease attenuated by EPS^{Bs} is GvHD, a severe and often lethal complication of hematopoietic stem cell transplantation, which is frequently used to treat leukemia. The devastating effects of GvHD are mediated by alloreactive donor T cells that recognize host antigens as foreign, become activated, and destroy host tissues and organs. Intraperitoneal injection of EPS^{Bs} (2.5 mg/kg) administered several times, 7, 5, and 3 days prior to induction of GvHD, increased survival of mice 80 days post GvHD from 10% to 70% (Figure 2). Kalinina et al. assessed inflammation in live mice during GvHD using a caspase-1 reporter mouse to measure inflammasome activation [7]. With this biosensor mouse model, they found that the administration of EPS^{Bs} prevented the activation of alloreactive donor T cells, explaining the increased survival of mice. The results showed that EPS^{Bs} did not directly affect alloreactive T cells. In mixed lymphocyte reactions (MLR) *in vitro*, EPS^{Bs}-treated BMDCs potently inhibited alloreactive T cells, suggesting that *in vivo*, EPS^{Bs} induces DCs or other innate cells to become inhibitory and prevent the activation of alloreactive T cells, thereby reducing GvHD.

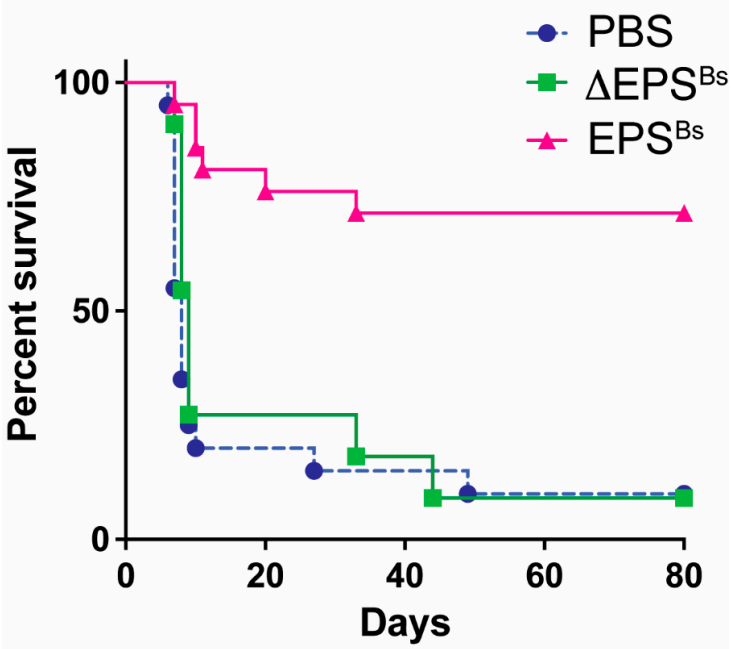

Figure 2. Kaplan–Meier survival curves of mice undergoing acute graft versus host disease (GvHD) after treatment with *B. subtilis* exopolysaccharide (EPS^{Bs}), or the negative control ΔEPS^{Bs}. ΔEPS^{Bs} is purified from a *B. subtilis* mutant *epsH*, which does not produce EPS associated with biofilms. Adapted from [7].

5. Mechanism by Which EPS^{Bs} Inhibits Inflammation

The mechanisms by which EPS from different bacteria ameliorate disease are likely to be highly variable, depending on the structure of the EPS. As indicated above, EPS^{Bs} affects innate cells, such as macrophages and DCs, converting them into anti-inflammatory cells. EPS^{Bs} does not directly affect T cells, either $CD4^+$ or $CD8^+$, even though inflammation in many diseases is caused by both pathogenic T cells. Instead, the effect of EPS^{Bs} on T cells occurs primarily through EPS^{Bs}-induced anti-inflammatory innate cells. Molecules, thus far, known to be associated with these processes include TLR4, TGF-β, PD-L1, and indoleamine 2,3 dioxygenase (IDO), as discussed below and are shown in Figure 3.

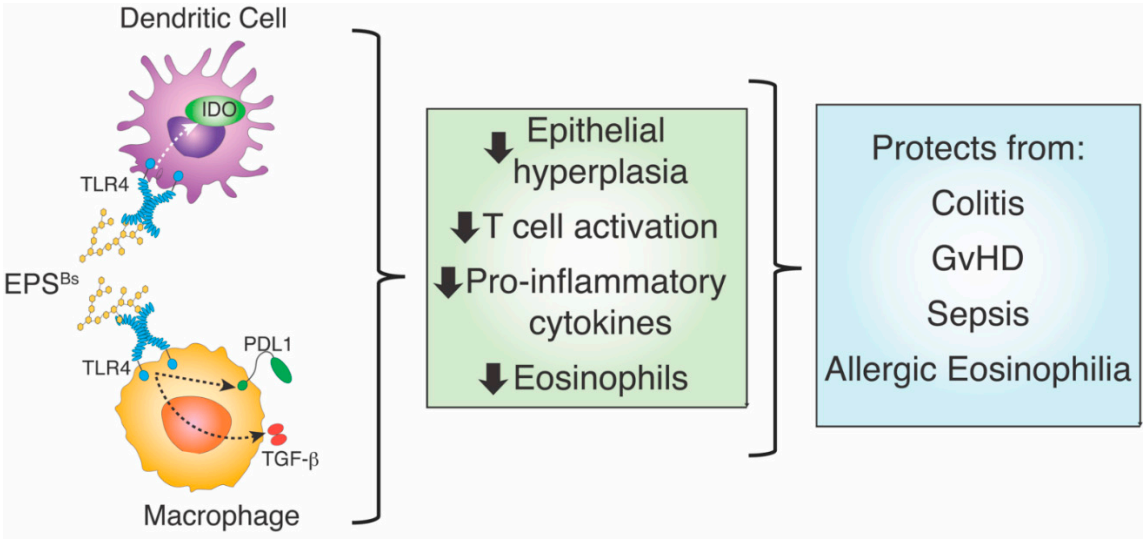

Figure 3. Summary of cellular and molecular effects of EPS^{Bs} on disease-induced inflammatory processes.

5.1. Cells

In all of the model systems tested, EPS^{Bs} inhibits the activation of T cells, but it does not directly affect them [7,10]. Instead, EPS^{Bs} induces anti-inflammatory M2-like macrophages and anti-inflammatory DCs, both of which have the capacity to inhibit T cell proliferation. Intraperitoneal injection of EPS^{Bs} leads to the induction of peritoneal M2-like macrophages that inhibit proliferation of activated T cells in the *C. rodentium* colitis model [10,12], as well as hybrid M1-M2-like macrophages in mice infected with *S. aureus* [11]. These M1-M2-like macrophages not only inhibit T cell activation by *S. aureus* superantigen but also upregulate ROS and have the capacity to growth arrest *S. aureus*. For DCs, *in vitro* stimulation of BMDCs with EPS^{Bs} results in anti-inflammatory cells that inhibit alloreactive T cells in MLR and presumably in GvHD [7]. Other cells affected by EPS^{Bs} include NK cells, although the mechanism by which this occurs remains a mystery [13].

5.2. Immune Regulator Molecules

1. TLR4. The anti-inflammatory effect of EPS^{Bs} requires TLR4, as shown in the disease models for colitis [12], bacterial sepsis [11], and GvHD [7]. The requirement for TLR4 resides in the macrophages and DCs [7,10,12], but TLR4 on other cell types, e.g., epithelial cells, mesenchymal, and NK cells, may also be required for, or contribute to, the protection by EPS^{Bs}. Experiments with cell-specific knockout mice will establish the identity of $TLR4^{+}$ cells required for the anti-inflammatory activity of EPS^{Bs}. The finding that EPS^{Bs} induces an anti-inflammatory effect through TLR4 is, of course surprising, because TLR4 signaling is associated with an inflammatory response due to LPS signaling. Although LPS is generally associated with pro-inflammatory responses, LPS can also induce tolerance, known as low dose tolerance or endotoxin tolerance. In this case, prolonged administration of LPS [74], or a single low dose of LPS, can dampen inflammation and induce tolerance. The mechanism by which this occurs remains under investigation [75]. Other TLR4-mediated anti-inflammatory responses have also been described and in these cases, the immunoregulatory effect is often mediated by other receptors. For example, the toll-like receptor family protein RP105/MD1 complex is involved in the immunoregulatory effect of EPS^{Lp} from Lactobacillus plantarum N14, which inhibits inflammatory TLR4 signaling [51]. Furthermore, Horvatinovich et al. showed that soluble CD83 inhibits T cell activation by binding to the TLR4/MD-2 complex on $CD14^{+}$ monocytes and altering the signaling cascade to induce the production of anti-inflammatory molecules IDO and IL-10 [76]. Gringhuis et al. showed that the lectin DC-SIGN modulates TLR signaling via Raf-1 kinase-dependent acetylation of the transcription factor NF-κB, which increased the anti-inflammatory response by increasing the transcription of IL-10 [77]. Furthermore, Yao et al. showed that leukadherin-1-mediated activation of CD11b inhibits LPS-induced pro-inflammatory responses in macrophages and protects mice from endotoxic shock by blocking LPS-TLR4 interaction [78]. Lastly, Li et al. showed that galectin-3 is a negative regulator of LPS-mediated inflammation [79]. We hypothesize that EPS^{Bs} also engages a receptor that negatively regulates TLR4-mediated signaling.

2. TGF-β and PD-L1. Paynich et al. showed that EPS^{Bs}-induced peritoneal M2 macrophages inhibit proliferation and activation of both $CD4^{+}$ and $CD8^{+}$ T cells activated with anti-CD3 and anti-CD28 antibodies, *in vitro* [10]. M2 macrophages are known to mediate an anti-inflammatory response by several molecules, including TGF-β, Arg-1, IL-10, PD-L1, and PD-L2. EPS^{Bs}-induced peritoneal M2 macrophages inhibited T cells in a contact-dependent manner, and this inhibition was dependent on TGF-β in the case of $CD4^{+}$ T cells and on TGF-β and PD-L1 in the case of $CD8^{+}$ T cells [10]. EPS^{Bs}-induced peritoneal M2 macrophages also upregulated Arg-1 and secretion of IL-10, and although these molecules were not required for the inhibition of T cell proliferation and activation *in vitro*, we predict that they are involved in the EPS^{Bs}-induced anti-inflammatory response *in vivo*.

3. IDO. Kalinina et al. showed that EPS^{Bs} induces inhibitory BMDCs that completely inhibited alloreactive T cell proliferation in an MLR [7]. This inhibition was neither depen-

dent on TGF-β or PD-L1, nor on other inhibitory molecules, such as Arg-1, IL-10, CTLA4, and PD-L2. Instead, the EPS^{Bs}-induced BMDCs inhibited alloreactive T cells in the MLR through the inhibitory molecule, IDO [7]. EPS^{Bs}-treated *ido1*$^{-/-}$ BMDCs do not inhibit T cell proliferation, and the addition of the IDO inhibitor 1-methyl-L-tryptophan to the MLR cultures with EPS^{Bs}-treated WT BMDCs restored T cell proliferation. IDO inhibition of T cell proliferation is due to degradation of the essential amino acid, tryptophan, and the production of tryptophan metabolites, kynurenines [80].

6. Translational Potential of EPS^{Bs}

B. subtilis has anti-inflammatory properties and is protective in several T cell-mediated diseases [7–13]. One molecule of *B. subtilis* that confers the anti-inflammatory responses is EPS^{Bs}, secreted as a part of a biofilm. Using *B. subtilis* mutants that overexpress and secrete EPS, large quantities of EPS^{Bs} can be easily purified for prophylactic or therapeutic administration. Injection of EPS^{Bs} confers protection similar to that shown by oral administration of *B. subtilis* spores [9], and currently, in collaboration with colleagues in Food Science at the University of Massachusetts, we plan to formulate capsules to optimize oral administration of EPS^{Bs}. Using an active molecule instead of live bacteria has many advantages: it is not dangerous for immunocompromised patients, it allows for more accurate dosage, its effects are temporary, and it will not alter the intestinal microbiome. Although there is no evidence that EPS^{Bs} is toxic to mice, clinical studies will be needed to confirm that it is also not toxic for humans.

Thus far, EPS^{Bs} has been used primarily as a prophylactic [7–13]. However, in studies with *C. rodentium*-induced colitis, EPS^{Bs} injected 3 days post infection also inhibited disease [10], indicating that EPS^{Bs} has potential as a therapeutic, especially for patients suffering from acute diarrhea, currently treated with fluid and electrolyte replacement.

Another potential use of EPS^{Bs} is for inflammatory bowel disease (IBD), where, in remission, most patients relapse and require treatment [81]. EPS^{Bs} could be used in combination with other anti-inflammatory agents not only to achieve remission but also could be used continuously during remission to prevent future relapses. Similarly, seasonal allergies and asthma induce inflammation after contact with allergens or molecular triggers. Because intranasal administration of EPS^{Bs} reduces allergic eosinophilia in a murine model [8], it could be used as a preventative medication to prevent allergic and asthma attacks in a prophylactic manner during allergy seasons or when exposure to allergens is unavoidable. This idea is in line with Strachan's hygiene hypothesis [70], which states that as human settings become free from bacteria, the incidence of allergies rises. By dosing individuals who suffer from allergies with EPS^{Bs}, the allergic inflammation flares may be reduced.

EPS^{Bs} also shows exciting promise to prevent GvHD where alloreactive T cells become activated and can initiate severe inflammation. In an *in vitro* MLR, human cells from different MHC types are cultured together, and alloreactive T cells become activated, as in GvHD. If BMDCs treated with EPS^{Bs} are added to the MLR cultures, T cell activation is inhibited, whereas with untreated BMDCs no inhibition is observed [7]. It may well be that the administration of EPS to the donor and/or recipient of the graft may limit inflammation and the resultant GvHD. Another possibility is to treat BMDCs *in vitro* with EPS^{Bs}, and then administer them along with the graft. This may be especially helpful for bone marrow transplants in patients with a hematologic malignancy, many of which require bone marrow transplants. Other disease candidates to test the clinical efficacy of EPS^{Bs} are chronic diseases, including atopic dermatitis, psoriasis, chronic sinusitis, and eosinophilic esophagitis. These are inflammatory disorders that cycle from remissions to flares or remain at low continuous inflammation. EPS^{Bs} can be easily administered for symptom relief.

7. Concluding Remarks

Since Strachan's [70] hygiene hypothesis, numerous studies have demonstrated the potential of bacteria, especially commensal bacteria to provide health benefits (reviewed in [3]). Such bacteria are now referred to as probiotics and are readily ingested by millions

of people in over-the-shelf supplements. As discussed above, most studies with probiotics utilize intact bacteria instead of identifying bioactive bacterial molecules and determining how they can provide health benefits. The advantage to identifying bioactive molecules, such as cell envelope and secreted molecules, is that they can be administered locally, systemically, and by intranasal or oral administration without the potential of developing sepsis, as might occur after the administration of live bacteria.

B. subtilis is one of the probiotics for which a bioactive molecule has been identified. Structural analysis for this and other probiotic molecules will provide the opportunity to synthesize molecules and chemically optimize them for biologic longevity, oral administration, and minimal toxicity. It is quite possible that other compounds, either secreted or derived from bacterial cell envelopes that modulate immune responses are yet to be discovered. We are optimistic that probiotic molecules will provide a new source of bioactive molecules that can prevent or treat numerous inflammatory diseases.

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References

1. Clarke, T.C.; Black, L.I.; Stussman, B.J.; Barnes, P.M.; Nahin, R.L. Trends in the use of complementary health approaches among adults: United States, 2002–2012. *Natl. Health Stat. Rep.* **2015**, *79*, 1–16.
2. Venugopalan, V.; Shriner, K.A.; Wong-Beringer, A. Regulatory oversight and safety of probiotic use. *Emerg. Infect. Dis.* **2010**, *16*, 1661–1665. [CrossRef] [PubMed]
3. Suez, J.; Zmora, N.; Segal, E.; Elinav, E. The pros, cons, and many unknowns of probiotics. *Nat. Med.* **2019**, *25*, 716–729. [CrossRef]
4. Ford, A.C.; Quigley, E.M.; Lacy, B.E.; Lembo, A.J.; Saito, Y.A.; Schiller, L.R.; Soffer, E.E.; Spiegel, B.M.; Moayyedi, P. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: Systematic review and meta-analysis. *Am. J. Gastroenterol.* **2014**, *109*, 1547–1561. [CrossRef]
5. Li, L.; Han, Z.; Niu, X.; Zhang, G.; Jia, Y.; Zhang, S.; He, C. Probiotic Supplementation for Prevention of Atopic Dermatitis in Infants and Children: A Systematic Review and Meta-analysis. *Am. J. Clin. Dermatol.* **2019**, *20*, 367–377. [CrossRef] [PubMed]
6. LeBlanc, J.G.; Chain, F.; Martín, R.; Bermúdez-Humarán, L.G.; Courau, S.; Langella, P. Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb. Cell Fact.* **2017**, *16*, 79. [CrossRef]
7. Kalinina, O.; Talley, S.; Zamora-Pineda, J.; Paik, W.; Campbell, E.M.; Knight, K.L. Amelioration of Graft-versus-Host Disease by Exopolysaccharide from a Commensal Bacterium. *J. Immunol.* **2021**, *206*, 2101–2108. [CrossRef]
8. Swartzendruber, J.A.; Incrocci, R.W.; Wolf, S.A.; Jung, A.; Knight, K.L. *Bacillus subtilis* exopolysaccharide prevents allergic eosinophilia. *Allergy* **2019**, *74*, 819–821. [CrossRef] [PubMed]
9. Jones, S.E.; Knight, K.L. Bacillus subtilis-mediated protection from Citrobacter rodentium-associated enteric disease requires espH and functional flagella. *Infect. Immun.* **2012**, *80*, 710–719. [CrossRef] [PubMed]
10. Paynich, M.L.; Jones-Burrage, S.E.; Knight, K.L. Exopolysaccharide from *Bacillus subtilis* Induces Anti-Inflammatory M2 Macrophages That Prevent T Cell-Mediated Disease. *J. Immunol.* **2017**, *198*, 2689–2698. [CrossRef]
11. Paik, W.; Alonzo, F., 3rd; Knight, K.L. Probiotic Exopolysaccharide Protects against Systemic *Staphylococcus aureus* Infection, Inducing Dual-Functioning Macrophages That Restrict Bacterial Growth and Limit Inflammation. *Infect. Immun.* **2019**, *87*, e00791-18. [CrossRef] [PubMed]
12. Jones, S.E.; Paynich, M.L.; Kearns, D.B.; Knight, K.L. Protection from intestinal inflammation by bacterial exopolysaccharides. *J. Immunol.* **2014**, *192*, 4813–4820. [CrossRef]
13. Paik, W.; Alonzo, F., 3rd; Knight, K.L. Suppression of *Staphylococcus aureus* Superantigen-Independent Interferon Gamma Response by a Probiotic Polysaccharide. *Infect. Immun* **2020**, *88*, e00661-19. [CrossRef] [PubMed]
14. Zhang, B.; Fang, L.; Wu, H.M.; Ding, P.S.; Xu, K.; Liu, R.Y. Mer receptor tyrosine kinase negatively regulates lipoteichoic acid-induced inflammatory response via PI3K/Akt and SOCS3. *Mol. Immunol.* **2016**, *76*, 98–107. [CrossRef] [PubMed]
15. Wang, Q.; McLoughlin, R.M.; Cobb, B.A.; Charrel-Dennis, M.; Zaleski, K.J.; Golenbock, D.; Tzianabos, A.O.; Kasper, D.L. A bacterial carbohydrate links innate and adaptive responses through Toll-like receptor 2. *J. Exp. Med.* **2006**, *203*, 2853–2863. [CrossRef]
16. Ochoa-Repáraz, J.; Mielcarz, D.W.; Ditrio, L.E.; Burroughs, A.R.; Begum-Haque, S.; Dasgupta, S.; Kasper, D.L.; Kasper, L.H. Central nervous system demyelinating disease protection by the human commensal *Bacteroides fragilis* depends on polysaccharide A expression. *J. Immunol.* **2010**, *185*, 4101–4108. [CrossRef] [PubMed]

17. Mazmanian, S.K.; Round, J.L.; Kasper, D.L. A microbial symbiosis factor prevents intestinal inflammatory disease. *Nature* **2008**, *453*, 620–625. [CrossRef] [PubMed]
18. Round, J.L.; Lee, S.M.; Li, J.; Tran, G.; Jabri, B.; Chatila, T.A.; Mazmanian, S.K. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* **2011**, *332*, 974–977. [CrossRef]
19. Ochoa-Repáraz, J.; Mielcarz, D.W.; Wang, Y.; Begum-Haque, S.; Dasgupta, S.; Kasper, D.L.; Kasper, L.H. A polysaccharide from the human commensal *Bacteroides fragilis* protects against CNS demyelinating disease. *Mucosal Immunol.* **2010**, *3*, 487–495. [CrossRef] [PubMed]
20. Stefan, K.L.; Kim, M.V.; Iwasaki, A.; Kasper, D.L. Commensal Microbiota Modulation of Natural Resistance to Virus Infection. *Cell* **2020**, *183*, 1312–1324.e10. [CrossRef]
21. An, D.; Oh, S.F.; Olszak, T.; Neves, J.F.; Avci, F.Y.; Erturk-Hasdemir, D.; Lu, X.; Zeissig, S.; Blumberg, R.S.; Kasper, D.L. Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. *Cell* **2014**, *156*, 123–133. [CrossRef]
22. Wieland Brown, L.C.; Penaranda, C.; Kashyap, P.C.; Williams, B.B.; Clardy, J.; Kronenberg, M.; Sonnenburg, J.L.; Comstock, L.E.; Bluestone, J.A.; Fischbach, M.A. Production of α-galactosylceramide by a prominent member of the human gut microbiota. *PLoS Biol.* **2013**, *11*, e1001610. [CrossRef] [PubMed]
23. Yu, R.; Zuo, F.; Ma, H.; Chen, S. Exopolysaccharide-Producing Bifidobacterium adolescentis Strains with Similar Adhesion Property Induce Differential Regulation of Inflammatory Immune Response in Treg/Th17 Axis of DSS-Colitis Mice. *Nutrients* **2019**, *11*, 782. [CrossRef] [PubMed]
24. Fanning, S.; Hall, L.J.; Cronin, M.; Zomer, A.; MacSharry, J.; Goulding, D.; Motherway, M.O.; Shanahan, F.; Nally, K.; Dougan, G.; et al. Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 2108–2113. [CrossRef]
25. Hickey, A.; Stamou, P.; Udayan, S.; Ramón-Vázquez, A.; Esteban-Torres, M.; Bottacini, F.; Woznicki, J.A.; Hughes, O.; Melgar, S.; Ventura, M.; et al. Bifidobacterium breve Exopolysaccharide Blocks Dendritic Cell Maturation and Activation of $CD4^+$ T Cells. *Front. Microbiol.* **2021**, *12*, 653587. [CrossRef]
26. Hughes, K.R.; Harnisch, L.C.; Alcon-Giner, C.; Mitra, S.; Wright, C.J.; Ketskemety, J.; van Sinderen, D.; Watson, A.J.; Hall, L.J. *Bifidobacterium breve* reduces apoptotic epithelial cell shedding in an exopolysaccharide and MyD88-dependent manner. *Open Biol.* **2017**, *7*, 160155. [CrossRef]
27. Luo, M.; Gan, M.; Yu, X.; Wu, X.; Xu, F. Study on the regulatory effects and mechanisms of action of bifidobacterial exopolysaccharides on anaphylaxes in mice. *Int. J. Biol. Macromol.* **2020**, *165*, 1447–1454. [CrossRef] [PubMed]
28. Ivanov, D.; Emonet, C.; Foata, F.; Affolter, M.; Delley, M.; Fisseha, M.; Blum-Sperisen, S.; Kochhar, S.; Arigoni, F. A serpin from the gut bacterium *Bifidobacterium longum* inhibits eukaryotic elastase-like serine proteases. *J. Biol. Chem.* **2006**, *281*, 17246–17252. [CrossRef] [PubMed]
29. McCarville, J.L.; Dong, J.; Caminero, A.; Bermudez-Brito, M.; Jury, J.; Murray, J.A.; Duboux, S.; Steinmann, M.; Delley, M.; Tangyu, M.; et al. A Commensal *Bifidobacterium longum* Strain Prevents Gluten-Related Immunopathology in Mice through Expression of a Serine Protease Inhibitor. *Appl. Environ. Microbiol.* **2017**, *83*, e01323-17. [CrossRef] [PubMed]
30. Schiavi, E.; Gleinser, M.; Molloy, E.; Groeger, D.; Frei, R.; Ferstl, R.; Rodriguez-Perez, N.; Ziegler, M.; Grant, R.; Moriarty, T.F.; et al. The Surface-Associated Exopolysaccharide of Bifidobacterium longum 35624 Plays an Essential Role in Dampening Host Proinflammatory Responses and Repressing Local TH17 Responses. *Appl. Environ. Microbiol.* **2016**, *82*, 7185–7196. [CrossRef]
31. Schiavi, E.; Plattner, S.; Rodriguez-Perez, N.; Barcik, W.; Frei, R.; Ferstl, R.; Kurnik-Lucka, M.; Groeger, D.; Grant, R.; Roper, J.; et al. Exopolysaccharide from *Bifidobacterium longum* subsp. longum 35624™ modulates murine allergic airway responses. *Benef. Microbes* **2018**, *9*, 761–773. [CrossRef] [PubMed]
32. Yan, S.; Yang, B.; Zhao, J.; Zhao, J.; Stanton, C.; Ross, R.P.; Zhang, H.; Chen, W. A ropy exopolysaccharide producing strain *Bifidobacterium longum* subsp. longum YS108R alleviates DSS-induced colitis by maintenance of the mucosal barrier and gut microbiota modulation. *Food Funct.* **2019**, *10*, 1595–1608. [CrossRef] [PubMed]
33. O'Connell Motherway, M.; Zomer, A.; Leahy, S.C.; Reunanen, J.; Bottacini, F.; Claesson, M.J.; O'Brien, F.; Flynn, K.; Casey, P.G.; Munoz, J.A.; et al. Functional genome analysis of *Bifidobacterium breve* UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 11217–11222. [CrossRef]
34. Vargas García, C.E.; Petrova, M.; Claes, I.J.; De Boeck, I.; Verhoeven, T.L.; Dilissen, E.; von Ossowski, I.; Palva, A.; Bullens, D.M.; Vanderleyden, J.; et al. Piliation of *Lactobacillus rhamnosus* GG promotes adhesion, phagocytosis, and cytokine modulation in macrophages. *Appl. Environ. Microbiol.* **2015**, *81*, 2050–2062. [CrossRef] [PubMed]
35. Turroni, F.; Serafini, F.; Foroni, E.; Duranti, S.; O'Connell Motherway, M.; Taverniti, V.; Mangifesta, M.; Milani, C.; Viappiani, A.; Roversi, T.; et al. Role of sortase-dependent pili of *Bifidobacterium bifidum* PRL2010 in modulating bacterium-host interactions. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 11151–11156. [CrossRef] [PubMed]
36. Schlee, M.; Wehkamp, J.; Altenhoefer, A.; Oelschlaeger, T.A.; Stange, E.F.; Fellermann, K. Induction of human beta-defensin 2 by the probiotic *Escherichia coli* Nissle 1917 is mediated through flagellin. *Infect. Immun.* **2007**, *75*, 2399–2407. [CrossRef]
37. Konstantinov, S.R.; Smidt, H.; de Vos, W.M.; Bruijns, S.C.; Singh, S.K.; Valence, F.; Molle, D.; Lortal, S.; Altermann, E.; Klaenhammer, T.R.; et al. S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 19474–19479. [CrossRef] [PubMed]

38. von Schillde, M.A.; Hörmannsperger, G.; Weiher, M.; Alpert, C.A.; Hahne, H.; Bäuerl, C.; van Huynegem, K.; Steidler, L.; Hrncir, T.; Pérez-Martínez, G.; et al. Lactocepin secreted by Lactobacillus exerts anti-inflammatory effects by selectively degrading proinflammatory chemokines. *Cell Host Microbe* **2012**, *11*, 387–396. [CrossRef] [PubMed]
39. Seth, A.; Yan, F.; Polk, D.B.; Rao, R.K. Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2008**, *294*, G1060–G1069. [CrossRef]
40. Yan, F.; Cao, H.; Cover, T.L.; Washington, M.K.; Shi, Y.; Liu, L.; Chaturvedi, R.; Peek, R.M., Jr.; Wilson, K.T.; Polk, D.B. Colon-specific delivery of a probiotic-derived soluble protein ameliorates intestinal inflammation in mice through an EGFR-dependent mechanism. *J. Clin. Investig.* **2011**, *121*, 2242–2253. [CrossRef]
41. Yan, F.; Cao, H.; Cover, T.L.; Whitehead, R.; Washington, M.K.; Polk, D.B. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* **2007**, *132*, 562–575. [CrossRef] [PubMed]
42. Yasuda, E.; Serata, M.; Sako, T. Suppressive effect on activation of macrophages by Lactobacillus casei strain Shirota genes determining the synthesis of cell wall-associated polysaccharides. *Appl. Environ. Microbiol.* **2008**, *74*, 4746–4755. [CrossRef] [PubMed]
43. Matsumoto, S.; Hara, T.; Hori, T.; Mitsuyama, K.; Nagaoka, M.; Tomiyasu, N.; Suzuki, A.; Sata, M. Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells. *Clin. Exp. Immunol.* **2005**, *140*, 417–426. [CrossRef]
44. Liu, Y.; Zheng, S.; Cui, J.; Guo, T.; Zhang, J.; Li, B. Alleviative Effects of Exopolysaccharide Produced by *Lactobacillus helveticus* KLDS1.8701 on Dextran Sulfate Sodium-Induced Colitis in Mice. *Microorganisms* **2021**, *9*, 2086. [CrossRef] [PubMed]
45. Vinderola, G.; Perdigón, G.; Duarte, J.; Farnworth, E.; Matar, C. Effects of the oral administration of the exopolysaccharide produced by *Lactobacillus kefiranofaciens* on the gut mucosal immunity. *Cytokine* **2006**, *36*, 254–260. [CrossRef]
46. Al-Hassi, H.O.; Mann, E.R.; Sanchez, B.; English, N.R.; Peake, S.T.; Landy, J.; Man, R.; Urdaci, M.; Hart, A.L.; Fernandez-Salazar, L.; et al. Altered human gut dendritic cell properties in ulcerative colitis are reversed by *Lactobacillus plantarum* extracellular encrypted peptide STp. *Mol. Nutr. Food Res.* **2014**, *58*, 1132–1143. [CrossRef] [PubMed]
47. Kim, Y.; Lee, Y.D.; Kim, M.; Kim, H.; Chung, D.K. Combination treatment with lipoteichoic acids isolated from Lactobacillus plantarum and *Staphylococcus aureus* alleviates atopic dermatitis via upregulation of CD55 and CD59. *Immunol. Lett.* **2019**, *214*, 23–29. [CrossRef] [PubMed]
48. Ahn, J.E.; Kim, H.; Chung, D.K. Lipoteichoic Acid Isolated from Lactobacillus plantarum Maintains Inflammatory Homeostasis through Regulation of Th1- and Th2-Induced Cytokines. *J. Microbiol. Biotechnol.* **2019**, *29*, 151–159. [CrossRef]
49. Jeon, B.; Kim, H.R.; Kim, H.; Chung, D.K. In vitro and in vivo downregulation of C3 by lipoteichoic acid isolated from *Lactobacillus plantarum* K8 suppressed cytokine-mediated complement system activation. *FEMS Microbiol. Lett.* **2016**, *363*, fnw140. [CrossRef] [PubMed]
50. Kwon, M.; Lee, J.; Park, S.; Kwon, O.H.; Seo, J.; Roh, S. Exopolysaccharide Isolated from *Lactobacillus plantarum* L-14 Has Anti-Inflammatory Effects via the Toll-Like Receptor 4 Pathway in LPS-Induced RAW 264.7 Cells. *Int. J. Mol. Sci.* **2020**, *21*, 9283. [CrossRef]
51. Murofushi, Y.; Villena, J.; Morie, K.; Kanmani, P.; Tohno, M.; Shimazu, T.; Aso, H.; Suda, Y.; Hashiguchi, K.; Saito, T.; et al. The toll-like receptor family protein RP105/MD1 complex is involved in the immunoregulatory effect of exopolysaccharides from *Lactobacillus plantarum* N14. *Mol. Immunol.* **2015**, *64*, 63–75. [CrossRef]
52. Lebeer, S.; Claes, I.; Tytgat, H.L.; Verhoeven, T.L.; Marien, E.; von Ossowski, I.; Reunanen, J.; Palva, A.; Vos, W.M.; Keersmaecker, S.C.; et al. Functional analysis of *Lactobacillus rhamnosus* GG pili in relation to adhesion and immunomodulatory interactions with intestinal epithelial cells. *Appl. Environ. Microbiol.* **2012**, *78*, 185–193. [CrossRef] [PubMed]
53. Claes, I.J.; Lebeer, S.; Shen, C.; Verhoeven, T.L.; Dilissen, E.; De Hertogh, G.; Bullens, D.M.; Ceuppens, J.L.; Van Assche, G.; Vermeire, S.; et al. Impact of lipoteichoic acid modification on the performance of the probiotic *Lactobacillus rhamnosus* GG in experimental colitis. *Clin. Exp. Immunol.* **2010**, *162*, 306–314. [CrossRef] [PubMed]
54. Harb, H.; van Tol, E.A.; Heine, H.; Braaksma, M.; Gross, G.; Overkamp, K.; Hennen, M.; Alrifai, M.; Conrad, M.L.; Renz, H.; et al. Neonatal supplementation of processed supernatant from *Lactobacillus rhamnosus* GG improves allergic airway inflammation in mice later in life. *Clin. Exp. Allergy* **2013**, *43*, 353–364. [CrossRef] [PubMed]
55. You, G.E.; Jung, B.J.; Kim, H.R.; Kim, H.G.; Kim, T.R.; Chung, D.K. *Lactobacillus sakei* lipoteichoic acid inhibits MMP-1 induced by UVA in normal dermal fibroblasts of human. *J. Microbiol. Biotechnol.* **2013**, *23*, 1357–1364. [CrossRef] [PubMed]
56. Macho Fernandez, E.; Valenti, V.; Rockel, C.; Hermann, C.; Pot, B.; Boneca, I.G.; Grangette, C. Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. *Gut* **2011**, *60*, 1050–1059. [CrossRef]
57. Kaji, R.; Kiyoshima-Shibata, J.; Nagaoka, M.; Nanno, M.; Shida, K. Bacterial teichoic acids reverse predominant IL-12 production induced by certain lactobacillus strains into predominant IL-10 production via TLR2-dependent ERK activation in macrophages. *J. Immunol.* **2010**, *184*, 3505–3513. [CrossRef]
58. Dinić, M.; Pecikoza, U.; Djokić, J.; Stepanović-Petrović, R.; Milenković, M.; Stevanović, M.; Filipović, N.; Begović, J.; Golić, N.; Lukić, J. Exopolysaccharide Produced by Probiotic Strain *Lactobacillus paraplantarum* BGCG11 Reduces Inflammatory Hyperalgesia in Rats. *Front. Pharmacol.* **2018**, *9*, 1. [CrossRef] [PubMed]
59. Zhou, X.; Qi, W.; Hong, T.; Xiong, T.; Gong, D.; Xie, M.; Nie, S. Exopolysaccharides from *Lactobacillus plantarum* NCU116 Regulate Intestinal Barrier Function via STAT3 Signaling Pathway. *J. Agric. Food Chem.* **2018**, *66*, 9719–9727. [CrossRef] [PubMed]

60. Li, J.; Li, Q.; Gao, N.; Wang, Z.; Li, F.; Li, J.; Shan, A. Exopolysaccharides produced by *Lactobacillus rhamnosus* GG alleviate hydrogen peroxide-induced intestinal oxidative damage and apoptosis through the Keap1/Nrf2 and Bax/Bcl-2 pathways in vitro. *Food Funct.* **2021**, *12*, 9632–9641. [CrossRef]
61. Riehl, T.E.; Alvarado, D.; Ee, X.; Zuckerman, A.; Foster, L.; Kapoor, V.; Thotala, D.; Ciorba, M.A.; Stenson, W.F. *Lactobacillus rhamnosus* GG protects the intestinal epithelium from radiation injury through release of lipoteichoic acid, macrophage activation and the migration of mesenchymal stem cells. *Gut* **2019**, *68*, 1003–1013. [CrossRef]
62. Nowak, B.; Śróttek, M.; Ciszek-Lenda, M.; Skałkowska, A.; Gamian, A.; Górska, S.; Marcinkiewicz, J. Exopolysaccharide from *Lactobacillus rhamnosus* KL37 Inhibits T Cell-dependent Immune Response in Mice. *Arch. Immunol. Ther. Exp.* **2020**, *68*, 17. [CrossRef] [PubMed]
63. Le Maréchal, C.; Peton, V.; Plé, C.; Vroland, C.; Jardin, J.; Briard-Bion, V.; Durant, G.; Chuat, V.; Loux, V.; Foligné, B.; et al. Surface proteins of *Propionibacterium freudenreichii* are involved in its anti-inflammatory properties. *J. Proteom.* **2015**, *113*, 447–461. [CrossRef]
64. Mazmanian, S.K.; Liu, C.H.; Tzianabos, A.O.; Kasper, D.L. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* **2005**, *122*, 107–118. [CrossRef] [PubMed]
65. Matsumoto, S.; Hara, T.; Nagaoka, M.; Mike, A.; Mitsuyama, K.; Sako, T.; Yamamoto, M.; Kado, S.; Takada, T. A component of polysaccharide peptidoglycan complex on Lactobacillus induced an improvement of murine model of inflammatory bowel disease and colitis-associated cancer. *Immunology* **2009**, *128*, e170–e180. [CrossRef] [PubMed]
66. Laiño, J.; Villena, J.; Kanmani, P.; Kitazawa, H. Immunoregulatory Effects Triggered by Lactic Acid Bacteria Exopolysaccharides: New Insights into Molecular Interactions with Host Cells. *Microorganisms* **2016**, *4*, 27. [CrossRef] [PubMed]
67. Divanovic, S.; Trompette, A.; Atabani, S.F.; Madan, R.; Golenbock, D.T.; Visintin, A.; Finberg, R.W.; Tarakhovsky, A.; Vogel, S.N.; Belkaid, Y.; et al. Inhibition of TLR-4/MD-2 signaling by RP105/MD-1. *J. Endotoxin Res.* **2005**, *11*, 363–368. [CrossRef] [PubMed]
68. Kearns, D.B.; Chu, F.; Branda, S.S.; Kolter, R.; Losick, R. A master regulator for biofilm formation by *Bacillus subtilis*. *Mol. Microbiol.* **2005**, *55*, 739–749. [CrossRef] [PubMed]
69. Flannagan, R.S.; Heit, B.; Heinrichs, D.E. Antimicrobial Mechanisms of Macrophages and the Immune Evasion Strategies of *Staphylococcus aureus*. *Pathogens* **2015**, *4*, 826–868. [CrossRef]
70. Strachan, D.P. Hay fever, hygiene, and household size. *BMJ* **1989**, *299*, 1259–1260. [CrossRef]
71. Stein, M.M.; Hrusch, C.L.; Gozdz, J.; Igartua, C.; Pivniouk, V.; Murray, S.E.; Ledford, J.G.; Marques Dos Santos, M.; Anderson, R.L.; Metwali, N.; et al. Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children. *N. Engl. J. Med.* **2016**, *375*, 411–421. [CrossRef]
72. Ober, C.; Sperling, A.I.; von Mutius, E.; Vercelli, D. Immune development and environment: Lessons from Amish and Hutterite children. *Curr. Opin. Immunol.* **2017**, *48*, 51–60. [CrossRef] [PubMed]
73. Okudaira, H.; Nogami, M.; Matsuzaki, G.; Dohi, M.; Suko, M.; Kasuya, S.; Takatsu, K. T-cell-dependent accumulation of eosinophils in the lung and its inhibition by monoclonal anti-interleukin-5. *Int. Arch. Allergy Appl. Immunol.* **1991**, *94*, 171–173. [CrossRef]
74. Biswas, S.K.; Lopez-Collazo, E. Endotoxin tolerance: New mechanisms, molecules and clinical significance. *Trends Immunol.* **2009**, *30*, 475–487. [CrossRef] [PubMed]
75. Seeley, J.J.; Ghosh, S. Molecular mechanisms of innate memory and tolerance to LPS. *J. Leukoc. Biol.* **2017**, *101*, 107–119. [CrossRef] [PubMed]
76. Horvatinovich, J.M.; Grogan, E.W.; Norris, M.; Steinkasserer, A.; Lemos, H.; Mellor, A.L.; Tcherepanova, I.Y.; Nicolette, C.A.; DeBenedette, M.A. Soluble CD83 Inhibits T Cell Activation by Binding to the TLR4/MD-2 Complex on $CD14^+$ Monocytes. *J. Immunol.* **2017**, *198*, 2286–2301. [CrossRef]
77. Gringhuis, S.I.; den Dunnen, J.; Litjens, M.; van Het Hof, B.; van Kooyk, Y.; Geijtenbeek, T.B. C-type lectin DC-SIGN modulates Toll-like receptor signaling via Raf-1 kinase-dependent acetylation of transcription factor NF-kappaB. *Immunity* **2007**, *26*, 605–616. [CrossRef]
78. Yao, X.; Dong, G.; Zhu, Y.; Yan, F.; Zhang, H.; Ma, Q.; Fu, X.; Li, X.; Zhang, Q.; Zhang, J.; et al. Leukadherin-1-Mediated Activation of CD11b Inhibits LPS-Induced Pro-inflammatory Response in Macrophages and Protects Mice Against Endotoxic Shock by Blocking LPS-TLR4 Interaction. *Front. Immunol.* **2019**, *10*, 215. [CrossRef]
79. Li, Y.; Komai-Koma, M.; Gilchrist, D.S.; Hsu, D.K.; Liu, F.T.; Springall, T.; Xu, D. Galectin-3 is a negative regulator of lipopolysaccharide-mediated inflammation. *J. Immunol.* **2008**, *181*, 2781–2789. [CrossRef]
80. Terness, P.; Bauer, T.M.; Röse, L.; Dufter, C.; Watzlik, A.; Simon, H.; Opelz, G. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: Mediation of suppression by tryptophan metabolites. *J. Exp. Med.* **2002**, *196*, 447–457. [CrossRef]
81. Bressler, B.; Marshall, J.K.; Bernstein, C.N.; Bitton, A.; Jones, J.; Leontiadis, G.I.; Panaccione, R.; Steinhart, A.H.; Tse, F.; Feagan, B. Clinical practice guidelines for the medical management of nonhospitalized ulcerative colitis: The Toronto consensus. *Gastroenterology* **2015**, *148*, 1035–1058.e3. [CrossRef] [PubMed]