Immune Modulatory Effects of Probiotic Streptococcus thermophilus on Human Monocytes

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Immune Modulatory Effects of Probiotic *Streptococcus thermophilus* on Human Monocytes

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Abstract: Ingesting probiotics contributes to the development of a healthy microflora in the GIT with established benefits to human health. Some of these beneficial effects may be through the modulation of the immune system. In addition, probiotics have become more common in the treatment of many inflammatory and immune disorders. Here, we demonstrate a range of immune modulating effects of *Streptococcus thermophilus* by human monocytes, including decreased mRNA expression of IL-1R, IL-18, IFNαR1, IFNγR1, CCL2, CCR5, TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, TLR-8, CD14, CD86, CD4, ITGAM, LYZ, TYK2, IFNR1, IRAK-1, NOD2, MYD88, SLC11A1, and increased expression of IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-23, IFNγ, TNFα, CSF-2. The routine administration of *Streptococcus thermophilus* in fermented dairy products and their consumption may be beneficial to the treatment/management of inflammatory and autoimmune diseases.

Keywords: probiotics; microbiome; lactic acid bacteria; *Streptococcus thermophilus*; peripheral blood mononuclear cells; monocyte; RNA; innate immune response; adaptive immune response; inflammation

Citation: Dargahi, N.; Johnson, J.C.; Apostolopoulos, V. Immune Modulatory Effects of Probiotic *Streptococcus thermophilus* on Human Monocytes. *Biologics* **2021**, *1*, 396–415. https://doi.org10.3390/biologics1030023

Academic Editor: Alex Loukas

Received: 9 September 2021
Accepted: 4 November 2021
Published: 19 November 2021

1. Introduction

The human body and, in particular, the gastrointestinal tract (GIT) hosts a variety of microbial populations collectively referred to as the microbiome [1]. The GIT microbiome plays a fundamental role in the maintenance of a healthy immune system [1,2], and any disruption to the microbiome can lead to serious ill health effects [3,4]. In order to maintain a healthy microbiome, regular ingestion of probiotic supplements as capsules or in fermented dairy products has been suggested. These practices have led to various improved health outcomes and treatment of ill health such as infections, constipation, and diarrhea [1,5,6].

The majority of probiotics belong to the lactic acid bacteria (LAB) family, Gram positive lactic acid producing microorganisms that include several genera such as bifidobacteria, lactobacilli streptococci, and enterococci [1]. The small and large intestines are highly populated with these microorganisms [7–9] and are routinely supplemented in foods as live strains due to their established beneficial effects to human health [1,2,9–14]. *Streptococcus* species such as exopolysaccharide-producing strains of *Streptococcus thermophilus* (ST) [11,15,16] are amongst those consumed. ST is used for fermentation of milk products and is recognized as an important species for its health benefits [17,18]. In fact, ST and *L. brevis* synergistically display health benefits which are well established. Moreover, ST is one of the bacteria in the VSL#3 probiotic mixture, which has been applied for the treatment of inflammatory conditions [19,20]. Furthermore, probiotics interact with the immune system where they exhibit immunomodulatory and anti-inflammatory effects [3,21,22].

The use of probiotic bacteria can increase the abundance of and concurrently modulate immune cells including B, T helper (Th)-1, Th-2, Th-17, and regulatory T (Treg) cells. In turn, this directly influences human health and modulates pathologies of immune/autoimmune

diseases [12]. In fact, primary macrophages co-cultured with ST bacteria have been shown to increase the production of anti-inflammatory Interleukin (IL)-10 and pro-inflammatory IL-12 cytokines [23]. ST1275 and *Bifidobacterium* (*B.*) *longum* BL536 induce the expression of high levels of transforming growth factor (TGF)-beta, a key factor in the differentiation of Treg and Th-17 cells by bulk peripheral blood mononuclear cell (PBMC) cultures [24]. Probiotic bacteria, however, can only confer these benefits through interaction with specific immune cells, primarily antigen presenting cells (APC), which include monocytes as mediators between bacteria/foreign agents and the immune system's effector adaptive immune cells [25].

In line with these findings, we previously noted that ST1342, ST1275, and ST285 modulated the U937 monocyte cell line by increasing IL-4, IL-10, GM-CSF, and CXCL8 production. In addition, the cell surface marker expression of CD11c, CD86, C206, CD209, and MHC-1 was upregulated, suggesting that ST bacteria have an influence on the immune system [1]. Furthermore, we recently showed that ST285 exerted an array of anti-inflammatory immune-modulatory properties to human PBMC [26]. In particular, ST285 has decreased the mRNA expression of IL-18, IFNγR1, CCR5, CXCL10, TLR-1, TLR-2, TLR-4, TLR-8, CD14, CD40, CD86, C3, GATA3, ITGAM, IRF7, NLP3, LYZ, TYK2, IFNR1, and upregulated IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-23, IFNγ, TNFα, CSF-2 [26]. The data demonstrated a predominant anti-inflammatory profile exhibited by ST285. Similarly, we showed that the exposure of ST285 to spleen cells of immunized mice with the agonist MBP_{83-99} peptide induced a substantial increase in the production of anti-inflammatory IL-4, IL-5, and IL-10 cytokines, and also reduced the expression of IL-1β and IFN-γ pro-inflammatory cytokines [27]. Due to the role of monocytes and their progeny in initiation as well as the maintenance of both innate and adaptive immune responses, we show at the present time the immune modulatory properties of ST285 on monocytes from healthy blood donors. The data pave the way for further work to determine the effects of ST285 in inflammatory disease models in vitro and in vivo such as multiple sclerosis, inflammatory bowel disease, and allergies.

2. Results

Among the 84 genes evaluated (Table S1), the expression of 30 genes was significantly altered by over 2-fold up or down regulations in monocyte samples (n = 3), following co-culture with ST285 compared to control monocytes (Figures 1 and S1). Similarly, the evaluation of secreted cytokine/chemokines IL-1β, IL-2, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, and TNF-α showed significant changes.

2.1. ST285 Alters Cytokine Gene Expression Levels of Monocytes

2.1.1. ST285 Causes Upregulation of IL-1α, IL-1β, IL-6, and IL-23 and Downregulation of IL-1R1

IL-1α was upregulated 4.66 ± 0.7 fold and IL-1β was upregulated 9.83 ± 0.49 fold. Moreover, IL-6 was upregulated 42.23 ± 0.32 fold and IL-23α was upregulated 3.8 ± 1.0 fold (Figure 2). However, IL-1R1 was downregulated 2.11 ± 0.36 fold (Figure 2), and neither IL-17A nor IL-2 or IL-10 were altered following the monocyte co-culture with ST285. Furthermore, cytokine production of IL-1β, IL-2, IL-6, and IL-10, respectively showed significant changes (Figure 3), and the monocyte samples showed a significant increase in secretion of IL-1β ($p < 0.01$), IL-2 ($p < 0.001$), IL-6 ($p < 0.001$), and IL-10 ($p < 0.05$), following co-culture with ST285 for 24 h.

A

Layout	01	02	03	04	05	06	07	08	09	10	11	12
A	APCS 1.05 C	C3 −1.67	CASP1 1.37	CCL2 −24.33	CCL5 1.72	CCR4 1.08 B	CCR5 −11.54	CCR6 1.25 B	CCR8 −1.09 B	CD14 −34.08	CD4 −7.14 B	CD40 1.08
B	CD40LG −1.01 B	CD80 −1.79	CD86 −10.16	CD8A −3.14 B	CRP 1.05 C	CSF2 63.82 A	CXCL10 1.09	CXCR3 −1.45 B	DDX58 −1.74	FASLG −1.30 A	FOXP3 −2.21 A	GATA3 −1.23 B
C	HLA-A −1.70	HLA-E −1.35	ICAM1 −1.17	IFNA1 −1.59 B	IFNAR1 −2.53	IFNB1 −1.14 B	IFNG 29.33 A	IFNGR1 −5.65	IL10 −1.12	IL13 −1.05 B	IL17A 1.05 C	IL18 −7.63 A
D	IL1A 4.66	IL1B 9.83	IL1R1 −2.11	IL2 −1.35 B	IL23A 11.79	IL4 1.33 B	IL5 1.10 B	IL6 45.23	CXCL8 9.18	IRAK1 −2.27	IRF3 −1.23	IRF7 −1.14
E	ITGAM −3.60 A	JAK2 −1.57	LY96 −1.81	LYZ −25.78	MAPK1 −1.68	MAPK8 −1.18	MBL2 1.05 C	MPO −3.71 B	MX1 −1.46	MYD88 −2.98	NFKB1 1.41	NFKBIA −1.23
F	NLRP3 −1.38	NOD1 −1.51 A	NOD2 −2.35	RAG1 1.01 B	RORC −1.29 B	SLC11A1 −4.70	STAT1 1.23	STAT3 −1.35	STAT4 1.14	STAT6 −1.31	TBX21 1.79	TICAM1 1.12
G	TLR −3.62	TLR2 −3.05	TLR3 −1.74 B	TLR4 −3.96	TLR5 −2.45 A	TLR6 −2.13 A	TLR7 −1.40 B	TLR8 −2.51	TLR9 −1.36 B	TNF 8.99	TRAF6 −1.42	TYK2 −2.19

B

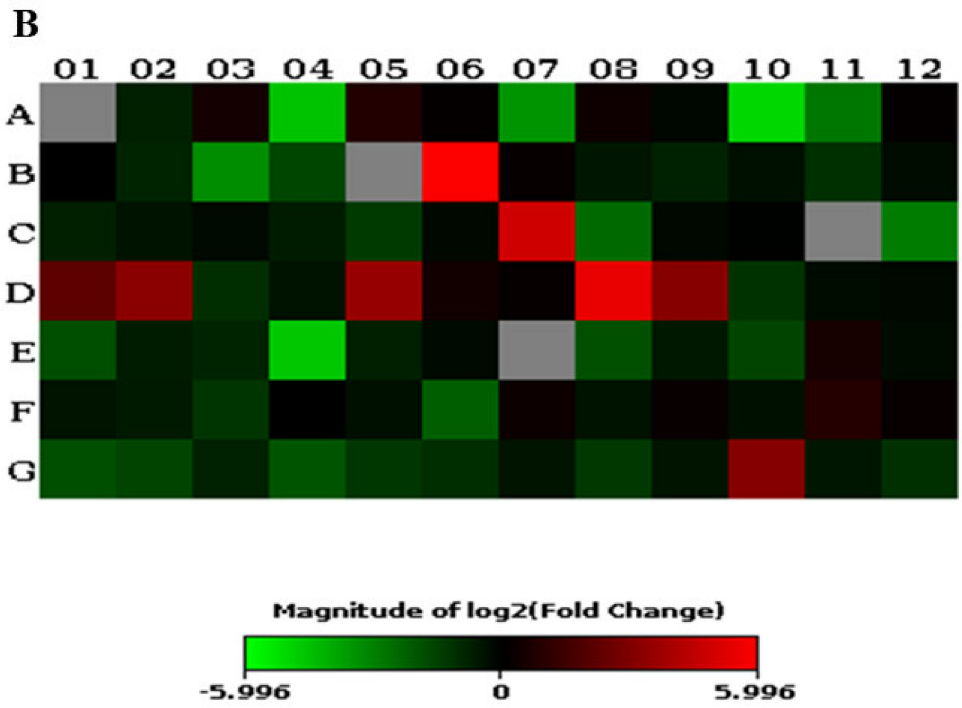

Figure 1. Effects of co-culturing ST285 with monocytes (n = 3) on gene/RNA expression compared to control monocytes after 24 h. (**A**) All of the 84 genes are shown including those with significant high up/down regulated genes (more than 2-fold) and those with no significant changes (less than 2-fold). The housekeeping genes (HKG) panel and other genes used for normalization of the raw data are not presented. HKG includes Hs.520640, Hs.534255, Hs.592355, Hs.412707, and Hs.546285, with respective symbols as ACTB, B2M, GAPDH, HPRT1, and RPLP0. In the case of no letter or comments, the expression of gene/s is relatively high in both the test and control group (threshold cycle (CT) is <30). Letter A specifies the gene's average threshold cycle to be reasonably high (>30) in the treated samples or controls and relatively low (<30) in the other/opposite samples. Therefore, in the case of presenting fold-changes with letter A, the estimated fold-change may be an underestimate. Letter B suggests a reasonably high (>30) gene average threshold cycle, that means a low level of average expression

of relevant gene in both the test/treated samples and untreated control samples, and the *p*-value for the fold-change might be relatively high ($p > 0.05$). Therefore, in the case of presenting fold-changes with letter B, the estimated fold-change may be slightly overestimated or unavailable. Letter C indicates that the gene's average threshold cycle is not determined or greater than the defined default 35 cut-off value in both the test/treated samples and control samples, suggesting that its expression was not detectable, resulting in the fold-change values being un-interpretable [28–30]. (**B**) Presentation of data as a heatmap of average gene/RNA expressions of monocytes ($n = 3$) co-cultured with ST285, compared to control monocytes. Green represents downregulated genes and red represents upregulated genes.

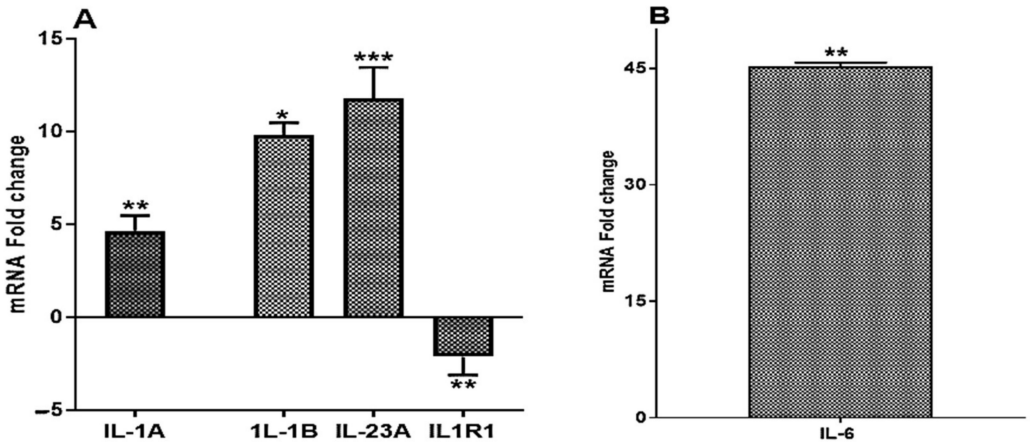

Figure 2. Effects of co-culturing ST285 with monocytes ($n = 3$) on gene/RNA expression compared to control monocytes after 24 h. (**A**) IL-1α, IL-1β, IL-23α, IL-1R1, and (**B**) IL-6 mRNA fold-change following a 24 h co-culture of ST285 with monocytes ($n = 3$), compared to control monocytes. The innate and adaptive RT^2 gene profiler arrays were used to determine changes in gene expression. The symbols represent the *p*-value for Tukey's test (one-way ANOVA) using the Statistical Package for the Social Sciences for Windows 25.0 (SPSS; Armonk, NY, USA: IBM Corp.), where *$p < 0.05$, ** $p < 0.04$ and *** $p < 0.02$.

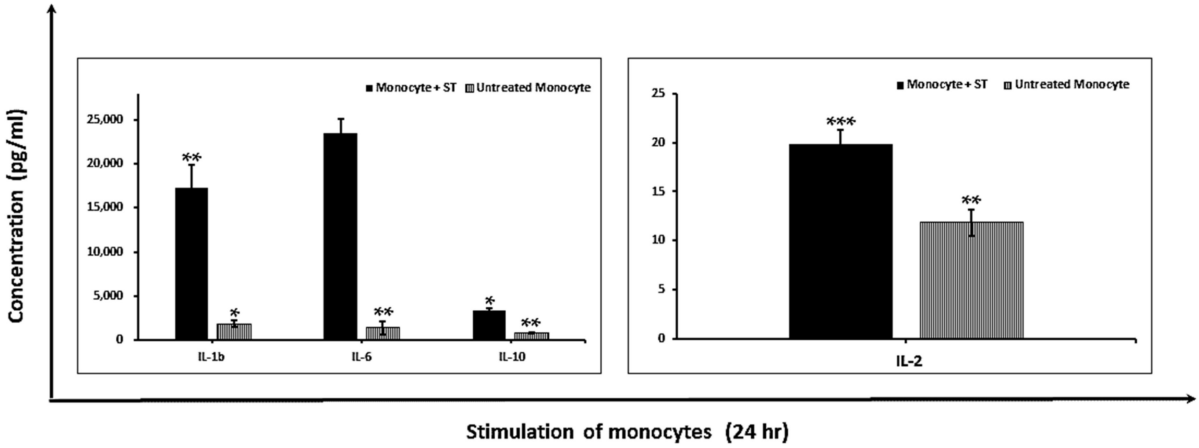

Figure 3. ST285 increases anti-inflammatory cytokine production by human monocytes. Human monocytes ($n = 3$) were stimulated with ST285 for 24 h and secretion of IL-1β, IL-2, IL-6, and IL-10 were measured. Treated human monocytes ($n = 3$) with ST285 probiotic bacteria are referred to as monocyte + ST, and human monocytes ($n = 3$) cultured in media without any ST285 probiotic bacteria are referred to as untreated monocytes. The means of two different readings of three replicate experiments were measured and analyzed. The means of readings for $n = 3$ monocytes were calculated and presented as plus or minus (±) the standard error of the mean. The symbols represent the *p*-value for Tukey's test (one-way ANOVA) where * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

2.1.2. Modulation of Pro-Inflammatory Cytokines

S. thermophilus (ST) ST285 induced the upregulation of IFNγ (29.33 ± 0.26 fold) (Figure 4). In addition, IL-18, a Th1 inducing pro-inflammatory cytokine was downregulated (7.63 ± 0.37 fold) (Figure 4). Moreover, IFNγR1, a transmembrane protein which interacts with IFNγ was downregulated 5.65 ± 0.05 fold. Furthermore, IFNAR1, which is involved in the defense against viruses was downregulated 2.53 ± 0.05 fold (Figure 4). Tumor-necrosis factor-alpha (TNFα), which is important in the defense against bacterial infections and in acute phase reactions was upregulated 8.99 ± 1.06 fold (Figure 4). Gene expressions of IFNA1, IFNB1, IL-4, IL-5, IL-12, and IL-13 cytokines were not significantly altered. Correspondingly, the ST285 probiotic induced a significant increase in IL-4 production ($p < 0.001$) as well as a significant increase in secretion of IFN-γ ($p < 0.001$) and TNFα ($p < 0.001$) by monocytes following exposure to ST285, compared to control monocytes (Figure 5).

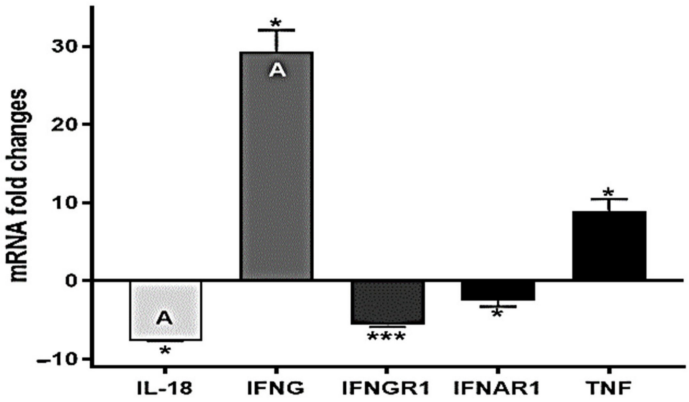

Figure 4. IL-18, IFNγ, IFNαR1, IFNγR1, and TNF mRNA fold-change following a 24 h co-culture of ST285 with monocytes ($n = 3$), compared to control monocytes. The innate and adaptive RT^2 gene profiler arrays were used to determine changes in gene expression. The symbols represent the p-value for Tukey's test (one-way ANOVA) where * $p < 0.05$ and *** $p < 0.02$.

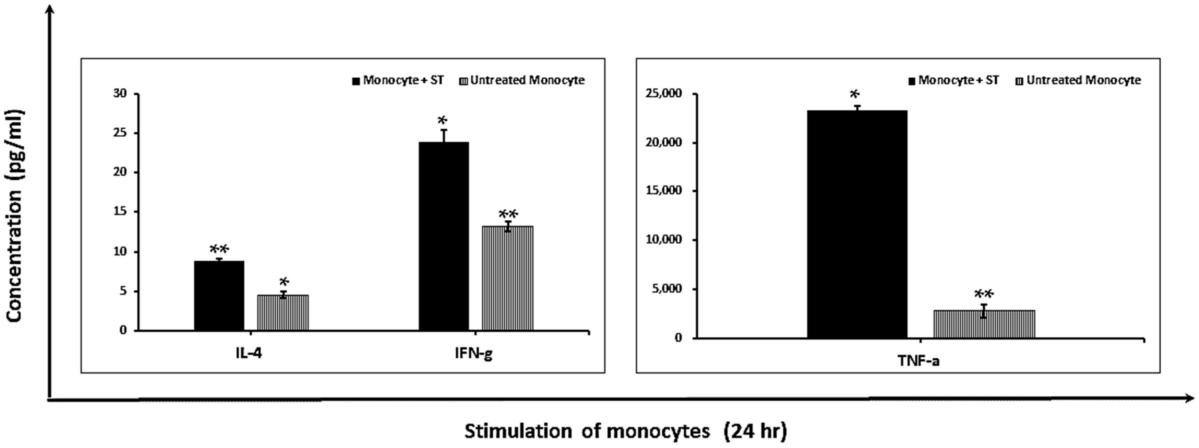

Figure 5. ST285 increases anti-inflammatory cytokine production by human monocytes. Human monocytes ($n = 3$) were stimulated with ST285 for 24 h and secretion of IL-4, IFN-γ, and TNF-α were measured. The treated human monocytes ($n = 3$) with ST285 probiotic bacteria are referred to as monocyte + ST and human monocytes ($n = 3$) cultured in media without any ST285 probiotic bacteria are referred to as untreated monocytes. The means of two different readings of three replicate experiments were measured and analyzed. The means of readings for $n = 3$ monocytes were calculated and presented as plus or minus (±) the standard error of the mean. The symbols represent the p-value for Tukey's test (one-way ANOVA) where * $p < 0.05$ and ** $p < 0.01$.

2.2. ST285 Alters Chemokine Gene Expression Levels of Monocytes

CCR5, which is a Th1 marker involved in immune response and CCL2 (MCP-1), which is involved in humoral immunity were downregulated 11.54 ± 0.23 and 24.33 ± 1.44 fold, respectively (Figure 6). Chemokine (CXCL8, IL-8), which is important in the innate immune system and stimulates chemotaxis was upregulated 9.18 ± 0.26 fold, following the ST285 co-culture with monocyte cells (Figure 6). However, no significant differences were noted for gene expressions of other chemokines including CXCL10 (INP10), CCL5 (RANTES), CCL8, CCR4, CCR8, and CXCR3, following the monocytes' exposure to ST285.

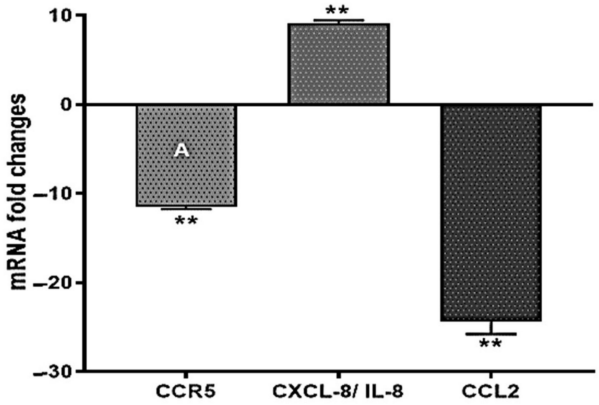

Figure 6. CCR5, CXCL8 (IL-8), and CCL2 mRNA fold-change following a 24 h co-culture of ST285 with monocytes (n = 3), compared to control monocytes. The innate and adaptive RT^2 gene profiler arrays were used to determine changes in gene expression. The symbol represents the p-value for Tukey's test (one-way ANOVA) where ** $p < 0.04$.

2.3. Significant Upregulation of Colony Stimulating Factor mRNA Expression Levels

Colony-stimulating factor (CSF)-2, which enables cell proliferation and differentiation of cells, was significantly increased by 63.82 ± 1.12 fold (Figure 7) after co-culturing monocytes with ST285 bacteria. In turn, secretion of the granulocyte-macrophage colony-stimulating factor (GM-CSF) showed a significant reduction following exposure of monocytes to the ST285 probiotic ($p < 0.05$) (Figure 8).

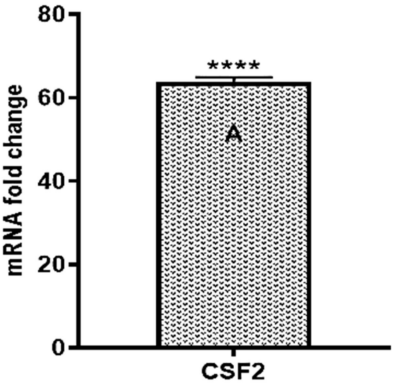

Figure 7. CSF-2 mRNA fold-change following a 24 h co-culture of ST285 with monocytes (n = 3), compared to control monocytes. The innate and adaptive RT^2 gene profiler arrays were used to determine changes in gene expression. The symbol represents the p-value for Tukey's test (one-way ANOVA) where **** $p < 0.01$.

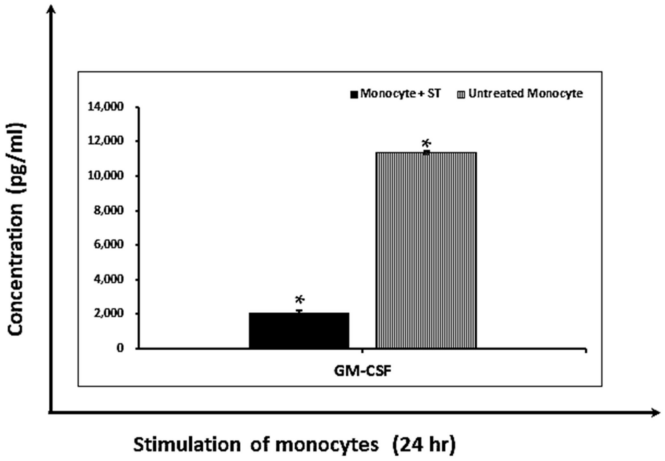

Figure 8. ST285 increases anti-inflammatory cytokine production by human monocytes. Human monocytes (n = 3) were stimulated with ST285 for 24 h and secretion of GMCSF was measured. The treated human monocytes (n = 3) with ST285 probiotic bacteria are referred to as monocyte + ST and human monocytes (n = 3) cultured in media without any ST285 probiotic bacteria are referred to as untreated monocytes. The means of two different readings of three replicate experiments were measured and analyzed. The means of readings for n = 3 monocytes were calculated and presented as plus or minus (±) the standard error of the mean. The symbols represent the p-value for Tukey's test (one-way ANOVA) where * $p < 0.05$.

2.4. ST285 Alters Toll-Like Receptor Gene Expression Levels of Monocytes

TLR (toll-like receptor)-1, TLR-2, TLR-4, TLR-5, TLR-6, and TLR-8 are part of the innate immune response and are involved in the defense response to bacteria. Monocytes co-cultured with ST285 induced a significant differential downregulation of TLRs, TLR-1 (-3.63 ± 0.14), TLR-2 (-3.05 ± 0.36 fold), TLR-4 (-3.96 ± 0.16 fold), TLR-5 (-2.45 ± 0.23 fold), TLR-6 (-2.13 ± 0.23 fold), and TLR-8 (-2.51 ± 0.12 fold) (Figure 9). However, changes to TLR-3 and TLR-9 were not significant.

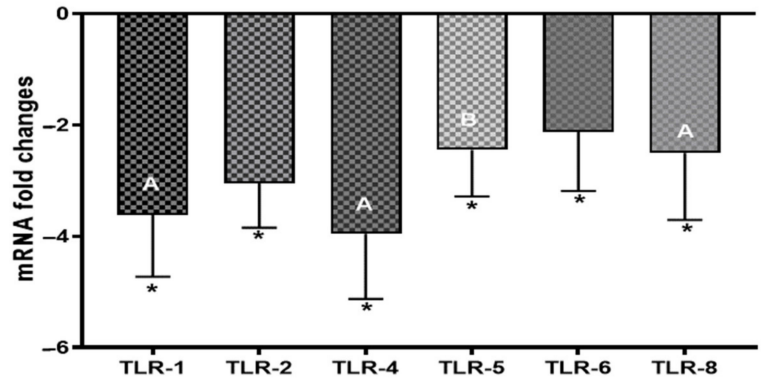

Figure 9. TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, and TLR-8 mRNA fold-change following a 24 h co-culture of ST285 with monocytes (n = 3), compared to control monocytes. The innate and adaptive RT^2 gene profiler arrays were used to determine changes in gene expression. The symbol represents the p-value for Tukey's test (one-way ANOVA) where * $p < 0.05$.

2.5. Cell Surface Markers CD14, CD86, and CD4 mRNA Expression Levels

Expression of the monocyte cell surface markers CD14 and CD86 was significantly downregulated 34.08 ± 3.42 and 10.16 ± 0.14 fold, respectively (Figure 10). CD4 is expressed by Th cells, monocytes, and macrophages (MQs), while dendritic cells (DCs) were downregulated 7.14 ± 0.41 fold. No significant changes were observed in the expression

of CD8A, CD40, CD80, GATA3, FOXP3, STAT3, CD40LG (TNFSF5), HLA-A, HLA-E, and RORC genes.

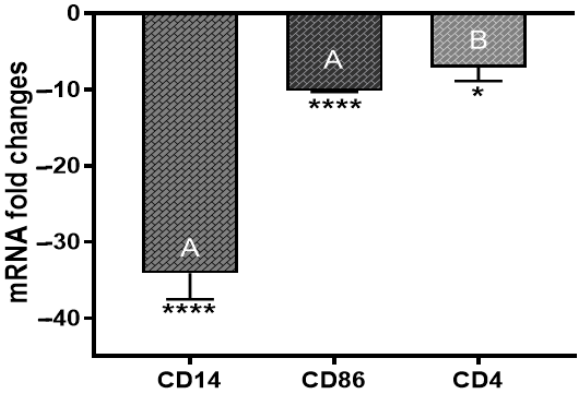

Figure 10. CD14, CD86, and CD4 mRNA fold-change following a 24 h co-culture of ST285 with monocytes (n = 3), compared to control monocytes. The innate and adaptive RT^2 gene profiler arrays were used to determine changes in gene expression. The symbols represent the p-value for Tukey's test (one-way ANOVA) where * $p < 0.05$ and **** $p < 0.02$.

2.6. Changes to Other Innate and Adaptive Molecules, mRNA Expression Levels

Altered levels of gene expression are noted in other genes following ST285 co-culture with monocytes. A significant downregulation of the following genes was noted: TYK2 (-2.19 ± 0.37), IRAK-1 (-2.27 ± 0.45), NOD2 (-2.35 ± 0.04), MYD88 (-2.98 ± 0.23), ITGAM (-3.6 ± 0.23), MPO (3.71 ± 0.12), SLC11A1 (-4.7 ± 0.17) (Figure 11A), and LYZ (25.78 ± 0.36) (Figure 11B). Other immune markers including FASLG (TNFSF6), ACTB, GATA3, complement component (C)-3, CRP, IFNAR1, JAK2, IL-1R1, MAPK8 (JNK1), IRF3, MBL2, NLRP3, NFKB1, MX1, ICAM1, MBL2, NOD1 (CARD4), DDX58 (RIG-I), RAG1, TICAM1 (TRIF), and IRF7 showed no significant mRNA gene changes in the levels of their expression.

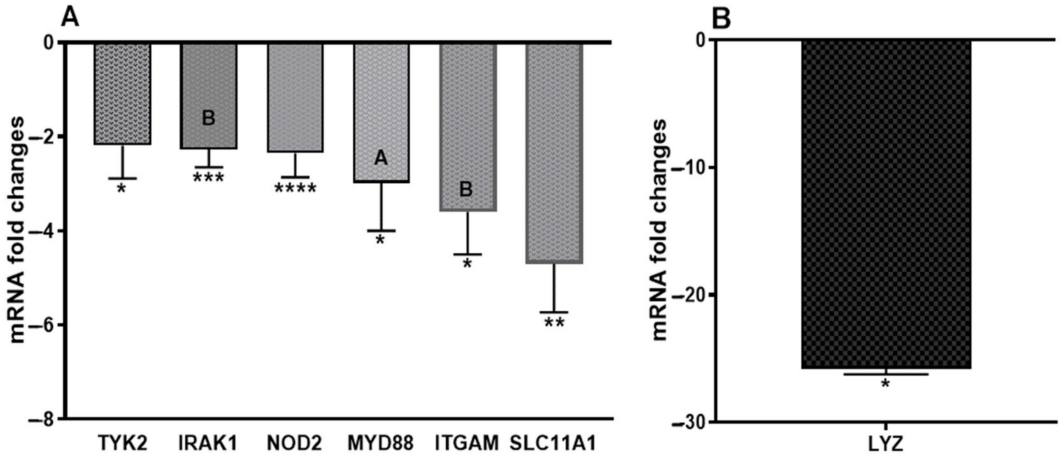

Figure 11. (**A**) TYK2, IRAK1, NOD2, MYD88, ITGAM, SLC11A1, and (**B**) LYZ and GATA3 mRNA fold-change following a 24 h co-culture of ST285 with monocytes (n = 3), compared to control monocytes. The innate and adaptive RT^2 gene profiler arrays were used to determine changes in gene expression. The symbols represent the p-value for Tukey's test (one-way ANOVA) where * $p < 0.05$, ** $p < 0.04$, *** $p < 0.02$, and **** $p < 0.01$.

3. Discussion

ST285 co-cultured with human monocytes resulted in significant changes to 30 genes associated with different immune responses of the innate and adaptive immunity, compared to control monocytes. In particular, mRNA gene expression of IL-1R, IL-18, IFNαR1, IFNγR1, CCL2, CCR5, TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, TLR-8, CD14, CD86, CD4,

ITGAM, LYZ, TYK2, IFNR1, IRAK-1, NOD2, MYD88, ITGAM, and SLC11A1 are downregulated. While ST285 increases the mRNA expression of IL-1α, IL-1β, IL-1αR, IL-6, IL-8, IL-23, IFNγ, TNFα, and CSF-2. Moreover, IL-1β, IL-2, IL-4, IL-6, IL-10, IFNγ, TNFα, and GMCSF produced by monocytes were altered following co-culture with ST285 probiotics. These results were broadly in agreement with our previous findings showing a predominant anti-inflammatory profile by human PBMCs upon co-culture with ST285 [26]. Likewise, our previous data showed a similar trend for a number of cytokines, chemokines, and cell surface markers for three different ST bacteria to the human U937 monocyte cell line, where ST285 was most effective [1].

3.1. ST285 Induces IL-1β and IL-6 and Downregulates IL-1R1

IL-1β secreted by DCs and MQs, usually initiates Th2 differentiation, while preventing polarization of Th1 cells [31]. IL-1β is secreted by monocytes and activated by MQs. In addition, it is involved in regulating immune and inflammatory responses in injuries and bacterial infections [32]. IL-6 is produced by activated immune cells including monocytes/MQs [33]. IL-1β and IL-6 are significantly upregulated, whereas IL-1R1 (CD121a), a key mediator associated with several inflammatory and immune responses is downregulated in monocytes after exposure to ST285. Similarly, a significant increase was noted in the secretion of bothIL-1β and IL-6 by monocytes in the presence of ST285. This is in accord with PBMCs co-cultured with ST285 [26] and the U937 monocyte cell line co-cultured with ST285 [2]. Co-culturing mouse spleen cells (mouse immunized with agonist MBP_{83-99} peptide) with ST285 led to the reduction of IL-1β expression [26]. The decreased expression of IL-1R1 gene may highlight the role of ST285 as a brake that controls the pro-inflammatory roles of both IL-6 and IL-1β.

IL-2 has been considered to play an important role mutually in the death and growth of T-cells such as mice with IL-2 and/or IL-2-receptor deficiency, and to develop fatal autoimmune disorders. Moreover, IL-2 is thought to be a key element for the maintenance of self-tolerance. A significant increase in the secretion of IL-2 was noted in the supernatant from the monocyte co-cultured with ST285. Although we previously showed that the IL-2 increased secretion by mouse splenocytes was caused by the MBP_{83-99} peptide in the positive control, it was marginally decreased to mouse splenocytes in the presence of ST285 [27]. Furthermore, co-culturing human PBMCs with ST285 downregulates the IL-2 mRNA expression [26].

Anti-inflammatory IL-10 cytokine is predominantly secreted by monocytes and is the most effective anti-inflammatory mediator that suppresses inflammatory immune factors including IFN-γ, TNF-α, IL-17, IL-1, and IL-23 by other immune cells [27]. Moreover, the monocyte samples showed increased secretion of IL-10 following co-culture with ST285. IL-10 indicates that ST285 could induce anti-inflammatory effects to monocytes. Although no significant changes were shown in the level of RNA expression of IL-10 in human monocytes following exposure to ST285, a significant increase in the level of IL-10 secreted by human monocytes was shown. In addition, we previously showed a significant increase in the IL-10 secretion levels by mice spleen cells when exposed to ST285. This was correspondingly noted in the U937 monocytic cell line upon co-culture with ST285 [1], as well as human PBMCs cultured in the presence of ST285 [26]. IL-6 acts as both pro- and anti-inflammatory cytokine [34]. In addition, its anti-inflammatory roles are associated with its inhibitory effects on IL-1 and TNF-α and activation of IL-10 and IL-1Ra [34,35]. On the other hand, the inhibitor of NF-κB kinase (IKK) governs the IL-6 mRNA stability (through phosphorylation of regnase-1), in response to IL-1R/TLR stimulation [36]. Therefore, *Lactobacillus paracasei* has been shown to reduce IL-6 production via prevention of NF-κB activation to the THP-1 cell line [37], which is in contrast with our findings. Whereas, the surface-associated exopolysaccharide (EPS) extracted from *L. paracasei* DG showed immune-stimulating properties to the human monocytic cell line THP-1, which is displayed through an increase of TNF-α and IL-6 gene expression that is in line with our findings [38]. In addition, human monocytes and monocyte-derived

DCs co-cultured with *Veillonella parvula*, *Escherichia (E.) coli*, *B. adolescentis*, and *L. plantarum* strains, stimulated a high level of IL-6 upon exposure to *V. parvula* and *E. coli*, but not *B. adolescentis* and *L. plantarum* [39].

3.2. ST285 Changes the Expression of Cytokines Involved in Inflammation and Defense against Bacteria

IL-18 is associated with severe inflammatory responses and plays a role in inflammatory and autoimmune disorders. Monocytes co-cultured with ST285 significantly reduced the gene expression of IL-18, which is in agreement with our recent study of ST285 co-cultured with PBMCs [26], suggesting an anti-inflammatory role for ST285 bacteria. IFNγ is an important activator of MQs, is secreted by monocytes, NK, and NKT cells, and is critical for functional innate and adaptive immune responses against viruses as well as some bacterial and protozoa infections [40].

Monocytes co-cultured with ST285 show increased gene expression of IFNγ suggesting an anti-bacterial response. In addition, a similar significant increased secretion of IFNγ is noted by monocyte cultures in the presence of ST285. Similarly, blood monocytes from healthy individuals who ingested a probiotic mixed of *S. thermophiles*, *L. bulgaricus*, and surplus *L. casei* DN114001 or a conventional yogurt containing the same probiotic mixture, showed increased production of IFNγ upon co-culturing monocyte cells ex vivo with LPS and phytohaemagglutinin. In another study, the effects of *L. casei* Shirota on the monocyte were shown indirectly, as the depletion of monocytes from PBMCs co-cultured with *L. casei* Shirota was associated with an absence of IFNγ and other cytokines, demonstrating the importance of monocytes against bacterial challenge [41]. Similarly, *L. plantarum* alone and mixed *L. plantarum* and *Helicobacter pylori* added to the monocytes (and lymphocytes) resulted in the production of high levels of IFNγ with *L. plantarum* alone, compared to the mixed cultures [42]. On the contrary, co-culturing ST285 with spleen cells from mouse immunized with agonist MBP_{83-99} peptide caused a reduced expression of IFN-γ cytokines [26].

TNFα, a pro-inflammatory cytokine is required against bacterial infections. In addition, it is involved in activating and recruiting T- and B-cells in the initiation of adaptive immune responses. Here, we show the upregulation of TNFα when human monocytes are co-cultured with ST285. Similarly, an increased secretion of TNFα was shown by the monocyte upon culture in the presence of ST285, which is in agreement with the observations of PBMCs [26] and the U937 monocyte cell line [26]. Isolated human monocytes and monocyte-derived DCs co-cultured with *V. parvula*, *E. coli*, *B. adolescentis*, and *L. plantarum* strains, similarly showed higher levels of TNFα [39]. In addition, EPS from *L. paracasei* DGs induced increased TNFα gene expression by the THP-1 monocyte cell line [38]. Although *L. paracasei* itself decreased the TNFα production by the THP-1 cell line via the inhibition of NF-κB activation [37]. Similarly, *L. plantarum* genomic DNA reduced the production of TNFα in THP-1 monocyte cells [43]. Moreover, the importance of monocytes in phagocytosis was shown using monocyte-depleted-PBMCs in co-culture with *L. casei* Shirota, which led to no secretion of TNFα [41].

IFNAR1 is a membrane protein and a receptor for both IFNα and IFNγ, associated with the defense against viruses. IFNAR1 signaling is involved in the production of pro-inflammatory cytokines [44], in which IFNAR1 knockout mice demonstrate reduced pro-inflammatory chemokines and cytokines [44]. IFNAR1 is significantly downregulated by monocytes following co-culture with ST285, supporting an anti-inflammatory role for ST285. The upregulation of IFNγ, IL-1β, and TNFα by monocytes following co-culture with ST285 suggests a powerful defense against invading pathogens induced by ST285 that could be advantageous in the defense against virus infections and tumors. Of interest, despite the upregulation of IFNγ, IL-1β, and TNFα considering the collective downregulation of IFNAR1, IFNGR1, and IL-18, our results might reveal an antagonistic effect of ST285 on pro-inflammatory IFNγ, IL-1β, and TNFα responses. This may lead to an overall downstream tolerance and even an ultimate anti-inflammatory outcome. One important anti-inflammatory cytokine is IL-4, which is required for reactions against inflammatory

conditions (such as allergies and multiple sclerosis (MS)). IL-4 production was significantly increased to monocyte cultures following co-culture with ST285.

3.3. ST285 Activates mRNA Expression of CXCL8 and Downregulates CCR5 and CCL2

IL-8, also known as CXCL8 is produced by MQs. In addition, it is an important innate immune system chemokine, associated with recruiting neutrophils and other granulocytes of innate immune defense [45]. Our findings show a significant increased IL-8 gene expression by monocytes after exposure to ST285. We previously noted that ST1342, ST1275, and ST285 stimulate the U937 monocyte cell line to secrete increased levels of IL-8 [1]. Similarly, we showed that the PBMC exposure to ST285 results in overexpression of IL-8 [26]. Correspondingly, EPS from the *L. paracasei* DGs probiotic displayed immune-stimulating effects to the human monocytic cell line THP-1 by increased expression of IL-8 [38]. In contrast, it was shown that dairy and soy fermented milks inoculated with *S. thermophilus* ST5 (ST5) mixed with *L. helveticus* R0052 (R0052) or *B. longum* R0175 (R0175) and added to the LPS-challenged THP-1 monocyte cell line decreased the IL-8 production only when co-cultured with ST5 + R0175 [46]. In addition, milk fermented with ST5 + R0052 or ST5 + R0175 did not alter the production of IL-8 by the U937 monocyte cell line, whilst soy ferment prepared with ST5 + R0175 downregulated the IL-8 production [46].

C-C chemokine receptor type 5 (CCR5, CD195) and chemokine (C-C motif) ligand (CCL) 2 are mainly expressed on monocytes, DCs, and MQs [47]. CCR5 is associated with Th1 immune responses and CCL2 with the pathogenicity of a number of inflammatory diseases including rheumatoid arthritis and psoriasis, categorized by monocytic infiltrates through chemo-attracting monocytes [48]. Monocytes co-cultured with ST285 significantly downregulated CCR5 and CCL2, which is similar to ST285 co-cultured with PBMCs [26], suggesting an anti-inflammatory influence of ST285.

Although the overexpression of IL-8 exclusively may be interpreted as an inflammatory effect, taking into account the largely upregulated anti-inflammatory cocktail of cytokines and mediators induced by ST285, it can in fact modulate this effect towards an anti-inflammatory profile for ST285. The upregulated IL-8 might be an initiating function of ST285 in order to trigger immune responses in the innate immune system, which then gets controlled by ST285 through reduction in the expression of CCR5. In turn, this may lead to reduced Th1 immune responses, as well as decreased CCL2 and subsequently resulting in a controlled recruitment of monocytes. These effects may again highlight the immunomodulatory effects of ST285 bacteria.

3.4. ST285 Significantly Upregulates mRNA Expression Level of Colony Stimulating Factor

Colony stimulating factor (CSF, GM-CSF) is secreted by monocyte/MQs. In addition, it supports and induces propagation, differentiation, and production of different immune cells, mainly monocyte/MQs which are fundamental in responses against infections. CSF is significantly increased (63.82 fold) by monocyte cultures in the presence of ST285, which is in alignment with our recent data showing increased CSF gene expression by PBMCs co-cultured with ST285 [26]. GM-CSF is significantly decreased by monocyte cultures in the presence of ST285. The reduced secretion of GM-CSF following exposure to monocytes and the ST285 probiotic is likely due to GM-CSF uptake by monocytes following their increased activity. This is in response to ST285 exposure and monocytes innate response to the presence of bacteria that might lead to monocytes proliferation [27]. Moreover, ST1275, ST1342, and ST285 were noted to induce high levels of GM-CSF production by the U937 monocyte cell line [1]. It is known that G-CSF induces the development of IL-10-producing cells [47,49]. Therefore, suggesting that ST285 may have an anti-inflammatory effect on the immune system.

3.5. ST285 Downregulates mRNA Expression Levels of Toll-Like Receptors

Toll-like receptors (TLRs) are mediators of innate immune responses primarily required in the defense against pathogens [50]. ST285 induced significant downregulation of

TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, and TLR-8, similar to our previous findings showing the reduction of several TLRs by PBMCs co-cultured with ST285 [26]. Activated TLRs (especially TLR-2 and TLR-4) together with other immune system factors can facilitate pro-inflammatory responses, as well as further stimulate innate immune system actions [51–53]. Therefore, an increased expression of TLR-2 and TLR-4 can lead to predominant inflammatory responses in the host, and their downregulation suggests a reduction in these pro-inflammatory responses. Moreover, TLR-5 activation leads to stimulation of NF-κB, which results in pro-inflammatory TNFα production [54]. Furthermore, its reduced expression in the monocyte co-cultured with ST285 may signify an anti-inflammatory role for the ST bacteria. Similar to our findings, another study has shown decreased expression of TLR-2, TLR-4, and TLR-9 using *L. plantarum* genomic DNA with THP-1 monocyte cells [43]. However, a study using human monocytes and monocyte-derived DCs exposed to UV-radiated *V. parvula*, *E. coli*, *B. adolescentis,* and *L. plantarum*, showed higher expression of TLR-2 on monocytes compared to DCs, while TLR-4 was not detectable on DCs [39]. Additionally, in the same study, it was shown that TLR-4 expression on monocytes was downregulated in response to exposure to *E. coli* or *L. plantarum*α [39]. Downregulation in mRNA expression of TLRs genes, specifically when it occurs across a wide range including TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, and TLR-8, designates anti-inflammatory properties for ST285.

3.6. ST285 Downregulates Cell Surface Markers CD14, CD86, and CD4

CD14 is expressed on the cell surface of monocytes, MQs, and DCs. In addition, it primarily binds to bacterial components [55–57]. CD14 was significantly downregulated when co-cultured with ST285 bacteria, suggesting an anti-inflammatory response. CD14 together with TLR-4 bind to bacterial components and both CD14 and TLR-4 were downregulated in the presence of ST285 bacteria. Moreover, the co-culture of PBMCs with ST285 led to the downregulated CD14 and TLR-4 expression [26]. However, the ST285 upregulated the expression of CD14 by the U937 monocyte cell line [1]. In accord with our findings, human monocytes isolated from PBMCs and exposed to *E. coli* or *L. plantarum* displayed the downregulated expression of CD14 [39]. CD86 (B7-2) is a co-stimulatory molecule necessary for initiating and maintaining T-cells. The expression of CD86 mRNA levels by the monocyte is significantly downregulated following co-culture with ST285, in line with our previous findings where CD86 was downregulated by bulk PBMC cultures [26]. Therefore, ST285 seems to induce an anti-inflammatory profile. Likewise, ST5 + R0052 or ST5 + R0175 milk or soy ferments also reduced the expression of CD86 [46]. However, *L. fermentum* GR1485 and *L. plantarum* WCFS1 increased the expression of CD86 by monocytes, inversely to *L. delbruekii* and *L. rhamnosus* that reduced CD86 expression [58]. Additionally, monocyte-derived immature DCs co-cultured with *L. lactis* subsp. cremoris ARH74, *B. breve* Bb99 and *S. thermophilus* THS increased the expression of CD86 [23]. The contrast in these findings is not surprising and may be due to the dissimilarities in the nature of experiments. In current study, the co-culture of monocytes only with ST285 bacteria while in the other study monocytes differentiated into immature DCs was co-cultured with several probiotics which lead dissimilarities related to the properties of different bacteria.

CD4 is an extracellular cell surface molecule expressed by monocytes, MQ, DCs, and Th cells. In addition, its acts as a co-receptor between T-cells and antigen presenting cells [59]. CD4 was significantly downregulated in monocyte cultures with ST285. In HIV-infected monocytes and MQs, CD4 is required for entry into the cell, and suggests that ST285 may have anti-viral properties.

Given the functional role of cell surface markers in immune responses, the CD14 involvement in native immune responses, the CD86 in T-cell activation, and the presence of CD4 on many cells underpinning innate and adaptive immunity, their downregulation in the presence of ST285 indicates an anti-inflammatory and anti-stimulatory profile for ST285. Additionally, due to the role of these cell surface markers in mediating innate and/or adaptive immune responses in the defense against bacteria, the downregulation of these markers could be suggestive of ST285 initiating self-tolerance via its immune modulation effects.

3.7. ST285 Differentially Downregulates mRNA Expression Level of Other Innate and Adaptive Immune Response Markers and Chemokines

Integrin alpha M (ITGAM) or CD11b is another innate immune response factor associated with several inflammatory reactions such as phagocytosis, cell-mediated cytotoxicity, and chemotaxis. In addition, lysozyme (LYZ) is an innate immune response mediator associated with several inflammatory actions. Moreover, it exists in mononuclear phagocytes such as MQs and performs as an antimicrobial enzyme. ITGAM and LYZ gene expressions are vastly downregulated in monocytes co-cultured with ST285, similarly to our recent findings showing downregulation of ITGAM and LYZ by PBMCs co-cultured with ST285 [26]. Conversely, in the U937 monocyte cell line, exposure to ST285 caused significant upregulation of CD11b/ITGAM [1]. The contrast could be related to the difference between monocytes from healthy blood donors compared to the monocyte cell line. MYD88 which is implicated in innate immunity is downregulated by monocytes in response to the ST285 co-culture. Moreover, IL1RA1 has been shown to interact with MYD88 (together with PIK3R1 and IL1RAP) [60] and is downregulated, which highlights an anti-inflammatory role for ST285. Non-receptor tyrosine-protein kinase (TYK2) is an enzyme involved in various cellular events. In addition, extensive studies of TYK2-deficient mice indicate compromised IFNα, IL-12, and IL-23 pathways [61] as well as IL-12/Th1 and IL-23/Th17 axes [62]. However, it is dispensable for the signaling pathways of IL-6 or IL-10 [61]. It is believed that TKY2 is associated with a broader cellular pathway in humans and it has a role in IL-12/Th1 and IL-23/Th17 axes involved in inflammatory/autoimmunity, highlighting the choice of TKY2 as an effective therapeutic approach for select autoimmune diseases [61]. TYK2 is significantly downregulated by monocytes upon co-culture with ST285. A similar trend was found in our results when PBMCs were co-cultured with ST285 recently [26], which mutually support an anti-inflammatory profile for ST285.

IL-1-receptor-associated kinase-1 (IRAK1) is involved in innate immunity. In addition, ST285 induced a significant downregulation of IRAK1 by the monocyte culture. Likewise, *L. paracasei* stimulated the expression of IRAK3, but not IRAK1 in the THP-1 cell line post differentiation with PMA. The IRAK4 inhibitor suppressed the expression of negative regulators [37]. In contrast, THP-1 monocyte cells treated with *L. plantarum* genomic DNA induced a slight increase in IRAK-1 production [43]. SLC11A1, which is a monocyte-MQ protein-1 involved in T-cell activation and inflammatory disorders such as type 1 diabetes [63,64], Crohn's disease [65], and rheumatoid arthritis [66] is downregulated by monocytes upon co-culture with ST285. Our previous findings using ST285 to co-culture with PBMCs similarly showed a reduced expression of SLC11A1 [26], again suggesting an anti-inflammatory role for ST285. The induced downregulation of IRAK1, MYD88, TKY2, ITGAM, NOD2, SLC11A1, and LYZ by monocytes due to the exposure to ST285 is suggestive of anti-inflammatory effects of ST285.

4. Material and Methods

4.1. Bacterial Strains

Pure bacterial cultures of ST285 were obtained from the Victoria University culture collection (Werribee, Melbourne, VIC, Australia). Stock cultures were stored in cryobeads at −80 °C. Prior to each experiment, the cultures were propagated in M17 broth (Oxoid, Melbourne, Australia) with 20 g/L lactose and incubated at 37 °C under aerobic conditions. In order to confirm Gram-positivity and to assess purity, morphology, and characteristics, the bacteria were cultured in M17 agar (1.5% *w*/*v* agar) with 20 g/L lactose (Oxoid, Melbourne, Australia), as well [1].

4.1.1. Preparation of Live Bacterial Suspensions

Prior to the experiments, the bacterial culture medium was prepared and autoclaved at 121 °C for 15 min and bacterial cultures were grown three times in M17 broth with 20 g/L lactose, at 37 °C aerobically for 18 h with a 1% inoculum transfer rate [67] at 37–42 °C [15]. Bacteria were harvested during the stationary growth phase on the day

of the experiment, centrifuged (6000× *g*) for 15 min at 4 °C, followed by washing twice with phosphate-buffered saline (PBS) (Invitrogen, Pty Ltd., Melbourne, Australia), and resuspended in the Roswell Park Memorial Institute (RPMI) 1640 culture media (Invitrogen, Pty Ltd., Melbourne, Australia), which constituted the live-bacteria suspensions.

4.1.2. Enumeration of Bacterial Cells

Prior to co-culturing with PBMCs, bacterial strains cultured in M17 broth were centrifuged and transferred into PBS (Invitrogen, Pty Ltd. Melbourne, Australia). In addition, they were adjusted to a final concentration of 10^8 colony forming units (cfu)/mL by measuring the optical density at 600 nm. Then, the strains were washed twice with PBS and resuspended in RPMI 1640 (Invitrogen, Pty Ltd., Melbourne, Australia) [1].

4.2. Isolation of Monocytes from Buffy Coats

Ethics approval (Ethical approval ID number: HRE16-058) was obtained from Victoria University and the Australian Red Cross blood bank ethics committees. Buffy coats were received from the Australian Red Cross blood bank in Melbourne, and PBMCs were isolated using the standard Ficoll-Paque density gradient centrifugation method, as previously described [68]. PBMC cells were resuspended at ~5 × 10^8 cells/mL in an adequate amount of Dulbecco's phosphate-buffered saline, D-PBS (D-PBS without Ca^{++} and Mg^{++}) supplemented with 2% FBS and 3 mM cell culture grade EDTA (Life Technologies; Thermofisher, Melbourne, Australia) prior to monocyte isolation. Monocytes were isolated using the EasySep Human Mono Isolation Kit (STEMCELL technology, Melbourne, Australia) [69]. The isolation method involved the use of the immunomagnetic negative selection method targeting $CD16^+$ monocytes, excluding non-monocyte cells, and platelets, yielding highly pure $CD14^+CD16^-$ monocytes. Therefore, the unwanted cell populations are labelled with specific cell surface marker antibodies and magnetic particles, and are removed following separation using an EasySep™ magnet (STEMCELL technology, Melbourne, Australia) according to the manufacturer's instructions [69]. Monocyte cells were added into a fresh tube, then checked for viability and purity.

4.3. Stimulation of Monocytes with ST285

Monocytes (~3–5 × 10^7 cells) isolated from three different donors were resuspended in RPMI 1640 media supplemented with 10% heat-inactivated FBS (Invitrogen, Pty Ltd., Melbourne, Australia), 1% antibiotic-antimycotic solution (Gibco®, Melbourne, Australia), and 2 mM L-glutamine (Gibco®, Melbourne, Australia) in cell culture flasks, into which 5 × 10^8 ST285 bacteria were added. The antibiotic-antimycotic solution is used to prevent bacterial/fungal contamination. In co-culturing cells with bacteria, antibiotic supplementation prevents bacterial overgrowth. Monocytes (~3–5 × 10^7 cells) minus the ST285 bacteria were used as a control and incubated at 37 °C, 5% CO_2 for 24 h [1]. In previous studies, we demonstrated that a 24 h co-culture was optimal for stimulation of the U937 monocyte cell line, and all of the incubations described herein were for 24 h [1]. Monocytes were harvested post the incubation period, snap frozen, and stored at −80 °C.

4.4. RNA Extraction from Monocytes

Total RNA was extracted from stimulated and unstimulated monocytes using the RNeasy® mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Briefly, monocytes were harvested using centrifugation, supernatants were removed, and RNA was extracted from each pellet by resuspending the pellet in lysis buffer supplemented with β-mercaptoethanol for cell disruption. Monocytes were lysed and each cell lysate was homogenized by passing through Qia-shredder columns (Qiagen, Hilden, Germany). Each monocyte lysate was then mixed 1:1 with 70% ethanol (equal volume) and transferred onto RNeasy mini-spin columns. DNA was eliminated using the DNase digestion/treatment using the RNase-Free DNase Set (Qiagen, Hilden, Germany) by adding it directly onto the columns. The RNA Integrity Number (RIN) of all the RNA samples was determined

using an Agilent 2100 Bioanalyzer and Agilent RNA 6000 nano kit (Agilent Technologies, Santa Clara, CA, USA). A minimum RIN of 8 was used as the standard for inclusion in the gene expression study. Subsequently, the concentration of each individual monocyte RNA sample was quantified using a Qubit RNA BR Assay (Invitrogen, Pty Ltd., Melbourne, Australia).

4.5. Assessing Changes in the Expression of Genes Associated with Innate and Adaptive Immunity

Using the RT^2 first strand kit (Qiagen, Hilden, Germany), adequate aliquots of each RNA sample was reverse-transcribed to produce complementary DNA (cDNA), according to the manufacturer's instructions. The quantitative real-time polymerase chain reaction (qRT-PCR) was carried out using the 'Human Innate and Adaptive immune Response' kit (Qiagen, Hilden, Germany) to assess the expression of genes/mRNA. Using a CFX Real-Time touch PCR System thermo-cycler (Biorad, Melbourne, Australia) and Qiagen prescribed cycle, the relative gene/mRNA expression of ST285-treated monocytes was analyzed in contrast to the control untreated monocytes. The RT^2 qPCR innate and adaptive immune response arrays targeted a set of 84 innate and adaptive immune-related genes, five housekeeping genes, an RT control, a positive PCR control, and a human genomic DNA contamination control (Table S1 and Figure S2) [70]. The relative gene expression was calculated using the Qiagen web portal PCR array data analysis web-based software (Qiagen, Germany). Differential expression (up- and downregulation) of the genes was identified using the criteria of a >2-fold increase/decrease in gene expressions in the treated monocytes, in comparison with those genes in control monocyte cultures.

4.6. Data Analysis

The Delta-Delta CT (ΔΔCT) method was used for calculating fold-changes [71]. Fold-regulation represents fold-change results in a biologically meaningful way. In these RT^2 profiler PCR array results, fold-change values > 1 indicate a positive (or an up) regulation. Actually, in the case of genes which are upregulated, the fold-regulation is equivalent to the fold-change. Fold-change rates < 1 indicate a negative (or a down) regulation. In the case of negative values, the fold-regulation is actually the negative inverse of the fold-change [72–74]. Data related to changes in the expression of the genes were estimated using the Qiagen RT^2 profiler data analysis web portal that uses the ΔΔCT method in calculating fold-changes. The raw CT values were uploaded to the Qiagen data analysis web portal with the lower limit of detection set for 35 cycles and three internal controls. For controls, RT efficiency, PCR array reproducibility, and genomic DNA contamination were assessed to ensure that all of the arrays successfully passed all of the control check-points. Normalization of the raw data was done using the incorporated housekeeping genes (HKG) panel. Then, using the ΔΔCT method, both the housekeeping gene references and controls (untreated monocytes in RPMI) were evaluated to determine the relative expression of mRNA.

4.7. Statistical Analysis

The *p*-values were calculated with the use of a Student's *t*-test of the Triplicate 2^ (−Delta CT) [(2^−ΔCT)] values for each gene in the treatment groups (monocyte co-cultured with ST) and the control group (monocyte in RPMI media) [72,73].

4.8. Cytokine Production Analysis

Cytokine production of monocyte in culture supernatants was evaluated using commercially available capture and detection antibodies in a Bioplex multiplex-bead-assay for a panel of nine human cytokines and chemokines, using a 9-plex kit (BioRad, Melbourne Australia) to quantity IL-1β, IL-2, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, and TNF-α. Cell-free supernatants were collected and the test procedures were conducted in accordance with the manufacturer's instructions. Briefly, 1× coupled beads were coated in a flat bottom 96-well plate and washed two times. The blanks, serially diluted standards, and samples were added to the allocated wells in triplicate. The plates were incubated and shaken at room temperature, then washed two times. Next, the 1× detection antibody was added,

incubated, and shaken at room temperature. The plates were washed three times, the stop solution 1× Streptavidin Phycoerythrin (SA-PE) was added, then incubated at room temperature, and washed. Data collection was conducted two times. Moreover, data were presented as the mean cytokine response minus the background (pg/mL) of each treatment from triplicate wells, plus or minus the standard error of the mean [27,75].

5. Conclusions

Commensal bacteria and probiotics have made their entry into the mainstream of healthcare. In addition, they contribute to immune homeostasis in the gastrointestinal tract as well as confer beneficial immunomodulatory properties that assist in the maintenance of a healthy immune system. ST is commonly applied in dairy products to ferment cheeses and yogurts and is thought to be beneficial to human health. We assessed the immune modulatory effects of ST285 on human monocytes and demonstrated that it delivers a range of potential immunomodulatory and anti-inflammatory properties. ST285 decreases the mRNA expression of IL-1R, IL-18, IFNαR1, IFNγR1, CCL2, CCR5, TLR-1, TLR-2, TLR-4, TLR-5, TLR-6, TLR-8, CD14, CD86, CD4, ITGAM, LYZ, TYK2, IFNR1, IRAK-1, NOD2, MYD88, ITGAM, and SLC11A1. Moreover, ST285 upregulates IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-23, IFNγ, TNFα, and CSF-2. No changes to the mRNA expression were noted with IL-4, IL-5, IL-13, CCL2, CCL5, CCL8, CCR4, CCR8, CXCR3, CXCL10, TLR-3, TLR-9, CD8A, CD40, CD80, IFNB1, MPO, FOXP3, GATA3, STAT3, CD40LG, HLA-A, HLA-E, and RORC. The data exhibit a predominant anti-inflammatory profile of cytokines, chemokines, and cell markers induced by ST285. Therefore, the use of ST285 may be an efficacious approach for the treatment of select autoimmune diseases, without the need of using the currently available treatments for autoimmune disorders which are mainly broad immunosuppressive medications. Supplementary work is required to determine whether the ST bacteria display similar anti-inflammatory effects in vitro and in vivo in compromised immune disorders/models such as inflammatory bowel disease, multiple sclerosis, and allergies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/biologics1030023/s1, Table S1: Full name Universal name, symbol and full name the genes and the house keeping gens (HKG) included in RT2 gene profiler arrays used to determine gene/RNA expression of monocytes post co-culturing with ST285 with monocytes, Figure S1: Effects of co-culturing ST285 with monocytes (n = 3) on gene/RNA expression compared to control monocytes after 24 h presented as Clustergram, Figure S2 Position of the genes and the house keeping gens (HKG) included in RT2 gene profiler arrays used to determine gene/RNA expression of monocytes post co-culturing with ST285 with monocytes.

Author Contributions: Conceptualization, N.D., J.C.J. and V.A.; methodology, N.D.; software, N.D.; validation, N.D., J.C.J. and V.A.; formal analysis, N.D.; writing—original draft preparation, N.D.; writing—review and editing, N.D., J.C.J. and V.A.; supervision, V.A and J.C.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Victoria University (Ethical approval number HRE16-058 date of approval 7 June 2017).

Informed Consent Statement: Australian Red Cross ethics committee and Victoria University ethics committee evaluated our ethics application for the use of human buffy coat in this research. Authors were not in direct contact with human participants. Australian Red Cross provided "buffy coat" for experiments.

Data Availability Statement: Data is contained within the article or Supplementary Materials.

Acknowledgments: All authors were supported by VU Research and in particular, Institute for Health and Sport and the Institute for Sustainable Industries and Liveable Cities at Victoria University. J.J. was supported by a VU Research Fellowship, Victoria University, Australia. The authors

acknowledge the help from Sarah Fraser and technical staff Mary Marshall, Charmaine DiQuattro, and Stacey Lloyd at Victoria University, Werribee campus.

Conflicts of Interest: The authors declare no conflict of interest.

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