The probiotic mixture VSL#3 mediates both pro- and anti-inflammatory responses in bone marrow derived dendritic cells from C57BL/6 and BALB/c mice

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**Short Title**

Immunomodulatory effects of probiotics on DC

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**Abstract**

Probiotic bacteria express a wide range of molecular structures that bind to receptors on innate immune cells and mediate health-promoting effects in the host. We have recently demonstrated in a colitis model that favourable effects of the probiotic mixture VSL#3 may in part be due to suppression of intestinal chemokine expression. To obtain more insight into the underlying mechanisms, we studied modulation of bone marrow derived dendritic cells (BM-DC) from BALB/c (Th2 biased) versus C57BL/6 (Th1 biased) mice. Our data show that VSL#3 differed from pure TLR ligands by inducing higher levels of various cytokines, including IL-12p70, IL-23 and IL-10. Dedicated TLR-arrays were employed to profile mRNA from BM-DC cultured with LPS, VSL#3, or a combination of both. This approach identified (I) a cluster of genes that was up-or down-regulated, irrespective of the stimulus, (II) a cluster of genes that was synergistically up-regulated by LPS and VSL#3in BM-DC from C57BL/6 mice, but not from BALB/c mice, (III) a cluster of LPS-induced genes that were suppressed by VSL#3, in particular chemokines. These data show therefore that this probiotic mixture has both pro- and anti-inflammatory effects on BM-DC, and suggest that their immune-modulating properties *in vivo* may depend on the genetic background of the host.

**Introduction**

Dendritic cells (DC) are bone marrow derived antigen-presenting cells capable of inducing protective adaptive immune responses or tolerance. DC are equipped with Toll-like receptors (TLR) and C-type lectin receptors that activate different signalling pathways in response to microorganisms(1). TLR expressed on the cell surface predominantly bind bacterial products, such as lipopeptides and peptidoglycan (TLR1, -2 and -6), lipopolysaccharide (LPS, ligand of TLR4) and flagellin (TLR5). TLR3, -7, -8 and -9 reside in intracellular organelles and recognise microbial nucleic acids(2). In the intestinal mucosa, various subsets of DC are in close contact with the intestinal microbiota and continuously migrate from the lamina propria to the mesenteric lymph nodes, ensuring a balance between immunity and tolerance(3). Lamina propria DC can be divided into two major classes: CD103- DC induce Th1 and Th17 responses, whereas CD103+ DC rather induce regulatory T cells(4). Different types of DC develop from bone marrow progenitors and therefore immature bone marrow derived DC (BM-DC) are widely used in *in vitro* studies. Such studies contribute to a better understanding of the mechanism by which microorganisms modulate immune responses *in vivo*. Although not identical to intestinal DC, it has been demonstrated that splenic DC from Th1-prone C57BL/6 mice and Th2-prone BALB/c mice respond differently to microbial stimuli(5-7) and this may in part be explained by differences in TLR expression. BALB/c mice express higher levels of TLR2, -4, and -5 mRNA, whereas C57BL/6 mice express more TLR9 mRNA(8). In addition, the expression of the macrophage mannose receptor on BM-DC is mouse-strain specific, and this phenomenon may influence antigen-uptakeby such cells(9).

Probiotic bacteria modulate DC via surface expression and/or secretion of products which function as ligands for TLR and C-type lectin receptors. The expression of these ligands depends on bacterial species, growth phases and available nutrients, and this explains in part that different species and strains of probiotics vary in their ability to induce cytokines and chemokines(10-12).

Recently, we demonstrated that treatment of BALB/c mice with a mixture of probiotic bacteria (i.e. VSL#3) has profound effects on gene expression in the colons of mice subjected to colitis induction, with favourable effects on the development of disease(13). However, substantial variability in the response of human subjects to probiotic interventions has been found(14), suggestive for an influence of genetic and environmental factors.

To gain more insight into the influence of genetic background, we choose to study BM-DC from two genetically distinct mouse strains and evaluate their response to the probiotic mixture VSL#3 *in vitro*, as compared to ultrapure TLR ligands. Our results, obtained by dedicated gene expression profiling of genes related to TLR-signalling and by studying cytokine production, suggest that VSL#3 may display both pro- and anti-inflammatory effects, dependent on mouse strain.

**Materials and methods**

*Mice*Seven- to eleven–week-old C57BL/6 mice (Charles River, Maastricht, Netherlands) and BALB/c mice (Janvier, St. Berthevin, France) were used in this study. All animal experiments were conducted with the approval of the Institutional Animal Welfare Committee, filed as number DEC2661, in compliance with European Community specifications regarding the use of laboratory animals.

*Isolation and culture of BM-DC*

BM-DC were isolated as described previously(15), with slight modifications. Briefly, bone marrow was flushed from femur and tibia. Cells were passed through nylon mesh to obtain a single cell suspension. After a single wash step, cells were cultured in RPMI 1640 medium containing 10% foetal bovine serum (Lonza, Verviers, Belgium), 2mM L-glutamine, 100 U/ml streptomycin, 100 μg/ml penicillin (Gibco, San Diego, CA, USA), 50μM β-mercaptoethanol (Sigma Adrich, Zwijndrecht, The Netherlands) in the presence of 20 ng/ml recombinant mGM-CSF (Peprotech, Rocky Hill, NJ, USA). Cells were cultured (106 cells/ml) at 37oC. Twothirds of the culture medium were refreshed on days 3 and 6. After 8 days of culture non-adherent and loosely adherent BM-DC were collected and used for stimulation experiments. Viability of cells (>95%) was assessed by trypan blue dye exclusion. Cells were cultured at a cell density of 106/ml and stimulated with TLR-ligands and/or probiotic bacteria at concentrations indicated in the figure legends.

*Reagents*

**Pam3CSK4, poly I:C, LPS** E. coli K12, **Flagellin** S.typhimurium, CpG**ODN1826,** Imiquimod and Peptidoglycan from *E. coli* 0111:B4 were purchased from Invivogen (San Diego, CA, USA). All of these ultra-pure TLR ligands were endotoxin free(<0.001 endotoxin units/μg), except for peptidoglycan which contained <125 endotoxin units /ml.

VSL#3, a mixture containing freeze-dried *B. longum, B. breve, B. infantis, L. acidophilus, L. plantarum, L. casei,L. bulgaricus and S. thermophilus*, was purchased from Ferring Pharmaceuticals (Berkshire, UK). *L. plantarum* NCIMB8826 and *B.animalis ssp. Lactis*BB-82 were grown at 37 oC in Mann-Rogusa Sharpe (MRS) broth (Scharlau Chemie, Barcelona, Spain) until mid-exponential density (OD600nm = 1). Bacteria were washed twice in phosphate-buffered saline (PBS, pH 7.4). Lyophilized bacteria were prepared by freezing bacterial pellets (-80oC) before overnight lyophilisation in a freeze-dryer under vacuum (40 mBar). Lyophilised bacteria were stored at -20oC until use.

*RNA isolation and RT-qPCR*

Total RNA from 106 cells was isolated using an RNAEasy Kit (Qiagen, Venlo, Netherlands) according to the manufacturer’s instructions. Five hundred ng RNA were reverse transcribed into single stranded cDNA using a High Capacity RNA-to-cDNA kit (Applied Biosystems (AB), Carlsbad, CaliforniaUSA) and incubated for 60 minutes at 37oC and 5 minutes at 95oC. cDNA was amplified employing a 7500 Fast Thermal cycler (AB, USA) using SYBRgreen PCR master mix (AB, USA). Thermal cycling parameters consisted of 1’ at 50oC, 10’ at 95oC, followed by 40 cycles of 15’’ at 95oC s and 1’at 60oC. Primer pairs were for *Il12p35*: 5′-CTG GAG CAT CCG AAT TGC A-3′ (sense, S) and 5′-CAT CCT CTG AGA TTT GAC GCT TT-3′ (anti-sense, AS); *Il23p19:* 5′-GGC AAC TTG GAC CTG AGG AG-3′ (S) and 5′-CAT GGG CTC TCG GTC CAT AG-3′ (AS); *Cxcl9*: 5′-CCT AGT GAT AAG GAA TGC ACG ATG-3′ (S) and 5′-CTA GGC AGG TTT GAT CTC CGT TC-3′ (AS); *Cxcl10*: 5′-ATC ATC CCT GCG AGC CTA TCC T-3′ (S) and 5′-GAC CTT TTT TGG CTA AAC GCT TTC-3′ (AS); *B2m:* 5′- acc gtg aaa aga tga tga ccc ag-3′ (S) and 5′- agc ctg gat ggc tac gta ca-3′ (AS); ); *Il12p40*: 5′-GGA AGC ACG GCA GCA GAA TA-3′ (S) and 5′-AAC TTG AGG GAG AAG TAG GAA TGG-3′ (AS). Gene expression levels were quantified according to the formula: 2-(Cti-Cta) where Cti is the cycle threshold of the gene of interest and Cta is the cycle threshold value for β-actin.

*PCR array*

Expression of genes involved in TLR pathways was studied by using the RT2-ProfilerPCR Array (Mouse TLR-Signaling Pathway) from SABiosciences (Frederick, MD, USA). This array combines the quantitative performance of SYBR Green-based real-time PCR with the multiple gene profiling capabilities of a microarray. 96-well plates containing gene-specific primer sets for 84 relevant TLR pathway genes, 5 housekeeping genes, and 2 negative controls were used. For each experimental condition, RNA was isolated as described above. Gene expression was normalized to internal controls (housekeeping genes) to determine the fold change in gene expression between test and control samples by ΔΔCt (SA Bioscience).

*Cytokine/chemokine analysis*

Cytokines were measured in the supernatants of BM-DC cultures 24 hours after cell stimulation using commercially available ELISA kits for IL-12p70, IL-12p40, IL-23, IL-10, IL-6, and TNF-α (Ebioscience, San Diego, CA, USA). Chemokines (CXCL-9 and CXCL-10) were quantified using a multiplex immunoassay (Invitrogen). Assays were performed according to the manufacturer’s instructions.

*Flowcytometryanalysis*

BM-DC were phenotypically characterized before and after 24 hours of stimulation. Cells were collected and washed with PBS. 2 x 105 cells were incubated (30 min, 4oC, protected from light) with monoclonal antibodies. The following dye-conjugated antibodies CD11c-PerCP/Cy5.5 (N418), CD86-FITC (B7-2GL-1), CD80-Pacific Blue (16-10A1), MHCII-PE (M5/114.15.2), CD317-APC (927), CD103-PE(2E7) were purchased from BD Biosciences (San Diego, CA, USA). Flowcytometric analyses were performed with a fluorescence-activated cell sorter (FACSCanto II, BD Biosciences) and analysed with FACSDiva Software 6.1.2. Gating of positive cells was based on the results obtained with isotype control antibodies.

*Statistical analysis*

Statistical analyses were performed with the Mann-Whitney U-test or Student’s t test if the samples passed the normality analyses by using RT² Profiler™ PCR Array Data Analysis.

**Results**

***BALB/c and C57BL/6 bone marrow progenitors differentiate into phenotypically distinct DC***

BM-DC from BALB/c and C57BL/6 mice were generated by 8 days of culture under identical conditions in the presence of GM-CSF. As shown in Figure 1A, BM-DC from BALB/c mice showed a higher expression of CD103 and CD317 as compared to BM-DC from C57BL/6 mice. Moreover, gene expression profiling of transcripts involved in TLR-signalling showed higher expression of *Tlr3* and *Ifnb* in cells from BALB/c mice (Figure 1B). BM-DC from BALB/c mice also showed significantly higher levels of mRNA encoding TLR5, -6 and -9, as compared to BM-DC from C57BL/6 mice (Figure 1C).

Before studying the response of these cells to probiotic bacteria we first evaluated their response to ultrapure TLR ligands. Flowcytometric analysis of the stimulated BM-DC confirmed similar up-regulation of CD80, CD86 and MHC class II expression, regardless of mouse strain or TLR-ligand (data not shown). However, BM-DC from BALB/c and C57BL/6 mice showed a different cytokine response to several TLR-ligands, as shown in supportive Figure 1. In particular, the IL-12p70 production in response to LPS or CpG was higher in C57BL/6 BM-DC. These data indicate that the genetic background strongly influences the phenotype and response of BM-DC to pure TLR ligands.

***The probiotic mixture VSL#3 and LPS synergistically induce IL-12p70 and IL-23 production in BM-DC from C57BL/6mice***

Probiotic bacteria are far more complex than ultrapure TLR ligands and therefore we studied to what extent the genetic background of BM-DC would influence their response to VSL#3.

We therefore stimulated BM-DC with increasing numbers of bacteria, in the absence or presence of LPS. Figure 2A shows the production of TNF-α, IL-6 and IL-12p70 upon stimulation of C57BL/6 BM-DC. All of these cytokines were stimulated by VSL#3 in a dose-dependent fashion. Simultaneously, VSL#3 strongly induced IL-10 production (supportive Figure 1). The combination of VSL#3 and LPS resulted in an additive effect on the secretion ofall of these cytokines. However, at a VSL#3:DC ratio of 10:1 a synergistic induction of IL-12p70 was observed.

Figure 2B shows that BM-DC from C57BL/6 mice showed a synergistic increase of IL-12p70 and IL-23in response to VSL#3 and LPS, whereas such an effect was not observed with BM-DC from BALB/c mice. This difference in response of BM-DC from C57BL/6 and BALB/c mice was confirmed for IL-12p70 with *L.plantarum,* one of the constituents of VSL#3 (Figure 2B).

Recently, it has been shown that TRIF- and MyD88-dependent TLR ligands act in synergy to induce the release of IL-12p70 in BM-DC(16). However, VSL#3 did not show synergy with the MyD88-coupled TLR-ligands CpG and Pam3CSK4, or the TRIF-coupled TLR ligand poly I:C with regard to the induction of IL-12p35 and IL-23p19 mRNA (Figure 2C).

Synergy in IL-12p70 and IL-23 induction was hence specific for the combination of VSL#3 and LPS, and only found with BM-DC from C57BL/6 mice.

**TLR gene array analysis of BM-DC identifies stimulating and blocking activities of VSL#3**

To identify mechanisms by which probiotic bacteria may modulate the innate immune response, we analysed mRNA expression of 84 genes implicated in TLR signalling. A time course study, revealed peak levels of mRNA encoding IL-12p35, IL-12p40, and IL-23p19 after 4 hours of stimulation with VSL#3 and LPS (data not shown). Therefore, RNA was isolated from BM-DC after 4 hours of culture with or without LPS, VSL#3, or a combination of both. Figure 3 shows the relative gene expression in BM-DC from C57BL/6 and BALB/c mice. Results of three individual mice per stimulus are each shown as a column in this heatmap. Hierarchical clustering of transcripts in C57BL/6 BM-DC revealed genes that are co-regulated. In BM-DC derived from both mouse strains, LPS and VSL#3 down-regulated a cluster of genes encoding TLR, e.g.*Tlr5, Tlr4* and *Tlr8*. Moreover, both VSL#3 and LPS induced a set of pro-inflammatory gene transcripts, such as *Tnf*, *Il1a* and *Il6*, regardless of mouse strain.

In addition to the synergistic induction of IL12 and IL23 (Figures 2B and 2C), the TLR signalling array revealed that the combination of VSL#3 and LPS also synergistically induced the expression of several other genes involved in innate immunity. In Figure 4, it is shown that 3 representatives of this cluster - *Lta*, *Ifng* and *Ifnb*– are synergistically induced in C57BL/6 BM-DC, but not in BALB/c BM-DC.

Importantly, a set of LPS-induced gene transcripts was suppressed by VSL#3 both in BALB/c BM-DC and in C57BL/6 BM-DC.In this cluster *Myd88*, *Cxcl9* and *Cxcl10* were the most important representatives.

The suppressive effect of VSL#3 on LPS-induced *Cxcl9* and *Cxcl10* expression was verified by quantitative RT-PCR (Figure 5A). Down-regulation was also confirmed on the protein level for CXCL-10 (Figure 5B); levels of CXCL-9 were below the detection limit.

VSL#3 is composed of different members of two lactic acid genera, i.e. Lactobacilli and Bifidobacteria. We therefore studied effects of representative members, i.e. *L.plantarum* and *B.lactis.* Whereas *L.plantarum* suppressed LPS-induced CXCL-10 both at the mRNA and protein level and CXCL-9 at the mRNA level, *B.lactis* failed in this respect (Figure 5C).

**Discussion**

Probiotic bacteria and prebiotic supplements have successfully been applied in the treatment of various inflammatory disorders, including allergies and intestinal disorders(17, 18). However, results of clinical trials are not consistent and variation in efficacy most likely depends on numerous factors, including type of bacterial strain (single strain versus a mixture of strains), dosing regimen, delivery method and host factors, such as genetics, age, diet, and disease state(19). Several trials have shown beneficial effects of lactobacilli, bifidobacteria and *E.coli* Nissle 1917 in the induction of remission as well as the maintenance of remission in patients with Ulcerative Colitis (20). However, the efficacy in Crohn's disease appears less evident(21). In the underlying study, we employed a simplified model system to evaluate the effects of VSL#3 - a complex mixture of TLR ligands - on BM-DC and identified both pro- and anti-inflammatory effects. These apparently contradictory findings may be due to the simultaneous presence of eight probiotic strains. On the other hand, similar contradictory effects were observed with *L.plantarum*, i.e. one single probiotic strain. In fact, even individual TLR ligands may induce a quick and transient production of pro-inflammatory cytokines such as IL-12, followed by the production of anti-inflammatory cytokines such as IL-10, as part of a suppressive mechanism essential to limit the extent of inflammation. The balance between (the levels) of these cytokines may differ between individuals and represents a major determinant for the severity of the subsequent inflammation process. Besides, probiotic bacteria may mediate additional mechanisms *in vivo* that control the extent of inflammation, e.g. the enhancement of intestinal barrier function, altered epithelial signaling, competition with pathogens and secretion of short chain fatty acids like acetate, propronate and butyrate, and have also effects on immune cells distinct from DC(22-26).

Although we allowed bone marrow progenitors from BALB/c versus C57BL/6 mice to differentiate *in vitro* into immature DC under controlled and identical conditions, we observed that VSL#3 and several TLR-ligands induced a more pronounced pro-inflammatory response in C57BL/6 mice, whereas BALB/c rather showed more IL-10 production. These observations are consistent with differences between the two mouse strains in terms of cellular and humoral immune responses to different pathogens(27-29). Our study did not show that levels of TLR gene expression could explain these differences. Moreover, we observed in BM-DC from C57BL/6 mice, but not from BALB/c mice, that VSL#3 augmented the induction of IL-12p70 and IL-23 by LPS in a synergistic fashion. Furthermore, several other genes involved in cellular immunity, were synergistically up-regulated after combined stimulation with VSL#3 and LPS. This suggests that this mixture of probiotic bacteria carries the potential to amplify inflammatory responses dependent on the genetic background of the host.

In parallel studies employing human DC, we demonstrated that LPS-induced phosphorylation of STAT-1 was inhibited by VSL#3, whereas phosphorylation of NF-κB was not influenced (Mariman et al., submitted for publication). A similar mechanism could explain the inhibition of chemokines, such as CXCL-9 and CXCL-10 by VSL#3, along with a robust induction of IL-12 and IL-23.

It should be noted that effects of VSL#3 are not representative for individual probiotic strains, as was shown by comparing *L.plantarum* and *B.lactis* in our *in vitro* studies.

Differential immune modulating capacities of specific probiotic bacterial species *in vivo* are likely to be caused by the distinct repertoire of microbe-associated molecular patterns or effector molecules, which are expressed in a strain-specific manner (30).

Our findings may also explain that certain probiotic strains were ineffective *in vivo* or even displayed adverse effects during ongoing inflammation(31, 32). On the other hand, our extensive characterization of gene expression during the induction of TNBS-colitis in BALB/c micedid not reveal that VSL#3 enhanced IL-12p40 or IL-23p19 mRNA levels in colon tissue (even not in acute phases of the model)(33). As mentioned above, this might be due to simultaneous induction of anti-inflammatory cytokines, or dampening of the local inflammatory response through mechanisms distinct from TLR-signaling.

Importantly, in this *in vitro* study we confirmed that probiotic bacteria may down-regulate the production of chemokines in agreement with our *in vivo* findings in the recurrent TNBS colitis model, where we also showed less recruitment of inflammatory cells and a lower disease severity(33).

Altogether, our data suggest that the net effect of probiotic bacteria *in vivo* depends on a complex of factors that vary between individuals and may depend on their genetic background and health status.

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**Financial interest**

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**Legends to Figures**

**Figure 1.**

**Flowcytometry and gene expression profiling reveals phenotypic differences between BM-DC from C57BL/6 and BALB/c mice**

1. Immature BM-DC generated in the presence of GM-CSF were stained with PE-labelled anti-CD103 and APC-labelled anti-CD317 and evaluated by flowcytometry. Filled histograms show the fluorescence intensity for each marker. Dotted lines reflect staining with isotype control antibodies. The percentage of cells expressing these markers is indicated in each panel.
2. mRNA isolated from immature BM-DC was used to evaluate the expression of genes involved in TLR signaling. Results (i.e. an algorithm expressing Ct of the gene of interest relative to the Ct of the housekeeping gene) for C57BL/6 BM-DC (Y-axis) were plotted against results for BALB/c BM-DC (X-axis). Genes that showed at least a 3-fold difference in expression between C57BL/6 or BALB/c BM-DC are indicated.
3. Differences in TLR gene expression between BM-DC from C57BL/6 and BALB/c mice. mRNA isolated from immature BM-DC was amplified by quantitative RT-PCR and Ct-values for each transcript were normalized to a panel of six housekeeping genes. These normalized Ct values are inversely correlated to the level of mRNA expression. Bars represent the normalized Ct values ± SD for BM-DC from 3 individual mice. Student’s t-test: \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

**Figure 2.**

**BM-DC from C57BL/6 and BALB/c mice differ in their response to probiotic bacteria and pure TLR agonists.**

**(A)** Production of IL-6, TNFα, and IL-12p70 by BM-DC from C57BL/6 mice, stimulated with various VSL#3 concentrations (CFU/ml) in the absence or presence of 1 µg/ml LPS.

**(B)** Synergistic induction of IL-12p70 and IL-23 depends on the genetic background of the bone marrow donor mouse. IL-12p70 and IL-23 secretion by BM-DC from C57BL/6 mice (white bars) or BALB/c mice (hatched bars) stimulated with bacteria, LPS or a combination of both, is shown as a percentage of cytokine levels secreted in response to LPS alone (C57BL/6: 138 pg/ml IL-12p70 and 117 pg/ml IL-23; BALB/c: 14 pg/ml IL-12p70 and 258 pg/ml IL-23) . Results represent the mean ± SD of 3 mice per group. Mann-Whitney U test: \* p<0.05, \*\* p<0.01, \*\*\* p<0.001. Data are representative for 2 to 3 independent experiments.

**(C)** Relative *Il12p35* and *Il23p19* gene expression by C57BL/6 BM-DC after stimulation with LPS (1 µg/ml), CpG (5 µg/ml), Pam3CSK4 (100 ng/ml) and poly I:C (50 ng/ml). Results are expressed as a percentage (mean ± SD of 3 individual mice) of mRNA expression, induced by stimulation with VSL#3 alone. Synergistic induction was only found with the combination of VSL#3 and LPS. Results were evaluated statistically using the Mann-Whitney U test; \* p<0.05.

**Figure 3.**

**Differences in gene expression between BM-DC from C57BL/6 mice and BALB/c mice.**

Clusterogram showing the supervised hierarchical clustering of genes related to TLR-signaling after stimulation of BM-DC with medium, LPS (1 μg/ml), VSL#3 (107 CFU/ml), or a combination of both. Relative expression levels for each individual gene are presented as minimum (green) and maximum (red). Columns represent gene expression profiles of BM-DC from C57BL/6 and BALB/c mice, 4 hours after stimulation (3 individual mice per strain and experimental condition).

**Figure 4**.

**VSL#3 and LPS show synergistic induction of a cluster of genes in BM-DC from C57BL/6 mice, but not from BALB/c mice.**

The expression level (i.e. Ctvalues obtained from Figure 3) of *Lta* (top), *Ifnb* (middle) and *Ifng* (bottom) in BM-DC from C57BL/6 or BALB/c mice was determined after stimulation with VSL#3 (107 CFU/ml), LPS (1 µg/ml) or a combination of both. Bar graphs represent the fold induction compared to unstimulated C57BL/6 BM-DC. Statistical evaluation was performed with the Student’s t-test; \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.

**Figure 5**. **Suppression of LPS-induced chemokine induction by VSL#3 and *L.plantarum*.**

1. mRNA expression level for *Cxcl9* and *Cxcl10* in BM-DC from C57BL/6 mice after stimulation with VSL#3, in the absence or presence of LPS (1 µg/ml). Data are presented relative (mean ± SD of three individual mice) to unstimulated cells. For statistical evaluation the Mann-Whitney U-test was used. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001. Data are representative for 3 independent experiments.
2. CXCL-10 production after 24 hours of stimulation was determined by multiplex assay. Mean concentrations ± SD measured in culture supernatants of BM-DC from 3 individual mice are presented. Mann-Whitney U-test; \*\*\* p<0.001.
3. Effect of *L.plantarum* and *B.lactis* on LPS-induced expression of *Cxcl9* and *Cxcl10* in BM-DC from C57BL/6 mice. Data represent mean fold change ±SD of three individual mice, compared to unstimulated cells. Results were evaluated statistically using the Mann-Whitney U test. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.
4. CXCL-10 production after 24 hours of stimulation was determined by multiplex assay.Mean concentrations ± SD in culture supernatants corresponding with three individual mice are presented. Mann-Whitney U-test; \*\* p<0.01.

**Supporting Figure 1.**

**BM-DC from C57BL/6 and BALB/c mice respond differently to TLR-ligands.**

Induction of key polarizing cytokines depends on the genetic background of the bone marrow donor mice. BM-DC were stimulated for 24 hours with 100 ng/ml Pam3CSK4, 50 μg/ml peptidoglycan (PG), 100 µg/ml poly I:C, 1 μg/ml LPS, 500 ng/ml Flagellin (Flag), 1 μM Imiquimod (Imiq), 5 μg/ml CpG or 107 CFU/ml VSL#3. Supernatants were evaluated by ELISA with regard to cytokine secretion for C57BL/6 mice (white bars) or BALB/c mice (hatched bars). Means concentrations ± SD of 3 individual mice are presented. Mann-Whitney U test: \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.