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1-12 *LACTOBACILLUS CASEI* STRAIN SHIROTA SELECTIVELY MODULATES MACROPHAGE SUBSET CYTOKINE PRODUCTION

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ABSTRACT: ***Probiotics confer health benefits through many mechanisms including modulation of the gut immune system. Gut macrophages regulate immune homeostasis, mounting tolerogenic responses to food and commensal bacteria or immune inflammatory responses to pathogens. Local environment and macrophage subset determine immune response and tolerance, associated with an M2-like phenotype and inflammatory activation with an M1-like phenotype. Subset predominance will determine immunomodulatory effects of probiotic species such as Lactobacillus casei strain Shirota (LcS). The aim of this study was to investigate differential regulatory effects of LcS on M1 and M2 macrophage subsets. PMA or vitamin D_3 differentiated THP-1 human monocytic cells were used to investigate heat-killed LcS and secreted protein immunoregulation of M1 and M2 cytokine production, respectively. Additionally, regulation of $CD14^{lo}$ M2 and $CD14^{hi}$ M1 function was investigated. Cytokine expression was measured by ELISA and NFkB activity by reporter assay. Both HK-LcS and SP-LcS augmented IL-1β, suppressed IL-6 and differentially regulated TNFα and IL-8, dependent on macrophage subset. HK-LcS and LcS-SP augmented $CD14^{hi}$ M1 TNFα whereas suppressed $CD14^{lo}$ M2 IL-6 and $CD14^{hi}$ M1 NFkB. In conclusion, LcS differentially regulates macrophage cytokines and NFkB activation, is subset-dependent and suggests a cautionary approach to probiotic treatment of mucosal inflammation***.

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13-16 *LACTOBACILLUS CASEI* SHIROTA MODULATION OF AMMONIA METABOLISM IN PHYSICAL EXERCISE

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ABSTRACT: ***Ammonia is a continuously produced metabolic toxic waste product. Decompensated liver disease patients have elevated ammonia levels and are prone to infection. Increased ammonia levels have been recorded following physical exertion. Mechanisms to control ammonia overspill are limited. Urea cycle disorders receive therapeutic phenylacetate to lower ammonia, captured as glutamine. Bacteria of the genus Lactobacillus synthesise phenylacetate and this may provide a useful mechanism to control ammonia overspill in athletes. A pilot study with male football players investigated the possibility that L. casei Shirota probiotic supplement could naturally generate phenylacetate, and contributes to ammonia removal through glutamine sequestration as phenylacetylglutamine via the kidneys. Volunteers were assigned to one of two groups: probiotic supplemented (x2/day) or controls. All subjects undertook an exhaustive exercise routine in a 9-station static exercise program. Urine samples were collected 4hr after the exercise program. The program was repeated after 1 month. Urinary phenyacetylglutamine and ammonia, corrected by creatinine levels, were measured. Expressed as the difference in urinary levels between the two sampling points. Phenylacetylglutamine was significantly increased in the probiotic group ($P<0.01$) ammonia***

levels were lower compared to the control group (P=0.064). Probiotic Lactobacillus supplementation may be useful in controlling exercise-generated ammonia.

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17-22 THE EFFECTS OF PROBIOTIC COMBINATION *LACTOBACILLUS RHAMNOSUS* GG AND *LACTOBACILLUS RHAMNOSUS* LC705 IN CYTOKINE AND CHEMOKINE RESPONSE IN HUMAN MACROPHAGES

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ABSTRACT: ***Mucosal tolerance is central to efficient gastrointestinal tract function, tolerating food and commensal bacteria, whilst maintaining immune responsiveness to pathogens. Mucosal macrophages play a pivotal role in tolerance; whereas in inflammatory bowel disease, dysfunctional macrophages lead to tolerance breakdown, whereby commensals perpetuate inflammation. Macrophage subsets however, determine effector function: M1s are pro-inflammatory whereas M2s are anti-inflammatory/regulatory. In addition to commensal bacteria, butyrate, a short chain fatty acid probiotic metabolite, may also modulate macrophage-mediated tolerance. The human monocytic cell line, THP-1, was used to investigate butyrate immunoregulation in M1 and M2 macrophages, generated by monocyte differentiation in the presence of PMA or vitamin D_3, respectively. Butyrate modulation of LPS- and PGN-induced TNFa, IL-1b, IL-10 and NFkB was measured by sandwich ELISA and reporter gene assay, respectively. Data indicated butyrate suppresses LPS- and PGN-induced monocyte and M2 production of IL-1b and TNFa, M1-induced TNFa and IL-10 but failed to modulate M1-induced IL-1b. Additionally, butyrate augmented M2 IL-10 production, LPS- and PGN-stimulated M1 and LPS-induced M2 NFkB activity but failed to regulate PGN-induced M2 NF-kB. In conclusion, butyrate differentially regulates macrophage cytokine production and NFkB activation, which is subset-dependent and suggestive of a cautionary approach to butyrate use in treatment of mucosal inflammation.***

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23-32 *LACTOBACILLUS FERMENTUM* NCIMB 5221 HAS A GREATER FERULIC ACID PRODUCTION COMPARED TO OTHER FERULIC ACID ESTERASE PRODUCING LACTOBACILLI

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ABSTRACT*: Ferulic acid (FA) is an antioxidant compound known to neutralize free radicals, such as reactive oxygen species (ROS). These free radicals have been shown to be involved in DNA damage, cancer and aging. The administration of FA, as an oral therapeutic is hampered by its absorption in the small intestine followed by its quick excretion. Colonic microbial enzymes have been shown to produce FA. In this article, selected Lactobacillus strains were screened for FA production by Ferulic Acid Esterase (FAE), as determined by the release of free FA from a natural substrate, ethyl ferulate (EFA). Using a MRS-EFA plate clearing assay, L. fermentum ATCC 11976, L. reuteri ATCC 23272 and L. fermentum NCIMB 5221 all showed clearance zones of 10mm in diameter, confirming FAE activity. Results show that L. fermentum NCIMB 5221 is the most efficient FA producing strain, producing 0.168±0.001mg/mL FA following 48 hours of incubation in 0.296mg/mL EFA. We also*

investigated the total antioxidant capacity of L. fermentum NCIMB 5221 when grown in culture. Results suggest that, due to its FA production, L. fermentum NCIMB 5221 has potential for use as a future therapeutic.

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33-38 EFFICACY OF *BACILLUS COAGULANS* STRAIN UNIQUE IS-2 IN THE TREATMENT OF PATIENTS WITH ACUTE DIARRHEA

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ABSTRACT: *The objective of this study was to evaluate the efficacy and safety of Bacillus coagulans strain Unique IS-2 in the treatment of patients with acute diarrhea. To this end, a total of 28 patients with acute diarrhea were included in a prospective, phase II clinical study upon obtaining consent and ethical committee approval. The trial was performed on patients of both sexes between 18 to 55 years of age and having ≥3 loose motions in last 24 hours for less than7 Days. All patients were assigned to receive capsule (containing 2 billion CFU of Bacillus coagulans strain Unique IS-2) two times a duration of 10 days. Efficacy was evaluated by assessment of duration of diarrhea (in minutes) and frequency of defecation (times per day), abdominal pain (3=severe; 2=moderate; 1=mild; 0=absent) and consistency of stool (1=normal, 2=hard, 3= semi liquid, and 4=loose). Safety aspects of capsule were evaluated by assessment of incidence, type of adverse events, physical examination, and clinical laboratory test values (CBC, SGPT, serum creatinine, stool routine and microscopy). Concomitant medications including rescue medications were monitored throughout the study. Efficacy assessment for duration of diarrhea, frequency of defecation, abdominal pain, consistency of stool and fever was done on 1, 3, 6 and 10 days. Mean values for, duration of diarrhea decreased from 35.60 ± 5.46 to 3.52 ± 2.69 min per day, frequency of defecation was decreased from 7.96 ± 3.89 to 0.76 ± 0.60 times per day, abdominal pain decreased from 3.16 ± 0.99 to 0.36 ± 0.49 and consistency of stool improved from 3.84 ± 0.55 to 1.00 ± 0.00. No significant change in safety parameters was observed during treatment. This trial demonstrates that utilization of B. coagulans Unique IS-2 strain is efficient and safe to treat the patients with acute diarrhea.*

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39-48 PROBIOTIC BACTERIAL DNA INDUCES INTERLEUKIN-10 PRODUCTION BY HUMAN DENDRITIC CELLS VIA TOLL-LIKE RECEPTOR-9

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ABSTRACT: *Purpose: Dendritic cells regulate immune responses to microbial products. We assessed effects of bacterial DNA in the clinically-effective probiotic preparation, VSL#3, on activation, cytokine production and T-cell stimulatory capacity of human dendritic cells. Methods: Whole blood and enriched dendritic cells were cultured with bacterial DNA in the presence or absence of Toll-like receptor antibodies. Expression of Toll-like receptor 9 and activation markers (CD40, CCR7) on $CD11c^+$ (myeloid) and $CD11c^-$ (plasmacytoid) dendritic cells was analysed by flow cytometry. Cytokine production was assessed by intracellular staining and ELISA. Activation of naïve allogeneic $CD4^+$ T-cells was measured in mixed leukocyte reactions. Results: Probiotic bacterial DNA activated plasmacytoid and myeloid*

dendritic cells as indicated by enhanced CD40 and CCR7 expression. Probiotic bacterial DNA enhanced interleukin-10 production by myeloid dendritic cells using pathways involving Toll-like receptor 9 and inhibited dendritic cells' ability to stimulate naïve T-cells. Conclusions: Probiotic bacterial DNA induces an immunoregulatory rather than a pro-inflammatory cytokine profile in dendritic cells, suggesting that beneficial effects of probiotic bacteria are mediated in part by their DNA. These results suggest that the release of bacterial DNA from the microflora as a result of physiologically occurring bacterial lysis may be involved in intestinal immune homeostasis.