Global Prebiotic Association Young Researcher Awards - Entry #337

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Please indicate which category you're applying for:

GPA Young Researcher Award for Fundamental Research (100 points possible)

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Please provide a summary of your research(limit 250 words)

Resistant starches (RS), acting as dietary fiber, have significant potential for immune system stimulation, providing a valuable strategy against antimicrobial resistant infections. Immune-stimulating food ingredients that can enhance the responses against pathogens are timely and necessary. RS type 3 (RS-3), having also prebiotic potential, can be produced with different physicochemical characteristics, i.e., chain length (DP), A- or B-type crystal, and polydispersity index (PI) to tailor their immune-stimulating effects. This can be achieved by activating human toll-like receptors (TLRs). Stimulating TLRs is the first line for pathogen recognition by the immune system. Moreover, antibiotics reduce the activity of TLRs, which could be prevented by food-mediated immune stimulation. We hypothesized that crystal type, DP and PI, alone or in combination, impact the recognition of RS-3 preparations by TLRs leading to different RS-3 immunomodulatory effects. We studied the activation of TLR2, TLR4, and TLR5 by newly synthesized RS-3 preparations with increasing DP, PI and crystal type. Our findings show a strong activation of TLR2-dependent NF-kB activation with PI <1.25 (narrow-disperse), DP 18 (mid-chain) as an A- or B-type crystal. Interestingly, TLR2 activation was higher with B-type than A-type crystal. We used molecular docking to illustrate the effect of crystal type on TLR2 binding. We showed that B-crystals facilitate

TLR2/1 dimerization, supporting the stronger effects of B-type crystals. RS-3 immune stimulation is predominantly TLR2-dependent, and activation can be tailored by managing crystallinity, chain length, and PI. These findings hold importance for infection prevention and recovery, and also for immune boosting in immunocompromised groups.

Please provide a summary of methods (limit 250 words)

We investigated the immune-stimulating effect in relation to the physicochemical properties of newly synthesized RS-3. RS-3 preparations with distinct chain lengths, crystal types (A or B), and polydispersity indices (PI) were enzymatically synthesized or obtained from debranched starch. Narrow-disperse (PI <1.25) RS-3 preparations were enzymatically manufactured with potato glucan phosphorylase and sucrose phosphorylase. Polydisperse preparations were produced by debranching with isoamylase. Crystallization was achieved by boiling and cooling down at 4 or 50 °C. The crystal types were determined through X-ray diffraction. A total of 6 narrow-disperse and 5 polydisperse preparations equivalent in crystal type and DP were tested for activation of cell surface TLRs. TLR2-, TLR4-, and TLR5-dependent NF-kB activation was evaluated in reporter cell lines (HEK-Blue) incubated with increasing RS-3 concentrations (0.5, 1, and 2 mg/mL). THP-1 reporter cells expressing different TLRs, were also used to evaluate RS-3-mediated NF-kB activation in immune cells. The TLRs activation was assessed by quantifying NF-kB/AP-1 activation through SEAP activity in cell culture supernatants. RS-3 preparations were also incubated with THP-1 cells (monocytes), and cytokine production (IL-8, TNFα, IL-1 RA) was quantified using a magnetic Luminex® Assay in cell supernatants. In silico predictions were obtained by molecular docking simulations between amylose-A/B crystals and TLR2. Docking simulations were performed using the HDOCK server, and the resulting complex structures were visualized and analyzed using Chimera software. Statistical analyses, including t-tests, ANOVA, and post-hoc tests, were performed using GraphPad Prism version 8.0, with significance set at $P < 0.05$.

Please provide a summary of your results (limit 250 words)

In this study, we found that mid-chain length (DP18) narrow-disperse (PI <1.25) in an A- and B-type crystal conformation, namely sG5-A and sG5-B, induced NF-kB activation in TLR2 expressing Hek-Blue cells. Interestingly, B-type crystal sG5 led to a higher activation than sG5-A type crystal. Moreover, a modest, yet significant activation of TLR4-mediated NF-kB was induced by B-type crystals with longer chain length. On the contrary, none of the studied RS-3 preparations activation TLR5. Furthermore, in THP-1-Blue cells and THP-1 monocytes from human origin, expressing an array of multiple TLRs, we showed that sG5-A and sG5-B induced a significant activation of NF-kB and associated cytokines, including IL-8, TNFα and IL-1RA. To gain further understanding of the role of crystal type in the binding to TLR2, we performed molecular docking analysis using Amylose-A and -B as representative of A and B-type crystal with equivalent DP and PI. Our predictions models indicate that both crystal types can bind to TLR2, but B-type crystal facilitate the dimerization of TLR2 with TLR1. This might explain the stronger response to sG5-B found in our in vitro experiments. We provided different predicted binding models of the potential interactions of amylose-A and amylose-B with human TLR2.

Please provide a statement about what, in your opinion, makes this paper outstanding and why it fits into the grant category you selected. (limit 250 words)

By using different in vitro experimental approaches and in silico analysis, this study shows that the physicochemical characteristics of newly produced RS-3, including DP, PI and crystal type, play a crucial role in the binding to TLRs. Particularly, the narrow-disperse sG5-A and sG5-B, with DP18 activated TLR2, suggesting that a combination of physicochemical traits are required for this effect on TLR2 and the activation of the associated pathway. This holds relevance in finding new immune-stimulating molecules that could be

instrumental to boost immunity in immunocompromised groups or during infections where TLR2 plays a role in bacterial clearance, the establishment of a fit immune response against pathogens and a faster recovery from infections. This could also help to reduce antibiotics requirements and tackle the ever-increasing antimicrobial resistant infections. Furthermore, this study provides evidence that modifying particular physicochemical properties of RS-3 during the manufacturing process can be instrumental to achieve different levels of responses depending on the requirements of specific disease-groups or different phases of the immune response. Noteworthy, the molecules here studied can be used in the food industry and consumers could incorporate these immune-stimulating molecules in their diet. It will be especially effective before and during taking antibiotics as it is known that some antibiotics downregulate TLR2 signaling. While our study delves into fundamental research on RS-3 immune stimulation, its translational potential is pivotal.

By typing your full name below and completing this application, you verify that you are the first author of this research and that this paper is original research.

Luis Silva Lagos