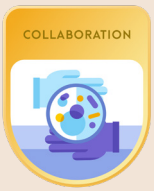
Enhancing innate immunity through gut microbiome-derived polysaccharide A



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The human body harbors a vast array of microorganisms such as bacteria, fungi, viruses, and other microbes collectively called the human microbiome. Diseases such as inflammatory bowel disease, rheumatoid arthritis, and immunodeficiency were all linked to dysregulated interaction between microbiome and host . Gut bacteria interact with cells in the intestine through the intestinal mucous layer, which is facilitated by polysaccharides produced by the bacteria. Past efforts to alter the intestinal immune response through a mix of commensal bacteria, probiotics, genetically engineered bacteria, or metabolites have not resulted in significant therapeutic benefits. Thus, we propose orally administering ingestible vesicles loaded with a known effector molecule, polysaccharide A (PSA). PSA produced by *Bacteroides fragilis*, a common gut bacteria, is shown to prevent intestinal inflammation in animal models. We propose to overexpress the gene responsible for producing PSA, produce the polysaccharide in vitro, and package it into vesicles for testing in mice. This approach is expected to increase the efficacy of PSA as the vesicles can fuse to intestinal mucosa and anchor the PSA for prolonged exposure. We expect this product to increase the human innate immune response through its secretion of crucial immunity mediating cells that act as the first defense against pathogens (Montalban-Arques 2018). We will assess the treatment effect on key intestinal innate immunity mediating cells such as intraepithelial lymphocytes and innate lymphoid cells. Once proven in mice, the same therapeutic approach can be adopted to humans to cure immune diseases or to enhance overall innate immunity.

Keywords: Innate immunity, microbiota, immunity, polysaccharide A, *Bacteroides fragilis*

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Watch a video introduction by the authors at https://youtu.be/nhemYcK-gaI

Background

Bacteria, fungi, viruses, and other microorganisms that live on and inside the human body, collectively known as the human microbiome,are the largest invisible organs of the human body. In fact, there are more microbial cells in the human body than human cells. It is estimated that 10-100 trillion bacteria of the total microbiota primarily live in the gut (Ursell et al., 2012). Research over the last decade has shown the key role these gut bacteria play in the health and disease of the hosting individual (Jiao et al., 2020). Autoimmune diseases such as rheumatoid arthritis and spondyloarthritis were all linked to the dysregulated microbiome and host interaction (Jiao et al., 2020).

Gut bacteria interact with intestinal cells through the intestinal mucous layer which is facilitated by molecules known as polysaccharides secreted by the gut bacteria. Although the human intestinal microbiota has more than 2172 bacterial species, *Bacteroides fragilis* is an important member of the gut microbiota that protects animals from experimentally induced colitis (Masmanian et al., 2008). This beneficial activity requires the immunomodulatory molecule PSA as *B. fragilis* largely depends on its highly complex and dynamic capsular PSA for its symbiotic interactions with the host. PSA is a zwitterionic polysaccharide that is made of repeating tetrasaccharide units each consisting of a positively charged amino group and a negatively charged carboxylate group (Ertuk-Hasdemir et al., 2019).

Bacterial capsules are extracellular structures typically composed of polysaccharides that are located outside the cell envelope. Most bacterial capsular polysaccharides are not zwitterionic thus making PSA very unique.

Past studies using murine models have demonstrated that *B. fragilis* and its produced PSA are effective in inhibiting colitis and experimental allergic encephalomyelitis (Wu & Wu, 2012). PSA induces regulatory T cells secreting the anti-inflammatory cytokine IL-10, which prevents pathogenic inflammation in the gut (Ramakrishna et al., 2019). PSA from *B. fragilis* was reported to protect against colorectal cancer (CRC) via TLR2 signaling as the immunomodulator inhibited CRC cell proliferation by controlling the cell cycle and impeding CRC cell migration and invasion (Sittipo et al., 2018). PSA was also shown to direct the maturation of the developing immune system in mice, including correction of systemic T cell deficiencies and Th1/Th2 imbalances in lymphoid tissues (Zheng et al., 2020).

Systems Level

We propose testing orally ingestible vesicle suspensions loaded with the immunomodulatory molecule PSA. By using a suspension, PSA's molecular structure is preserved avoiding further degradation as compared to a capsule or tablet in which excipients are generally added which could act to lower PSA potency. This approach aims to increase the efficacy of the molecule as the vesicles can fuse to the intestinal mucosa and anchor the PSA for prolonged exposure. A longer PSA exposure is expected to elicit the beneficial effects of the bacteria by inducing intraepithelial lymphocytes and innate lymphoid cells without the bacteria (Figure 1). PSA treatment that fuses through its vesicle is also expected to avoid a host immune reaction when the bacteria is present as a whole organism (Figure 1). Additionally, by using PSA vesicles, degradation in the stomach is avoided. Vesicles are expected to survive stomach acid as stomach acid mostly acts on water-soluble structures rather than the oily PSA vesicle.

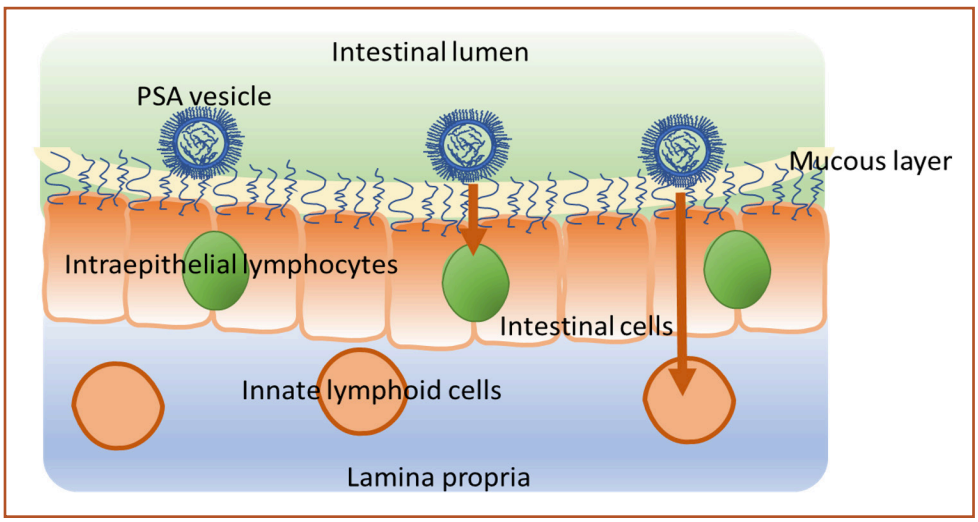

Figure 1. *This construct is the proposed mechanism of action for the PSA vesicles. The engineered vesicle contains PSA which will induce the production of intraepithelial lymphocytes and innate lymphoid cells*

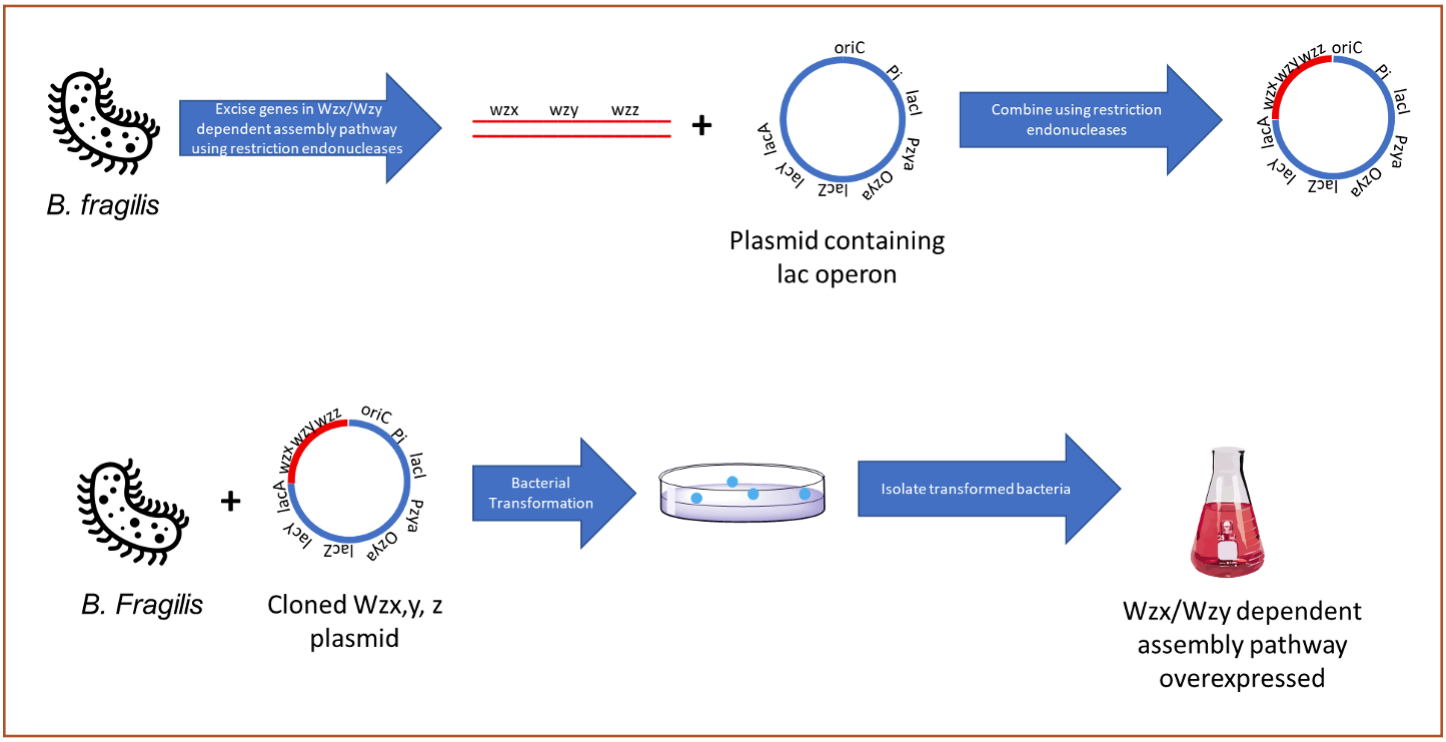

Figure 2. *Inducing overexpression of PSA in B. fragilis. This construct is composed of four genetic parts, further explained in the parts level.*

Device Level

Using the Wzx/Wzy-dependent assembly pathway known to synthesize bacterial polysaccharides, the genes Wzx flippase, Wzy polymerase, and Wzz chain-length regulator proteins will be excised and loaded onto a plasmid (Zeidan et al., 2017). Adding this plasmid to the bacterial culture will then be assessed to determine if the culture can successfully digest lactose (Figure 2).

Parts Level

Our proposed system will utilize the *B. fragilis* strain NCTC9343 (Microbiologics, 2021). PSA in *B. fragilis* will be overexpressed by the Wzx/Wzy-dependent assembly pathway which contains the genes Wzx flippase, Wzy polymerase, and Wzz chain-length regulator proteins (Islam & Lam, 2014). We propose excising the Wzx/Wzy-dependent assembly pathway from the *B. fragilis* genome using PCR and restriction endonucleases. Then, we will also load the Wzx, Wzy, and Wzz genes onto a plasmid containing the lac operon and appropriate restriction endonucleases and ligases such as EcoRV and DNA ligase. This engineered plasmid will then be added to the overnight *B. fragilis* culture which does not have the ability to digest lactose (Parker & Smith, 2012) (Figure 2). Properly transformed *B. fragilis* will have the ability to metabolize lactose. Successfully transformed bacteria will be isolated on agar plates using the chemical compound X-gal, which will turn the bacteria blue if the lac operon is functional. The transformed cells can therefore overexpress the stronger and inducible lac operon promoter coupled Wzx/Wzy-dependent pathway when lactose is present in the growth media which will then lead to excess PSA production.

PSA overexpressing bacteria will be grown in a fermenter in brain heart infusion (BHI) broth. Bacterial pellets will be harvested, and soluble material will be isolated by phenol/chloroform extraction. Nucleic acids and proteins will be digested with DNase/RNase and Pronase K, respectively. PSA will be purified using column chromatography. The purified PSA will be dissolved in phosphate- buffered saline (PBS) to obtain a stock solution of 1 mg/mL. Phosphate-buffered saline (PBS) will be used as a control treatment for this study. The molecular structure of PSA will be confirmed by Nuclear Magnetic Resonance (NMR) analysis. Pure PSA will be encapsulated into lipid vesicles.

Safety

Lipid vesicle suspensions containing PSA or a phosphate-buffered saline (PBS) placebo will be administered

orally in mice for 8 weeks. Animals will be sacrificed after 8 weeks, and intestinal cells will be harvested and analyzed for intraepithelial lymphocytes and innate lymphoid cells. Intraepithelial lymphocytes and innate lymphoid cells will be isolated and characterized through their markers, and induction will be measured by flow cytometry. PSA-treated cells are expected to have a higher number of lymphocytes and lymphoid cells which in turn should enhance innate immunity. We will also assess IL-10 gene induction and protein levels – the IL-10 gene prevents pathogenic inflammation in the gut (Ramakrishna et al., 2019). Induction of IL-10 will be analyzed by real-time PCR (qPCR) with primers specific to cDNA of IL-10. Intestinal cell IL-10 levels will be assessed by the IL-10 ELISA kit. IL-10 gene and protein levels are expected to increase in animal-treated cells conferring anti-inflammatory effects.

The methods used are all BSL-2 standard lab procedures and standard aseptic safety protocols will be followed for all experiments. All live bacteria will be autoclaved prior to dispensing. More importantly, *B. fragilis* is a commensal bacterium in the human colon and is not pathogenic. PSA produced by *B. fragilis* is a naturally occurring polysaccharide with no known toxicity. There are no known hazardous chemicals or pathogenic biologic drugs administered to mice.

Discussions

The discovery of microbiome-host interactions within the last decade presents an opportunity to address various immune diseases and raise innate immunity in general. The field of synthetic biology enables us to utilize the principles of molecular biology and metabolic engineering to design biological circuits that can beneficially influence the host response. Our approach eliminates the need to use whole bacteria, genetically engineered bacteria, or even bacterial metabolites. Using a naturally occurring molecule in a lipid vesicle suspension is a key advantage of this approach. Once proven effective in mice, the same therapeutic approach can be adopted to humans to cure immune diseases or to enhance overall innate immunity.

Next Steps

With the completion of our background research, we would need to run a variety of tests to determine if our selected Wzx/Wzy-dependent assembly pathway will be effective to increase PSA production. After further testing is completed and confirms this approach will work, it could be developed into the therapeutic we hope to create.

In a high school environment, we recognize that there are evident limitations in regards to how far we can investigate. Although we may not be able to conduct advanced experimentation, we hope that, with the help of our mentors, we could find resources to help further our research. By using concept experimentation and the equipment that we can access, we hope to be able to integrate our ideas into real life to help alleviate and provide a cure for immune diseases.

Author Contributions

K.D. took the lead in creating the diagrams and centering the project's focus on the immunomodulatory molecule PSA. A.M. contributed to researching and providing feedback. A.M. also provided a critical role in the revising and citations process.

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