Review

Could certain strains of gut bacteria play a role in the prevention and potential treatment of COVID-19 infections?

Christine Bishara, MD[1*], Françoise Sidime, PhD[2], Jon Bass, MD[3], Jonathan Blaize, PhD[4]

[1]From Within Medical, New York, NY, 10010
[2]Helene Fuld College of Nursing, Department of Science, New York, NY, 10035
[3]Summit Medical group, Berkeley Heights, NJ, 07922
[4]Wagner College, Department of Biological Sciences, 1 Campus Road, Staten Island, NY, 10301

***Correspondence:** Christine Bishara, MD, From Within Medical, New York, NY, 10010
Email: info@fromwithinmedical.com

ABSTRACT

It has been established that high risk groups are more vulnerable to COVID-19 infections and succumb to the illness more frequently than those in the general population. The pertinent risk factors (age over 65, diabetes mellitus, insulin resistance & obesity (BMI >30)) contribute to poor clinical outcomes and are aligned with chronic inflammatory disorders. Inflammation is regulated by the immune system and compelling evidence suggests that certain gastrointestinal flora act as anti-inflammatory mediators. With few exceptions, children have been spared from severe COVID-19 related illnesses. Recent studies reveal that children (age<10) have elevated gut concentrations of a specific microbe, Bifidobacterium, which diminishes significantly with age. This reduction contributes to immune dysregulation and correlates to pathological onset of the forenamed conditions. Thus, we predict that Bifidobacterium functions as a key immunoprotective agent and prevents complications associated with COVID-19. The following review is offered to : 1) Better define the relationship between Bifidobacteria concentration and immunoprotection; 2) Consider how fluctuation in certain bacterial strains affects the young and high-risk populations; 3) Consider the potential mechanisms that negatively impact immune status in those who are high-risk; 4) Provide evidence that administration of Bifidobacteria leads to improvement of numerous medical conditions while suggesting its administration is a viable option for prevention and treatment of COVID-19 infections. (Am J Transl Med 2020. 4:75-94).

Keywords: COVID-19, interleukin, Bifidobacterium, Cytokine Storm, Gut

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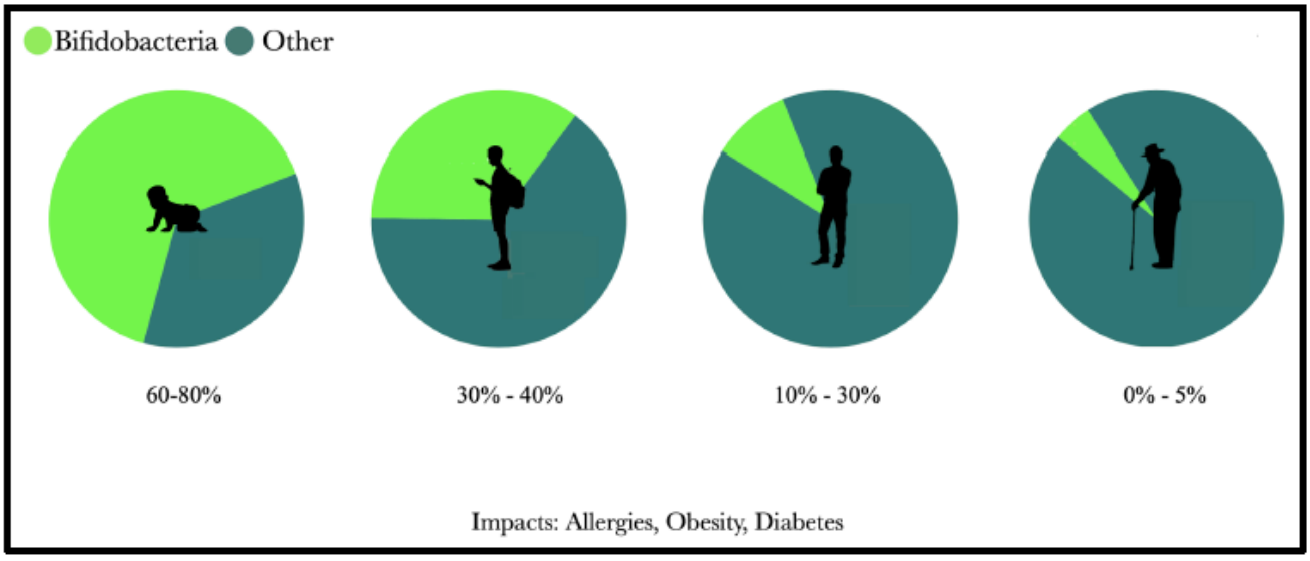
INTRODUCTION

The vast majority of children and adolescents infected with the COVID-19 virus have had minimal or mild symptoms. Aside from the vulnerable elderly population and those with comorbidities, current observations of hospitalized patients reveal that more than 60% of adults being treated in the ICU are either diabetic, insulin resistant or have a BMI over 30. According to GlobalData Healthcare, obesity is the most prevalent underlying condition in 18-64 year olds hospitalized with COVID infections.

What is causing this protective immunity afforded to the young? Is the presence of higher levels of Bifidobacterium in the gut microbiome of healthy children and young adults providing this protection? The role of Bifidobacterium as an immunoprotective agent is significant in this context since it has been shown to regulate a number of pro and anti-inflammatory cytokines including interleukin-6 (IL-6) and interleukin-10 (IL-10), respectively. IL-6 is well characterized as a pro inflammatory mediator, with chronic presence indicating a less favorable role in overall host immunity. IL-6 levels are known to be higher at baseline in the high risk group noted and represent a subclinical, chronic inflammatory state. IL-6 is among the interleukins shown to initiate the hyper exaggerated response seen in the "cytokine storm" present in many hospitalized COVID-19 patients. The use of IL-6 inhibitor medications has shown positive results as has hydroxychloroquine, which also appears to moderately inhibit IL-6 (Jang et al., 2006). Furthermore, the recent application of histamine 2 blocker medication, Famotidine (Pepcid) works to block the production of histamine, a known inducer of IL-6 synthesis by mast cell stimulation.

Our literature findings will illustrate the immuno-protective capacity of Bifidobacterium observed within young persons via appropriate modulation of inflammatory markers including IL-6, IL-10, interferon -γ (IFN-γ) and (TNF-α), while concurrently demonstrating that diminished concentrations of the forenamed microbe in high risk and aged populations



(Figure 1: Variations of Bifidobacterium concentrations among age groups Bifidobacterium levels range up to 80% concentration compared to other bacteria in children. The level of this bacterium declines with age (Arboleya, et al., 2016; Turroni, *et al.* 2012; Voreades, et al., 2014).

correlates to disease vulnerability.

The role of Bifidobacterium in children and adults

A comparison of gut species of children and adults shows a significant disparity between the percentage of Bifidobacterium species among the age groups. Overall, Bifidobacterium levels decline with age, becoming significantly reduced in the adult population and virtually absent in the high-risk groups mentioned (**Figure 1**) (Voreidas et al., 2014). Estimates of the total Bifidobacterium percentage in the gut microbiome of children ranges from 60-80% compared to 20-40% in adults and less than 10% in elderly and high-risk groups, with some as low as 1%.

Equally evident is the difference in the specific strains among the age groups. B. longum, B. infantis, B. catenulatum, and B. breve are the abundant species in babies and healthy children (Arboleya et al., 2019; Turroni et al., 2012). B. breve is highest in breast fed infants and has shown significant anti-inflammatory properties (Lawson et al., 2018). B. infantis has also shown significant positive immunoprotective effects (Henrick et al., 2019).

The majority of the adult gut Bifidobacterum, on the other hand, shows less colonization of these strains and is mainly colonized by B. angulatum and B. adolescentis (Arboleya et al., 2019). Many studies have linked age related changes in microbiota to decreased immunity (Sovran et al., 2019). Of particular interest, in a study of Chinese and Italian centenarians, the level of Bifidobacterium was higher than that

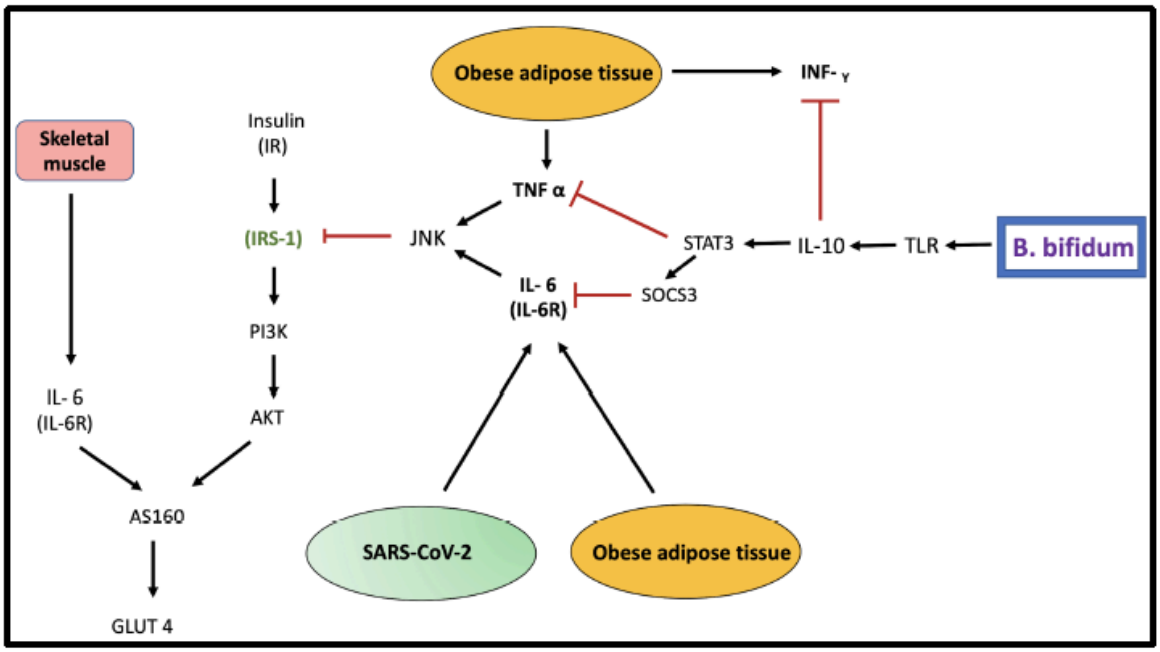


Figure 2: Bifidobacterium breve suppresses the expression of IL-6, INFγ and TNF α : IL-6 is acted upon by several signaling molecules, triggering the inflammatory response seen amongst COVID, SARS and MERS patients. Comorbidities such as obesity impede recovery from COVID as excessive adipose tissue contributes to elevated serum IL-6, augmenting inflammation. Bifidobacterium breve is believed to activate select TLRs which promote IL-10 synthesis and secretion. In turn, IL-10 receptor activation prompts nuclear translocation of STAT3 to promote expression of suppressor of cytokine signaling 3 (SOCS3), a known inflammation signal inhibitor.

found in the younger elderly population, suggesting that longevity may be partly attributed to a higher level of this flora. (Zhao et al., 2011). Diet has been implicated as an important factor driving gut microbe selection, though many other variables including genetics are likely to play a role.

The Role of IL-6 on the immune response

Interleukins are a subset of the larger group of cytokines which act as cellular messengers. They are secreted in response to a stimulus such as an infectious agent or a perceived intruder in the body. There are approximately 18 subtypes and while the roles of each is complex, there has been an established link of elevated subclinical levels of IL-6 in the high-risk group mentioned, indicating a chronic inflammatory state (Eder et al., 2009). In recent years, IL-6 has also been implicated in pathological processes associated with a number of autoimmune and inflammatory disorders. Studies have demonstrated a correlation between elevated levels of IL-6 in the serum, various tissues and disease activity in inflammatory conditions (Nieto-Vasquez et. al, 2018; Schett et al., 2018) (**Figure 2**). IL-6 has been established as an inflammatory mediator present in adipose tissue. The presence of these inflammatory mediators has reflected severity of disease and predicted future events. The results of a study (Roytblat et al., 2000) indicated that serum IL - 6 levels were significantly increased in obese patients with and without obesity hypoventilation syndrome (OHS) or Obstructive Sleep Apnea Syndrome (OSAS) (Eder et al., 2009; Khoaidair et al., 2004).

Table 1: Effects of Bifidobacterium on various conditions

Species/strains tested	Population	Observations	Reference
Interventional studies			
B. lactis BB12	Healthy adults	A four-week consumption of yogurt with this strain led to a decreased expression of TNF-α.	Meng, et al., 2017
B. lactis Bi-07	Healthy elderly adults	Four-week consumption showed increased phagocytic activity of monocytes and granulocytes.	Maneerat, et al., 2014
B. breve B-3 powder	Obese individuals	Administration of oral B breve led to a significant decrease in BMI after 8-12 weeks.	Minami, et al., 2018
B. longum BB536	Elderly individuals	Administration to individuals over 65 years of age alongside influenza vaccinations resulted in stimulation of neutrophil phagocytes, natural killer cells, immunoglobulin A secretion and a reduction in flu symptoms.	Akatsu, et al., 2013; Namba, et al., 2010
B. infantis EVC001	Infants	Administration led to decrease in levels of pro-inflammatory cytokines,calprotectin and endotoxin,reducing intestinal inflammation.	Henrick, et al., 2019
B. lactis HN019	Patients with metabolic syndrome	Decrease in TNFα and IL6 as well as lower BMI and cholesterol levels. These all decrease risk factors for Coronary artery disease.	Bernini, et al., 2016
B. breve BR03 and B. breve B632	Cystic fibrosis/children	The two strains were given for 90 days and led to reduced proinflammatory marker TNF-α.	Klemenak, et al., 2015
B. breve M-16V and B. longum BB536	Pregnant females one-month prior delivery and infants in first 6 months of life	Decreased incidence of eczema.	Enomoto, et al., 2014
B. longum BB536, B. infantis M-63, B. breve M-16V mixture	Children with allergic Asthma	Improvement of symptoms after 4 weeks of the probiotic combination.	Miraglia Del Fiudice, et al., 2017
B. longum 35624	Ulcerative Colitis, Psoriasis and Chronic fatigue Syndrome diagnosed patients.	Levels of CRP, TNFα, and IL6 decreased following an eight-week course.	Groeger, et al., 2013
B. longum CECT 7347	Children with celiac disease	Reduction of peripheral CD3+ lymphocytes, TNFα, and sIgA in stools after 90 days.	Olivares, et al., 2014
Observational studies			
Bifidobacterium spp. and B. adolescentis	Adults with allergic asthma.	Adults with long term asthma symptoms show reduced levels of B. adolescentis.	Hevia, et al., 2016
B. pseudocatenulatum	Gout patients	B. pseudocatenulatum depletion in this group.	Guo, et al., 2016

Importance of the role of IL-10 in suppression of inflammatory cytokines

IL-10 is an important anti-inflammatory cytokine. Reduced serum IL-10 and or IL-10 insensitivity has been linked, repeatedly, to physiological disruption of the gastrointestinal and endocrine systems, manifesting as inflammatory bowel diseases and diabetes, respectively (Esposito et al., 2005; Burmeister & Marriot, 2018; Couper et al., 2008; Shouval et al., 2014; Shukla et al., 2018). Clinical trials demonstrate the therapeutic efficacy of administering IL-10 to mitigate inflammation brought on by dermatological lesions and arthritis. Schmulson et al show that low IL-10 concentration is a reliable predictor of inflammatory bowel syndrome (IBS) (Schmulson et al., 2012). These findings are supported by laboratory mutagenesis experiments where spontaneous enterocolitis can be produced in double negative IL-10 mice (Shouval et al., 2014). Autoimmune disorders such as multiple sclerosis is exacerbated when IL-10 activity is compromised, demonstrating its importance within the central nervous system (Ozenci et al., 1999). While IL-10 contributes to many immunomodulatory behaviors, including those that are immunostimulatory, these findings support the hypothesis that this pleiotropic cytokine is a necessary mediator of anti-inflammation and overall health. IL-10 inhibits proinflammatory lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), interleukins 1**β**, 12, 6 (IL-1**β**, IL-12 and IL-6), cyclooxygenase (COX2), matrix metalloproteinases (MMPs) and IFN-**γ** through activation of the phosphoinositol 3 kinase (PI3K) and Janus kinases/signal transducer and activators of transcription (JAK/STAT) pathways (Chang et al., 2019: Moore et al., 2001; Williams et al, 2003; Bousoik et al., 2018; Cianciulli et al., 2016; Furman et al., 2017; Martin et al., 2003).

IL-10 is a member of the type-II cytokine family expressed by immune system cells (B, T, monocytes, macrophages and dendritic) and intestinal epithelia that support mucosal homeostasis (Shouval et al., 2014; Medzhitov et al., 2010). Activation of the IL-10 receptor (IL-10R) causes phosphorylation of the transcription factor STAT3, enabling nuclear translocation and expression of protein suppressor of cytokine signaling (SOCS3) (**Figure 2**).

SOCS3 is the primary regulator of IL-6 activity and inhibits this pro-inflammatory cytokine upon recognition of its receptor (IL-6R). Support of SOCS3's role in suppressing inflammation has been well documented, and mounting evidence illustrates STAT3 contributions to virtually all anti-inflammatory events induced by IL-10 receptor activation (White et a., 2011; Williams et al., 2003; Babon et al., 2015; Ahmed et al., 2000; Antoniv et al., 2011; Okada et al., 2009). For example, a recent study evaluating the anti-inflammatory effects of Benznidazole during treatment of Chagas disease revealed that inhibition of SOCS3 with siRNA abolishes protection offered by the anti-parasite drug (Cevey et al., 2019; Eder et al., 2009). Anti- inflammation is partially achieved via non-enzymatic allosteric regulation of the IL-6 by SOCS3, whereby the latter binds the gp130 subunit of the IL-6 receptor (Cevey et al., 2019). IL-6 activation of downstream, pro-inflammatory cascades (including STAT3) is inhibited in a time dependent fashion and the paradoxical, contradictory behavior of the forenamed signalling molecules appears to be significantly influenced by timing. Additionally, SOCS3 upregulation blocks the regulatory transcription factor NF-KB from binding DNA domains of pro- inflammatory genes (Cevey et al., 2019).

The role of Bifidobacterium in immune modulation and immunoprotection

A 2009 study (Zhang et al., 2013) investigated the role

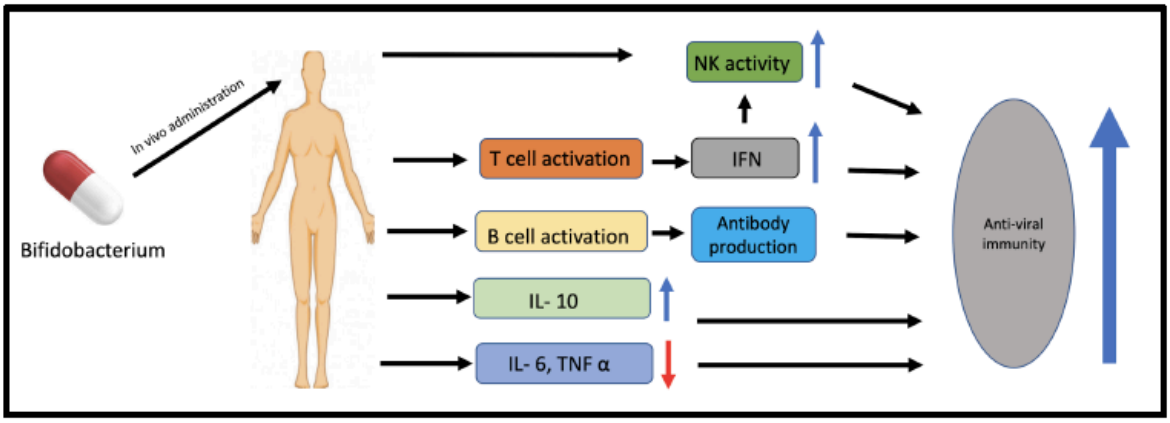


Figure 3: Administration of Bifidobacterium increases antiviral activity. The administration of Bifidobacterium has been reported to activate various immuno-protective pathways. T cell activation increases IFN, subsequently increasing NK cell activity. Antibody production rises as a result of B cell activation. Suppression of both IL-6 and TNF-α along with stimulation in the production of IL-10 have collectively augmented antiviral activity.

of administration of viable Bifidobacterium supplement to patients undergoing colon cancer resection and examined its effect on the gut flora, inflammatory response and prognosis of these patients. Sixty patients with colorectal cancer were randomized into a treatment group (n=30) and control group (n=30). Patients in the treatment group received oral viable Bifidobacterium with routine enteral nutrition and patients in the control group received only routine enteral nutrition. The intestinal flora of stool was analyzed and stool IgA (SIgA), serum IgG, IgM, IgA, IL-6, and C-reactive protein (CRP) were detected. Post-operative (9 days) stool samples returned higher SIgA in the treatment group, while serum IgG, IgM, IgA IL-6, CRP in the treatment group were lower, signifying a decreased inflammatory state.

In a 2019 study at UC Davis, researchers found that infants who received B. infantis produced significantly lower levels of inflammatory markers, compared to infants in the control group. B infantis was shown to have the following benefits:

- Production of short chain fatty acids. Infantis digests human milk oligosaccharides (HMO ' s), producing short-chain fatty acids which provide energy and help control yeast and fungus growth.

- Strengthening gut integrity. Infantis allows gut cells in infants to produce proteins that fill gaps between intestinal cells. These gaps can be dangerous as they may allow toxins to get into the bloodstream.

- Replacing bad bacteria in the gut. Infantis consumes HMOs, thereby usurping the space in the gut so potentially dangerous bacteria cannot multiply and cause problems. (Henrick et al., 2019)

Additionally, Bifidobacteriaceae, specifically gut colonization of B infantis in infancy, has been shown to improve the development of the host immune system by enhancing CD4 T cell responses (Henrick et al., 2019). The following table by Ruiz, et al., (**Table 1**) highlights the vast impact of bifidobacteria on the immune response.

Potential immunoprotection by B. breve through Toll-like receptor activation

Toll-like receptors are a family of immunostimulatory

proteins. The complete molecular underpinnings of B. breve mediated support are unknown; however, its capacity to engage members of the forenamed family are worthy of further clinical and empirical investigation. B. breve surfactants and supernatant have been shown to engage TLR upstream of both IL-6 and TNFα, two cornerstones of the inflammatory response (Plantinga et al., 2011; Citar et al., 2014; Fujie et al., 2011; Hoarau et al., 2006, 2007). Plantinga et al. demonstrate that induction of TLR9 and inhibition of TLR2 by breve resulted in elevated production of IL-10, the universally accepted inflammation suppressing cytokine, accompanied by maturation and survival of dendritic cells, in vitro (Plantinga et al., 2011; Butcher et al., 2018). Enhancement of IL-10 has been achieved by others simulating various microenvironmental conditions, including a study conducted by Yonagawa who was able to re-stimulate cultured dendritic cell TLR to augment IL-10 production (Yanagawa & Onoe, 2007; Abreu, et al., 2003; O'neill, et al., 2008, Teixeira-Coelho, et al., 2013).

The inherent antiviral properties associated with elevated interferon expression are also well documented. These chemokines, particularly type-I family IFNs, block viral replication, activate natural killer cells, and contribute to suppression of systemic inflammation making them an appealing therapeutic model (**Figure 3**). Interestingly, lactis induced activation of plasmacytoid dendritic cells (pDC) was observed in the volunteers benefiting from H1N1 protection described previously (**Figure 4**). pDC's are key regulators of the immune response that contribute to pathogen recognition via TLR activation. Kanauchi et al., specifically describe the activation of TLR9 by microbial DNA CpG motifs, and TLR7 activation by microbial RNA or analogous synthetic guanosine (Akatsu et al., 2013; Kanauchi et al, 2013); their evidence demonstrating lactis stimulation of these TLRs suggest a non-pathogen related mechanism for elevating immunoprotective agents. This represents one of many potential pathways of probiotic mediated protection, we propose a similar mechanism for Bifidobactierum in this review.

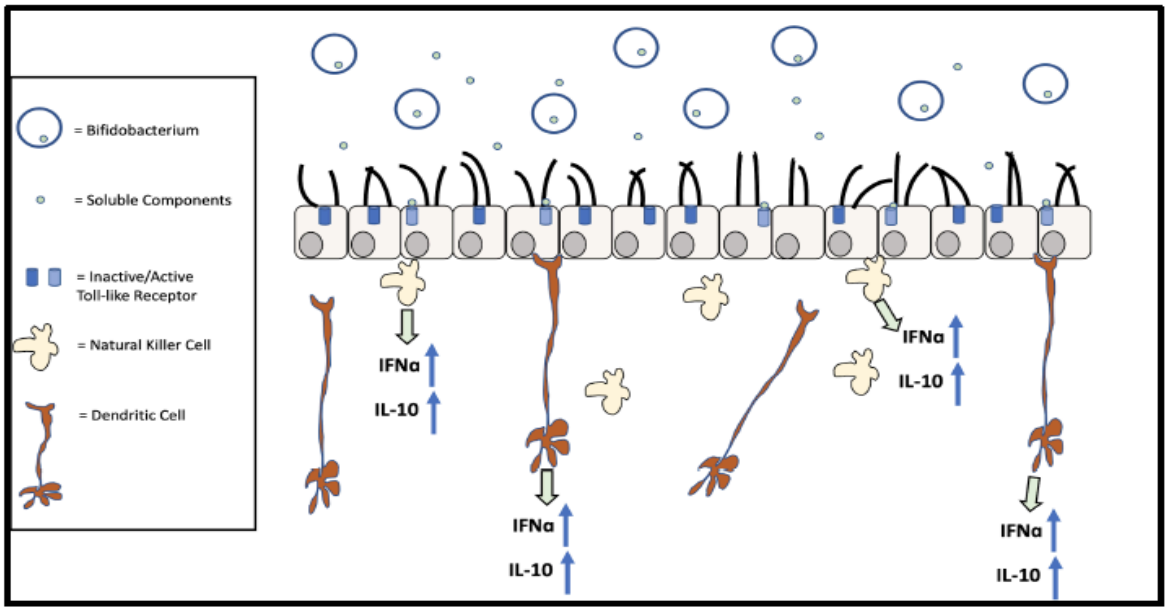


Figure 4: Microbial surfactant and supernatant activate toll-like receptors Bifidobacterium stimulates dendritic cells and macrophages to produce anti-inflammatory cytokines IL-10 and IFN-α, *in vitro*.

The role of B. bifidum in decreasing Pro-inflammatory markers and increasing anti-inflammatory markers in intestinal mucosa

Many microbes including B.breve are shown to quickly trigger induction of IL-10 which subsequently suppresses IL-6 to promote a beneficial anti-inflammatory state (Sanin et al., 2015, Peres et al., 2013) (**Figure 2**). For example, Bifidobacterium animalis taken from colonic and ileal mucosa exhibits simultaneous downregulation of IL-6 and IL-12 alongside upregulation of IL-10 (Citar et al., 2014) The synergistic action of IL-10 and IFN-1α when induced by TLR, particularly within intestinal epithelia and antigen presenting cells, represents a potential molecular chain of events that confers or (at minimum) delays pathological onset.

The role of gut health in the obese and prediabetic/diabetic population and relationship to immune response

The use of B. breve has been investigated as a therapeutic option in certain pediatric ailments including but not limited to necrotizing enterocolitis and has been strongly supported by significant and solid outcomes. Its use is not limited to pediatric supplementation, but it is also involved in improving many health and weight related conditions and associated liver disease.

The Minami laboratory investigated the use of B. breve B-3 at a daily dosage (Minami et al., 2018) of 5×10^{10} CFU/capsule for 12 weeks in adults with a tendency for obesity. A significant decrease of the fat mass and an improvement of blood parameters were observed. In particular, markers of liver injury and high-sensitivity C-reactive protein were significantly reduced (Minami et al., 2018) Another study demonstrated that a high fat diet changed the intestinal microbiota composition; in particular, the number of Bifidobacterium spp. was reduced (Kalliomaki et al., 2008). It also observed that fecal Bifidobacterium spp. counts were higher in children who remained at normal weight at the age of seven, while this was not the case in overweight children.

The role of Bifidobacterium in asthma, obesity, influenza and other viral infections

Numerous public datasets that showcase the use of Bifidobacteria as probiotics for therapeutic purposes are available. At the time of this report, more than 50 clinical trials were recruiting participants to continue studying the aforementioned tandem (Clinicaltrials. gov).

In addition to findings related to B. breve, lack of B. infantis, another principal gut microbe in children, can contribute to gut dysbiosis which has been linked to chronic health conditions such as obesity and diabetes (Henrick et al., 2018).

Respiratory and GI infections

Bifidobacterium strains belonging to the animalis (BB-12 strain) and longum species were shown to increase the immune response and decrease duration of disease in children hospitalized with rotavirus induced diarrhea (Chau et al., 2015). One study noted that Bifidobacterium breve UCC2003 which contains a cell surface exopolysaccharide (EPS) is able to play an important role in immunomodulation in B cell response. B. breve YIT4064 strain, taken from the feces of a healthy breast-fed infant, was orally administered to mice inoculated with an influenza virus and was able to increase anti-influenza virus IgG levels in serum, thus protecting mice against infection. The study concluded that the oral administration of this strain may enhance antigen-specific IgG against some

pathogenic antigens and lead to protection against various viral infections (Yasui et al., 1999). Bifidobacterium longum strain (BB536) shows numerous immunoprotective properties. Oral and intranasal administration of B. longum to influenza infected murine conferred cellular immunity and diminished traditional influenza symptoms (Iwabuchi et al., 2009-2010). This finding was supported clinically through administration of the same strain to aged persons alongside influenza vaccinations resulting in stimulation of neutrophil phagocytes, natural killer cells, immunoglobulin A secretion and a reduction in influenza symptoms (Akatsu et al., 2013; Namba et al., 2010). Host immune response within the elderly population is generally weaker, compromising vaccine efficacy, thus increasing their vulnerability to infection. Taken together, BB536 may enhance resistance to pathogens amongst those above the age of 65, and holds promise as a supplement to influenza vaccines (Wong et al., 2019) (**Table** 1).

Asthma

B. breve MRx0004 isolated from feces of healthy humans also exhibited protective benefits in a severe asthma condition. Following administration of B. breve MRx0004, one study showed a decrease of both neutrophil and eosinophil infiltration in lung bronchoalveolar lavage fluid in a mouse model with severe asthma (Cionci et al., 2018). This result demonstrated a reduction of pro-inflammatory cytokines that are involved in neutrophil mobilization, proving that B. breve MRx0004 was an effective option in reducing the aforementioned inflammatory condition and can be considered as a viable treatment alternative of management of severe asthma (Raftis et al., 2018).

Another study (Akay et al., 2014) aimed to compare the fecal Bifidobacterium species of children with allergic asthma to those of healthy children. Stool samples were obtained from 99 children between 0 and 3 years of age who showed clinical symptoms of allergic asthma and other forms of atopy. Samples were also obtained from 102 healthy children of similar age and sex. Bifidobacteria were isolated by culture and identified at the genus level by API 20 A. Bifidobacterium longum was detected in 11% of the allergic children and in 30% of the healthy children. Statistical analysis revealed a significant difference in the prevalence of B. longum between these two groups ($X2: 11.2$, $p < 0.001$). The significant difference in the presence of B. longum in the two groups suggests that perhaps it plays a role in prevention of the development of asthma (Akay et al., 2014).

Obesity

Body weight maintenance and control of energy expenditure are the product of chemical, behavioral and psychological harmony; thus, deregulation within any or all of forenamed systems can manifest as a morbidity. Rising obesity rates in the United States have been associated with physiological maladies including chronic inflammation, cardiovascular disease and diabetes mellitus. Specifically, two hormones- leptin and ghrelin- have been implicated as key mediators of body weight whereby the former is secreted by adipose tissue to suppress appetite and the latter by gastrointestinal cells to promote hunger (Cava 2017; Duranti et al., 2017). Leptin has the distinction of being an adipokine since it also promotes secretion of pro-inflammatory cytokines IL-6 and TNF-α, the pro-inflammatory chemokine IFN-**γ** in dendritic cells, macrophages and T-helper cells (Klok, MD 2007; Yin, et al., 2010; Esposito et al., 2005).

It comes as little surprise that several studies demonstrate a reduction in adipose tissue, serum triglycerides and inflammation when obese and pre-obese patients are given B. breve. A recent study by Minami et al shows a significant reduction in body fat mass among non-placebo participants 8-12 weeks post daily treatment with B. breve B-3 capsules

($2x10^{10}$/cfu). Yin et al demonstrate a strain dependent relationship with Bifidobacterium energy homeostasis and fat distribution (Minami, et al., 2018). Finally, Esposito et al present an association of obesity alongside low serum concentrations of IL-10 among obese women suffering from metabolic syndrome. No direct link between B. breve and leptin has been established; however, it is likely that suppression of IL-6 via previously described pathway is a contributing factor (Yin et al., 2010).

Obese adipose tissue over expresses TNF-α, IL-6 and IFN-γ

In lean non-diabetic patients, IL-6 from skeletal muscle has been reported to aid in the intake of glucose through two pathways (LKB1/AMPK) and PI3K/AKT (Nieto-Vazquez et al., 2008). Conversely, in obese/diabetic patients, adipose tissue overly expresses TNF-α, IL-6 and IFN-γ (Hotamisligil et al., 1995; Kern et al., 2001; Nieto-Vazquez et al., 2008) (**Figure 2**). The release of the cytokines not only causes insulin resistance in these patients (Fernandez-Veledo et al., 2006; Nieto-Vazquez et al., 2007; Nieto-Vazquez et al., 2008; De Alvaro et al., 2004), but increases the levels of these cytokines in the body (Nieto-Vazquez et al., 2008). The combined effect of IL-6 released from non-adipose and adipose tissue manifest as both local and systemic inflammation.

Obesity raises risk of COVID-19 complications

A recent NYU analysis conducted by Lighter and colleagues confirms that obesity is a risk factor for COVID-19 hospitalized patients under the age of 60. The team analyzed the BMI of 3,615 patients that were admitted into their institution during a one-month time frame. Individuals with a BMI of 18 to 25 were considered normal weight, 25 to 30 were reported as overweight, and a BMI over 30 was considered obese. The authors reported that patients who were under the age of 60 years with a BMI between 30 to 34 were 2.0 times (95% confidence interval [CI], 1.6 to 2.6, $P < 0.0001$) and 1.8 times (95% CI, 1.2 to 2.7, $P = 0.006$) were more likely to be admitted in a critical care setting, respectively, than the individuals with a BMI of 30 or below. For patients with a BMI over 35, the risk was 2.2 and 3.6 times higher, respectively. This has raised concerns as 40% of American adults under the age of 60 have a BMI of 30 or higher. According to the authors, this makes obesity a significant risk factor for COVID-19 hospitalizations (Lighter et al., 2020). Another study analyzed data of 5,700 COVID-19 patients admitted between March 1 and April 4, 2020 across a dozen hospitals in New York City, Long Island and Westchester County. The data analysis highlighted the most common comorbidities to be hypertension, (3026; 56.6%), obesity (1737; 40.17%), and diabetes (1808; 33.8%) (Richardson et al., 2020). A New York Times article made reference to smaller reports obtained from the New York City area hospitals also highlighting obesity as a complicating factor. The hypothesis was that obesity causes chronic, low grade inflammation. This inflammation leads to an increase in the circulating, pro-inflammatory cytokines that may result in the worst COVID-19 outcomes (Rabin R. 2020).

HYPOTHESIS

Effects of COVID-19 on the immune system and how it relates to increased IL-6, TNF-α IFN-γ and Cytokine Storm:

COVID-19 is born of the virus belonging to the Coronaviridae family and contains the largest known genome of all RNA viruses. It consists of a ~30kb positive-sense single-stranded RNA polymer (+ssRNA)

enclosed within a nucleocapsid (Cascella et al., 2020; Shi et al., 2020). Infection is mediated by a class I viral fusion spike glycoprotein (S) found extruding from the viral envelope that shows a high affinity to human angiotensin-converting enzyme 2 (hACE2). Subsequent conformational changes to (S) facilitate membrane fusion of the virus to the host plasma membrane, followed by endocytosis of the viral-bound ACE2 enabling infiltration (Hoffman et al., 2020). The virus stimulates secretion of pro-inflammatory cytokines IL-1b and IL-6 through toll-like receptors resulting in an inflammatory immune response in lung tissue. (Russell et al., 2020)

Recent reports of patients exhibiting more serious COVID-19 complications show a cytokine storm picture evidenced by an increase in certain inflammatory markers (D-dimer, ferritin) as well as pro-inflammatory cytokines (Yang et al., 2020; Herold et al., 2020). The cytokine storm is a triggered response that attracts immune cells to the region in an effort to attack the virus, causing localized inflammation. In some of the high-risk patients, most likely due to an already present inflammatory state, an excessive and uncontrolled cytokine release occurs, generating a hyperimmune response which leads to further tissue damage (Liu et al., 2020). IL- 6 is a marker of disease severity in COVID-19 patients and is responsible for initiating this cytokine release syndrome. Physicians treating these patients are now testing levels of IL-6, along with elevated ferritin, and C-reactive protein. In a study conducted by Russell et al, the serum IL-6 levels were found to be markedly elevated and predictors of severity of pneumonia. Additionally, the risk for respiratory failure was observed when levels of IL-6 exceeded 80 pg/ml and was twenty-two times higher than individuals with lower IL-6 levels. Elevated IL-6 levels were also strongly associated with the need for a mechanical respirator (Herold et al., 2020). This suggests that the increased mortality of these patients might be attributed to the hyper-inflammatory immune reaction. Evidence of increased IFN-**γ**, and TNF-α have also been well documented in COVID-19 positive patients (Herold et al., 2020; Liu et al., 2020; Yang et al., 2020). Along with IL-6, TNF-α, and IFN-γ are also involved in lung tissue damage leading to increased vascular permeability, leakage of plasma and in many cases, the disseminated intravascular coagulation (DIC) picture seen in compromised patients. Unfortunately, as a result of this cascade of events, the virus is able to diffuse into the bloodstream more quickly, further increasing morbidity. Inhibition of IL-6 with the use of medications such as Tocilizumab has shown promise in these cases (Monteleone et al., 2020).

Furthermore, supportive evidence shows that cytotoxic lymphocytes such as natural killer cells (NK) are low or exhausted among COVID-19 patients with mild symptoms and severe symptoms (respectively) (Zheng et al., 2020). Given the impact on both innate and adaptive immunity, lymphocyte and antibody replenishment via convalescence therapy has become an effective treatment strategy for those suffering from COVID-19 infection (Shen et al., 2020; Liang et al., 2020). This trail of logic concludes with the supposition that individuals with a populous gut microbiome, replete with Bifidobacterium will also have higher serum IFNα and presumably elevated NK cells capable of mitigating infections (Akatsu et al., 2013) (**Figures 3 & 4**). Decreasing concentrations of B. longum, B.infantis & changing NK cell phenotypes have been reported with age, while enhancement of NK activity in aged persons who are given the forenamed microbes has been reported (Kanauchi et al., 2013; Zheng, et al., 2020; Przemska-Kosicka, et al., 2018). Furthermore, while lymphocyte and antibody replenishment serve as a costly, transient response to viral load, the reestablishment of the gut microbiome via daily supplementation is a practical and effective method for viral remediation.

SUMMARY AND CONCLUSION

The evidence presented here supports the hypothesis that modulation of the immune response by gut flora, and in particular Bifidobacterium species prevents the very opportunistic attack seen in those with COVID-19 infections. Additionally, the evidence of inflammatory dysregulation provided within this review proves to be a significant contributor to disease severity and morbidity. The illustrated positive effects of Bifidobacterium in controlling this dysregulation cannot be discounted. In support of these findings, the administration of concentrated strains of certain Bifidobacterium species, in particular those prevalent in the pediatric population, should be utilized as viable and low risk strategies in prevention and treatment of not only COVID-19 infections, but potentially other viral illnesses which attack the host in the same manner (**Figure 5**).

Assessing the concentration of both cytoplasmic and media suspended cytokines, pre- and post-stimulation of leukocytes with Bifidobacterium supernatant or surfactant in vitro will confirm if the previously described pathways behave as we predict here. Analysis of Bifidobacterial secretions followed by membrane docking simulations can further the aforementioned studies and enable characterization of the subsequent cellular signaling cascade. If data harvested from this investigation supports our hypotheses, it would represent a major step towards uncovering prevention and treatment options in COVID-19 infections. In light of these findings, a clinical trial analyzing the gut microbiome of currently infected and high-risk patients could prove beneficial. While the populations discussed have proven less vulnerability to COVID-19, there has been a small subset of the pediatric population diagnosed with COVID-19 related Multisystem Inflammatory Disorder. Further investigation of impacts should be explored and our hypotheses would be further supported should gut microbiome profiling reveal lower concentrations of Bifidobacterium in these patients.

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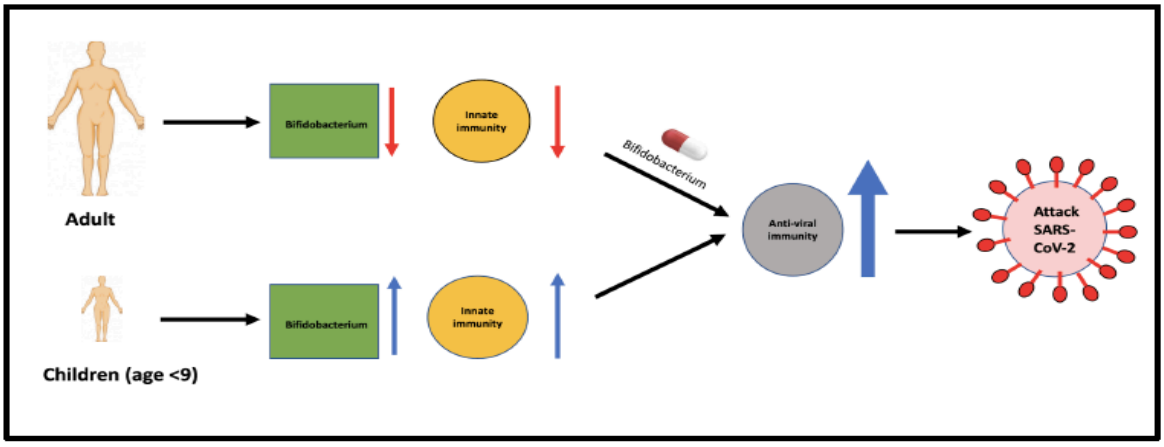

Figure 5: Increase in Bifidobacterium contributes to the increase in innate immunity and could subsequently aid in the fight against SARS-CoV-2 virus Higher levels of Bifidobacteria in children have been proposed to improve innate immunity. These levels which decline in adults may contribute to the reduced immunity and subsequent inability to adequately fight the virus.

DECLARATION OF CONFLICTS OF INTERESTS

The authors of this work do not declare any conflicts of interest.

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