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Vaccines and autoimmunity during the COVID-19 pandemic

Tsvetelina Velikova

To control the pandemic, efficient vaccines must be applied to the population, including patients with autoimmune diseases. Therefore, one can expect that coronavirus disease 2019 (COVID-19) vaccines may influence the underlying autoimmune processes in these patients. Additionally, it is essential to understand whether COVID-19 vaccines would be effective, safe, and provide long-lasting immunological protection and memory. However, the currently available and approved COVID-19 vaccines turned out to be safe, effective, and reliable in patients with autoimmune inflammatory and rheumatic diseases. Furthermore, most patients said they felt safer after getting vaccinations for COVID-19 and reported enhanced overall quality of life and psychological wellbeing. In general, the COVID-19 vaccines have been highly tolerated by autoimmune patients. Such findings might comfort patients who are reluctant to use COVID-19 vaccines and assist doctors in guiding their patients into receiving vaccinations more easily and quickly.

Key Words: SARS-CoV-2; COVID-19; Immune response; COVID-19 vaccine; Immune memory; Autoimmunity; Autoimmune diseases; Relapse

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Core Tip: Coronavirus disease 2019 (COVID-19) vaccines have created concerns about their efficacy and safety, notably in autoimmune patients. Which vaccine adverse events are related to the underlying autoimmunity is unclear. Additional data is needed to evaluate the immunological impact of COVID-19 vaccines in terms of effectiveness and immune-driven adverse effects that might provoke a disease flare in individuals with a history of autoimmune-related symptoms. However, the risk of autoimmune disease flare after vaccination was considered low, while the immune responses after vaccination showed great immunogenicity for these patients. In addition, vaccination will considerably decrease related morbidity and mortality from COVID-19 in autoimmune patients.

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INTRODUCTION

We still do not know all the mechanisms involved in the immune system - severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) interaction during coronavirus disease 2019 (COVID-19) infection. However, it was demonstrated that the virus possesses a tremendous ability to inhibit the immune mechanisms, both innate and adaptive[1]. Nevertheless, there are still controversial data on which immunity is better - naturally acquired or vaccine[3].

There are concerns regarding people living with autoimmune diseases as well. In patients with autoimmune diseases, the body’s immune system is overactive and destroys its own cells through various mechanisms, including autoantibodies and immune cells[4,5]; therefore, one can expect that COVID-19 vaccines may influence the autoimmune processes in these patients. Additionally, it is essential to understand whether COVID-19 vaccines would be effective and safe in patients with autoimmune diseases and whether vaccines will provide long-lasting immunological protection and memory[6]. However, in order to take control of the pandemic, the medical community has stressed that efficient vaccines must be applied to the population. This approach includes vaccinating patients with autoimmune diseases.

CAN THE COVID-19 VACCINES CAUSE AUTOIMMUNITY?

Data showed that the immune hyperactivation and cytokine-excessive release in patients with COVID-19 resulted in multi-organ failure and death[7]. In line with this, patients with already activated immune system could be more prone to severe SARS-CoV-2; however, this was not proven for patients with autoimmune diseases. The main concerns are severe outcomes for patients on immunosuppressive therapy or developing severe clinical complications[8]. Indeed, it was shown that SARS-CoV-2 could induce a robust immune response in immunocompromised patients[9,10].

On the other hand, COVID-19 vaccines have also created concerns about their efficacy and safety, notably in autoimmune patients. We recently published a paper addressing the known pros and cons of vaccinating patients with autoimmune disorders, stressing the absence of data on the advantages and disadvantages of newly discovered COVID-19 in patients with autoinflammatory and rheumatic diseases[11]. Various pathways that contribute to the increase in acute autoimmune responses have been suggested[12]. For example, molecular mimicry, i.e., antibodies against SARS-CoV-2 spike glycoproteins, has the theoretical potential to trigger autoimmunity, as Vojdani and Kharrazian[13] recently demonstrated. Talotta[14] further suggested that an injectable nucleic acid vaccination might put young women in danger of undesired, unexpected immunological side effects, especially those already susceptible to autoimmune or auto-inflammatory disease. However, even in the autoimmune population, serious adverse events are rare[15,16].

Akinosoglou et al[7] further hypothesize that immunization with COVID-19 is not the cause for de novo immune-mediated adverse events. In contrast, the immunological reaction might lead to dysregulation of the pre-existing underlying pathways. This might result from the polyclonal expansion of the B cells leading to the development of immunological features of autoimmunity. It should be noted that autoimmune disorders can be provoked in genetically sensitive individuals through various autoimmune mechanisms, including epitope spreading and bystander activation[17]. Which vaccine adverse events are related to the underlying autoimmunity is unclear. An unsolved issue remains whether to provide a second dosage after such reactions in patients with rheumatic diseases. Additional data is needed to evaluate the immunological impact of COVID-19 vaccines in terms of effectiveness and immune-driven adverse effects that might provoke a disease flare in individuals with a history of
autoimmune-related symptoms[7].

### COVID-19 VACCINES AND PATIENTS WITH AUTOIMMUNE DISEASES

As demonstrated previously, elderly populations with chronic disorders such as diabetes, asthma, and cardiovascular disease are especially susceptible to severe SARS-CoV-2[18]. The same concerns were raised regarding patients with autoimmune inflammatory rheumatic diseases (AIIRDs)[19,20]. However, recent studies demonstrated that patients receiving immunosuppressive therapy for AIIRDs produced sufficient and protective immune response after SARS-CoV-2 mRNA vaccination without experiencing severe side effects or flares[21].

Since AIIRD patients are usually not included in phase III clinical trials of vaccines, immunological response to COVID-19 vaccination in AIIRD patients under the immunosuppression treatment remains unknown. Although the COVID-19 vaccine efficacy was demonstrated between 60%-95% with acceptable safety, uncertainty in AIIRD patients for the COVID-19 vaccines, especially the novel RNA and viral vector vaccines, led to hesitancy in both physicians and patients[22,23]. However, the currently available and approved COVID-19 vaccines turned out to be safe, effective, and reliable in patients with AIIRD. Furthermore, unless contraindicated for medical conditions, such as previous allergy/anaphylaxis to the COVID-19 vaccine or its ingredients, any patient with AIIRD should receive one of the available COVID-19 vaccines.

Patients have to continue immunosuppressive therapy for their underlying AIIRD, which may include biological and selective synthetic disease-modifying anti-rheumatic medications. Korean College of Rheumatology issued guidelines recommending limiting corticosteroids to the lowest possible dosage without exacerbating AIIRD. Methotrexate may be deferred for 1-2 wk following each injection to increase vaccine response. The duration of rituximab and abatacept infusions may also be adjusted[21]. The overall vaccine benefits exceed possible vaccine dangers, as the study showed. Additionally, the risk of disease flare of AIIRD after vaccination is low. However, the currently accepted surrogate markers for the immune response after vaccination (i.e., antibodies against SARS-CoV-2 and activated T cells) showed great immunogenicity of the vaccines in these patients[21].

Another concern that must be discussed is assessing vaccine effectiveness in the IV phase, a.k.a. the real-world studies. Clinical studies investigating high-risk for infection people, i.e., healthcare workers, showed that the absolute risk of testing positive for SARS-CoV-2 after vaccination with mRNA vaccine in a cohort of healthcare workers was 0.97%-1.19%[24]. One must consider that the healthcare staff was younger and more susceptible to SARS-CoV-2 than the clinical trial participants. As stated above, hesitancy in autoimmune patients may have arisen because these individuals were mainly omitted from vaccination studies of COVID-19. Boekel et al[18] have already shown that more than one-third of autoimmune patients are reluctant to get vaccinations against COVID-19. The primary concerns are the anticipated side effects and the lack of long-term studies.

Additionally, there are currently very little data on the safety of COVID-19 vaccines in patients with autoimmune disorders, and no research available can compare the impact of different types of vaccinations between patients and healthy controls. For example, worldwide vaccination recommendations for COVID-19 for autoimmune illness patients is based on experts' opinion[25]. In their previous study, Boekel et al[26] presented the results from a survey that evaluated the adverse events following COVID-19 vaccinations in systemic AIIRD patients and healthy control (Netherlands Trial Register, trial ID NL8513 and NCT04498286). Of all participants, 1780 patients and 660 controls filled out the questionnaire, whereas 46% and 41% of patients received ChAdOx1 nCoV-19 (AstraZeneca) and BNT162b2 (Pfizer/BioNTech), respectively. Thirteen percent of patients were vaccinated with Moderna. Half of the patients and controls reported at least one mild adverse event, and about 20% of all participants had moderate adverse events. Severe adverse events remained below 1%, with no serious adverse events. Complaints of joints and bones were stated more frequently by patients with AIIRD than controls (10% vs 1%, respectively). Fortunately, only 2% of patients reported flare or deterioration of the disease up to 2 mo after COVID-19 vaccination[26].

The results from the survey show that, regardless of the kind of vaccine, adverse effects of immunization with COVID-19 in patients with autoimmune disorders are equivalent to controls. The adverse effects included also predicted local or systemic hyperreactivity responses, which were largely self-limiting. The incidence of individuals who reported adverse events in the clinical trials was lower than the number stated[25], similar to the national study of COVID-19 adverse events in the United Kingdom general population[27]. In conclusion, the survey demonstrated that the vaccines against COVID-19 do not tend to induce autoimmunity flares, as shown in previous limited studies that evaluated mRNA vaccines' impact on patients with autoimmune diseases[20,28].

Known pathophysiological effects mRNA may be both immunostimulatory and immunosuppressive to the innate immune system as COVID-19 vaccines are the first to be widely applied, and prospective, monitored studies of the long-run effects of COVID-19 vaccines on their activities require robust conclusions[11]. Nevertheless, most participants said they felt safer after getting vaccinations for COVID-19, and 20% of individuals with autoimmune disorders reported enhanced overall quality of life.
Autoimmunity and autoimmune diseases associated with SARS-CoV-2 infection

- Antiphospholipid antibodies, associated with anti-phospholipid syndrome
- Antinuclear antibodies, antineutrophil-cytoplasmic antibodies
- Glian-Barré syndrome
- Kawasaki disease
- Autoimmune hemolytic anemia
- Miller Fisher syndrome
- Polyneuritis cranialis
- Thyroid function (Graves’ disease, etc.)
- Vasculitis
- Type 1 diabetes
- Immune thrombocytopenic purpura
- Systemic lupus erythematosus
- Myasthenia gravis

Side effects associated with COVID-19 vaccines

- Allergic reactions
- Injection site reactions
- Fever or chills
- Myalgia
- Fatigue
- Headache
- Increased disease activity
- Joint complaints

Figure 1 Many features and characteristics of severe acute respiratory syndrome coronavirus-2 are associated with the development of autoantibodies and autoimmune phenomena. In some patients, autoimmune disease is developed after coronavirus disease 2019 (COVID-19). On the other hand, COVID-19 vaccines proved their efficacy, effectiveness and safety in patients with autoimmune diseases. SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; COVID-19: Coronavirus disease 2019.

and psychological wellbeing[26]. If we compare these adverse effects associated with the application of COVID-19 vaccines with autoimmune complications during SARS-CoV-2 infection[29], the benefits of vaccines significantly outweigh the side effects of vaccination. This comparison is presented in Figure 1.

CONCLUSION

In general, the COVID-19 vaccines have been highly tolerated by autoimmune patients. Such findings might comfort patients who are reluctant to use COVID-19 vaccines and assist doctors in guiding their patients in vaccination timely. Therefore, the therapy and management of COVID-19 should be given priority to reduce the catastrophic effect of COVID-19 in autoimmune patients, and SARS-CoV-2 immunization is one of the most effective protection against infection. Additionally, significant research with the acquisition of new data is required to assess the safety and efficiency of COVID-19 vaccines in immunocompromised patients. In addition, medical practitioners should counsel their immunocompromised patients to support SARS-CoV-2 vaccinations, as this might considerably decrease related morbidity and mortality from COVID-19.

FOOTNOTES

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Probiotic treatment of inflammatory bowel disease: Its extent and intensity

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Abstract

Free radicals (reactive oxygen species, superoxides and hydroxyl radicals) lead to the development of oxidative stress because of imbalance in the amount of antioxidants. Continued development of oxidative stress leads to chronic diseases in humans. The instability in the antioxidant activities and accumulation of oxidative stress due to free radicals may occur in diseases like inflammatory bowel disease (IBD). Antioxidants are substances that inhibit or delay the mechanism of oxidation of molecules mediated by free radicals and also transform into lesser-active derivatives. Probiotics are defined as live microorganisms that show beneficial effects on inflamed intestine and balance the inflammatory immune responses in the gut. Probiotic strains have been reported to scavenge hydroxyl radicals and superoxide anions that are abundantly produced during oxidative stress. The most widely studied probiotic strains are *Streptococcus*, *Bifidobacterium* and *Lactobacillus*. Probiotics cultured in broth have shown some amount of antioxidant activities. Fermented milk and soy milk, which possess starter microorganisms (probiotics), tends to increase the antioxidant activities many-fold. This review aims to discuss the *in vivo* and *in vitro* antioxidant activities of specific probiotics with various assays with respect to IBD.

Key Words: Oxidative stress; Inflammatory bowel disease; Probiotics; Therapy; Antioxidative activity

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Core tip: Inflammatory bowel diseases (IBDs) are degenerative diseases that cause chronic inflammation in the intestine. The most prevalent therapy for IBD is conventional antibiotic therapy. Keeping the adverse effects of antibiotics in mind, researchers have shown that Streptococcus, Lactobacillus and Bifidobacterium are some of the most efficient antioxidative agents with respect to in vitro and in vivo activities. Probiotics individually or in combination play an important role in regulating superoxide dismutase activity, which is always dysregulated due to oxidative stress caused in IBD. The mechanism of antioxidation of probiotics using NRF2-antioxidative response element pathway, nuclear factor-B and protein kinase C pathway may be activated to contribute to the reduction of oxidative-stress-induced IBD. The review focuses on the antioxidative activities of the specific bacterial strains as therapeutic molecules in IBD. Multiple combinations of probiotic strains have still not been adequately studied. We are currently researching the antioxidative effect of Streptococcus thermophilus, Lactobacillus acidophilus and Bifidobacterium bifidum combination.

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INTRODUCTION

Inflammatory bowel disease (IBD) is an umbrella term used to describe chronic inflammation in the human digestive tract. IBDs are characterized by diarrhea, rectal bleeding, abdominal pain, fatigue and weight loss. IBDs are prevalent in western countries, although they are on the rising track in the Asian countries, which mimics the prevalence in American and European countries. When the burden of IBD is compared between eastern and western countries, the prevalence of IBD in India, which is one of the eastern countries, is found to be the highest. The imbalance in pro-oxidants and antioxidants in the gut leads to inflammation. Despite having antibiotic medication, the prevalence of IBD is still high worldwide. Thus, there is a need to investigate small molecule therapeutic approaches to stop the increase in the number of cases of IBD. In humans, reactive oxygen species (ROS) function as regulators and mediators to ensure correct cell functioning[1,2]. Overproduction of ROS can easily induce damage to proteins, nucleic acids or lipids through free radical reactions. Therefore, in the event of excess ROS production, protective antioxidant mechanisms are required to prevent oxidative stress[1,2]. ROS include superoxides, nitric oxides (NO), hydroxyl radicals, singlet oxygen and hydrogen peroxide (H2O2 ) that contributes to cellular damage, leading to inflammation. IBD is known for the occurrence of oxidative stress. Ulcerative colitis (UC), which is one type of IBD, leads to the increased generation of highly toxic ROS that exceeds the capacity of the limited intestinal antioxidative defense system[3,4]. Oxidative stress in IBD is the key factor for progression of inflammation and is identified by the increased production of ROS, decreased antioxidant molecules and enzymes (beta-carotene, vitamin C and vitamin E) and enhanced lipid peroxidation in the intestine[5].

In the inflammatory processes, intestinal cells of inflamed tissue in response to chemical agents or pathogens, produce high levels of ROS and superoxide anions[6]. Exposure to antigens for a short period of time does not cause any harm because of the adequate first-line defense system producing antioxidative enzymes for protection[6]. However, in chronic intestinal inflammation, there is persistent high ROS production. This process damages the intestinal epithelial barrier, enhances inflammation and injures the intestinal epithelium[6]. Lipid peroxidation is another process that involves a source of secondary free radicals, which directly interact with other biomolecules. The lipid peroxidation depends on the number of double bonds; therefore, polyunsaturated fatty acids are the most susceptible to oxidation. Lipid peroxidation occurs on polyunsaturated fatty acids located on the cell membrane[7]. Superoxide anion radicals, H2O2, and hydroxyl radicals secreted by neutrophils and other phagocytes, causes cell membrane to be impaired, eventually leading to cell death by lipid peroxidation[6]. Enhanced free radicals in the gut can exert peroxidation of membrane phospholipids of intestinal epithelial cells, resulting in the release of toxic products like malondialdehyde (MDA) that can cause damage and cellular stress. MDA is the key breakdown product of lipid peroxides, which is present in the plasma of IBD patients[6]. Increased level of MDA in plasma of Crohn’s disease (CD) patients is considered to be an oxidative stress marker[6]. Decreased superoxide dismutase (SOD)-2 expression is one of the identification markers in colitis-induced mice.

The current preferred therapies for IBD include 5-aminosalicylate, steroids, corticosteroids and azathioprine[6]. The limitations of IBD therapy include the clinical adverse effects of antibiotics, corticosteroids and immunomodulators, which revolves around nausea, vomiting, stomach pain, diarrhea, headaches, respiratory infections, acne, weight gain, insomnia, dizziness, muscle or joint cramps and pathological side effects, causing some pathogenic bacteria to become resistant in IBD.
Surgery is generally costly and unaffordable to many people in remote areas. Also it can cause harm to many organs. Thus, the literature reviews have confirmed the apparent need for improvised treatment using small molecules, like probiotics. Nowadays, 60%–80% of the world population relies on alternative medication to cure IBD. Probiotics are preferably of human origin: they have to be safe for the host, genetically stable and capable of surviving throughout the gastrointestinal tract. Probiotics are generally applicable for viable cells, whereas, postbiotics are soluble factors (either secreted by live bacteria or released after bacterial cell lysis), which are beneficial to human hosts. Probiotics have recently been emerged as one of the powerful novel therapeutic small molecules against IBD. They have been shown to have a positive effect on oxidative stress by promoting the potency of the antioxidative defense system, and in turn may lower the risk of several inflammatory disorders such as IBD. Various known probiotics play an important role in antioxidative activity. Probiotics could be a possible intervention for reducing ROS and lipid peroxidation and thereby increasing SOD activity. Our goal was to review on the in vivo and in vitro antioxidative activities of probiotics. Antioxidative activities of probiotics like Streptococcus, Bifidobacterium and Lactobacilli against oxidative stress in IBD are the main focus of the review.

**MECHANISM OF OXIDATIVE REACTION INSIDE A CELL**

Oxidative stress occurs due to an imbalance between free radical production and antioxidant defense, resulting in hydroxylation of DNA, denaturation of proteins, peroxidation of lipid, and apoptosis, ultimately compromising cell viability. An excess of oxidative stress can lead to the oxidation of lipids and proteins, which is associated with changes in their structure and function. H$_2$O$_2$ is formed by dismutation of superoxides or direct reduction of oxygen. H$_2$O$_2$ can penetrate most of the cell membranes and react with iron in the cell to form hydroxyl radicals. Therefore, hydrogen peroxides are more cytotoxic than superoxide anion radicals. The oxidative modification of lipids, proteins, nucleic acids and carbohydrates is induced and mediated by both free radicals and nonradical activities of reactive species. Superoxides are unreactive molecules but undergo dismutation or enzymatic catalysis to form H$_2$O$_2$. Hydroxyl radicals are thought to initiate ROS and remove hydrogen atoms. This form of radical is extremely reactive and attack most cellular components (Figure 1).

**MECHANISM OF ANTIOXIDANT MOLECULES**

To neutralize the damaging effect of oxidative stress, we need supplements that possess some antioxidative activities. Antioxidants are proteins or enzymes in nature. Antioxidants inhibit cellular damage mainly through their radical scavenging properties. The principle micronutrients that can scavenge free radicals are vitamin E, Vitamin C and beta-carotene. Humans cannot produce these antioxidant micronutrients. So, they must be supplied through the diet. SOD catalyzes the breakdown of superoxide anions into oxygen and H$_2$O using Zn/Cu, Fe/Mn and Ni as cofactors. Only a few species of Lactobacillus, Lactobacillus casei, Lactobacillus paraplantarum, Lactobacillus buchneri and Lactobacillus rhamnosus exhibit SOD activity. Catalases are the common enzymes found in all living organisms, which are frequently used by cells to catalyze the decomposition of H$_2$O$_2$ to water and less reactive gaseous oxygen.

The nicotinamide adenine dinucleotide phosphate (NADP) oxidase/NADP peroxidase enzyme system prevents oxygen accumulation in bacterial cells by formation of H$_2$O$_2$ followed by water. This maintains an intracellular redox balance. Antioxidants work by scavenging free radicals, preventing production of free radicals and improving levels of endogenous antioxidants. Scavenging antioxidants remove active species rapidly, before they react with biologically essential molecules in the body. This antioxidants function by scavenging active free radicals before they attack biologically essential molecules by donating hydrogen atoms to give stable compounds.

**PROBIOTICS AS ANTIOXIDANT SMALL MOLECULES**

When the antioxidative capacity of damaged mucosa is compromised, various natural substances can act as antioxidant molecules to inhibit ROS generation, cell damages and improve the activity of antioxidative enzymes in cells. A food can be considered as functional, when it is demonstrated to provide nutritional effects for health and well-being and reduction of the risk of disease. Ingredients that make foods functional are: dietary fibers, vitamins, minerals, antioxidants and essential fatty acids. One of the novel approaches as therapy against oxidative stress are the development of probiotics. Probiotics are the functional foods that possess antioxidant properties. Several studies have highlighted that the ability of probiotics are to enhance antioxidative properties. For probiotics growth, milk can be used as a substrate for starter microorganisms. Naturally, milk has its own antioxidant activities due to the

[Figure 1]
Figure 1 Illustration of the role of oxidative stress and inflammation in the pathophysiology of inflammatory bowel diseases. Inflammation enhances the oxidative stress by stimulating inducible nitric oxide synthase and myeloperoxidase from inflammatory cells. Simultaneously, oxidative stress involves in the secretion of the inflammatory cytokines, like nuclear factor-B and other cytokines developing IBDs. IBD: Inflammatory bowel disease; iNOS: Inducible nitric oxide synthase; MPO: Myeloperoxidase.

presence of bioactive compounds of whey proteins, caseins, lactoferrin, urate, ascorbate, alphatocopherol, beta-carotene as well as enzymes like SOD, catalase and glutathione peroxidase. Fermented milk with probiotic microorganisms has further improved antioxidant potential[18]. Furthermore, the fermentation of soybean extract using probiotic cultures of lactic acid bacteria possesses superoxide radical scavenging and reducing activities. Soybeans contain SOD, which possesses the superoxide anion scavenging effect. Soymilk obtained from soybean is also expected to possess SOD. The fermented soymilk has an increased superoxide-anion-scavenging effect due to the production of secretory byproducts in the presence of lactic acid bacteria[19].

MODES OF ANTIOXIDATIVE ACTIONS OF PROBIOTICS

Probiotics can directly act to neutralize oxidants by the production of antioxidant enzymes. The antioxidant mechanism of probiotics could be assigned to ROS scavenging, chelation of metal ions, enzyme inhibition and their reducing ability. Probiotics have an antioxidant effect by scavenging of oxidants or by prevention of generation of free radicals in the intestine. Probiotics can upregulate the intracellular activity of SOD, catalase and glutathione peroxidase to protect the cells from intracellular damage. Pro-oxidative metal ions are capable of initiating decomposition of $H_2O_2$ into radicals and triggering lipid peroxidation. Certain chelators are normally detected in probiotics, stating the chelating capacity of probiotics[8,18]. According to reviews, Lactobacillus rhamnosus and Lactobacillus paracasei have significantly inhibited the production of hydrogen peroxide, whereas, L. casei also possess high antioxidant activity via chelating Fe$^{2+}$[10,21]. Different in vitro and in vivo studies have reported that probiotic bacteria can protect against oxidative stress through regulation of the Nrf2 (Nuclear factor erythroid 2-related factor 2)–Keap1–antioxidant response element (ARE) pathway, protein kinase C (PKC) pathway and nuclear factor (NF)-B pathway[7,10,22].

The Nrf2–Keap1–ARE system transmits signal into the nucleus. Under normal conditions, Keap1 is associated with Nrf2. However, in ROS infiltration in cells, the bond between Keap1 and Nrf2 is cleaved and Nrf2 eventually enters the nucleus and binds to ARE and enhances the production of the antioxidative enzymes $H_2O_2$ into radicals and triggering lipid peroxidation. Thus, the probiotic formulations (Lactobacillus spp., Bifidobacterium spp. and Streptococcus spp.) are able to inhibit NF-B activation in colonicephithelial cells[10,24] (Figures 2 and 3). PKCs are the family of protein kinases that are the target for redox modifications. Administration of L. plantarum improved the oxidative stress in a rat model of obstructive jaundice by strengthening the expression and activity of the PKCpathway[10,24,25].

IN VITRO AND IN VIVO ANTIOXIDATIVE ACTIVITY

Not all the probiotics have antioxidant activity due to high strain heterogeneity. Bacillus proteolyticus shows the highest 1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activity[26]. Zeng et al[26] reported that Bacillus amyoliquefaciens could significantly increase the antioxidative
capacity of epithelial cells to reduce induced oxidative stress in pigs. *Bacillus subtilis* and *L. casei* can scavenge free radicals (*in vitro*) and reduce oxidative damage by improving lipid metabolism followed by reduction in lipid peroxidation. *Streptococcus thermophilus* (YIT 2001) showed the highest *in vitro* antioxidative activity against lipid peroxidation[27]. *Lactobacillus* and *S. thermophilus* showed the highest TAA (total antioxidant activity against linoleic acid oxidation) and TAA (total antioxidant activity against ascorbate auto-oxidation). The cell-free extracts and intact cells of *Lactobacillus acidophilus* (ATCC4356) demonstrated an increased inhibition of linoleic acid peroxidation from 38% to 48%. This indicates a strong antioxidative activity[14]. *Bifidobacterium longum* was also investigated for inhibition of lipid peroxidation activity.

**In-vitro cell based antioxidative activity**

Stress induced HT29 cells, *i.e.*, H$_2$O$_2$-stimulated HT29 cells showed a reduced amount of intracellular SOD, catalase and increased ROS activity. The cultured cells were treated with probiotics for 24 h. The

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**Figure 2** Cellular antioxidative regulations of probiotics. Antioxidative effect of probiotic on cellular receptor and regulation of cellular cascade is portrayed. SOD: Superoxide dismutase; CAT: Catalase; ARE: Antioxidant response element.

**Figure 3** Modulation of antioxidation by probiotics. SOD: Superoxide dismutase; CAT: Catalase; ROS: Reactive oxygen species; PKC: Protein kinase C.
supernatant of the cells was collected to study the presence of the antioxidative enzyme activity of SOD and catalase[28]. The Bifidobacterium bifidum treated cell line showed increased catalase activity. SOD and catalase production by B. bifidum can decrease oxidative stress. Moreover, in vitro studies have showed that strains like L. acidophilus and Lactobacillus delbrueckii displayed highest superoxide anion radical dismutation. L. plantarum showed increased ability to degrade chemically pure H$_2$O$_2$ and demonstrated the highest catalase activity[29]. SOD activity was found in Lactococcus, S. thermophilus and Bifidobacterium, with significantly higher activity in Lactococcus than in S. thermophilus[14]. SOD activity of cell-free extracts of the above-mentioned probiotics was studied by the amount of inhibition of reduction of nitrobluetetrazolium[14]. Greatest SOD activity was demonstrated by Lactococcus strains. Glutathione was analyzed in deproteinized bacterial cell-free extract using a commercial kit that showed that the Lactococcus group had the highest inhibitory effect[14]. However, S.thermophilus, Lactococcus lactis and Bifidobacterium animalis also contained relevant amounts of intracellular reduced and oxidized forms of glutathione. Total glutathione measurement was carried out in presence of glutathione reductase and NADP[14].

**In vivo probiotic antioxidative activity**

In an animal model of IBD, it was observed that L. acidophilus with dismutase-like activity was more effective than L. plantarum in suppressing the inflammatory process[29]. In vivo studies have also revealed that L. plantarum 08 and L. acidophilus has the highest catalase activity and highest dismutase-like activity respectively. Male Wister rats were administered with probiotic formulation (mixture of B.animalis, L. acidophilus DSMZ 23033 and Lactobacillus brevis DSMZ 23034) after acclimatization of rats in cages. After 18 d of probiotics supplementation, blood plasma was collected to study the antioxidant status[14]. Reactive oxygen metabolite (ROM) concentration of plasma was evaluated as studied by d-ROM test. Plasma total antioxidant activity (TAA) was spectrophotometrically measured in the presence of 2-binamine-di-3-ethylbenzothiazolin-6-sulfonic acid (ABTS) radical by evaluating the decoloration and reduction of radical cations of ABTS[14]. Plasma ROM concentration was inversely related to the dose of administered probiotics[14]. TAA was significantly related to the dose of administered probiotics. In another study, oral administration of Bifidobacterium breve yakult appeared to prevent transepidermal water loss and significantly suppress oxidation of lipids, proteins and H$_2$O$_2$ levels[31].

The antioxidative activity of buffalo milk fermented with B.bifidum and Acidophilus was evaluated. Control groups included mice fed with standard dahi without probiotic enrichment and another with fermented milk. Catalase and SOD activity in blood was analyzed[27,31]. SOD activity in red blood cells increased exclusively after probiotic dahi administration. Dahi supplemented by L.casei NCDC19 and L.acidophilus NCDC14 inhibited lipid peroxidation and maintained the activity of glutathione peroxidase, SOD and catalaseinstreptozotocin-induced oxidative stress in rats[32,33]. Lactobacillus fermentum (LF1) was studied to assess its antioxidative properties, and confirmed the enhanced expression of NRF2 and MDA inhibition in HT29 cells under stress[34]. In another study it was shown that S.thermophilus YIT2001 decreased the amount of lipid peroxide in colonic mucosa and improved the symptoms of DSS-induced colitis in mice[27].

**QUANTIFIABLE PARAMETERS THAT INDICATE ANTIOXIDATIVE ACTIVITY**

Scavenging activity of ROS is one of the antioxidative properties of probiotics. The Reactive Oxygen Species are used to include both oxygen centered radicals and nonradical derivatives of oxygen. There is the scavenging activity of probiotics occurs in conditions where there is abundance of ROS, hydroxyl radicals and H$_2$O$_2$.

**DPPH RADICAL SCAVENGING ACTIVITY**

To evaluate the antioxidative activity of probiotics, DPPH solution was mixed with methanol and probiotic sample and incubated at 37 degree Celsius for 30 min in the dark. The DPPH radical scavenging activity was calculated by measuring the absorbance of the sample and blank at 517 nm. The radical scavenging activity was calculated as follows: [1-(A$_{517}$ sample)/ A$_{517}$ (blank)]× 100%. According to Das and Goyal, DPPH radical scavenging activity was higher in L. plantarum and L. acidophilus. Scavenging activity of Bacillus ranged from 46% to 190%. B. proteolyticus showed the highest DPPH radical scavenging activity, whereas, B. amylobiofaciens had the weakest DPPH radical scavenging activity[36]. Probiotic strains such as S. therophilus and L. delbrueckii can scavenge ROS, hydroxyl radicals and H$_2$O$_2$[37]. Cell-free supernatants of Lactobacillus exhibit strong DPPH radical scavenging activity[37]. Moreover, the crude peptides extracted from L. acidophilus, L. casei and L. paracasei have radical scavenging activities for DPPH in vitro.
LIPID PEROXIDATION INHIBITION

To study the effectiveness of antioxidants, inhibition of lipid peroxidation is commonly studied. Bacterial strains (L. acidophilus and B. longum) and the intracellular cell-free extract indicated an inhibitory rate on linoleic acid peroxidation that ranged from 33% to 46%[38]. L. acidophilus and B. longum demonstrated a high antioxidant activity for inhibiting lipid peroxidation. Inhibitory rate of different strains of L. acidophilus ranged from 34.9% to 46.3%[37]. Cell-free supernatants of Lactobacillus show higher inhibitory effect than MRS broth cell culture. Intact cells or intracellular cell-free extracts of L. acidophilus and B. longum were investigated for their antioxidative effects, which demonstrated that inhibition of linoleic acid peroxidation ranged from 38% to 48%[34,39]. Levilactobacillus brevis exhibited greater radical scavenging activity and lipid peroxidation inhibitory activity than Pediococcus pentascaceus [35]. Many studies related to lipid peroxidation have chosen linoleic acid as the source of unsaturated fatty acids. Unsaturated fatty acids such as linoleic acid, methyl linoleate and arachidonic acid are typically used. The protocol for lipid peroxidation assay using linoleic acid has been standardized to study the inhibition of linoleic acid peroxidation. Egg homogenate is generally not used for lipid peroxidation inhibition studies in the presence of probiotics. Thus, lipid peroxidation assay using egg homogenate can be used to investigate the inhibition of lipid peroxidation by probiotics.

REDUCING ACTIVITY

Reducing power is based on the kinetics of reduction of Fe³⁺ to Fe²⁺ to prevent the oxidation reaction [37]. Ferric-reducing antioxidant power allows estimation of the ability to reduce pro-oxidant metal ions. The fermented black soybean broths of B. subtilis have shown a potent reducing power as compared to positive controls i.e., -tocopherol and Butylated hydroxytoluene[39]. Cell-free supernatants of Lactobacillus strains showed significantly higher reducing power than MRS broth containing Lactobacillus[38]. Ferric ion reducing antioxidant power assay was performed for the fermented milk with Lactobacillus spp., S. thermophilus and Bifidobacteriumsp. in the presence of green tea supplementation[15]. Fermented milk with 15% green tea infusion (GTI) shows the highest anti-oxidative power as compared to 10% or 5% GTI[15].

SUPEROXIDE ANION SCAVENGING ACTIVITY

Superoxides are radicals with free electrons located on oxygen[16]. These radicals initiate lipid oxidation as the superoxides and H₂O₂ are precursors of singlet oxygen and hydroxyl radicals[17]. Assays can measure the ability to scavenge superoxide anion radicals. S. thermophilus containing fermented milk accounts for the highest superoxide anion scavenging effect as compared to L. acidophilus. Archibald and Fridovich showed that S. thermophilus was able to produce SOD, while Lacidophilus was not. Fermented soy milk with L. acidophilus+Bifidobacterium infantis, L. acidophilus+B. longum, S. thermophilus+B. infantis, or S. thermophilus+B. longum shows higher superoxide anion scavenging activity than reducing activity [17]. The cell-free supernatant of L. plantarum and L. acidophilus showed a potent inhibitory superoxide radical scavenging activity with increasing concentration compared to ascorbic acid[40]. Xing et al[36] had studied an enhanced superoxide radical scavenging activity in co-fermentation conditions in milk (with B. infantis, L. plantarum, B. animalis and S. thermophilus). S. thermophilus exhibited only 58.34% activity, whereas co-fermentation increased the superoxide scavenging activity to 65%.

SCAVENGING OF HYDROGEN PEROXIDE ACTIVITY

H₂O₂ can be generated in biological system in oxidative stress conditions. Being a non-radical oxygen containing reactive agent, it can form hydroxyl radicals (the most highly oxygen radical known). Soymilk fermented with Bifidobacterium alone accumulated the largest amount of H₂O₂, whereas, fermented soymilk with Bifidobacterium and lactic acid bacteria simultaneously reduced H₂O₂[17].

HYDROXYL RADICAL SCAVENGING ACTIVITY

Among reactive oxygen species, hydroxyl radicals are the most reactive species. It can react with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to the cells. Venkatesan et al stated that different concentrations of probiotic species of Bifidobacterium and Lactobacillus showed strongest radical scavenging activities. The hydroxyl radical scavenging activity of cell-free supernatant of L. plantarum and L. acidophilus showed potent hydroxyl radical scavenging activity
when compared to positive control ascorbic acid. These two specific strains have shown a better DPPH and hydroxyl radical scavenging activity. The radical scavenging activity was calculated as follows: \[ \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{blank}} - A_{\text{control}}} \times 100\% \]. Cell-free supernatants of various Lactobacillus strains (L. rhamnosus, L. casei, L. plantarum, L. reuteri, L. acidophilus, Lactobacillus fermenti and Lactobacillus paracasei) were studied through in vitro cell-free hydroxyl radical assay. It was concluded that all the Lactobacillus strains showed a better scavenging than hydroxyl radical scavenging activities.

### ASSESSING THE POTENCY OF PROBIOTICS AS ANTIOXIDANTS

Generally, antioxidants are molecules that interact with the free radicals generated in the cells and terminate the chain reaction before damage is done to the vital molecules. In recent years, researchers have witnessed a beneficial effect of probiotics, especially in regulating the oxidative stress in IBD. Lactobacillus, Streptococcus and Bifidobacterium have been shown to have antioxidative activity that can easily scavenge oxidative stress inducing molecules inside a cell.

### CONCLUSION

From this review, it can be concluded that, in IBD, high levels of oxidative stress induce intestinal tissue damage. Oxidative stress is defined as an imbalance between pro-oxidants and antioxidants, and is tightly associated with the exacerbation of IBD. This disturbs the cellular homeostasis by causing cell injury and increased permeability of the mucosal barrier. Probiotics are equipped with antioxidative defense mechanisms, not only to protect their own survival but also to confer protection to the host cell against oxidative stress during colitis. Probiotics are used to combat IBD by reducing ROS generation and lipid peroxidation and by increasing production of antioxidative enzymes (SOD, catalases and peroxidases). The most common strains studied, Bifidobacterium and Lactobacillus are reported to secrete SOD and antioxidant molecules that can alleviate oxidative stress in inflamed intestine. Accumulation of probiotic strains in inflamed colon results in some protective effects like, metal-chelating activities, antioxidant enzymes (SOD), eventually showing free-radical scavenging activities by restoring the gut microbiota during colitis. Different in vitro studies have suggested that combination of probiotics in fermented milk improve its antioxidative activity. An enhanced superoxide radical scavenging activity of soy milk containing Bifidobacterium was observed. Multiple in vivo and in vitro studies have demonstrated that Lactobacillus, Streptococcus and Bifidobacterium possess outstanding antioxidant activities like DPPH, hydroxyl, superoxide radical scavenging and reducing activities. The important mechanism of antioxidant activities used by probiotics is to reduce oxidative stress, which includes, redox signaling of Nrf2 leading to increase in antioxidant enzyme levels and scavenging of Reactive Oxygen Species. Moreover, it can also be concluded that multiple probiotic strains in combination is much more effective than single probiotic strain with respect to antioxidative studies. Antioxidant probiotic strains can be selected and investigated as promising candidate against IBD. Thus, to develop a novel probiotic combination product with the potential for preventing the oxidative stress, there remains a need to search for particular probiotic strains that can be effective in mitigation of oxidative stress in IBD. The molecular mechanism of the reviewed probiotic strains (Streptococcus, Lactobacillus and Bifidobacterium) by which they regulate the oxidative stress based cellular cascade in IBD conditions needs to be investigated in detail and validate these antioxidative properties in specific in vivo models. Likewise, our novel combination probiotics (S. thermophilus, L. acidophilus and B. bifidum) are under investigation with respect to their antioxidative properties.

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