Review

Does intestinal dysbiosis contribute to an aberrant inflammatory response to severe acute respiratory syndrome coronavirus 2 in frail patients?

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ABSTRACT

In a few months, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has become the main health problem worldwide. Epidemiologic studies revealed that populations have different vulnerabilities to SARS-CoV-2. Severe outcomes of the coronavirus disease 2019 (COVID-19) with an increased risk of death are observed in patients with metabolic syndrome, as well as diabetic and heart conditions (frail population). Excessive proinflammatory cytokine storm could be the main cause of increased vulnerability in this frail population. In patients with diabetes and/or heart disease, a low inflammatory state is often associated with gut dysbiosis. The increase amount of microbial metabolites (i.e., trimethylamine N-oxide and lipopolysaccharide), which generate an inflammatory microenvironment, is probably associated with an improved risk of severe illness from COVID-19. Nutritional interventions aimed at restoring the gut microbial balance could represent preventive strategies to protect the frail population from COVID-19. This narrative review presents the possible molecular mechanisms by which intestinal dysbiosis that enhances the inflammatory state could promote the spread of SARS-CoV-2 infection. Some nutritional strategies to counteract inflammation in frail patients are also analyzed.

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Coronavirus disease 2019 pandemic

On March 11, 2020, the World Health Organization declared the coronavirus disease 2019 (COVID-19) a pandemic [1]. Just 3 mo ago (December 2019), the first cases of unexplained severe acute respiratory syndrome and pneumonia were reported in the province of Hubei, China [2], and subsequently this respiratory syndrome was associated with infections related to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; previously named 2019-nCoV) infection [3], a novel human coronavirus that belongs to the β-coronavirus cluster that also includes SARS-CoV and Middle East respiratory syndrome CoV [3]. In the first week of May, approximately 4,000,000 individuals were infected, and 290,000 subjects had died in 208 countries [1]. These numbers highlight the global severity of this epidemic for health and economic development worldwide.

The cornerstone of the COVID-19 pandemic management is the early detection of positive patients and their isolation to prevent further infections, considering that SARS-CoV-2 spreads through respiratory droplets. The clinical strategy for COVID-19 treatment is represented by symptomatic treatments, because no specific protocols, antiviral drugs, or vaccines have been developed to date [4].

Epidemiologic studies indicate that the elderly, who are above all other subjects affected by metabolic syndrome, diabetes, and/or cardiovascular diseases (especially hypertension), are more susceptible to SARS-CoV-2 infection as highlighted by their increased symptom severity, disease rate progression, and mortality [2,5].

Given the high number of infected people who need intensive care and the ineffectiveness and aspecificity of currently adopted therapies, many countries are now committed to limiting SARS-CoV-2's exponential growth by making extraordinary decisions, including the lockdown of certain regions or entire countries with severe economic impact [6].

While countries work to contain the COVID-19 pandemic and until a vaccine is developed, the problem of higher SARS-CoV-2-associated risk and mortality in frail subpopulations (eg, diabetic patients and patients with metabolic syndrome or cardiovascular diseases) remains. Some authors have strongly advised the implementation of blood glucose management in diabetic patients, using online services, and avoiding person-to-person contact [7]. Others

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have suggested a tighter nutritional control to avoid malnutrition conditions [8].

However, to protect frail subjects, investigating the mechanisms involved in the aberrant immune response triggered by SARS-Cov-2 and identifying possible therapeutic targets to counteract the infection are necessary.

Aberrant inflammatory response induction and SARS-CoV-2

SARS-CoV-2 activates intracellular stress pathways, and induces and takes advantage of aberrant responses by the host that are innate and adaptive to the immune system, which plays a crucial role in causing fatal pneumonia and death [1]. SARS-CoV-2 (as SARS-CoV) enters the host target cells by binding to the angiotensin-converting enzyme 2 (ACE2) protein [9]. The inflammatory process begins after the interaction between SARS-CoV-2 and the toll-like receptor (TLR), whose activation triggers a sequence of responses from leads to interleukin (IL) synthesis to leads the synthesis of interleukins (ILs) [10] and inflammasome activation [11].

Previous robust data show how SARS-CoV, whose sequence homology with SARS-CoV-2 is 96% [12], interacts with the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, indicating NLRP3 as a coronavirus inflammatory target [13,14]. NLRP3 inflammasome is a multiprotein complex that consists of NLRP3, adaptor protein apoptosis-associated speck-like protein-containing CARD adapter (ASC) molecule, and pro-caspase 1 effector molecule [15]. After an inflammatory insult, the assembly of these 3 complexes induces caspase-1 activation by cleavage of pro-caspase-1 and then pro-IL-1 and pro-IL-18 conversion in their active forms IL-1 and IL-18 [15–18]. NLRP3 inflammasome is associated with various autoimmune, inflammatory, and metabolic diseases, and its formation is responsible for pyroptosis due to the loss of the ionic gradient after the formation of pores on the cell membrane [16].

NLRP3 activation requires 2 steps, both generated by stressful conditions [15,17,18]: First, the transcriptional activation of inflammasome-related proteins (including NLRP3, pro-IL-1 beta, and pro-IL-18) and then their subsequent assembly and activation. The priming step occurs after nuclear factor kappa B (NF-kB) induction enhanced by the interaction between several microbial molecules or endogenous cytokines with different receptors, including TLRs [19]. The binding of lipopolysaccharide (LPS), the major structural component of Gram-negative bacteria membrane, to the TLR4 receptor represents a strong stimulus for NF-kB activation [20]. LPS−TLR4 binding, enhancing c-Jun N-terminal kinase and I-κB kinase intracellular signaling, leads to NF-kB activation through the dissociation of its inhibitor (Fig. 1). This process culminates in NF-kB translocation into the nucleus and the consequent mRNA expression of all inflammasome subunits [21].

Recent data suggest that TLR4 could also play an important role in the viral infection immune response [21]. TLR4 overstimulation is associated with an excessive inflammatory response and an unfavorable outcome of viral infections [22]. Olejnik et al. demonstrated that TLR4 signaling inhibition reduces excessive proinflammatory responses during Ebola virus infection [23]. In the same manner, TLR4 and LPS antagonists, which inhibit LPS−TLR4 binding, prevent receptor activation and significantly reduce the inflammatory state during respiratory syncytial virus infection [24]. On the other hand, other authors have suggested that a low degree of TLR4 activation could have positive effects on establishing a protective immune response during viral infection and that TLR4[(-/-)] mice are more sensitive to SARS-CoV compared with wild-type mice [25]. These data suggest that the TLR4 activation degree is crucial in SARS-CoV infection, and TLR4 overactivation is
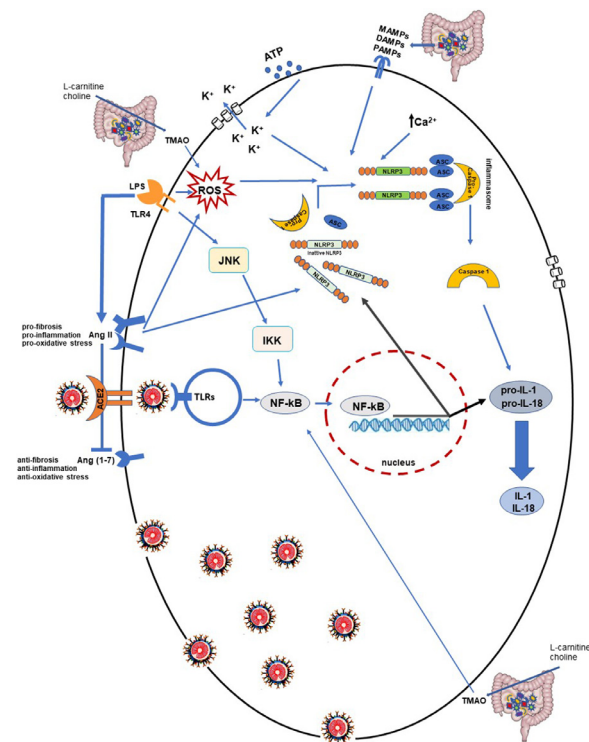

Fig. 1. Possible molecular mechanisms involved in aberrant inflammatory response to severe acute respiratory syndrome coronavirus 2 in frail patients. The binding of severe acute respiratory syndrome coronavirus 2 to angiotensin-converting enzyme 2 and its interaction with intracellular toll-like receptors (TLRs) triggers a sequence of responses, which leads to nuclear factor kappa B activation and interleukin synthesis. Host cell inflammation could be exacerbated by NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation and proinflammatory cytokine production. NLRP3 inflammasome activation requires 2 steps generated by stressful conditions: Transcription of NLRP3 and adaptor protein apoptosis-associated speck-like protein-containing CARD adapter, pro-caspase-1, and their assembly and activation. The first step is consequent to nuclear factor kappa B activation and translocation into the nucleus, stimulated by lipopolysaccharide−TLR4 binding through c-Jun N-terminal kinase and IκB kinase intracellular signaling. The second step is induced by an abnormal mitochondrial reactive oxygen species production, or by pathogen-associated molecular patterns, damage-associated molecular patterns, and microbial-associated molecular pattern stimuli related to gut dysbiosis. Moreover, the K^+ outflow, due to adenosine triphosphate extracellular stimulus, or intracellular and cytosolic Ca^{2+} fluxes imbalance induces NLRP3 inflammasome assembly. NLRP3 activation is also regulated by AngII overexpression stimulated by lipopolysaccharide−TRL4 interaction and gut dysbiosis-derived metabolite trimethylamine N-oxide.

associated with a poor prognosis. In this context, concomitant pathologies that excessively activate TLR4 could conceivably aggravate the COVID-19 condition.

The second step of NLRP3 activation is represented by inflammasome subunits (NLRP3, ASC, and pro-caspase 1) assembling, which is essential for caspase-1 supply and IL-1 beta and IL-18 production and secretion [15,26]. This process is stimulated by different cellular stress conditions, including adenosine triphosphate extracellular stimulus, that determines an outflow of K^+ and membrane pores formation or fluxes of intracellular and cytosolic Ca^{2+} (ie, mitochondrial dysfunction due to Ca^{2+} overload) [18,27]. In the same way, aberrant reactive oxygen species production, pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs) induce NLRP3 assembly (Fig. 1) [28].

Of note, NLRP3 is also regulated by angiotensin II (AngII) [29], a key hormone of the renin-angiotensin system involved in blood pressure regulation [30]. AngII is highly responsible for oxidative stress induction, inflammation, and the fibrosis process

characterizing numerous diseases, including hypertension [31] and insulin resistance [32]. These metabolic alterations predispose to metabolic syndrome and type 2 diabetes mellitus, which are associated with an increased risk of heart disease and lung dysfunction [33,34]. Numerous studies have shown that AngII stimulates NLRP3 inflammasome, promoting proinflammatory cytokines expression [31,35]. Interestingly, some authors have observed that the LPS–TLR4 interaction is involved in AngII overexpression and consequently in NLRP3/NF-kB activation (Fig. 1) [36,37]. This leads to the deduction that pathogenetic stimuli, capable of increasing NLRP3 activation, contribute to the worsening of proinflammatory conditions trigged by SARS-CoV-2.

Recent accumulating evidence indicates that Ang-(1-7), derived from AngII degradation by the ACE2 enzyme, is a counter-regulator for the AngII prooxidative and NLRP3 inflammasome activation [38]. Different authors have demonstrated that an increased Ang-(1-7) level, through exogenous administration or by molecules enhancing Ang-(1-7) signaling, improves lung pathology in mice models [39–41]. Of note, cardiovascular and pulmonary health is closely related to the AngII/Ang-(1-7) axis controlled by ACE2 and the SARS-2-CoV-2 functional receptor, and in patients infected with COVID-19, the ACE2/Ang-(1-7) pathway is downregulated [42]. In patients infected with COVID-19 who are affected by metabolic syndrome, diabetes, or cardiovascular diseases, SARS-CoV-2 exacerbates the ACE2/Ang-(1-7) axis, and the alteration worsens the inflammation state and causes multi-organ damage (Fig. 1).

During the first weeks of the pandemic, some authors and social media outlets speculated that ACE inhibitors and AngII type 1 receptor blockers (ARBs) used for the treatment of hypertension facilitate SARS-CoV-2 infection by increasing ACE2 expression. However, the data supporting this hypothesis are conflicting.

Although ACE and ACE2 enzymes are highly homologous, they catalyze different reactions of the renin-angiotensin system and ACE inhibitors are not able to inhibit ACE2. Moreover, different studies report that ARBs increase ACE2 expression, but ARB action is dose-related and tissue-specific. Inappropriate information about these antihypertensive agents causes stress and anxiety in patients and some have discontinued treatment, probably aggravating their condition.

Currently, ACE inhibitors or ARB correlation with SARS-CoV-2 severe illness is not clinically proven. Indeed, as mentioned, elevated Ang-(1-7) probably has a protective pulmonary action. For this reason, therapy with renin-angiotensin system blockers should not be withdrawn or modified to prevent SARS-CoV-2 infection [43,44].

Possible link between gut dysbiosis and COVID-19 severe illness

The gut microbiome has received growing attention over the last decades and accumulating data have shown how gut alterations are associated with physiological and pathologic conditions [45–47]. In particular, in diabetic patients and those with heart disease, gut microbiota is characterized by a decrease in microbiologic diversity associated with an altered ratio between Firmicutes and Bacteroidetes, the 2 principal phyla in intestinal microflora, in favor of Firmicutes [48–50].

An altered Firmicutes/Bacteroidetes ratio is known to decrease intestinal permeability by promoting PAMPs, DAMPs, and microbial-associated molecular pattern productions and contributing to the proinflammatory state [51]. Moreover, gut dysbiosis is associated with an increased level of trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite that is processed after choline and L-carnitine ingestion [52,53]. This molecule has been receiving interest as a mediator in systemic inflammation and

atherosclerosis condition [54–56]. Both observational and experimental studies suggest that TMAO causes endothelial inflammatory injury [55,56]. Remarkably, recent studies have shown that TMAO recruits NLRP3 inflammasome by activating NF-kB and reactive oxygen species signaling, induces proinflammatory cytokines release, and promotes over-inflammatory conditions [57,58]. Therefore, high TMAO levels are correlated with an abnormal inflammation condition that could be exacerbated by a SARS-CoV-2 infection (Fig. 1). However, other elements seem to be involved.

LPS–TLR4 interaction [19,20] plays a role in the pathogenesis of insulin resistance [59,60]. Higher levels of plasma LPS concentration were found in obese and elderly patients, as well as those with type 2 diabetes mellitus, compared with healthy subjects [60]. LPS–TLR4 binding not only triggers inflammatory conditions, but also inhibits insulin-stimulated IRS1 phosphorylation and impairs glucose homeostasis control [61]. To confirm the LPS–TLR4 role in insulin resistance, recent evidence has revealed that NLRP3 activation represents an essential mechanism in metabolic inflammation and insulin-resistance induction [61], and its inhibition could prevent ischemic stroke in diabetic patients [62]. Furthermore, as previously reported, the LPS–TLR4 axis increases AngII overexpression that contributes to the destruction of the ACE2/Ang-(1-7) balance [36,37]. Of note, inflammation induced by gut microbiota-altered composition represents an important mediator in the cardiometabolic and diabetic pathogenesis and could contribute to aggravated SARS-CoV-2 infection in frail patients.

As further support for this hypothesis, high plasma LPS levels are correlated with lung diseases and IL-6 overproduction. Several studies have shown that LPS, which promotes IL-6 production, is strongly involved in lung pathologies that accelerate neutrophil recruitment and cell infiltration [63,64]. Chen et al. highlighted IL-6's important pathophysiological role in COVID-19, and proposed IL-6 serum levels as a biomarker for SARS-CoV-2 prognosis. High IL-6 levels are associated with a poor prognosis [65]. Different groups have proposed to counteract IL-6 overproduction and tocilizumab (IL-6 inhibitor usually used to treat rheumatoid arthritis) has already been administered to Chinese patients infected with COVID-19. In Italy, a clinical trial is evaluating tocilizumab safety and efficacy, and the U.S. Food and Drug Administration has formally approved a tocilizumab phase 3 trial for patients who are severely ill with COVID-19 [66,67].

Recently, different authors have emphasized the important relationship between the gut microbiome and the lung assuming a gut–lung axis. The gut microbiome is crucial for immune homeostasis and above all pulmonary innate immunity against infections. The altered microbiota composition of mice, which was induced by treatment with antibiotic cocktails, is associated with an ineffective T- and B-cell immunity after an influenza virus infection. These animals displayed an impaired capacity to reduce viral replication and consequently increased morbidity and mortality [68,69].

Nutritional strategies to improve gut dysbiosis and reduce low chronic inflammation state

Intestinal dysbiosis could contribute to the aberrant inflammatory response triggered by SARS-CoV-2. Nutraceutical strategies aimed at restoring eubiosis conditions could represent a valid adjuvant intervention for COVID-19 management. To reduce TMAO production, limiting the ingestion of choline and carnitine, from which TMAO derives, is an option [70], but these molecules are essential to maintain a healthy state. Choline has a structural role and is a neurotransmitter precursor, while several groups, including ours, have demonstrated that L-carnitine improves

musculoskeletal function [71,72] and has a crucial antioxidant action on the heart, in particular during a myocardial infarction [73].

Alternatively, the use of prebiotic and probiotic agents could be convenient to decrease TMAO production. Martin et al. demonstrated that *Lactobacillus paracasei* ingestion counteracts TMAO synthesis [74]. Moreover, Qui et al. studied the beneficial effect of *Enterobacter aerogenes* ZDY01 and *Lactobacillus plantarum* ZDY04 administration on TMAO levels [75,76]. Other authors obtained encouraging results promoting gut colonization with methanogenic archaea [77]. In addition, resveratrol [78] and berberine [79] nutraceutical supplementation appears able to modulate gut composition and decrease TMAO production. Moreover, a diet enriched with polyphenols ameliorates the metabolic syndrome that enhances gut eubiosis. In particular, Roopchand et al. studied the effect of concord grape polyphenols on a high-fat diet and observed that this nutraceutical intervention attenuated the production of inflammatory markers, including IL-6 and LPS. Moreover, grape polyphenols modify the Firmicutes/Bacteroidetes ratio, decreasing the growth of Firmicutes and increasing *Akkermansia muciniphila* bacteria [80].

The positive role of *A. muciniphila* on the metabolic syndrome and immune diseases has been well investigated in numerous animal models and human studies [81]. In obese mice, the administration of *A. muciniphila* decreases hepatic lipid accumulation and IL-6 expression, and ameliorates the altered Firmicutes/Bacteroidetes ratio [82]. Several studies have shown that inulin (an important soluble dietary fiber) or oligofructose consumption are involved in LPS reduction and the maintenance of the gut barrier integrity. Zhang et al. demonstrated that inulin decreases Desulfovibrio abundance and reduces LPS and IL-6 levels in mice models [83,84]. This inulin action was replicated in human clinical trials [85,86].

In addition, oligofructose consumption with/without inulin enrichment exerts a positive action on gut dysbiosis and inflammation. Parnell observed that 12 wk of oligofructose supplementation induces a significant plasma LPS reduction in overweight and obese adults [87]. In addition, oligofructose-enriched inulin treatment alleviates metabolic endotoxemia in diabetic women [88]. Oligofructose intake has been reported to increase the amount of Roseburia in the intestinal gut. Different studies demonstrated that Roseburia increased the differentiation of regulatory T cells and inhibited intestinal inflammation [59]. Moreover, probiotic and prebiotic food compounds can selectively modify the gut microbial community, enhancing the growth of health-promoting bacteria (ie, Lactobacillus spp., *Bifidobacterium breve*, and Bacteroides−Prevotella spp.). Recently, Milajerdi et al. performed a meta-analysis and observed how probiotic supplementation significantly reduced several cytokines productions, including IL-6 [89]. In obese mice, *Lactobacillus rhamnosus* LS-8 and *Lactobacillus crustorum* MN047 supplementation ameliorated the inflammatory state and gut dysbiosis, reducing Bacteroides and Desulfovibrio bacteria and increasing the Lactobacillus and Bifidobacterium population [90]. Supplementation with *Bifidobacterium animalis* subsp. lactis V9 inhibits the hepatic expression of TLR4 and NLRP3, reducing the secretion of inflammatory cytokines (IL-6, IL-1 beta, and TNF-α) [91].

A clinical trial performed by Ried et al. for 3 mo established that Kyolic-aged garlic extract has an important antihypertensive action, but above all decreases TNF-α and IL-6 and improved gut diversity, increasing the immune-stimulating bacteria Lactobacillus and Clostridia species [92]. Accumulated data confirm the beneficial effects of probiotic/prebiotic oral supplementation on inflammatory conditions. Many of these compounds are already marketed and personalized dietary regimens may be developed for patients who are most vulnerable to SARS-CoV-2 infection [93,94].

Conclusions

Overall, clinical data exist to show that patients with obesity, hypertension, diabetes, and general metabolic syndrome (i.e., frail patients) are at a higher risk for COVID-19 infection. As known, the interconnection between these pathologies and gut microbiota composition promotes an aberrant secretion of bacterial products, including TMAO and LPS. LPS binding to TRL4 induces a low-grade inflammation state. Data reported in the literature suggest that SARS-CoV-2 interacting with TRL activates NLRP3 inflammasome, which can also be activated by TMAO (Fig. 1), thereby worsening the host cellular proinflammatory microenvironment.

Pending the development of specific drugs or a vaccine, effective strategies to ensure good health for patients with metabolic syndrome must be identified. Modulation of the microbiome through diet or probiotic/prebiotic agents is well known to ameliorate an inflammation state. In this work, we reviewed some nutritional interventions, focusing on gut microbiota, aimed at improving low-grade inflammation conditions. Currently, the consumption of probiotic/prebiotic agents, polyphenols, or oligofructose/inulin counteracts gut dysbiosis and ameliorates the inflammation state. Future advances on the knowledge of COVID-19 molecular mechanisms would lead to an improved understanding of interactions between SARS-CoV-2 and gut microbiota. In perspective, after microbiota analyses, a novel personalized nutritional approach can be developed to restore a healthy gut microbiota and avoid SARS-CoV-2-induced exacerbated inflammation in frail patients.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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