COVID-19 Perfect Storm (Part I): Cytokine Release Syndrome in Aged People

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IMPACT STATEMENT

1. We certify that this work is novel

2. What this research specifically adds to the literature: SARS-CoV-2 represents a public health emergency of international concern, but no effective therapy against this virus is currently available. Therefore, studies investigating the mechanisms involved in the pathogenesis of this infection and the possible strategy for its treatment are strongly requested. We have taken advantage from studies carried out in patients suffering from some autoimmune diseases, like Rheumatoid Arthritis and Lupus Erytematosus Systemicus. Furthermore, it has to be considered

that a proper function of Immune System requires the presence of adequate concentrations of several micronutrients, like Vitamin A, D, E and C.

ABSTRACT

OBJECTIVES. In December 2019 a novel human-infecting coronavirus, SARS-CoV-2, has emerged. The WHO has stated the epidemic as a "public health emergency of international concern". A drammatic situation has emerged with thousands of deaths, occurring mainly in the aged and very ill people. Epidemiological studies suggest that immune system function is impaired in elderly individuals and these subjects often present a severe deficiency in nutrients as fatsoluble and hydrosoluble vitamins.

DESIGN. In this first part of the review about Cov2 in aged people, we searched for reviews describing the characteristics of autoimmune diseases and the available therapeutic protocols for their treatment. We sed them as a paradigm with the purpose to retrieve pathogenetic mechanisms in common among these pathological conditions and SARS-CoV-2 infection, as well as the alteration induced in immune system function by this virus, or by its homologous SARS-CoV.

RESULTS. SARS-CoV-2 infection induces an important immune system dysfunction with the development of an exhuberant proinflammatory response in the host, and with the development of a life-threatening condition defined as Cytokine Release Syndrome (CRS). This leads to the Acute Respiratory Syndrome (ARDS), mainly in the aged people. High mortality and lethality rates have been observed in the elderly subjects with CoV-2-related infection.

CONCLUSION. These diseases may serve as a paradigm for the study of CRS emerging in the course of SARS CoV-2 infection. This review discusses about the possible activity of Vitamin A, D, E and C in restoring normal antiviral Immune System function or the potential therapeutic role of these micronutrients as a part of a multi-treatment strategy against SARS-CoV-2 infection.

Keywords: SARS; CoV-2; COVID-19; immune system; cytokine

INTRODUCTION

In December 2019 a novel human-infecting coronavirus, defined SARS-CoV-2 or 2019-nCoV, has been recognized as a very severe life-threatening health problem in Wuhan, Hubei Province in China. This infectious condition is now known as "coronavirus disease 2019" (abbreviated "COVID-19"), its most frequent manifestation is represented by the development of pneumonias with different forms of severity [1]. However, less common symptoms, like diarrhea, headache, myalgia or arthralgia, chills, nausea or vomiting, nasal congestion and conjunctival congestion have been described in infected individuals. From China, the epidemic has spread worldwide, and the number of subjects infected with the virus is progressively growing every day. On January 30, 2020, the International Health Regulations Emergency Committee of the World Health Organization has stated the epidemic as a "public health emergency of international concern" [2]. A dramatic situation is progressively emerging in Italy with an increasing number of infected subjects, who suffer from severe forms of interstitial pneumonia and are at a high risk of mortality. Therefore, the enhancing need for intensive care beds with the purpose to provide an effective treatment of these patients can cause the collapse of the Italian Health System and induce similar consequences very quickly also in other countries, even in the most developed nations. Unfortunately, neither a vaccine nor a proved specific therapy against this virus are currently available. Therefore, new strategies of treatment are strongly needed to efficaciously contrast SARS-CoV-2 and to establish effective antiviral schedules. As a wide range of viruses, SARS-CoV-2 also is able to interact with host, influencing the antiviral immune response and determining its pathogenesis. It is well known that proteins produced by a large series of viruses may directly or indirectly modify the amounts of cell RNA transcripts (and consequently of the codified cell proteins) by binding to specific cell DNA sequences or indirectly by activating cytoplasmatic cell signalling pathways. In particular, HBx, NS3, NS5A and NS5B have been demonstrated to dysregulate the expression profile of some cellular cytoskeletal genes with

these mechanisms [3] or to interact with distinct elements of cytoskeleton (microfilaments, microtubules, intermediate filaments and actin stress fibers) [4-7]. Furthermore, some cell nuclear factors may bind to the promoter regions of several viral genes and influence the rate of viral proteins production and viral replication [8, 9].

Virion and genome structure of SARS-CoV-2

SARS-CoV-2 as well as MERS-CoV and SARS-CoV consists of an enveloped spherical betacoronavirus with spike proteins projecting from surface of the viral particles [10]. The envelope is composed of a lipid bilayer originated from the host cell membrane, whereas its genome is characterized by a positive-sense, single-stranded and non segmented RNA [11]. It is 5'-capped and 3'-polyadenylated RNA, encoding four viral structural proteins: the spike (S) glycoprotein, the matrix (M) protein, the small envelope (E) protein and the nucleocapsid (N) protein. It also includes multiple open reading frames (ORFs), codifying accessory proteins interposed among the structural genes (**Figure 1**).

The N protein is detectable in the core of the viral particle, it interacts with the CoV RNA, playing a crucial role in the transcription of viral RNA and in the viral nucleocapsid assembly to generate the mature virions [12]. The S protein consists of a heavily glycosylated protein generating homotrimeric spikes on the surface of the viral particles and modulate the process of viral entry into host cells [13]. Two large overlapping ORFs (ORF1a and ORF1b) encode the coronavirus replicase and occupy about two-thirds of the genome. Furthermore, viral genome includes also several accessory proteins, like ORF 3a, 3 b, 4a, 4b, 5a, 5b, 7 and 7a. They exert pleiotropic and not well-known effects in the infected hosts. These proteins have resulted able to inhibit type I interferon production and type I downstream signaling [14]. These enzymes are

directly translated from the genomic RNA, whereas the structural and accessory genes are generated from viral subgenomic RNAs (sgRNAs). These fragments are produced during viral genome transcription/replication [15].

SARS-CoV-2 immunopathogenesis and role of the different viral proteins in the modulation of inflammatory process

On the basis of the immunopathogenetic hypothesis by Gu and colleagues [16], and on the assumption that the genome of 2019-nCoV is characterized by about 89% of nucleotide identity with the bat SARS-like-CoVZXC21 and around 82% with that of human SARS-CoV [17], it may be suggested that SARS-CoV-2 immunopathogenesis shares very common aspects with the immunopatogenetic events, observed during SARS-CoV infection. Therefore, it may be assumed that an inadequate and inappropriate host immune response against this virus may explain the severity of the disease, detectable in a part of patients suffering from this pathological condition and sometimes leading to a severe distress acute respiratory syndrome (ARDS) with an unfavourable outcome [18, 19]**.** Crucial immunopathogenetic characteristics of the infectious disease caused by SARS-CoV-2 are represented by the induction of an important immune system dysfunction with the development of a strong proinflammatory host response, following the synthesis and the release of some viral antigens; this event may induce the emergence of a life-threatening condition defined as Cytokine Release Syndrome [20]**.** The inhibition or the dysregulation of the immune system protective responses against SARS-CoV-2 as well as the induction of inefficient activities in different types of immune cells prevent the effective control of this virus and may induce harmful immune responses, eventually leading to a dismal outcome. Therefore, an adequate knowledge of the immunopathogenesis of this disease is a pressing need and may identify potentially useful therapeutic targets to improve the quality and efficiency of immune response. On the basis of Gu's hypothesis [16] and of genome homology between CoV

and CoV-2 [17, 18], it may be suggested that also the CoV-2 virus enters the respiratory tract, involving the epithelial cells of the trachea, bronchi, bronchioles, and lungs. However, the colonization of resident, infiltrating, and circulating immune cells represents the key event in the pathogenesis of this infectious disease. This pathogen is carried by the infected circulating immune cells and reach the Nasal associated lymphoid tissue (NALT), the Bronchus-associated lymphoid tissue (BALT) and the mucosa-associated lymphoid tissue (MALT), spreading to the lymphoid tissue of other organs as well as to the mucosa of the intestine, the epithelium of the renal distal tubules and the neurons of the brain. Overall, these events lead to a serious impairment of immune system activities. The severity of the immune cell damage more than the extent of the lesions detectable in the lungs suggests that the patient's immune status and his lymphocyte count probably represent the main predictors of his clinical evolution. Viral load also may exert a crucial impact on the strength and efficacy of the patient's immune response.

Immune response in aged people and in patients with chronic diseases

Patients with chronic diseases, including Alzheimer's Disease, Obesity, Chronic Obstructive Pulmonary Disease, Chronic Viral Hepatitis, Diabetes Mellitus and Cardiovascular Diseases [21-24] as well as old-individuals present some quali/quantitative dysfunctions and defects in their immune response with a persistent low-grade inflammatory state, characterized by the establishment of an imbalance in the cytokine pattern with the production of proinflammatory cytokines (IL-1α, IL-2, IL-6, IL-8, IL-12, IFN-γ), which become prevalent in comparison with anti-inflammatory ones (IL-1 Ra, IL-4, IL-10, TGF-β) (**Figure 2**) [25]. In brief, the strength of the immune response decreases with the age with a modification in the composition and activity of lymphocites in secondary lymphoid tissues. Furthermore, a large series of deficits emerge in B lymphocytes. They show a decreased ability to respond to viral infections like influenza and to produce antibodies with decreased binding activities against the antigens. CD4 positive T helper

cells present dysfunctions in their activation and increased differentiation into T helper 17 lineage. CD8 positive T cells show an impaired activity, exhibiting oliglonal expansion and loss of CD 28. Increased concentration of inflammatory cytokines may be produced by stromal elements, dendritic cells, or aging B and T cells. Additional modifications are represented by the enhanced number of memory cells that persistently colonize tissue niches and induce an inflammatory milieu. These events compromise the ability of naive B and T cells to migrate from the bone marrow and thymus towards the peripheral host tissues. Overall, these modifications result in decreased immune activities in the elderly [26-28].

Serum levels of fatsoluble a, d, e and watersoluble c vitamins in aged people and their role in proper function of normal immune system

Serum levels of different fatsoluble A, D, E, watersoluble C vitamins and other nutrition-related parameters have been associated with frailty in aged people [29, 30]. The prevalence of low vitamin D status represents a global health problem not only in the oldest individuals, but also in all age groups, even in geographical areas with sun exposure all year round [31-33].

It has been reported an association between Vitamin D deficiency and a higher risk of intensive care admission [34] and mortality in subjects with more severe forms of pneumonia [35]**.** Furthermore, Vitamin D deficiency has been commonly observed in patients with ARDS, following different causes, including pneumonia, sepsis, pancreatitis, chest trauma or aortic aneurysm repair [36]**.** Some studies have assessed vitamin A, E and C serum levels in different geographical areas, including Italy, both in institutionalized and non-instituzionalized elderly people and have detected deficits of different extent in the amount of fatsoluble and watersoluble vitamins. In particular, about 10-20% and 50% of subjects over 70 years, who were included in the Italian research, had low serum levels of vitamin E and ascorbic acid respectively [37]**.** Similar results of vitamin deficiency in elderly people have been described in other reports [38, 39].

The plasma levels of vitamin A, C, E and D were significantly lower in patients with metabolic syndrome compared to healthy subjects [40, 41]. Fatsoluble and watersoluble vitamins are needed for a proper function and activity of the immune system and their immunoregulatory and immunomodulatory role have been observed and described both in animal and human models in healthy individuals and in patients with different diseases. The deficiency of these micronutrients have been demonstrated to impair normal functions of the immune system in mankind [42-46]**.** These deficits can be corrected in vivo by providing the supplementation of these vitamins. Several studies have been focused on the understanding of the emergence, development, activity and regulation of dendritic cells (DCs), macrophages, natural killer (NK) cells, T cells, and B cell and on the modulatory effects of vitamins on the specific immune response [47, 48].

AIM OF THE REVIEW

On the basis of the available epidemiological data concerning the current outbreak of the novel SARS-CoV-2 in Italy, the infectious disease caused by this virus represents a very severe health problem for individuals over 60, with aged-people at higher risk of severe forms of disease and of death. About 85% of individuals who died from COVID-19 infection were over the age of 60 (https://www.epicentro.iss.it/, Istituto Superiore Sanità, accessed on 25/3/2020)

Taking advantage from all these epidemiological data, observations, assumptions and hypotheses, we have performed this review, with the aim to examine the possible aspects of the complex loop which can develop between host immune response and SARS-CoV-2, the factors and mechanisms involved in this intricate process.

RESULTS

Role of the different viral proteins of CoVs in the modulation of inflammatory process

It has been reported that some viral proteins of SARS-CoV are able to modulate the inflammatory process directely or indirectely, by influencing the expression of a large series of cytokines. According to our hypothesis, it is conceivable that SARS-CoV-2 may have common pathonegetic mechanisms with CoV. A lot of host and viral components have been reported to affect this very complex process. In particular, the induction of the inflammatory response in the patients suffering from SARS-CoV-2 seem to be characterized by a strength, an extent and a duration of cytokine release of different degrees and with different outcomes [49, 50]**.** According to our knowledge, it is generally thought that, about 5-7 days after the infection, SARS-CoV-2 is able to cause the activtion of a robust immune response in the affected individuals with the production and release of a wide spectrum of proinflammatory cytokines and chemokines [2], leading to the event called Cytokine Storm and to the clinical condition known Cytokine Release Syndrome [51]**.** According to available epidemiological data in the current situation as well as to Gu's hypothesis, these events seem to emerge in about 15-20% of infected subjects and produce a critical impact on the degree of tissue damage, resulting in the stimulation of its remodelling. Substantial alteration in the anatomy of organs and tissues may emerge and induce considerable modifications in their activities [16, 18]. Overall, in SARS-CoV and probably in SARS-CoV-2 patient's expression of proinflammatory cytokines, including IL-1, IL-2, IL-6, IL-8 and IL-17, and chemokines such as CXCL10 and CCL2 are increased both in peripheral blood and lungs of individuals infected with CoV-SARS and are associated with disease severity [52]. Several signal transduction cascades are activated during SARS-CoV and SARS-CoV-2, inducing the synthesis and release of proinflammatory cytokines. Among the mediators involved in this process, AP-1 (activating protein), NF-κB (nuclear factor kB) and NF-AT (nuclear factor of activated T cells) are the most studied and known [53]. Furthermore, several NF-AT binding sites have been identified on TNF-α promoter. Overall, these components of inflammatory cascades contribute to amplify the pro-inflammatory response detectable in patients with SARS-CoV infection. All

these events have a crucial impact on the final outcome of the infection and may explain the differences in the clinical severity of lung damage. Some viral proteins have been proved to stimulate this type of robust pro-inflammatory responses, during SARS-CoV infection.

SARS-CoV N protein is able**:** 1) to activate COX-2 gene expression via the direct interaction with regulatory binding sites (nuclear factor-kappa B or NF-kB and CCAAT/enhancer binding protein or C/EBP) detectable in the promoter of this gene [54]. The transcription of COX-2 gene, in response to several cell biochemical mediators such as cytokines, originates an enzyme isoform, involved in the active production and release of prostaglandins. The result of this event produces the up-regulation of inflammatory process, through the activation of multiple COX-2 signaling cascades and the modification of their function [55]. The induction of COX-2 and release of Prostaglandin E-2 (PGE-2) represent critical events for an efficient viral replication in infected hosts. The inhibition of COX-2 decreases viral progeny levels in virus-expressing cultured cell models [56]. In addition, PGE-2 exerts an immune-suppressive effect, modulating both the innate and adaptive components of the immune system [57]. Furthermore, PGE2 exerts a complex regulatory activity on IL-8 gene expression. This function correlates with the concentration of this chemokine as well as with the cell specificity. It acts as a strong chemoattractant for neutrophils into local inflammatory microenvironment. A large spectrum of cells produce IL-8 in response to different stimuli and contribute to influence the extent of inflammatory response **[58, 59]**.

2) To activate IL-1 gene transcription**:** Interleukin (IL)-1 is a cytokine with pro-inflammatory properties. It is composed of two polypeptides: IL-1a and IL-1b. Its activity is controlled by a natural competitive inhibitor, IL-1 receptor antagonist (IL-1RN) [60]. According to available data, no NF-kappa B binding within the cell IL-1 alpha promoter have been detected, whereas several AP-1-binding site for Jun and Fos proteins have been isolated. The development of the inflam-

matory process is characterized by an increased binding of AP-1 proteins to the specific sequences on DNA, resulting in IL-1 alfa production with consequent pro-inflammatory effects [61].

A recent study has evaluated the induction of pro-inflammatory cytokines and lung inflammation during SARS-CoV-2 infection. The binding of COVI-19 to the Toll Like Receptor (TLR) of immune cells causes the release of pro-IL-1β. This element is cleaved by caspase-1 and induces the inflammasome activation and release of active mature IL-1β. This element mediates the development of lung inflammation, fever and fibrosis [62].

3) To activate IL-6, IL-8 and TNF-α gene transcription. Elevated serum levels of IL-6, IL-8 and TNF-α have also been detected in individuals suffering from SARS-CoV infection during the acute stage (cytokine storm) of the disease in association with lung lesions [63]. It has been reported that SARS-coronavirus nucleocapsid and/or spike viral proteins may directly bind to DNA specific motifs on the promoters of a wide series of genes encoding proinflammatory cytokines or may interact with specific subdomains in NF-kB protein and activate it. NF-kB is one of the main mediators involved in the generation of the inflammatory process. Therefore, nucleocapsid and/or spike viral proteins may directly or indirectly induce the synthesis of IL-1, IL-6, IL-8, IL-17 and TNF-α during the development of SARS-CoV-2-caused infection [49]. The final effect of these events is the induction of a proinflammatory pattern leading to a self-maintaining loop with the possible progressive impairment of clinical conditions [49, 50]. It may be hypothesized that the viral antigen load may have an important impact on the amounts of released interleukins and, consequently, on the extent of the inflammation.

Moreover, ORF 3a, 3 b, 4a, 4b, 5a, 5b, 7 and 7a, as well as M, N and S have resulted able to inhibit both type I interferon production and its downstream signaling and to suppress the antiviral response of the host [52, 64].

Clinical signs, symptoms and anatomopathological features in patients with SARS-CoV-2 infection

Up to now the specific factors and pathogenic mechanisms during SARS-CoV-2-mediated infection and transmission route in human host are not well-understood. According to the available studies SARS-CoV and SARS-CoV-2 are considered respiratory viruses, associated with elevated morbidity and mortality [65]. The respiratory tract is the main site of infection and the clinical features during the disease caused by SARS-CoV2 range from an acute respiratory illness with fever, cough, shortness of breath to more severe forms, like Acute Lung Injury and in some cases to Acute Respiratory Distress Syndrome with a fatal outcome until to septic shock [19, 66-68]. Furthermore SARS-CoV-2 might infect also the cells of the intestinal mucosa, the epithelium of the renal distal tubules and the neurons of the brain and macrophages in different organs, as suggested by Gu's in subjects with SARS-CoV. The presence of symptoms like diarrhea, hematuria, headache and parestesias are often observed in patients with SARS-CoV-2 and seem to confirm these two viruses share common pathogenetic mechanisms [16]. Autoptic lung samples from subjects who died because of SARS are characterized by some tissue alterations. The most frequent changes include: extensive cell infiltrates involving the interstitium and alveoli, with alveolar damage (DAD) and hemorrage/edema, hyaline membrane development, fibrin exudation, epithelial necrosis with thickening of alveolar septa in the earlier stages of the disease and the emergence of fibrosis in septa and alveoli in the later ones. In particular, DAD is a critical and very important histological feature observed in the lungs from patient, who died because of a SARS-CoV-induced infection [16]. Viral genome and antigens have been detected in the epithelial cells of upper airway and alveoli as well as in vascular endothelial cells, neutrophils, macrophages, monocytes and lymphocytes in specimens from individuals and from animal models [16, 69].

The current knowledge on the possible activity of the liposoluble and hydrosoluble vitamins alone or in association with other drugs against viral infection

Our current knowledge concerning the immunopathogenesis of SARS-CoV- and SARS-CoV-2- mediated disease suggests that both the type and the quality of the immune response against these pathogens represent crucial factors during the course of this disease and may have a strong impact on the final outcome of the affected subjects [62]. Therefore, the re-modulation and the regulation of the inappropriate and exhuberant pro-inflammatory response observed during SARS-CoV-2 infection may be a key point in the strategy to counteract this virus and to prevent its life-threatening effects. All these considerations may contribute to explain the differences in clinical course and severity of illness in patients with COVID-19 infection and may constitute a conceptual basis for the development of novel therapeutic and/or preventive approaches. As previously reported, in the last years a large series of epidemiological studies have underlined that some fatsoluble and watersoluble vitamins (A, D, E and C) are essential elements for the normal immune system function [43-46, 70, 71] and in individuals suffering from a wide spectrum of diseases, including chronic viral infections. It has been suggested that the deficiency of these micronutrients may impair the host's antiviral defenses and favour the persistence of several viruses-mediated infections [21]. In particular, some studies have been carried out in patients with some chronic viral infections, like HBV, HCV and HIV. These trials have shown that in chronically infected patients: a) serum concentrations of vitamin A, E, D and C are decreased [72, 73]; b) Vitamin A, D, E and C deficiency is associated with higher levels of viral replication as well as with higher titres of pro-inflammatory cytokines, like IL-6 and TNF-α, or severity of illness in some studies carried out in different virus-associated diseases [72-79]; c) Vitamins may suppress or reduce viral replication or load in different virus infections both in adults and in children [80-84].

In the second part of this review (entitled "**COVID-19 perfect storm (part II): role of vitamins as therapy or preventive strategy in aged people)** the possible efficacy of vitamins A, D, E and C in improving the immune system function and their possible activities against viruses will be discussed.

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FIGURE LEGENDS

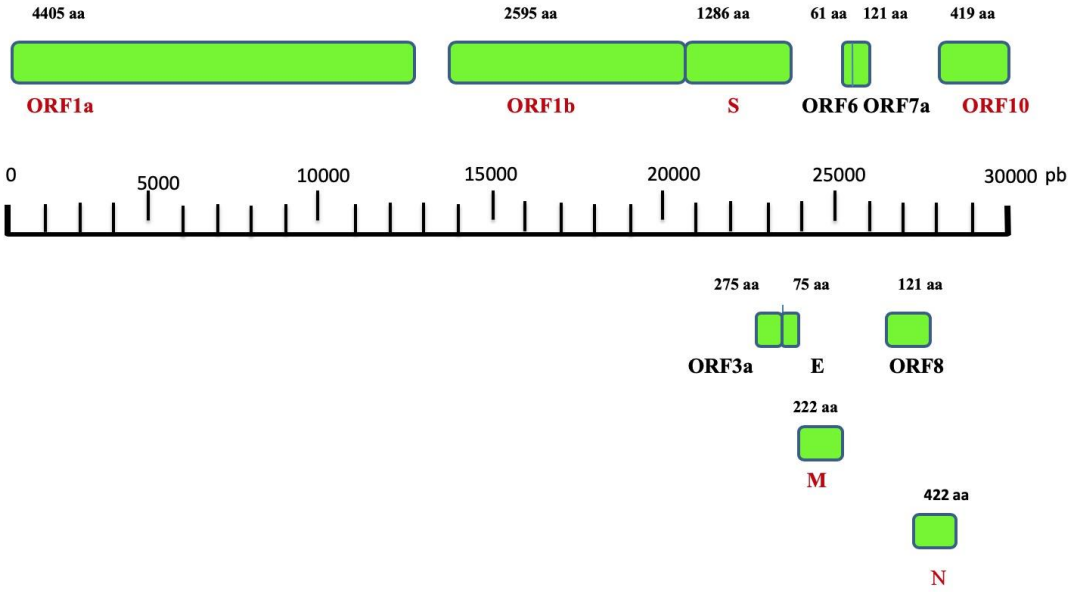

Figure 1. Coronavirus genoma with its structural and nonstructural proteins (see the text) are shown. N and S viral proteins, involved in the Cytokine Release Syndrome are reported.

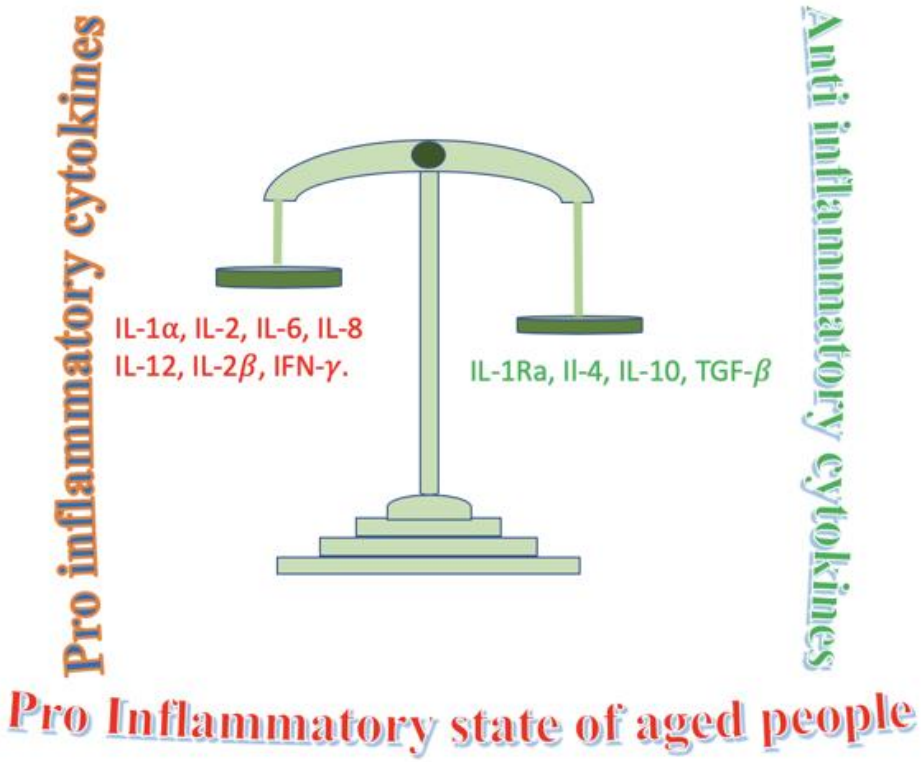

Figure 2. Old individuals present some quali/quantitative dysfunctions and defects in their immune response with the establishment of a persistent low-grade inflammatory state. A pro-inflammatory cytokine pattern is generally present in the aged people. It is characterized by the emergence of an imbalance in the cytokine pattern with the production of proinflammatory cytokines (IL-1α, IL-2, IL-6, IL-8, IL-12, IFN-γ), which become prevalent in comparison with anti-inflammatory ones (IL-1 Ra, IL-4, IL-10, TGF-β). The strength of the immune response decreases with the age with a modification in the composition and activity of lymphocites in secondary lymphoid tissues. Furthermore, a large series of deficits emerge in B lymphocytes. They show a decreased ability to respond to viral infections like influenza and to produce antibodies with decreased binding activities against the antigens. CD4 positive T-helper cells present dysfunctions in their activation and increased differentiation into Th-17 lineage. Overall, these modifications result in decreased immune activities in the elderly.