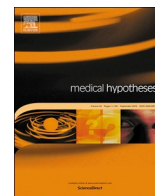




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COVID-19: Endogenous Retinoic Acid Theory and Retinoic Acid Depletion Syndrome



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ABSTRACT

This study presents two new concepts and definitions to the medical literature. One of those is “endogenous retinoic acid theory” and the other “retinoic acid depletion syndrome”. A new classification will be provided for the immune system: “retinoic acid-dependent component” and “retinoic acid non-dependent component”. If this theory is verified, all the diseases where the retinoic acid metabolism is defective and retinoic acid levels are low will be identified and new approaches will be developed for treating such diseases. When the need for retinoic acids increases, such as acute infection, high fever, severe catabolic process, or chronic antigenic stimulation, cytochrome oxidase enzymes are inhibited by drugs or internal mechanisms. Metabolism and excretion of retinoic acids stored in the liver are prevented. In this way, retinoic acid levels in the blood are raised to therapeutic levels. This is called “Endogenous Retinoic Acid Theory”. Retinoic acids also manage their metabolism through feedback mechanisms. Despite compensatory mechanisms, causes such as high fever, serious catabolic process and excessively large viral genome (SARS-CoV-2), excessive use of RIG-I and Type I interferon synthesis pathway using retinoic acid causes emptying of retinoic acid stores. As a result, the RIG-I pathway becomes ineffective, Type I IFN synthesis stops, and the congenital immune system collapses. Then the immune mechanism passes to TLR3, TLR7, TLR8, TLR9, MDA5 and UPS pathways in the monocyte, macrophage, neutrophil and dendritic cells of the adaptive immune defense system that do not require retinoic acid. This leads to excessive TNF α and cytokine discharge from the pathway. With the depletion of retinoic acid stores as a result of this overuse, the immune defense mechanism switches from the congenital immune system to the adaptive immune system, where retinoic acids cannot be used. As a result of this depletion of retinoic acids, the shift of the immune system to the NF κ B arm, which causes excessive cytokine release, is called “retinoic acid depletion syndrome”. COVID-19 and previously defined sepsis, SIRS and ARDS are each retinoic acid depletion syndrome. We claim that retinoic acid metabolism is defective in most inflammatory diseases, particularly COVID-19 (cytokine storm) sepsis, SIRS and ARDS. Finding a solution to this mechanism will bring a new perspective and treatment approach to such diseases.

Introduction

In this study, the pathophysiological processes involved in COVID-19 and retinoic acid metabolism were examined. The indispensable role of retinol (vitamin A-retinoic acid) in the immune system is clearly demonstrated here. Based on the literature findings and observations, it is understood that retinoic acids have a central regulatory function in the immune system. This study reveals the central and indispensable regulatory role of retinoic acids on key molecules such as Type-I IFN synthesis, transcription factors, DNA and proteasomes. Everything that happens in the periphery depends on whether the retinoic acid regulation in the center is working properly. Considering in terms of structure and function, retinol (vitamin A) is not only a vitamin but also

a hormone. In fact, retinol is a major hormone and regulator of the immune system. Zinc plays a role as a cofactor in the functioning of retinol in the immune system.

Retinol is converted into active RA derivatives such as all-trans RA, 9-Cis trans RA and 13-Cis trans RA by taking it into the cell in cases where the need for host increases such as acute infection. These active derivatives of retinol mediate the synthesis of Type-I IFN, the most powerful antiviral mediator of host defense, through nuclear retinoic acid receptors (RAR and RXR). Synthesized Type-I IFN (α and β) cleans the virus from the body by strengthening the cellular and humoral immune system. It also allows the development of a permanent immune response against the virus. Once the hypothesis asserted here is supported with clinical studies, the scientific community will agree that

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vitamin A deserves the definition of growth factor or hormone A, which was its designation when it was first discovered. Most importantly, we will have a new and simple option for the treatment of COVID-19.

The reason that this mechanism was not noticed until now was the assumption that retinoic acid, which is an endogenous ligand, can always be found in the medium. However, the amount of retinoic acid in the human body is limited and is approximately at levels that can last for three months for a person [1]. Retinoic acid can be consumed rapidly due to reasons such as extreme viral load, high fever and extreme catabolic degradation, particularly continuous and long RIG-I stimulation. Retinoic acids can also limit biological effects very rapidly due being metabolized prevalently and rapidly [1,2]. Retinoic acid metabolism is performed by the cytochrome P450 (CYP26) enzymes [2–4].

High fever observed in acute infections, extreme catabolic process, the extremely large genome as in SARS-CoV-2 and heavy viral load lead to fast depletion of the retinoic acids stored in the liver. Retinoic acid levels have previously been found to be severely reduced and depleted during viral infections such as measles and RVS [5–7].

The Hypothesis: Endogenous retinoic acid theory and retinoic acid depletion syndrome

This study presents two new concepts and definitions of medical literature. One is “*endogenous retinoic acid theory*” and the other is “*retinoic acid depletion syndrome*”. With this study; the immune system will obtain a new classification as “retinoic acid-dependent component” and “retinoic acid non-dependent component”. Moreover, it will provide a new perspective for other viral infections, particularly in the treatment of COVID-19, and certain bacterial infectious diseases, immune system and autoimmune diseases, vaccine and adjuvant molecules, sepsis and cytokine storm, allograft reactions degenerative neurological diseases and cancer physiopathology.

When the need for retinoic acids such as acute infection increases. With drugs or internal mechanisms, liver cytochrome oxidase enzymes are inhibited and excretion of retinoic acids stored in the liver is prevented. In this way, raising retinoic acids to therapeutic levels is called “*Endogenous Retinoic Acid Theory*” (TERA). Retinoic acids also manage their own metabolisms with feedback mechanisms. Despite such compensatory mechanisms, the retinol stores of the body quickly get depleted as a result of overuse of the RIG-I pathway, which includes retinoic acid receptors, due to reasons such as high fever, severe catabolic process and oversized viral genome (SARS-CoV-2). As a result, the RIG-I pathway is passivized and the immune defense mechanism shifts to the TLR3, TLR7, TLR8, TLR9, MDA5 and UPS pathways found in neutrophil, macrophage and dendritic cells belonging to the adaptive immune component elements, and causes over-discharge of cytokine (cytokine storm) through the NFκB arm. Such over immune response results in severe clinical presentations. Retinoic acid stores get quickly depleted as a result of overuse of retinoic acids in the RIG-I pathway and the Type-I Interferon synthesis pathway. Then the immune defense mechanism shifts to the NFκB pathway, where retinoic acid cannot be used and which results in cytokine release, is called “*Retinoic Acid Depletion Syndrome*” (RADS).

Such over-release of cytokine causes severe clinical presentations that may further lead to endothelial damage, hypoxia, necrosis and multiorgan damage (cytokine storm, SIRS, ARDS). COVID-19 and the previously described sepsis, SIRS and ARDS and many inflammatory events are each a retinoic acid depletion syndrome.

We claim that retinoic acid metabolism is defective in COVID-19 (cytokine storm) and most inflammatory diseases such as sepsis, SIRS and ARDS. It is asserted that the RIG-I pathway does not function healthily and retinoic acid metabolism is defective in certain diseases such as severe viral and bacterial infection diseases, including COVID-19, chronic autoimmune diseases, sepsis, cytokine storm, SIRS and ARDS and chronic degenerative neurological diseases. Finding a solution to this mechanism will require a new perspective and treatment

approach to such diseases.

It is very easy to prove or disprove the thesis put forward here. It will be sufficient to examine the retinol levels in the serum of severe COVID-19 patients. Serum retinol levels of COVID-19 patients have not yet been studied anywhere in the world. Our clinical trial is still ongoing to determine serum retinol levels in COVID-19 patients. The symptoms and findings observed in COVID-19 patients that support this thesis will be listed below. COVID-19 patients have many entities, symptoms and findings regarding the regulation of endogenous retinoic acids. Most of these are currently based on observations. The findings observed particularly in COVID-19 patients with severe clinical presentation, dramatically resemble the symptoms and findings of vitamin A (retinol) deficiency.

COVID-19 pathogenesis and retinoic acid depletion syndrome

Caused by SARS-CoV-2, this disease was named as COVID-19 by the World Health Organization (WHO) and quickly spreads all over the world [8]. The COVID-19 outbreak was declared an epidemic by the World Health Organization after causing the death of tens of thousands of people [9]. Millions of people infected and more than 600,000 died in the outbreak [9,10]. Currently, there is no approved specific treatment for this virus yet. A vaccine is also not yet developed against the coronavirus. The COVID-19 epidemic is continuing to be a major problem for the whole world [8,11].

As COVID-19, the largest epidemic of modern times turned into a pandemic, an urgent search was started throughout the world for a therapeutic drug against COVID-19 to control the epidemic and decrease the high mortality rates. Due to the time-consuming processes of developing new medications, the fastest solution to this pandemic was the idea to reposition existing medications. This search put focus once again on vitamin A (retinol) and its active derivatives retinoic acids, which were used in the past during the measles epidemics but then got forgotten in time [5].

The congenital immune response of the host is important in controlling the infection. It achieves this through Tip I interferon by increasing the immune response [14]. SARS-CoV-2 is an enveloped single-strand RNA virus with its largest genome. The genome size of RNA viruses is generally less than 10 kB, but the genome length of SARS-CoV-2 is 30 kB [12]. As the SARS-CoV-2 genome has a single-stranded RNA structure, the immune response developed against it essentially functions through RIG-I, which is the congenital immune system component [15]. RIG-I is the major receptor of the immune system that identifies viral, single-stranded RNA ligands [15,16]. RIG-I is activated after the viral RNA ligand binds [17,18]. The RIG-I pathway, which is the most studied and most well-known component of the congenital immune system, functions as dependent on retinoic acid [17,18].

Some viruses are recognized by the host as RIG-I dependent. These viruses include the West Nile virus, the Japanese Encephalitis virus, Influenza A virus, the Sendai virus, Flavivirus and Coronaviruses [15,16]. The extremely large size of the SARS-CoV-2 genome (30 kB) and high virulence, causes a lot more RNA fragments to be scattered from the virus when it is degraded during the defense of the host. Over-stimulation of RIG-I due to the high viral load also causes the retinoic acid stores of the body to rapidly deplete due to high fever and severe catabolic process. The RIG-I pathway, where viral ssRNA ligands are first recognized, retinoic acid and retinoic acid receptors are used are very important in viral infections and are perhaps the most active pathway in the body until retinoic acids are depleted.

The RIG-I pathway is deactivated after retinoic acids are depleted. In the following process, the immune mechanism shifts to the NFκB pathway that causes over-release of TNFα and cytokine through neutrophil where retinoic acid is not used and the TLR3, TLR7, TLR8, TLR9, MDA5 receptors and UPS (ubiquitin/proteasome system) pathways in the macrophage and dendritic cells. Over-discharge of TNFα and cytokine (cytokine storm) takes place with the activation of this

mechanism. The UPS degradation mechanism gains activity in the absence of retinoic acids. UPS degradation covers are opened.

During SARS-CoV-2 infection, IL-1 α is transported and released on the cell surface due to apoptosis and inflammation. It initiates sterile inflammation in adjacent cells. Other chemokines released from macrophages due to viral replication or inflammation is required for IL-1 β release. After binding to IL-1 α and IL-1 β receptors, they stimulate the release of inflammatory cytokines and TNF- α via the NF- κ B pathway. IL-1 release; It causes fever, hyperferritinemia, vasodilation, hematopoiesis inhibition, as well as the release of chemokines, acute phase proteins, adhesion molecules and cytokines, especially IL-6. IL-6 plays a central role in cytokine storm [21].

Chemokines are chemotactic cytokines generated by leukocytes and other cell types. Chemokines are a large molecule family that direct leukocytes to the infection area and play a role in lymphocyte migration. IL5, IL-8, IL-10 and granulocyte-macrophage-colony stimulating factor (GM-CSF) also continuously increase during the cytokine storm and play a role in the emergence of the pathological response [20,21].

In COVID-19, the reason why the disease was very mild in some people and very severe in others is thought to be related to the amount of retinol previously stored in the patient's liver. Whether there is enough retinol in the patient's liver seems to be an effective factor in the patient's response to the disease. In COVID-19, the falling levels of retinoic acids, which are rapidly depleted in the body, and the resulting excessive cytokine release syndrome, are responsible for most of the disease, severe clinical and symptoms. Two separate clinical studies from Egypt reported to the US NIH clinical trials are underway to determine the effectiveness of Isotretinoin, a retinoic acid derivative, in COVID-19 [22].

COVID-19, retinoic acids, ocular and nervous system

The clinical picture, which is common in severe COVID-19 patients and is referred to as "pink eye" [23,24], is nothing more than conjunctivitis seen in severe vitamin A deficiency. Most likely, retinitis and other vision problems in these patients also develop due to atrophy and necrosis caused by a lack of retinoic acid in nerve cells in the retina.

Although attempts are made to explain the taste and olfactory disorders in COVID-19 patients with ACE2 receptors it is clear that it takes place through retinoic acid receptors [25]. It is interesting that vitamin A deficiency also leads to taste and olfactory disorders. This also suggest that vitamin A deficiency also develops in COVID-19. The findings and symptoms that arise in the nervous system and eyes of COVID-19 patients are nothing but the results of retinoic acid deficiency manifested through retinoid acid receptors.

Dizziness, headache, impaired consciousness, acute cerebrovascular disorder, ataxia and epilepsy are observed in COVID-19 patients as central nervous system involvement and hypogeusia, hyposmia, hypopsia and neuralgia are observed as peripheral nervous system involvement. Muscle involvement was also observed in patients [26,54]. Acute hemorrhagic necrotizing encephalopathy cases related to COVID-19 were also reported. Hypodensity in bilateral medial thalamuses was observed in the unenhanced cranial BT taken in the patients [35]. Likewise, this area is one where retinoic acid receptors are densely located [27]. The first Guillain-Barré syndrome that could be related to COVID-19 was reported by Zhao et al [28].

Retinoic acids play a central role in increasing neuroplasticity and neurogenesis. Retinoic acids are vital for hippocampus and the hypothalamus that control memory and alertness. All-trans retinoic acid (atRA) can be created from the retinoic acid in the brain. This is important for long-term potentiation (LTP). Vitamin A deficiency also causes circadian dysfunction. Cognitive dysfunction is also frequently observed [27,29].

Components of the metabolic pathways for retinoids have been clearly defined for the adult brain [29]. All-trans-retinoic acid was shown to be synthesizable in certain areas of the brain. Certain

neuronal-specific genes contain recognition sequences for retinoid receptors and can be arranged directly by retinoids. Retinoid receptors have a prevalent distribution in the adult nervous system. This distribution is different from that observed during embryonic development and suggests that retinoid signaling could play a physiological role in the adult cortex, amygdala, hypothalamus hippocampus, striatum and related areas of the brain [29,34].

Disruption of retinoid signal pathways in rodent models resulted in disruption in synaptic plasticity, learning and memory behaviors. Retinoid signal pathways also play a role in the pathophysiology of Alzheimer's disease, schizophrenia and depression [29].

COVID-19, retinoic acids and ARDS

SARS-CoV-2 binds to the receptors on the alveolar and gastrointestinal epithelium cells and activates these cells in the natural and acquired immune system to cause the release of high amounts of cytokines, particularly IL-6 [20,21]. The inflammatory response generated by the over cytokine release observed with T-cell and monocyte/macrophage activation causes an increase in vascular permeability and exudative liquid accumulation in the alveoli and thereby cause release (ARDS). Multiorgan damages and cardiovascular complications are added to the clinical presentation as the situation gets more severe (SIRS) [20,21,36].

In COVID-19, development of ARDS is essentially associated with inflammatory cytokine release and the exudative liquid accumulation that is caused as a result. The effect should also not be ignored on ARDS pathogenesis of the lack of retinol derivatives lecithin, colin and inositol in the medium and the potential disruption of surfactant synthesis, subject to depletion of retinoic acids in the body due to severe catabolic status and severe infection. These retinol derivatives that have a significant place in the structure of surfactant are important topics that need to be investigated regarding the development and severity of the ARDS presentation.

Surfactant deficiency has a very significant ratio in Respiratory Distress Syndrome (RDS) observed in newborns. Respiratory distress syndrome observed in newborns due to surfactant insufficiency constitutes one-fourth of the infant mortalities in developed countries [38]. Death due to pneumonia in newborn measles was shown to be reduced by 50% with vitamin A fortification, for the first time in 1952 [5]. The function of lecithin, colin and inositol synthesized from vitamin A and added to the structure of surfactant in reducing deaths caused by pneumonia should be taken into consideration.

Retinoids play key roles in the formation and continued functioning of lung alveoli. Retinoids must play a role in the functioning of the mature lung because they are proving to be pharmacologically useful in treating certain lung diseases. The lung is a major tissue for the storage of retinol as retinyl esters. Exogenous RA can stimulate retinol uptake and storage in the lung; for example, when neonatal rats were treated with retinol combined with retinoic acid (RA; 9-*cis*-RA; Am580, an analog of RA) lung retinyl esters increased approximately 5–7 times more than after an equal amount of retinol alone. Thus, retinoids are stored in the lung and active retinoids regulate the level of precursor storage [39].

COVID-19, retinoic acids and autoimmunity

Toll Like receptors recognize molecules bound to the pathogen and cause pathogen-specific innate and adaptive immune responses in the host. These receptors can also be stimulated by host DNA and RNA fragments released as a result of apoptosis or due to lysis of infected cells or mitochondria degradation [40,41]. Of the 11 TLRs identified in humans, TLR3, TLR7, TLR8 and TLR9 are expressed in endolysosomes. These recognize viral DNA, RNA and synthetic nucleic acids [20,41]. Tol-like receptors constitute the TIR (Toll-IL-1) receptor area with the interleukin-1 receptors. RA (atRA), plays a role in immune homeostasis

in a steady state. However, atRA activates pathogenic T cells under inflammation conditions. Thus, atRA induces effector T cell responses also during infections or autoimmune diseases [42].

The adaptive immune system cells case TNF α and inflammatory cytokine discharge through NF κ B over the TLR3, TLR7, TLR8, TLR9 MDA5 and UPS pathways found in monocyte, macrophage and dendritic cells [20,41]. The TLR7, TLR8, TLR9 and MDA5 receptors located in the neutrophil, macrophage and dendritic cells of the adaptive immune system, were determined to recognize the self-DNA fragments of the host in autoimmune diseases such as lupus, psoriasis, arthritis and multiple sclerosis [43]. This mechanism triggers the release of pro-inflammatory cytokines that contribute to the autoimmune disease pathogenesis [43].

It could be considered that the reason for observing autoimmune diseases such as Kawasaki in COVID-19 is related to this mechanism. Clarification of the pathophysiological mechanism here will be instructive for us in the treatment of tens of autoimmune diseases, particularly Type-I diabetes. The first Guillain-Barré syndrome that could be related to COVID-19 was reported by Zhao et al [28]. It is suspected that the fibrinogen and heat shock proteins (HSP) of the host bind to the toll-like receptors [44]. This may increase the tendency to thrombosis in addition to hypoxia and endothelial damage in COVID-19.

Toll-like receptors (TLR3, TLR7 and TLR8) play an important role in the activation of the immune system and their agonists can thus function as promising vaccine adjuvants. However, TLR over-stimulation causes chronic immune activation [45]. The presentation of SSPE after the measles vaccine may be a dramatic clinical picture that develops through the retinoic acid receptors in the brain as a result of the depletion of retinoic acids as a result of continuous stimulation of the RIG-I or TLR pathway with vaccine antigens. The reason for this thought is that the antigen is able to constantly stimulate receptors, and the measles virus lowers vitamin A (retinoic acid) levels during infection.

It is predicted that if retinoic acid levels are at normal limits and the RIG-I pathway functions healthily, endogenous antigens of the host can be prevented from being presented to the TLR7, TLR8, TLR9, MDA5 receptors and UPS pathway of the adaptive immune system. It can be that failure of the RIG-I pathway to function healthily or defective retinoic acid metabolism may play a role in the pathophysiological process in autoimmune diseases.

Retinoic acids and inhibitors of retinoic acid metabolism are used in dermatological diseases such as ichthyosis and psoriasis and good results are obtained. Similarly, these observations and evaluations suggest that retinoic acid metabolism may be defective in such dermatological diseases [2,31].

COVID-19, RIG-I and retinoic acid depletion syndrome mechanism

RIG-I (retinoic acid-inducible gene-I) and MDA-5 (melanoma differentiation-associated gene-5) are cytoplasmic RNA helicases. RIG-I, as the name implies, is a cytosolic receptor synthesized due to retinoic acids. Critical for antiviral responses in the host. RIG-I is an important molecule in the innate immune system for identifying the viruses inside the cell.

Coronaviruses are recognized by the host as dependent on RIG-I [15,16,18]. The RIG-I pathway, which is the most studied and most well-known component of the congenital immune system, functions as dependent on retinoic acid [18]. RIG-I is the major receptor of the immune system that identifies viral, single-stranded RNA ligands. RIG-I is activated after the viral RNA ligand binds [17,18]. This activation also activates retinoic acid receptors and initiates its own (RIG-I) synthesis from the DNA DDX58 promoter gene area. At the same time as the activation of RIG-I, the immune defense cascade that leads up to Type-I IFN release also starts to operate [18].

The RAR and RXR receptors, which mediate the nuclear transcription of RIG-I, the most important pathway in the immune response developed against single-stranded RNA viruses, get activated with

retinoic acid. Retinoic acid receptors (RXR/RAR) are not active before retinoic acid binds [17,22]. Retinoic acid increases RIG-I synthesis and activity by binding to the DNA through the nuclear receptors [18,22].

Apart from the RIG-I synthesis step, another mechanism that consumes retinoic acid in the cell is the stages in which Type-I interferon is synthesized by CREB (cyclicAMP-response element binding protein) and kinase activation. In the CREB stage, retinoic acid participates in interferon synthesis through retinoic acid receptors in the structure of transcription factors. In the kinase activation phase, retinoic acids can provide interferon synthesis by kinase activation without the need for a transcription complex [13]. Apart from the RIG-I synthesis step, excessive use of these two pathways also consumes retinoic acids. Another way in which retinoic acids are used and consumed is UPS suppression. Retinoic acids inhibit NF κ B activation by preventing degradation [33].

RIG-I and MDA-5 also detect double-stranded RNA (dsRNA) which is a replication intermediate substance for RNA viruses [17,18]. SsRNA viruses (such as SARS-CoV-2) are typically not detected as ssRNA but are detected through intermittent replication products in the form of dsRNA [17,15]. The dsRNA ligand may come from single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA) viruses [18,15]. RIG-I can also detect a dsRNA copied from dsDNA by a DNA-dependent RNA polymerase III (Pol III) [46,47]. Some DNA viruses are identified by RIG-I through this mechanism. The identification of certain DNA viruses including HSV-1, EBV, VACV and Adenovirus by the host also depends on RIG-I through this mechanism [46,47].

The viral 5' dsRNA terminal acts as a RIG-I stimulant [48]. 5'-diphosphateRNA (5'ppRNA) fragments found in viruses increase the expression of RIG-I by binding to RIG-I in in-vitro environments [18]. 5'ppp RNA sequences (RIG-I agonists) synthesized through transcription or chemical synthesis were shown to provide better RIG-I activation [18,49]. These agonists have broad-spectrum antiviral potential. They can also be optimized as vaccine adjuvants [50,51]. 5'-triphosphate RNA is a strong ligand for RIG-I [49].

Viral RNA ligand starts an inflammatory response through RIG-I. Upon binding of the RNA ligand to RIG-I, RIG-I undergoes a series of conformational and posttranslational changes to ensure full activation. Double helix RNA viruses are identified through RIG-I and IPS-1 (interferon promotor stimulator-1) [18]. The short double helix triphosphate RNA pattern (5'pppRNA/dsRNA) located in the Viral RNA 5'-terminal, binds to the RIG-I to activate the mitochondrial adapter protein, the mitochondrial antiviral signaling protein and IPS-1 (interferon promotor stimulator / RIG-I adapter trigger). These activated mediators activate the primary immune defense by initiating Type-I IFN and proinflammatory cytokine release. With the interaction between RIG-I, and ASC and Caspase-1 from another path and the stimulation of IL β 1 release, adapter proteins ensure that the activation of CARD-9, Bcl10, Mitochondrial antiviral signal protein (MAVS) and nuclear factor kappaB (NF- κ B) [18]. This arm, which increases inflammation, works when retinoic acid is exhausted.

When retinoic acid is depleted due to the extremely large RNA genome, high fever and severe catabolic process, the RIG-I and IPS-1 pathway, which are components of the congenital immune defense system, cease to function and the mechanism shifts to adaptive immune system cells to continue with the NF κ B pathway through neutrophil, macrophage and dendritic cells. This results in release of TNF α and inflammatory cytokine.

However, if retinoic acid is present, the mechanism will continue to produce Type-I IFN via RIG-I / IRF3-7. And these changes result in an increase in the release of Type-I IFN. After Type-I Interferons (IFN-I; IFN α and IFN β) leave the cell, they bind to the IFN-I receptors on the surface of the cell they came from or other proximal cell receptors [52]. This increases antiviral activity and ensures that more IFN-I is produced. IFN-I also activates the JAK-STAT pathways and increases the expression of the genes stimulated with IFN (ISGs) [52].

Type-I IFNs have three main functions. They prevent the virus from multiplying and limit its spread to host cells. It induces the natural

immune response, the especially cytotoxic effect on the virus through inflammatory responses. In particular, it activates secondary immune responses that result in a permanent immune response [52,53]. In some COVID-19 patients, the cause of inadequate antibody response may be due to retinol depletion. Adequate antibody response does not occur as a result of both cellular and humoral immune system collapse as a result of the discontinuation of Type-I IFN synthesis.

The RIG-I pathway, where viral ssRNA ligands are first recognized and retinoic acid and retinoic acid receptors used is very important in viral infections and is perhaps the most active pathway in the body until retinoic acids are depleted. Overstimulation of RIG-I due to the high viral load also causes the retinoic acid stores of the body to rapidly deplete due to high fever and severe catabolic process.

The RIG-I pathway is deactivated after retinoic acids are depleted. In the following process, the immune mechanism shifts to the NF κ B arm, which causes TNF α and excessive cytokine release via TLR3, TLR7, TLR8, TLR9, MDA5 and UPS pathways of the neutrophil, macrophage and dendritic cells of the adaptive immune system without retinoic acid. Over-discharge of TNF α and cytokine (cytokine storm) takes place with the activation of this mechanism.

Another mechanism that aggravates the picture is that host DNA and RNA fragments from apoptosis and mitochondria lysis cause excessive cytokine discharge from the NF κ B arm through adaptive immune system cells with MDA5 and TLR3, TLR7, TLR8, TLR8, TLR9 receptors. This is due to toll receptors and MDA5 can also be stimulated by DNA fragments released as a result of apoptosis or due to lysis of infected cells or mitochondria degradation [17,41]. This vicious cycle aggravates the situation even further.

Vitamin A deficiency and viral infections

Vitamin A (Retinol) is required for the healthy functioning of the immune system. Decreased vitamin A during infection weakens the host defense. The level of vitamin A drops even further particularly during viral infections [37].

Infectious diseases depress circulating retinol and contribute to vitamin A depletion. Enteric infections may alter the absorptive surface area, compete for absorption-binding sites, and increase urinary loss. Febrile systemic infections also increase urinary loss and metabolic utilization rates and may reduce apparent retinol stores if fever occurs frequently. A great deal of evidence supports an association of Vitamin A Deficiency (VAD) with the severity of infection once acquired, except for respiratory diseases, which are non-responsive to treatment. The severity of pneumonia associated with measles, however, is an exception because it decreases with the treatment of vitamin A supplementation. Measles virus infection is especially devastating to vitamin A metabolism, adversely interfering with both efficiencies of utilization and conservation [55].

Vitamin A deficiency significantly increases mortality in measles. Vitamin A deficiency is an important problem that impacts millions of children in developing countries. Vitamin A deficiency can be considered to be reported only in third-world countries, however, in a study conducted in California, vitamin A deficiency was found in 50% of the children with measles. The mortality rate in newborns was shown to be reducible by 50% with vitamin A fortification, for the first time in 1932 [5,37].

Respiratory Syncytial Virus (RSV) is today the most frequently encountered virus that causes infection in the respiratory tract. It is a very frequent cause of respiratory diseases in small children. Vitamin A level is low in children infected with RSV. In addition, low vitamin A has a relation with the disease similar to that mentioned for measles [7]. Vitamin A treatment is an effective option in newborn RSV infections. This is because it is a low-cost, prevalent, applicable and readily available treatment [7]. Vitamin A deficiency increases mortality in AIDS. Vitamin A replacement also provides benefit for other infections in AIDS. Vitamin A deficiency is encountered frequently in HIV

infection. This deficiency is related to the adjuvant T lymphocyte decrease in circulation, which is distinctive for HIV. Vitamin A deficiency increases mortality in AIDS [56].

The World Health Organization (WHO) gives high-dose (200,000 I.U.) vitamin A supplements as prophylactic to children once every six months to prevent vitamin A deficiency in underdeveloped countries [57]. The risk of measles is reduced due to the effective vaccination program. However, vitamin A treatment is an important requirement in the treatment of other viral infections during childhood [5].

The level of vitamin A decreases significantly in infections caused by the respiratory syncytial virus and the measles virus. These two viruses use the RIG-I pathway in the immune response mechanism of the host [17]. In addition, it should be noted that both of these viruses have single-stranded RNA like SARS-CoV-2.

Retinoic acid is obtained from tissues depending on the specific need of the cell. Retinol, which is one of the most active metabolites of retinoids, is found at a low concentration in the blood. The normal vitamin A (retinol) range is 28–86 μ g / dL. Vitamin A deficiency is defined as serum retinol levels below 28 μ g / dL [55].

Retinoic acids, immun system and IgA

Malabsorption, protein-energy malnutrition, liver diseases, zinc deficiency, viral infections, high fever, severe catabolic process, hyperthyroidism and abetalipoproteinemia caused by bile acid or pancreatic disorders lead to vitamin A deficiency [19,34,57].

Infectious diseases that induce the acute-phase response also impair the assessment of vitamin A status by transiently depressing serum retinol concentrations. Vitamin A deficiency impairs innate immunity by impeding normal regeneration of mucosal barriers damaged by infection, and by diminishing the function of neutrophils, macrophages, and natural killer cells. Vitamin A is also required for adaptive immunity and plays a role in the development of T both-helper (Th) cells and B-cells. In particular, vitamin A deficiency diminishes antibody-mediated responses directed by Th2 cells, although some aspects of Th1-mediated immunity are also diminished [37,39].

Vitamin A deficiency is related to immune system failure. Its deficiency causes deterioration in effective antibody response, a decrease in the number of T-helper cells and a disruption in the mucous barrier of the gastrointestinal tract, genitourinary system and respiratory system [57].

Persons with vitamin A deficiency are more prone to infections and have higher mortality rates. Furthermore, "vitamin A stores get depleted during the course of the infection" [55,57]. This causes a negative vicious cycle. The frequency of Measles, Chicken Pox, RSV, AIDS and pneumonia increase in vitamin A deficiency [57].

Vitamin A (retinol) is essential in ensuring the continuity of the functioning and integrity of skin and mucosal cells and defends the body against infections through the mucosal mechanical barrier and humoral IgA. Retinoic acids (RA), which are the active metabolite of vitamin A, are today considered as a significant factor in the normal development and regulation of the immune system [39,55]. Retinoic acid develops its role in the immune system through specific receptors. Carotenoids usually modulate T-cell proliferation. Increasing normal cell activity is another important objective of carotenoids. "Vitamin A deficiency increases sensitivity against diseases including measles, diarrhea and lung infections" [37].

RA stimulates the differentiation of adjuvant T-cells (Th2) and regulatory T-cells (Treg) and inhibits the differentiation of Th1 and Th17 [42]. RA also stimulates the differentiation of B-cells and ensures antibody production [58]. When retinoic acid is absent in the body, antibodies, especially Ig A, cannot be produced. One study showed that RA had an effect through RAR α , which directly affects IgA synthesis and secretion on B-cells [37,58]. The vitamin A metabolite atRA, plays a key role in mucosal immune responses. atRA also regulates the differentiation of the Foxp3 (+) regulating T-Cell (T-reg) and the Th17

Table 1

Drugs included in the COVID-19 treatment protocol and their stimulation / inhibition status on the CYP P450 system.

Drug	Cytochrome Enzyme-CYP			Sti. / Inh
Hydroxychloroquine	CYP2A4	CYP2C8	CYP3A4	Inhibition SPS:refid::bib30 [30]
	CYPA5	CYP2D6		
Lopinavir/Ritonavir	CYP2D6	CYP2C19	CYP3A4	Inhibition [30]
	CYP2A4	CYP2C8	CYP2C9	
Favipiravir	OAT1/OAT3	CYP2E1	P-GP	Inhibition [32]
Oseltamivir			CYP3A4	Inhibition [30]
Clarithromycin		CYP2D6	CYP3A4	Inhibition [30]

effector T-Cell. Therefore, although atRA can be used as an effective “mucosal adjuvant” in vaccines, it is also considered as necessary for creating the gut immune tolerance [42,50].

All-trans RA (atRA), is produced in macrophage and dendritic cells that can express retinal dehydrogenase from vitamin A. atRA binds to nuclear retinoic acid receptors that are expressed in lymphoid cells and function as transcription factors to regulate cell homing and differentiation. atRA, which is produced by CD103 (+) dendritic cells and alveolar macrophages, induce the transformation of pure T-Cells into Foxp3 (+) regulating T-Cells and thereby, work with TGF γ to protect the mucosal tolerance [59]. atRA plays a role in immune homeostasis in a steady state. However, it activates pathogenic T cells under conditions of inflammation. Therefore, atRA induces effector T-Cell responses during infections or autoimmune diseases [42]. The possible reason for this is that atRa activates only the RAR receptor, whereas 9-Cis-RA can activate both RXR and RAR receptors. The role of retinoic acid in the production of immunoglobulin A could be presented as a result of a broad review consisting of 151 articles. Immunoglobulin A is inhibited in the lack of retinoic acid [60].

The current treatment of COVID-19 and the cytochrome P450 system

The cytochrome oxidase enzyme system (CYP450) is a system that is responsible for the metabolism and detoxification of the toxins and drugs particularly found in the endoplasmic reticulum of liver cells. Only three of these (CYP1, CYP2, CYP3) are responsible for drug metabolism [30]. CYP3A4 accounts for 40% of the cytochrome oxidase system and is the main mono-oxidase enzyme responsible for drug metabolism [30].

The P450 mono-oxidase system shows heterogeneity among humans. The activity of this enzymatic system varies between individuals and societies. This heterogeneity is the reason that the biotransformation of drugs and compounds differs among individuals and societies [30,61]. The P450 system is an important area of drug-drug, drug-diet and drug-disease-condition interactions. The functional change in this system has significant consequences regarding an insufficient therapeutic response or increased toxicity. Selecting specific P450 enzymes when starting drug therapy will provide rational drug development, more effective clinical trial evaluation and better therapeutic approaches in patients requiring special attention [30,61].

Enzyme synthesis increases when induction occurs in the P450 system. The biotransformation of the drug that metabolizes with this enzyme increases accordingly. The serum level thus decreases. As for inhibition, in contrast with induction, the enzyme synthesis decreases and the serum level of the concerned compound increases. Enzyme inhibition occurs very rapidly and the blood level of the metabolized drug quickly increases. In such a case, the pharmacological effects of the drug or endogenous compound increase [30,61].

With the inhibition of the cytochrome oxidase system, the serum retinoic acid levels are increased by preventing the excretion of retinol esters previously stored in the liver and the retinoids taken with food. Retinoid acids increasing in the serum modulate the RIG-I pathway

through nuclear receptors to operate the primary immune system [18,22].

The mechanism of action of these drugs, particularly Hydroxychloroquine, used in the treatment of COVID-19, on SARS-CoV-2 is not clearly known. Therefore, although recovery is observed in patients, it is not certain that such recovery is directly related to these drugs. It is likely that such therapeutic efficacy is achieved by retinoic acid derivatives whose metabolism is halted and serum levels are increased as a result of inhibition of the cytochrome P450 oxidase system by the drugs used in the treatment. The real reason for success in cases with early treatment is also probably due to the prevention of the depletion of retinoic acid stores in the liver by early treatment.

All the drugs bound to a certain protocol by the COVID-19 Science and Advisory Board in our country, as in many countries throughout the world, and used in COVID-19 treatment, inhibit the cytochrome P450 system (Table 1). The table below shows the drugs included in the COVID-19 treatment protocol and their stimulation / inhibition status on the CYP P450 system.

The results of the studies conducted on Remdesivir in the USA were disclosed recently. A success ratio of 30% is mentioned there for Remdesivir in -American society [36]. The study conducted in China found this drug to be unsuccessful against COVID-19 [36]. The likely reason for this is the cytochrome P450 system that varies in terms of activity among individuals and societies.

Alcohol (Ethanol), decreases serum levels by increasing the metabolism of retinoic acids by stimulating cytochrome oxidase enzymes. In this respect, the use of alcohol has a negative impact on COVID-19 prognosis. Ethanol activates the P450 system while red wine inhibits it [30]. French people were consuming high amounts of red wine on the first days of the COVID-19 pandemic. It was stated that red wine (not white wine) consumption protected people from COVID-19 and that it was even good for the patients. This has some truth to it due to two reasons. Red wine inhibits the cytochrome P450 system through CYP3A4 and also the flavonoids in red wine have a similar structure to retinoic acids and can show a regulatory effect on the immune system [30,61].

The reason why COVID-19 progresses more in males than females (ignoring smoking) is that the cytochrome oxidase system is less inhibited in males than females. This is because estradiol inhibits the P450 system much more effectively and prevalently with respect to testosterone. Estradiol inhibits CYP1A2, CYP2A6, CYP3A4 whereas testosterone only inhibits CYP2D6 [30,62].

Conclusion

An effective immune response against RNA viruses proceeds based on the innate primary immune system and the adaptive immune system. The first path here is the innate immune system mediated by RIG-I, which functions as dependent on retinoic acids and proceeds through retinoic acid receptors (RXR-RAR). Retinoic acids are used in the regulation of this mechanism. The second path is the adaptive immune system which does not include retinoic acids but includes TLR3,TLR7,TLR8,TLR9 and MDA5 receptors in the neutrophil,

monocyte, macrophage and dendritic cells in the lungs and guts and continues through NF κ B to result in TNF α and over cytokine release.

Another way is UPS-NF κ B shunt. Here, retinoic acids inhibit proteasomal degradation via the UPS system. In case retinoic acids are depleted, this inhibition mechanism disappears and proteasomal degradation takes place. Proteasomal degradation causes NF κ B activation and causes TNF α and excessive cytokine secretion.

According to the hypothesis, in acute situations such as severe infection, retinoic acids are depleted and the defense system of the host shifts into the adaptive immune system, which gives an acute and extremely inflammatory response. On the one side, the RIG-I / IRF3-7 pathway of the congenital immune system and the production of Type-I interferon, on the other side, the UPS-NF κ B pathway of the adaptive regulatory immune system that secretes TNF α and cytokine, these two systems must be in balance. Production of Type-I interferon through the RIG-I and IRF3-7 pathway enhances strong immune defense and a permanent immune response in the host. If the balance shifts to the side of the UPS / NF κ B and cytokine discharge, inflammatory pathogenetic mechanisms are triggered in the disease-causing host.

If a sufficient amount of retinoic acid is available in the body, the immune response developed against viruses will take place through the RIG-I pathway that belongs to the innate immune system. The healthy functioning of this pathway will clear the infectious agent from the body and improve the immune response through Type I IFN. Healthy functioning of this pathway is particularly dependent on the availability of a sufficient amount of retinoic acid derivatives. Having enough retinoic acid will provide permanent immunity. However, rapid depletion of retinoic acids in the body during the acute infection process leads to the response of the adaptive immune system. This leads to the development of severe and serious clinical presentations and complications that may result in death. Therefore, having enough retinol in the body is vital and necessary.

What provides the balance between these two systems? Does the metabolic defect of retinoic acids and depletion of endogenous retinoic acids disrupt this balance? Can replacing retinoic acids restore this impaired balance? The answer to these questions will provide an understanding of the pathogenesis of all acute and chronic, benign and malignant, inflammatory and granulomatous, autoimmune and degenerative diseases listed above, and perhaps offer us new and simpler treatment options.

In this case, all drugs and compounds that inhibit the liver cytochrome P450 oxidase system may be effective against COVID-19 by preventing retinoic acid metabolism. In fact, it is not difficult to predict that even Ketoconazole, which is used as an antifungal, can have significant efficacy in COVID-19 treatment as a strong cytochrome oxidase P450 inhibitor (if sufficient retinoic acid is available in the body) [30]. Retinoic acid metabolism inhibitors (RAMBA) that block endogenous retinoic acid metabolism and provide therapeutic efficacy by increasing endogenous retinoic acid levels in order to avoid the teratogen side effect of retinoic acids have started being used in dermatological indications in recent years [31]. Retinoic acids, zinc and RAMBAs may work against COVID-19, which continues with full intensity.

The evaluations and observations regarding the above mentioned retinoic acid metabolism also support our hypothesis about endogenous retinoic acids. Considering the fact that COVID-19 turned into a pandemic and the socio-economic consequences of the disease, the endogenous retinoic acid theory, the retinoic acid depletion syndrome that we assert and the information, findings and observations they are based on should not be ignored. An important conclusion to be drawn from this is the presence of much information, documents and observations that retinoic acid derivatives and zinc could be effective in the treatment of COVID-19. The success achieved with vitamin A in measles and other viral infections should be kept in mind. Therefore, it is necessary to focus on these drugs for COVID-19 treatment and urgently start clinical studies with these drugs. The inclusion of these drugs into the COVID-19 treatment protocol as a result of clinical

studies will provide significant benefits both in terms of public health and socio-economic perspective.

The endogenous retinoic acid theory and retinoic acid depletion syndrome we obtained as a result of this study will be widely discussed in the medical community. There are almost no organ systems in the human body, without retinoic acids. For now, immune system-dependent retinoic acid mechanisms are highlighted. Studies on the retinoic acid mechanism of action will continue to increase in the coming years.

Declaration of Competing Interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2020.110250>.

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