

Review

## **Potential utility of melatonin in deadly infectious diseases related to the overreaction of innate immune response and destructive inflammation: focus on COVID-19**

**Dun-Xian Tan<sup>1\*</sup>, Rüdiger Hardeland<sup>2</sup>**

<sup>1</sup>S.T. Bio-Life, San Antonio, Texas, USA

<sup>2</sup> Johann Friedrich Blumenbach Institute of Zoology and Anthropology, University of Göttingen, Germany

\*Correspondence: tan@uthscsa.edu, Tel: +012103318816

**Running title:** Melatonin in deadly virus infection

Received: March 5, 2020; Accepted: March 22, 2020

### **ABSTRACT**

The high mortality of deadly virus infectious diseases including SARS, MERS, COVID-19, and avian flu is often caused by the uncontrolled innate immune response and destructive inflammation. The majority of viral diseases are self-limiting under the help of the activated adaptive immune system. This activity is cell proliferation dependent and thus, it requires several weeks to develop. Patients are vulnerable and mortality usually occurs during this window period. To control the innate immune response and reduce the inflammation during this period will increase the tolerance of patients and lowers the mortality in the deadly virus infection. Melatonin is a molecule that displays respective properties, since it downregulates the overreaction of the innate immune response and overshooting inflammation, but also promotes the adaptive immune activity. Many studies have reported the beneficial effects of melatonin on deadly virus infections in different animal models and its therapeutic efficacy in septic shock patients. Furthermore, melatonin has a great safety margin without serious adverse effects. We suggest the use of melatonin as an adjunctive or even regular therapy for deadly viral diseases, especially if no efficient direct anti-viral treatment is available.

**Key words:** melatonin, virus, innate immunity, inflammation, COVID-19, SARS, MERS, Avian flu, cytokines.

---

### **1. INTRODUCTION**

In relation to repeated epidemic outbreaks of deadly infectious diseases caused by several RNA viruses, actually fueled by the concern of a possible COVID-19 pandemic, the questions of how to combat these threats and how to reduce lethality of infected individuals has come into public focus. Solutions are especially urgent, as long as no specific therapies are available. The gold standard of vaccination requires time for development and is not immediately applicable after an initial outbreak, as is the case in COVID-19. But even in long-known viruses, their genetic variability leads to several subtypes that differ in their appearance from year to year and causes

additional problems. This is especially the case in the seasonal flu, which requires repeated adaptations of the vaccines. A poorly adapted vaccine results in higher incidence of infections and, thus, higher numbers of deaths in particularly vulnerable patients, as found among elderly individuals and persons with comorbidities. Alternatives to the development of antibodies do not always exist in conventional medicine, because this requires the identification of a drug-sensitive Achilles heel in the virus. While the surface molecules of a virus, especially spike head proteins, are typically accessible to antibodies, other anti-viral drugs have to interfere with intracellular or other host cell processes, such as 1). facilitation of host cell binding by host cell proteases; 2). fusion of the viral envelope with host cell membranes, which can be inhibited by preventing proteolytic activation of fusion proteins or increasing the endosomal pH; 3). virus replication; 4). release of virions, accessible by direct or indirect inhibition of protein channels required for RNP release (1-4). However, with regard to the viral diversity, drugs with required properties are not generally available or applicable. In the case of COVID-19, chloroquine, otherwise known as an anti-malarial drug, has been reported to be helpful (4), findings awaiting further support. Further research is also required for camostat mesilate, an inhibitor of the host cell surface protease TMPRSS2 (5), which is necessary for the endocytic entrance of the SARS-CoV-2 virus after its attachment to the spike head receptor, the host's ACE2 receptor. On the other hand, the limitations of identifying directly acting anti-viral drugs should by no means be perceived as a reason for pessimistic judgments. It seems important to also consider the host's responses to viral infections, especially with regard to severe disease progressions. In these more serious cases, the inflammatory responses can become life threatening. These phases of overshooting inflammation can be caused by damage responses of the host's innate immune system. However, in several cases, a direct viral contribution to inflammation has become evident. For instance, the viroporin protein ORF3a of SARS-CoV has been shown to activate the NLRP3 inflammasome, via TRAF3-dependent ubiquitination and processing of p105 (6, 7). As agents are known that prevent NLRP3 activation, anti-inflammatory treatments seem to be promising. However, the use of anti-inflammatory drugs with immune-suppressive properties such as glucocorticoids and also various nonsteroidal anti-inflammatory drugs (NSAIDs) (8) is not recommendable for the combat against an infectious disease. Instead, compounds are desired which combine anti-inflammatory effects with immune stimulatory actions, especially in the arm of adaptive immunity. An agent possessing these dual properties is melatonin (9).

## **2. MELATONIN**

Melatonin (*N*-acetyl-5-methoxytryptamine) is a derivative of the essential amino acid tryptophan. In vertebrates, it is only synthesized from tryptophan as a starting material. Limited supply of tryptophan will inevitably result in reduced melatonin production in vertebrates. Fortunately, many foods contain high level of tryptophan including beans, cheese, milk, and turkey meat. Consumption of these foodstuffs guarantees the tryptophan supply for melatonin synthesis, provided the enzymatic biosynthesis is not affected. In addition, almost all foodstuffs also contain melatonin. These include rice, wheat, corn, fruits, meat, fish, egg, milk, wine, beer, coffee, tea, etc. Consumption of some of these food stuff increases melatonin levels in vertebrates<sup>10</sup> and also in humans (11, 12). The dual ways of melatonin supply from its endogenous synthesis and extraction from the daily food consumption seem to maintain the persistent melatonin circadian rhythm and levels. This is not always the case as expected. For instance, melatonin levels are significantly reduced with aging in humans and numerous other organisms studied (13). The underling

mechanisms for this phenomenon are only partially known and deserve further clarification. Several factors such as functional declines in the circadian system, especially in the suprachiasmatic nucleus (SCN), pineal calcification and, perhaps, also increased melatonin degradation may contribute. Moreover, numerous diseases and disorders lead to reduced melatonin levels (14). Melatonin is a potent antioxidant and reacts with a variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (15, 16). Many studies have documented the increased productions of ROS and RNS in organisms with aging (17, 18). Melatonin as the first line antioxidant defense molecule will be exhausted with the increased oxidative stress with aging. This can be deduced from the results of animal studies or clinical observations. For example, when rats were exogenously given melatonin to increase their serum melatonin level, followed by administration of dioxin, the resulting oxidative stress from increased ROS production in the tissues caused immediate drops in the serum melatonin level compared to controls. This decrease is clearly not associated with reduced melatonin synthesis, but rather consumption (19-21). Other, indirect evidence may be deduced from clinical reports. In patients with disorders which are associated with oxidative stress such as Alzheimer's disease (AD), cardiovascular disease, their serum melatonin levels are always lower than that of their age matched controls (22-25). However, oxidative stress seems to be only one of several causes. Other diseases and disorders that are not associated with strongly enhanced oxidative stress lead also to decreased melatonin levels. Moreover, in AD as well as in normal aging, degenerative processes in SCN, pineal gland and their connections have to be taken into consideration (14, 26). This has, in particular, considerable consequences to the melatonin synthetic capacity. It has been reported that the gene expression of AANAT, a rate limiting enzyme of melatonin synthesis, is downregulated with aging in mammals (27) or its phosphorylation is impeded. The role of AANAT phosphorylation is of predominant importance in primates and ungulates, in which the enzyme is mainly post-translationally regulated, but less in rodents, in which AANAT is largely transcriptionally controlled (28, 29). Dephosphorylation inhibits the binding of the stabilizing 14-3-3 protein and, thus, leads to enhanced proteasomal AANAT degradation (28, 29). Notably, melatonin is a pleiotropically acting regulator, which also modulates the immune system in multiple ways (30). In summary, whatever the specific causes of decreased melatonin levels are, the consequences of reduced melatonergic functionality in countless melatonin-controlled physiological and cell biological parameters have to be losses of organismal fitness, resilience and resistance.

While most of the facts discussed above concern primarily the pineal gland and circulating melatonin, it is important to note that melatonin is also synthesized in most, presumably all tissues. However, extrapineally formed melatonin contributes in mammals, under normal conditions, only poorly to circulating levels. The widespread formation is not that much surprising, as melatonin has been shown to be synthesized in mitochondria (31, 32). In mammals, mitochondrial melatonin formation had recently been demonstrated in the rat brain (33). To which extent mitochondrially synthesized melatonin contributes to the overall amount present in the human body remains to be studied in detail. Tissue melatonin can often exceed the circulating levels by far (30, 34, 35), but the size of its fraction of mitochondrial origin requires further clarification. The presence of mitochondria is *per se* no evidence for melatonin synthesis, since the expression of nuclear genes encoding mitochondrially targeted proteins is highly tissue specific. Nevertheless, the formation of melatonin in numerous cell types may be in favor of a mitochondrial origin. On the other hand, it is important to remain aware that extramitochondrial melatonin formation is not ruled out, most importantly in the pineal gland, in which the machineries for AANAT regulation are primarily cytosolic or associated with the plasma membrane (29). Presence in the cytosol does not exclude

localization in the intermembrane space, but formation in the mitochondrial matrix would require functional analogs (36). Moreover, the mitochondrially formed melatonin in the rat brain did not exhibit circadian rhythmicity (33).

Mitochondria are particularly vulnerable to oxidative stress and mutation. The mutation accumulation of mitochondria increases with aging and finally will jeopardize their functions. Dysfunctional mitochondria not only produce less ATP, but can be also assumed to synthesize lower amounts of melatonin, another possible contribution to its aging-related decline.

Melatonin's primary function serves as the first line antioxidative defense in organisms (37) and other functions are acquired during evolution (38). For example, its earliest acquired function may be regulation of innate immunity. Primitive forms of innate immunity have been demonstrated in invertebrate animals and even amoebae, as a means of host defense against intruding pathogens (39-42). The modulation of immune responses by melatonin has been observed decades ago (43). Melatonin impacts both innate and adaptive immunities. These have been extensively reviewed by several authors (9, 44-46). Recently, the focus has been given to the anti-inflammatory activity of melatonin (47-49). Inflammation largely belongs to the innate immune response. Meanwhile, several studies have reported antiviral and antibacterial activities of melatonin in animals and in plants (50-53). These reports are challenging with regard to the questions of whether and under which conditions melatonin enhances or suppresses the innate immunity in organisms. This review tries to clarify this issue based on the published literature and our understanding on the basic function of melatonin in organisms.

### **3. INNATE AND ADAPTIVE IMMUNITIES**

Immunity against foreigners' invasion is one of the most complex processes in organisms. Defense against invaders can be traced back to early life forms in the evolution. Prokaryotic organisms have already developed immune responses, in a broader sense, toward virus infections (54). This is known since quite some time for bacteria, but has meanwhile also been demonstrated for archaea. Despite the considerable differences between bacterial and archaeal viruses (55, 56), similar defense strategies are present in either prokaryotic domain. In both of them, clustered regularly interspaced short palindromic repeats (CRISPRs) are the basis for eliminating undesired genetic elements of foreign origin, whereas the CRISPR-binding proteins (Cas proteins) vary between species. CRISPR/Cas systems are, thus, regarded as examples of anti-viral immunity in prokaryotes (57-59). However, in mechanistical terms, CRISPR/Cas systems differ from conventional processes of vertebrate innate immunity. Nevertheless, they show a relationship to eukaryotic noncoding RNAs, as CRISPR RNAs are related to piRNAs (PIWI-binding RNAs), which are capable of silencing transposable elements (60, 61). Therefore, the piRNA-directed mechanism has been regarded as a form of eukaryotic immunity (61). Remarkably diverse mechanisms of defense against viral or bacterial intruders are already present in eukaryotic unicells (62). Upon the appearance of multicellular organisms, the innate immune system has expanded from intracellular to intercellular mechanisms. This was accompanied by a considerably increase in the number of secretory factors, from antimicrobial peptides, which are found in both unicellular and multicellular organisms, to additional factors that either attack pathogens in the extracellular space or numerous regulatory molecules, which often bridge between the innate and adaptive systems. The intercellular innate immunity is primarily based on specific cell types, such as various forms of phagocytes and, in vertebrates, NK cells. However, non-lymphocytic immune cells that are traditionally regarded as part of the innate system can, in fact, be also directed via antibodies by the adaptive system. Innate immune cells act as first-line protectors and also orchestrate defense

machineries to protect against potentially multiple invasions and afford clearance of pathogens before they enter inside cells. In case that some pathogens intrude cells, the intracellular innate immunity will be activated. These innate immune activities respond to any pathogen they detect and are versatile with regard to targets. This does not imply a general lack of specificity, but works mainly on the basis of identification of molecular non-self characteristics, such as presence of double-stranded RNA, non-self RNA modifications or secondary structures, oxidatively damaged DNA, atypical methylation patterns, circular DNA, or formylated N-termini of proteins or peptides (63-67). Adaptive immune systems have evolved in multicellular organisms, with highest complexity in vertebrates (39, 68, 69). This system can accurately target the specific pathogens by antibodies and cytotoxic T-cells, directs by virtue of antibodies innate immune cells and the classic pathway of the complement system, and generates memory cells that allow rapid responses after re-infection. Both innate and adaptive immune systems perform coordinately to track, block and finally destroy invaded pathogens including viruses, bacteria, pathogenic fungi and parasitic animals.

In multicellular organisms, the physical and chemical barricades are the first defense of innate immune machineries (which are not discussed in this paper); however, from time to time, the pathogens may occasionally breach these barriers while entering organisms. In this case, it is up to the intercellular innate and the adaptive immune systems to respond. Invaders immediately face the rapid response of intercellular innate immune cells mainly including macrophages and other monocytes, dendritic cells, basophils/mast cells, neutrophils and natural killer (NK) cells. In part, these cells fulfill important functions by interacting with the adaptive immune system. Dendritic cells and macrophages function as antigen-presenting cells. Even NK cells influence the adaptive immune system by interacting with dendritic cells (70, 71). With the exception of NK cells, other leukocytes that are conventionally regarded as part of the innate immune system are, in fact, often directed by the adaptive system via binding or recognizing antibodies. However, these cells are also capable of acting independently of the adaptive system. While NK cells detect infected or transformed via absent or strongly reduced MHC class I surface expression (“missing self”) or, alternately, by several invariable target cell recognition receptors (72-74). These mechanisms are of particular importance because several viruses are able to suppress the surface expression of MHC class I molecules and presentation of the virus-derived peptides, which would otherwise be detected by cytotoxic T-cells (75). In the membranes of infected, transformed or severely stressed cells, pattern recognition receptors (PRRs) are expressed, such as Toll-like receptors (TLRs), Nod-like receptors (NODs, NLRs), C-type lectin receptors, etc. These receptors, mainly the TLRs, recognize the particular molecular patterns common to pathogens but absent in hosts (76). This is referred to as pathogen-associated molecular pattern (PAMP), which includes virus RNA, viral and bacterial associated proteins and DNAs as well as membrane constituents of bacteria such as lipopolysaccharide (LPS). Upon recognition of these pathogens, macrophages and neutrophils are activated to eliminate the pathogens by phagocytosis and chemical attack. These innate immune cells also secrete cytokines, chemokines and other signaling compounds to organize inflammatory responses that accelerate pathogen clearance and healing processes. Rarely, some pathogens do escape the intercellular innate immune attack and invade inside cells. In this situation, the intracellular innate immune response will act against the intracellular intruders. Generally, this intracellular innate immune response is not limited to the immune cells, but exists in almost all cell types. Intracellular PAMP receptors initiate the attack on these intruders by autophagy, apoptosis, or inflammasome-induced pyroptosis. Several inflammasome types of different composition and specificity exist, among which the relatively versatile NLRP3 has been studied



most frequently. The detector molecules also differ with regard to their specificity. For example, TLR9 and cGAS recognize foreign double-stranded DNA (dsDNA) (77), TLR7 and TLR8 purine-rich single-stranded RNA (ssRNA) (78, 79), TLR3, RIG1 and NODs double-stranded RNA (dsRNA)(80-81), respectively. Corresponding differences exist among the inflammasomes, which are also differently expressed in the various cell types. The AIM2 (absent in melanoma 2) inflammasome responds to cytosolic dsDNA of viral, bacterial or aberrant host origin (82-84), whereas IFI16 detects nuclear dsDNA of viral origin (85). The innate immune response occurs quickly after having encountered the pathogens. At the same time, via cross-talking between the innate immune and adaptive immune systems, the signal processing cells of innate immune system such as macrophages and dendritic cells pass the specific molecular patterns of the pathogen to Tc and B lymphocytes to initiate the adaptive immune response. Since the latter requires cell proliferation and needs weeks to develop full functionality, the clearance of remaining pathogens that have escaped the innate immune attack takes considerable time.

#### **4. OVERREACTION OF THE INNATE IMMUNE-RESPONSES, A DOUBLE-EDGED SWORD FOR HOSTS**

In humans, the immune system is essential for protection against infectious diseases. However, in some cases, unbalanced or overreacting innate immune responses will do more harm than good to the body. This is primarily occurring in deadly virus infections. For example, the innate immune overreaction in SARS, MERS, COVID-19 and avian flu virus infections causes serious cell, organ and tissue damage and leads to unnecessarily, and sometimes (e.g., Ebola) extremely, high mortality. The world-wide mortality for these highly contagious viral diseases are as high as from 11 to 51% (86-88), compared to 0.0537% (53.7/100,000) in seasonal influenza (89). However, such comparisons are strongly biased under several aspects: 1). the availability or lack of vaccines; 2). the temporal variation in the occurrence of viral subtypes of differing pathogenicity; 3). high numbers of unreported cases that vary from disease to disease; 4). differences in target cell specificity and organ failure; 5). the contribution of cytopathic properties of viral constituents and of coagulopathies, especially evident in hemorrhagic viral diseases caused by filoviruses such as Ebola and Marburg viruses (90, 91). Theoretically, in most cases, it is not the virus *per se* killing the hosts, but the innate immune overreaction that leads to the so-called “cytokine storm”(92, 93) and causes host cells entering a suicide mission. Biologically, the best outcome for the intruders and host cells is their coexistence or symbiosis. The best example for this symbiosis is the evolution of mitochondria whose ancestors have originally been intruders. Destroying the host is not the primary purpose of the virus and is also against the natural selective law for their survival. In contrary, for the host cells, having the unwelcome “guests” is not a comfortable situation and the host cells will try desperately to eliminate intruders’ spreading and finally get rid of them. For example, in corona virus invasion (in addition to the virus proteins), its ssRNA will be recognized as the PAMP, particularly by the RIGs and TLR7. The RIGs or TLR7 will direct this ssRNA to lysosomes for digestion by RNases. At the same time, the RIGs and TLR7 will also trigger the type 1 interferon reaction and release of many other proinflammatory cytokines via respective pathways. In some hypersensitive cases, the cytokine storm is initiated that consists of excessive production of cytokines, chemokines and other factors. This cytokine storm magnifies the danger signal of the virus invasion, but also leads to destructive inflammation and host cell damage. In turn, the components released from damaged cells, particularly from stressed mitochondria, including mitochondrial DNA, cardiolipin, cytochrome C and also segments of nuclear DNA (nDNA) will be recognized as damage associated molecular patterns (DAMPs) by intra and

intercellular immune molecules including TLR4/7/9, cGAS triggering a further large-scale proinflammatory cytokine release known as the “secondary cytokine storm”. The induction of inflammatory reaction cascades by PAMPs and deriving DAMPs leads to a vicious cycle. If this vicious cycle is not broken, it can result in widespread apoptosis, pyroptosis and necrosis even in non-infected cells. Self-sacrifice of cells has already evolved in the unicellular organisms, in which the suicide of infected individuals probably slows down the pathogen spreading within the population (94). This seems to be a suitable strategy for unicellular organisms, since they lack the advanced adaptive immune system and are often unable to achieve effective pathogen clearance. In multicellular organisms, particularly in mammals, the situation is entirely different, since mammals have evolved a sophisticated adaptive immune system which can specifically target pathogens with much higher efficiency than the innate one. The activation of the adaptive immune system including clonal expansion of T and B lymphocytes and production of specific antibodies require weeks after exposure to a new pathogen. This time period can limit the individual survival, in cases of attack by potentially deadly viruses such as SARS-CoV, SARS-CoV-2, MERS-CoV, MARV, EBOV and related filoviruses. In principle, these diseases can be self-limiting. If patients are able to generate sufficient amounts of virus-directed antibodies before inflammation and its consequences like fever and cell damage have severely aggravated the health status, they have a good chance to survive. Thus, a feasible strategy to decrease the mortality from these deadly infections is to break the destructive inflammation vicious cycle, i.e., to suppress the overactivity of the innate immune response. In this way, the patients will gain the precious time required to fully establish the adaptive immune response and attain sufficient amounts of specific antibodies.

It appears that bats, which are reservoirs of various corona- and filoviruses that are deadly to humans, have evolved a strategy to tolerate these viruses by preventing an overshooting innate immune response (95). For instance, they express interferon subtypes such as IFN- $\omega$  variants that compete with more strongly proinflammatory IFNs; they also express NK cell receptor variants such as NKG2-like homologs that contain inhibitory motifs in the cytosolic domain; they overexpress MHC class I molecules and, thereby, seem to circumvent the missing self (95). Different from other mammals, bats take advantage of a strategy of virus tolerance by avoiding an overreactive innate immune system, instead of an enhanced antiviral defense.

In humans, such a strategy of virus tolerance does not seem feasible, for genetic reasons. Instead, anti-inflammatory treatments are recommendable. This idea had been occasionally followed. For example, during the SARS breakout in 2003, glucocorticoids were used for this purpose, but, when used as monotherapy, were only tested in very small cohorts of limited statistical value, whereas combination treatments with virostatic drugs were moderately more successful, but did not allow profound conclusions (96). However, as mentioned in the introduction, the additional immune suppressive and other serious side effects of the steroids remain a matter of concern (97). To identify remedies which can modify the innate immune response without serious side effects is necessary and urgent. As to our knowledge, melatonin is such a molecule which can reduce the overreacting innate immune response and, at the same time, promote the adaptive immunity. Additionally, it has been shown to interfere with replication and pathogenicity of some other viruses. These properties will be discussed in detail below.

## **5. MELATONIN ON INNATE AND ADAPTIVE IMMUNITY**

The impact of melatonin on immunity was reported decades ago (98, 99). After the discovery of melatonin as a potent free radical scavenger and antioxidant (15), scientists have started to

investigate the potential anti-inflammatory activity of melatonin since the inflammation involves the free radical reaction. Cuzzocrea *et al.* (100) in 1997 first reported the protective effects of melatonin in carrageenan-induced local inflammation and attributed this anti-inflammatory effect to its antioxidant activity by scavenging peroxynitrite. In the follow-up studies, the group also observed that melatonin not only suppresses the non-specific local but also the systemic inflammations induced by zymosan in rats (101, 102). Thereafter, the anti-inflammatory activity of melatonin in different inducers, animal models and even in human clinical trials has been documented (103-106). Inflammation is always manifested by neutrophil infiltration, increased microvascular leakage, tissue swelling and fever. Normal inflammation helps the tissue to recover from the infections or non-infection injury. However, excessive inflammation can enhance or accelerate tissue injuries. The excessive inflammation is primarily the overreaction of the innate immune system on pathogen (PAMP) and further magnified by cell damage (DAMP). Both PAMP and DAMP related innate immune overreactions can be effectively regulated by melatonin (107, 108). Toll-like receptors (TLRs) play a key role in the innate immune system and they are expressed on immune or non-immune cells. They recognize structurally conserved molecules derived from pathogens to initiate the inflammatory reactions. Melatonin either downregulates the expression of *TLR2*, *TLR4* and *TLR9* or inhibit their downstream pathways under the inflammation but not under normal conditions. For example, in *H. pylori* infected mice, melatonin suppresses the TLR2/MyD88/p-ERK pathway to reduce the IL-2, IL-6, IL-10, IL-17, IFN- $\gamma$  and TFN- $\alpha$  production and subsides the inflammation (109). In LPS treated neonatal rats, melatonin reduced the innate immune response and, thus, inflammation by inhibiting the TLR4/ MAPK/NF- $\kappa$ B pathway (110). These effects are mediated by the melatonin membrane receptors MT1 and MT2, since the anti-inflammatory effect was reduced by blocking these receptors (111, 112). In contrast, the suppressive action of melatonin on the TLR9-mediated innate immune response involves inhibition of the ERK1/2 and AKT pathways, which have been reported to be melatonin receptor-independent (113). NOD-like receptors (NLRs) are intracellular sensors of PAMPs and they cooperate with TLRs participating in regulation of intracellular innate immune responses, such as inflammation and apoptosis. NLRP3 belongs to subfamily of NLRs and the NLRP3 inflammasome is a caspase-1 activating complex, which is inactive under normal conditions. When it is activated by PAMP and DAMP, the caspase-1 within the activated NLRP3 inflammasome in turn activates the inflammatory cytokine, IL-1 $\beta$  (114). In septic mice, melatonin treatment was reported to abolish the NLRP3 inflammasome activation, an effect that was ascribed to ROR $\alpha$  (115), which is now known to not bind melatonin and, therefore, to be no melatonin nuclear receptor (116, 117). The molecular pathway consists in blunting of the NF- $\kappa$ B/NLRP3 connection by melatonin, which results in an erasure of the sepsis-induced information (118). Therefore, melatonin possesses the capacity to suppress the NLRP3 inflammasome activation induced by different activators including cigarette smoking, LPS, radiotherapy, endoplasmic reticulum stress and cadmium toxicity (119-123). Not only the exogenously applied but also the endogenously produced melatonin inhibits the NLRP3 inflammasome activation. In a murine model of ovalbumin (OVA) induced allergic asthma, CpG-ODN (GpC-rich oligodeoxynucleotide) suppressed NLRP3 inflammasome activation and airway leukocytes infiltration, goblet cell hyperplasia and Th2 cytokines production (124). These effects by CpG-ODN were attributed to upregulation of melatonin synthetic enzymes, increased melatonin production, and abolished by the melatonin receptor antagonist, luzindole (124). Moreover, exogenous melatonin mimicked the anti-allergic actions of CpG-ODN (124). Actually, melatonin is known to be synthesized by mitochondria and these organelles are also important players in innate immunity and guardians of the inflammatory



response (125). Mitochondrially produced reactive oxygen species (ROS) stimulate the innate immune signaling cascade and intensify inflammation upon cytotoxic stimuli beyond microbial infection (126). Mitochondria have originated from  $\alpha$ -proteobacteria and still retain some features of their ancestors including the circular CpG-unmethylated DNA, *N*-formyl peptides, cytochrome C, membrane cardiolipin, elevated succinate and ATP. Upon their release from mitochondria under stressful conditions, all of them can serve as DAMPs to initiate the innate immune responses including autophagy, NLRP3 inflammasome activation and apoptosis (127). High levels of melatonin generated by mitochondria may provide on-site protection. Melatonin has been shown to preserve the mitochondrial membrane potential by regulating the mitochondrial permeability transition pore (mPTP) and thus, to prevent the release of mitochondrial contents (128, 129). In this way, melatonin blocks the secondary inflammatory cytokine storm caused by DAMPs of mitochondrial origin. Melatonin's anti-inflammatory actions that can be mainly attributed to the modulation of the innate immune system have been shown to involve numerous additional mechanisms, as summarized elsewhere (9). These effects comprise reduction of cyclooxygenase-2 and NO-mediated activation of phagocytes and microglia; promotion of Nrf2 signaling, which counteracts NF- $\kappa$ B stimulated prooxidant and pro-inflammatory actions; upregulation of sirtuin-1 expression, a regulator that also displays anti-inflammatory effects, including inhibition of the TLR4 activator HMGB1 (high-mobility group box 1), of mTORC1 (mechanistic target of rapamycin receptor complex 1), NF- $\kappa$ B, NLRP3, NICD (notch intracellular domain) in macrophages, and expression of the proinflammatory noncoding RNA lncRNA-CCL2. Moreover, melatonin has been recently reported to shift macrophage polarization from the proinflammatory type M1 to the anti-inflammatory M2 (49). Corresponding effects can be assumed for tissue-resident macrophage-like cells including microglia, in which the M1/M2 polarity also exists.

As to the adaptive immunity, melatonin exhibits various positive effects. One of the important targets of melatonin on adaptive immune system is the thymus which is experiencing atrophy with aging, leading to reduced thymus-specific cytokine production and decreased capacity for maturation, positive and negative selection of T lymphocytes (130). Melatonin delays and diminishes thymic involution and promotes the regeneration of the thymocytes (131, 132). Melatonin, thus, promotes T-cell activation and differentiation including Th17, Treg cells and also memory T-cells. Several cell signaling pathways, including ERK1/2-C/EBP $\alpha$ , are involved in the regulatory roles of melatonin in T-cell biology (133). The influence of melatonin in T-cell biology is primarily mediated by its membrane receptors (134) as well as receptor-independent mechanisms, for example, its direct antioxidant activity. MT1, MT2 receptors have been identified in the thymus and T-lymphocytes (135). Additional claims concerning nuclear receptors (135) remain to be revisited, notwithstanding the fact that the presence of RORs is not disputed (136). In addition, melatonin positively regulates the B lymphocyte activities. It promotes the B-lymphocyte proliferation in birds (137) and in human tonsillar tissue (138). In mice, melatonin administration inhibits the apoptosis of the precursor B cells in bone marrow and substantially promotes the survival of newly formed B cells mediating humoral immunity (139). Melatonin injection in sheep boosts the antibody titer (140) and serum IgG level. As a result, melatonin is suggested to be used as the adjuvant of vaccine to increase the antibody production (141). In fact, this led to substantially higher antibody production and also sustained it for a longer duration than that without melatonin adjuvant (142). Interestingly, the immune systems including thymus, bone marrow, lymphocytes, and other immune cells have the capacity to biosynthesize melatonin (143-146). Melatonin can serve as a hormone, paracrine, autocrine or tissue factor to coordinate the function of the immune system. Therefore, melatonin deficiency significantly impacts its function.

This has consequences to the vulnerability to severe infectious diseases in elderly patients as seen in COVID-19, because a well-functioning immune system is believed to be the strongest predictor of human longevity and healthy aging, as summarized elsewhere (147).

## 6. MELATONIN IN INFECTIOUS DISEASES

The potential utility of melatonin in parasite, bacterial and virus infections have been discussed in several excellent reviews (46, 148, 149). Here, we will focus on the potential effects of melatonin on deadly virus infections such as SARS, MERS, COVID-19, avian flu and filoviral hemorrhagic fevers. As mentioned above, melatonin is a phylogenic ancient molecule and serves as a signaling molecule of stress for numerous organisms. Therefore, melatonin cannot be expected to directly kill the parasites, bacteria, pathogenic fungi and viruses but can protect them against many forms of biotic or abiotic stress as a universal antioxidant and regulator. For example, melatonin treatment modulates the bacterial and fungal communities in agricultural soils under abiotic stress. This treatment even increases the populations of some microbes as indicated by the increased operational taxonomic units (OUT) which are suppressed under abiotic stress (150). Recently Madigan *et al.* have also observed that high doses of melatonin application increase the abiotic stress tolerance to phytopathogenic fungi in an *in vitro* study (Melatonin Research, in press). In its property as a stress signal molecule, melatonin is assumed to cause the microbes to hold excessive proliferation in favor of survival in adverse environments. Under a non-stressful condition (normal condition), holding of proliferation reduces the microbial vulnerability and can be expected to also result in response to a stress signal transmitted by exogenous melatonin. The first antibiotic effect of melatonin on bacteria was reported by Tekbas *et al.* (151). They observed that melatonin inhibited the growth of common pathogenic bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* by prolonging the lag phase of bacterial growth, another indication of melatonin's anti-proliferative actions in bacteria. Eukaryotic hosts and many microbes, too, possess the capacity of synthesizing melatonin for their respective own benefit. During evolution, some parasitic microbes have established symbiotic relationships to their hosts. One beneficial effect of this symbiosis seems to be that parasitic microbes or their descendants provide more melatonin to the hosts. The mitochondrion is one of the examples. In some cells, the majority of melatonin has been found to be generated by the mitochondria (32, 152, 153). However, the body of evidence is still small and not based on mitochondrial vs. cytosolic comparisons. In the choroid plexus, conclusions were drawn from AANAT/MitoTracker colocalization (153). During oocyte maturation, mitochondrial synthesis was demonstrated in mitochondrial cultures (152). Other data have been obtained in plants (154). Although the basis for generalizations and applicability to humans is still insufficient, the emerging consequences, if documented on a broader scale, would be highly fascinating. They would imply that mitochondria do not only provide ATP to the host cell, but also by delivering melatonin convey another important function (32). Some further examples are known from the botanical field and shall not be discussed here in detail. Both additional melatonin supply by an endophytic bacterium (155) and reduction of host melatonin synthesis by phytopathogenic bacteria (156) have been reported, as well as counteractions of exogenous melatonin (157).

The association of melatonin with mitochondria is also of potential interest to virus infections. Viruses have been shown to directly modulate mitochondrial structure and function and, thereby, to impact the innate immune responses of the host cells (158, 159). It is not unlikely that virus-induced mitochondrial dysfunction can reduce melatonin production of host cells. It may be an additional strategy of viruses to attack the melatonin synthetic capacity. This assumption would

be in agreement with clinical data on reduced salivary (160) and serum melatonin levels (161) in HIV infected patients. This phenomenon was also observed in the patients of hemorrhagic fever with renal syndrome (HFRS) which occurs after infection with hantavirus [(-) ssRNA (negative-sense single-stranded RNA)]. In the acute stage, plasma melatonin levels of these patients were significantly lower than those of the normal controls. Moreover, melatonin levels were negatively correlated with WBC counts at acute and convalescence stages. The authors concluded that melatonin might be involved in the regulation of inflammatory responses in HFRS patients, with consequences to pathogenesis and disease progression (162).

For the hosts, melatonin improves the defense system by upregulating the expression of antioxidant enzymes such as SOD, CAT, and glutathione peroxidase (163), effects that reduce the oxidative tissue damage caused by the pathogens. Most importantly, melatonin redirects the host immune response, i.e., by controlling the potentially overreactive innate response and promoting the adaptive immune response. The above-discussed cytokine storm may be stronger if pathogens have downregulated melatonin formation. This overreaction by innate immune cells may be induced by PAMP/DAMP-initiated and TLR-mediated activation of the NF $\kappa$ B and AP-1 signaling pathways (164), but can be also directly induced by viruses, e.g., by SARS-CoV protein ORF3a (165). The released proinflammatory cytokines include especially TNF $\alpha$ , IFN $\alpha/\gamma$ , IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-17A, IL-23, and various chemokines, such as monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 $\beta$  (MIP-1 $\beta$ ), RANTES, interferon-inducible protein 1 (IP-1), and IFN $\gamma$ -induced CXC chemokines. Elevated melatonin levels significantly decrease the first and secondary cytokine storms and, thereby, lead to the reduced tissue and organ damage as well as increases in the survival rate in septic animal models (166) and in human subjects (103). The beneficial effects of melatonin on multiple bacterial sepsis are by downplaying the innate immune response to suppress the phagocytic activities of neutrophils; however, promotes the development of the neutrophil extracellular trap (NET) to control the bacterial spreading out (166). Moreover, the downregulation of inducible NO synthase, reportedly with particular importance of a mitochondrially targeted subform, mt-iNOS, has been found to be a decisive advantage of melatonin treatment in rescuing animals from bacterial sepsis (167-171). Further references are summarized elsewhere (9, 172).

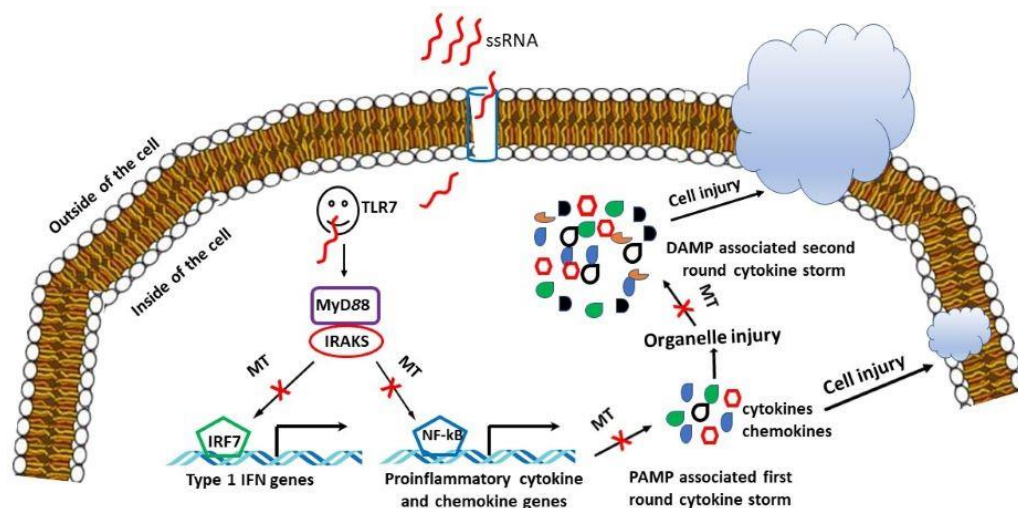
Concerning virus infections, melatonin has been successfully used to treat different diseases in animal models. In minks afflicted by parvovirus (a single-stranded DNA virus), melatonin significantly reduced death rate (173). Similar results have been observed in infections by Semliki Forest virus [(+) ssRNA virus (positive-sense single-stranded RNA virus)] (174), Venezuelan equine encephalomyelitis virus [(+) ssRNA virus] (175), LP-BM5 retrovirus (176), respiratory syncytial virus [(-) ssRNA (negative-sense single-stranded RNA) virus] (177), rabbit hemorrhagic disease virus [(+) ssRNA virus] (178). According to these findings, melatonin can convey protection against all virus types, as far as the differences in nucleic acid genomes are concerned. This indicates that melatonin's actions are not a matter of virus specificity, but rather of inflammation suppression. Coronaviruses, on which this article is centered, are (+) ssRNA viruses.

## **7. COULD MELATONIN BE USED AS A VERSATILE ADJUNCT TREATMENT FOR DEADLY VIRAL DISEASES? FOCUS ON CORONA AND INFLUENZA VIRUSES**

The purpose of this section is not to advocate the replacement of conventional therapies, as far as vaccines and specific anti-viral drugs are available. However, this is not always the case, especially when new zoonotic viruses or new subtypes of viruses appear. Moreover, as outlined above, building up a sufficient immune protection via the adaptive system, either by vaccination

or in the course of disease progression, takes considerable time. Before this state has been attained, a viral disease with potential for a deadly outcome may have already caused a life-threatening crisis. Moreover, some anti-viral drugs have serious side effects. Outbreaks of deadly viral diseases often appear suddenly and unexpectedly, especially in zoonoses. This was the case with several coronaviruses, such as SARS-CoV, SARS-CoV-2, MERS-CoV and zoonotic influenza viruses, in particular, A/H5N1 (avian influenza virus). In the past, several of these infectious diseases disappeared relatively soon, but there is no guarantee for this in newly appearing diseases, such as COVID-19 caused by SARS-CoV-2. Both corona and influenza viruses are present in countless forms in the majority of domestic and wild mammals and birds. SARS-CoV-2 seems to have its origin in bats, but may already be the result of recombination and/or mutations. Its genome has an identity of 96.2% of a SARS-related virus, SARSr-CoV RaTG13, but is more unsimilar to SARS-CoV and MERS-CoV (179). Moreover, the functional sites of the SARS-CoV-2 spike protein closely resemble those of pangolin coronaviruses (180, 181). Two major types of SARS-CoV-2 have been identified, type L and type S, and within the months since outbreak, a quantitative shift in favor of the S type has already occurred (179). These findings demonstrate the variability of viruses, which generate new types and change their proportions within short periods of time. Variability and changing types is already a classic in animal and human influenza viruses (182, 183). All this shows how laborious, time and money consuming the repeated adaptation of vaccines to the changing requirements of virus control have to be. If vaccines are available at all, a certain proportion of individuals will not receive them and remains in this regard unprotected. Whether anti-viral drugs may be applicable, depends on the specific virus or its subtype. For all these reasons, a more generalized and less virus-specific therapy is urgently needed, especially to prevent lethal outcomes. This is the more required as anti-viral medicine has not been developed for the purpose of preventing the massive and potentially fatal tissue damage caused by the overreacting innate immune system. Moreover, it may be also decisive to bridge the weeks of the window period, until the host adaptive immune system is sufficiently effective. Therefore, a major necessity should be seen in the availability of an anti-inflammatory medication, which is not associated with simultaneous suppression but rather support of the adaptive immune system. Such a treatment can serve as the basic complementary remedy to reduce the irreversible tissue damage and mortality. Melatonin appears to be such a remedy. As summarized in the preceding section, melatonin has already been shown to be effective in various viral diseases and can rescue organisms from high-grade inflammation in sepsis. Moreover, a recent network-based approach has identified melatonin as one of the top candidates for treatment of viral diseases, with particular focus on coronaviruses (184). These considerations may be equally applicable to severe cases of disease progression in seasonal flu, which also leads to thousands of deaths yearly, and to zoonotic influenza infections, especially avian flu. Finally, it should be noted that melatonin shows an excellent tolerability profile, which has allowed to give 300 mg daily for 2 years to severely ill ALS patients, without any negative side effects (185). Depending on the health status and disease progression, melatonin may be given in the respective viral diseases either as an adjunct therapy, along with anti-viral medication, or as a regular systemic treatment starting with early signs of infection. The potential mechanisms of melatonin for therapeutic purposes are summarized in Figure 1.





**Fig.1. Illustration of the potential effects of melatonin on the overreaction of the innate immune response induced by deadly virus infection.**

*SARS-CoV, SARS-CoV-2, and MERS-CoV are all single-stranded RNA (ssRNA) viruses. Here, an ssRNA virus is selected to serve as an example. To simplify the process, only ssRNA is used as the PAMP. TLR7 is the receptor to recognize the virus ssRNA as the PAMP. MT: melatonin, X: blocking effect.*

## 8. APPLICABILITY TO OTHER VIRAL DISEASES?

The question of whether conclusions obtained from corona and influenza viruses can be translated to other deadly viral diseases has to consider the often substantial differences between virus particles, their cytopathic constituents, and types of disease progression. This would be of particular interest several filoviruses of the (-) ssRNA category that cause hemorrhagic fevers, such as Ebola viruses, among which three of the five known forms are highly lethal (EBOV, SUDV, BDVB), and Marburg virus (MARV) as well as its related Ravn virus (RAVV). In these hemorrhagic fevers, disease progression and pathological changes differ considerably from those observed in corona or influenza viruses. The symptoms appear typically in a biphasic sequence. In the initial phase, symptoms are reminiscent of a flu, while inflammatory responses in the organs are in the beginning moderate, but become progressively serious and lead to conjunctivitis, diarrhea and other symptoms. These diseases, in particular, the lethal forms of Ebola, are also characterized by leukopenia and lymphopenia, followed by thrombopenia, endothelial leakage with hemorrhage and coagulopathy, which contribute to multi-organ failure (186).

An important difference to corona and influenza virus diseases concerns the changes in the immune status. In addition to cells of various organs, EBOV also infects macrophages, monocytes, dendritic cells and lymphocytes, which leads to changes in cytokine levels and, also, to overactivation of the extrinsic pathway of coagulation, which may be the cause of disseminated intravascular coagulation (186). The infection of macrophages and monocytes leads to aberrant patterns of cytokine and chemokine release, with transient elevations of IL-1 $\beta$ , IL-6, TNF $\alpha$  and MIP-1 $\beta$  in the early phase, followed by increased circulating interleukin antagonists and receptors (IL-1RA, sTNF-R, sIL-6R) in the later phase and after recovery. Surprisingly, a fatal outcome was associated with poorly elevated levels of IL-6 and TNF $\alpha$ , but high levels the anti-inflammatory factors IL-10, IL-1RA and sTNFR, low concentrations of MIP-1 $\alpha$  and MIP-1 $\beta$  and undetectable



IL-1 $\beta$ , whereas normal or elevated levels of the proinflammatory cytokines IL-1 $\beta$  and IL-6 were indicators of a non-fatal outcome (187). In other words, the lethal consequences in Ebola do not seem to be related to an overactivated innate immune system, but rather to its failure due to infection of macrophages and monocytes. Therefore, treatments that suppress the innate immune system are not recommendable in the late phases of disease. Whether they may be useful in earlier stages, remains to be investigated.

Melatonin has been suggested to be used in Ebola disease (188-190). Its suitability has been confirmed in a recent experimental study in cultured endothelial cells and engineered vessels (191), which showed that melatonin protected against albumin leakage induced by Ebola virus-like particles. Based on the molecular concentrations, the efficacy of melatonin seemed to be 10-fold higher than that of FX06, a specific Ebola disease medicine. The high efficacy of melatonin conforms to observations in another viral disease, Venezuelan equine encephalomyelitis (VEE) caused by the Togavirus VEEV, another (+) ssRNA virus. The disease is rarely transmitted by mosquitoes to humans, but can be lethal. When mice were infected by VEEV, melatonin's efficacy was, again, impressive. If melatonin was given at 1mg/kg subcutaneously 3 days before and 10 consecutive days after inoculation, the mortality of the infected mice was reduced from 100% in untreated animals to 16%. Moreover, very high titers of IgM antibodies were found from three to seven weeks after virus inoculation (175). The dose of 1mg/kg is for a 70 kg body weight human subject easily achievable and has no or only small side effects for several months of use in humans (192).

## **9. CONCLUSION**

Virus infections can attack the melatonin synthetic system resulting in reduced melatonin levels in hosts. This often leads to altered immune responses, specifically, with the overreaction of the innate immune response. The uncontrolled innate immune response promotes the massive inflammatory reaction and causes irreversible tissue damage and mortality of the hosts. Melatonin as a potent antioxidant and immune regulator not only suppresses the oxidative stress but also controls innate immune response and promotes the adaptive immune response. Collectively, melatonin administration subsides the excessive inflammation and increase the tolerance of host to the deadly virus infection and finally the hosts will recover from the infections. These properties should be used in all those deadly viral diseases that are associated with an emerging overactivity of the innate immune system, whereas other diseases with a breakdown of the innate arm due to leukocyte infections have to be considered with caution and will require further research on the suitability in the early phase. Especially in cases in which no specifically effective treatments of the deadly virus diseases are available, the high safety margin of the melatonin may be seen as an option with low risk. We think that melatonin is a best candidate to serve as complementary and, perhaps, regular conventional treatment for deadly viral diseases.

## **ACKNOWLEDGEMENTS**

The authors thank the reviewers for their valuable comments on this manuscript.

## **AUTHORSHIP**

DXT and RH both wrote and edited this article.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

## REFERENCES

1. Chen CC, *et al.* (2011) ORF 8a of severe acute respiratory syndrome coronavirus forms an ion channel: experiments and molecular dynamics simulations. *Biochim. Biophys. Acta* **1808**: 572–579.
2. Millet JK, Whittaker GR (2015) Host cell proteases: critical determinants of coronavirus tropism and pathogenesis. *Virus Res.* **202**: 120–134.
3. de Wilde AH, Snijder EJ, Kikkert M, van Hemert MJ (2018) Host factors in coronavirus replication. *Curr. Top. Microbiol. Immunol.* **419**: 1–42.
4. Gao J, Tian Z, Yang X (2020) Breakthrough: Chloroquine phosphate has shown apparent efficiency in treatment of COVID-19 associated pneumonia in clinical studies. *BioSci. Trends* **14** (1): 72–73. doi: 10.5582/bst.2020.01047.
5. Hoffmann M, *et al.* (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically-proven protease inhibitor. *Cell*. doi: 10.1016/j.cell.2020.02.052.
6. Chen IY, *et al.* (2019) Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. *Front. Microbiol.* **10**: 50.
7. Siu KL, *et al.* (2019) Severe acute respiratory syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. *FASEB J.* **33**: 8865–8877.
8. Bancos S, Bernard MP, Topham DJ, Phipps RP (2009) Ibuprofen and other widely used non-steroidal anti-inflammatory drugs inhibit antibody production in human cells. *Cell. Immunol.* **258**: 18–28.
9. Hardeland R (2018) Melatonin and inflammation — Story of a double-edged blade. *J. Pineal Res.* **65**: e12525.
10. Hattori A, *et al.* (1995) Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. *Biochem. Mol. Biol. Int.* **35**: 627–634.
11. Oba S, Nakamura K, Sahashi Y, Hattori A, Nagata C (2008) Consumption of vegetables alters morning urinary 6-sulfatoxymelatonin concentration. *J. Pineal Res.* **45**: 17–23.
12. Howatson G, *et al.* (2012) Effect of tart cherry juice (*Prunus cerasus*) on melatonin levels and enhanced sleep quality. *Eur. J. Nutr.* **51**: 909–916.
13. Reiter RJ (1991) Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr. Rev.* **12**, 151–180.
14. Hardeland R (2012) Melatonin in aging and disease — multiple consequences of reduced secretion, options and limits of treatment. *Aging Dis.* **3**: 194–225.
15. Tan DX, Chen L, Poeggeler B, Manchester LC, Reiter JR (1993) Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocr. J.* **1**: 57–63.
16. Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ (2007) One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.* **42**: 28–42.
17. Davalli P, Mitic T, Caporali A, Lauriola A, D’Arca D (2016) ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. *Oxid. Med. Cell. Longev.* **2016**: 1–18.

18. Baumann CW, Kwak D, Liu HM, Thompson LV (2016) Age-induced oxidative stress: how does it influence skeletal muscle quantity and quality? *J. Appl. Physiol.* **121**: 1047–1052.
19. Pohjanvirta R, Tuomisto J, Linden J, Laitinen J (1989) TCDD Reduces serum melatonin levels in long-evans rats. *Pharmacol. Toxicol.* **65**: 239–240.
20. Linden J, Pohjanvirta R, Rahko T, Tuomisto J TCDD (1991) Decreases rapidly and persistently serum melatonin concentration without morphologically affecting the pineal gland in TCDD-resistant Han/Wistar rats. *Pharmacol. Toxicol.* **69**: 427–432.
21. Pohjanvirta R, *et al.* (1996) Mechanism by which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) reduces circulating melatonin levels in the rat. *Toxicology* **107**: 85–97.
22. Ozcankaya, Delibas N (2002) Malondialdehyde, superoxide dismutase, melatonin, iron, copper, and zinc blood concentrations in patients with Alzheimer disease: cross-sectional study. *Croat. Med. J.* **43**: 28–32.
23. Uchida K, Okamoto N, Ohara K, Morita Y (1996) Daily rhythm of serum melatonin in patients with dementia of the degenerate type. *Brain Res.* **717**: 154–159.
24. Dominguez-Rodriguez A, Abreu-Gonzalez P, Arroyo-Ucar E, Reiter RJ (2012) Decreased level of melatonin in serum predicts left ventricular remodelling after acute myocardial infarction. *J. Pineal Res.* **53**: 319–323.
25. Dzida G, *et al.* (2013) Alteration in diurnal and nocturnal melatonin serum level in patients with chronic heart failure. *Ann. Agric. Environ. Med.* **20**: 745–748.
26. Hardeland R (2012) Neurobiology, pathophysiology, and treatment of melatonin deficiency and dysfunction. *Scientific World Journal* **2012**: 640389.
27. Uz T, Qu T, Sugaya K, Manev H (2002) Neuronal expression of arylalkylamine N-acetyltransferase (AANAT) mRNA in the rat brain. *Neurosci. Res.* **42**: 309–316.
28. Klein DC, *et al.* (2002) 14-3-3 Proteins and photoneuroendocrine transduction: role in controlling the daily rhythm in melatonin. *Biochem. Soc. Trans.* **30**: 365–373.
29. Hardeland R (2008) Melatonin, hormone of darkness and more – occurrence, control mechanisms, actions and bioactive metabolites. *Cell. Mol. Life Sci.* **65**: 2001–2018.
30. Hardeland R, *et al.* (2011) Melatonin – A pleiotropic, orchestrating regulator molecule. *Prog. Neurobiol.* **93**: 350–384.
31. Tan DX, *et al.* (2013) Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin’s primary function and evolution in eukaryotes. *J. Pineal Res.* **54**: 127–138.
32. Tan DX, Reiter RJ (2019) Mitochondria: the birth place, battle ground and the site of melatonin metabolism in cells. *Melatonin Res.* **2**: 44–66.
33. Suofu Y, *et al.* (2017) Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc. Natl. Acad. Sci.* **114**: E7997–E8006.
34. Huether G (1993) The contribution of extrapineal sites of melatonin synthesis to circulating melatonin levels in higher vertebrates. *Experientia* **49**: 665–670.
35. Bubenik GA (2002) Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig. Dis. Sci.* **47**: 2336–2348.
36. Hardeland R (2017) Melatonin and the electron transport chain. *Cell. Mol. Life Sci.* **74**: 3883–3896.
37. Hardeland R, *et al.* (1995) On the primary functions of melatonin in evolution: Mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. *J. Pineal Res.* **18**: 104–111.
38. Tan DX, *et al.* (2010) The changing biological roles of melatonin during evolution: from an

- antioxidant to signals of darkness, sexual selection and fitness. *Biol. Rev. Camb. Philos. Soc.* **85**: 607–623.
39. Boehm T, Swann JB (2014) Origin and evolution of adaptive immunity. *Annu. Rev. Anim. Biosci.* **2**: 259–283.
  40. Buchon N, *et al.* (2014) Immunity in *Drosophila melanogaster*—from microbial recognition to whole-organism physiology. *Nat. Rev. Immunol.* **14**: 796–810.
  41. Zhang X, Soldati T (2016) Of amoebae and men: Extracellular DNA traps as an ancient cell-intrinsic defense mechanism. *Front. Immunol.* **7**: 269.
  42. Tan DX (2019) Aging: An evolutionary competition between host cells and mitochondria. *Med. Hypotheses* **127**: 120–128.
  43. Csaba G (2013) The pineal regulation of the immune system: 40 years since the discovery. *Acta Microbiol. Immunol. Hung.* **60**: 77–91.
  44. Mańka S, Majewska E (2016) Immunoregulatory action of melatonin. The mechanism of action and the effect on inflammatory cells. *Postepy Hig. Med. Dosw.* **70**: 1059–1067.
  45. Markus RP, Fernandes PA, Kinker GS, da Silveira Cruz-Machado S, Marçola M (2018) Immune-pineal axis - acute inflammatory responses coordinate melatonin synthesis by pinealocytes and phagocytes. *Br. J. Pharmacol.* **175**: 3239–3250.
  46. Boga, JA, Coto-Montes A, Rosales-Corral SA, Tan DX, Reiter RJ (2012) Beneficial actions of melatonin in the management of viral infections: a new use for this “molecular handyman”? *Rev. Med. Virol.* **22**: 323–338.
  47. Hardeland R, Cardinali DP, Brown GM, Pandi-Perumal SR (2015) Melatonin and brain inflammaging. *Prog. Neurobiol.* **127–128**: 46–63.
  48. Hardeland R. (2019) Aging, melatonin, and the pro- and anti-inflammatory networks. *Int. J. Mol. Sci.* **20**: 1223.
  49. Xia Y, *et al.* (2019) Melatonin in macrophage biology: Current understanding and future perspectives. *J. Pineal Res.* **66**: e12547.
  50. Lu R, *et al.* (2019) Melatonin is responsible for rice resistance to rice stripe virus infection through a nitric oxide-dependent pathway. *Virol. J.* **16**: 141.
  51. Ouyang H, *et al.* (2019) Inhibitory effect of melatonin on Mst1 ameliorates myocarditis through attenuating ER stress and mitochondrial dysfunction. *J. Mol. Histol.* **50**: 405–415.
  52. Kılınçel Ö, *et al.* (2019) The effect of melatonin on antifungal susceptibility in planktonic and biofilm forms of *Candida* strains isolated from clinical samples. *Med. Mycol.* **57**: 45–51.
  53. Bishayi B, Adhikary R, Nandi A, Sultana S (2016) Beneficial Effects of exogenous melatonin in acute staphylococcus aureus and escherichia coli infection-induced inflammation and associated behavioral response in mice after exposure to short photoperiod. *Inflammation* **39**: 2072–2093.
  54. Safari F, *et al.* (2020) The interaction of phages and bacteria: the co-evolutionary arms race. *Crit. Rev. Biotechnol.* **40**: 119–137.
  55. Prangishvili D, *et al.* (2017) The enigmatic archaeal virosphere. *Nat. Rev. Microbiol.* **15**, 724–739.
  56. Aiweisakun P, *et al.* (2018) Evaluation of the genomic diversity of viruses infecting bacteria, archaea and eukaryotes using a common bioinformatic platform: steps towards a unified taxonomy. *J. Gen. Virol.* **99**: 1331–1343.
  57. Horvath P, Barrangou R (2010) CRISPR/Cas, the immune system of bacteria and archaea. *Science* **327**: 167–170.
  58. Bhaya D, Davison M, Barrangou R (2011) CRISPR-Cas systems in bacteria and archaea:

- versatile small RNAs for adaptive defense and regulation. *Annu. Rev. Genet.* **45**: 273–297.
59. Hampton HG, Watson BNJ, Fineran PC (2020) The arms race between bacteria and their phage foes. *Nature* **577**: 327–336.
  60. Kumar MS, Chen KC (2012) Evolution of animal Piwi-interacting RNAs and prokaryotic CRISPRs. Evolution of animal Piwi-interacting RNAs and prokaryotic CRISPRs. *Brief Funct. Genomics* **11**: 277–288.
  61. Ophinni Y, Palatini U, Hayashi Y, Parrish NF (2019) piRNA-guided CRISPR-like immunity in eukaryotes. *Trends Immunol.* **40**: 998–1010.
  62. Danilova N (2006) The evolution of immune mechanisms. *J. Exp. Zool. Part B Mol. Dev. Evol.* **306B**: 496–520.
  63. Amano A, Nakagawa I, Yoshimori T (2006) Autophagy in innate immunity against intracellular bacteria. *J. Biochem.* **140**: 161–166.
  64. Shimada K, *et al.* (2012) Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* **36**: 401–414.
  65. Glück S, Ablasser A (2018) Innate immunosensing of DNA in cellular senescence. *Curr. Opin. Immunol.* **56**: 31–36.
  66. Ma Z, Ni G, Damania B (2018) Innate sensing of DNA virus genomes. *Annu. Rev. Virol.* **5**: 341–362.
  67. Rongvaux A (2018) Innate immunity and tolerance toward mitochondria. *Mitochondrion* **41**: 14–20.
  68. Boehm T, *et al.* (2018) Evolution of alternative adaptive immune systems in vertebrates. *Annu. Rev. Immunol.* **36**: 19–42.
  69. Netea MG, Schlitzer A, Placek K, Joosten LAB, Schultz, JL (2019) Innate and adaptive immune memory: an evolutionary continuum in the host's response to pathogens. *Cell Host Microbe* **25**: 13–26.
  70. Brilot F, Strowig T, Munz C (2008) NK cells interactions with dendritic cells shape innate and adaptive immunity. *Front. Biosci.* **13**: 6443–6454.
  71. Reschner A, *et al.* (2008) Innate lymphocyte and dendritic cell cross-talk: a key factor in the regulation of the immune response. *Clin. Exp. Immunol.* **152**: 219–226.
  72. Moretta L, *et al.* (2006) Surface NK receptors and their ligands on tumor cells. *Semin. Immunol.* **18**: 151–158.
  73. Rajalingam R (2018) Diversity of killer cell immunoglobulin-like receptors and disease. *Clin. Lab. Med.* **38**: 637–653.
  74. Kumar V, McNerney ME (2005) A new self: MHC-class-I-independent Natural-killer-cell self-tolerance. *Nat. Rev. Immunol.* **5**, 363–374.
  75. Lodoen MB, Lanier LL (2005) Viral modulation of NK cell immunity. *Nat. Rev. Microbiol.* **3**: 59–69.
  76. Werling D, Jungi TW (2003) TOLL-like receptors linking innate and adaptive immune response. *Vet. Immunol. Immunopathol.* **91**: 1–12.
  77. Luecke S, Paludan SR (2015) Innate recognition of alphaherpesvirus DNA. *Adv. Virus Res.* **92**: 63–100.
  78. Sepehri Z, Kiani Z, Alavian SM, Arababadi MK, Kennedy D (2016) The link between TLR7 signaling and hepatitis B virus infection. *Life Sci.* **158**: 63–69.
  79. Petes C, Odoardi N, Gee K (2017) The toll for trafficking: toll-like receptor 7 delivery to the endosome. *Front. Immunol.* **8**: 1075.
  80. Takeuchi O, Akira S (2009) Innate immunity to virus infection. *Immunol. Rev.* **227**: 75–86.



81. Zhou L, *et al.* (2015) Induction of interferon- $\lambda$  contributes to TLR3 and RIG-I activation-mediated inhibition of herpes simplex virus type 2 replication in human cervical epithelial cells. *Mol. Hum. Reprod.* **21**: 917–929.
82. Muruve DA, *et al.* (2008) The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* **452**: 103–107.
83. Roberts TL, *et al.* (2009) HIN-200 proteins regulate caspase activation in response to foreign cytoplasmic DNA. *Science* **323**: 1057–1060.
84. Broz P, Dixit VM (2016) Inflammasomes: mechanism of assembly, regulation and signalling. *Nat. Rev. Immunol.* **16**: 407–420.
85. Winsor N, *et al.* (2019) Canonical and noncanonical inflammasomes in intestinal epithelial cells. *Cell. Microbiol.* **21**: e13079.
86. Al-Omari A, Rabaan AA, Salih S, Al-Tawfiq JA, Memish ZA (2019) MERS coronavirus outbreak: Implications for emerging viral infections. *Diagn. Microbiol. Infect. Dis.* **93**: 265–285.
87. Rojek AM, *et al.* (2019) A systematic review and meta-analysis of patient data from the West Africa (2013–16) Ebola virus disease epidemic. *Clin. Microbiol. Infect.* **25**: 1307–1314.
88. Chen J, Subbarao K (2007) The Immunobiology of SARS. *Annu. Rev. Immunol.* **25**: 443–472.
89. Paget J, *et al.* (2019) Global mortality associated with seasonal influenza epidemics: New burden estimates and predictors from the GLAMOR Project. *J. Glob. Health* **9**: 020421.
90. Messaoudi I, Amarasinghe GK, Basler CF (2015) Filovirus pathogenesis and immune evasion: insights from Ebola virus and Marburg virus. *Nat. Rev. Microbiol.* **13**: 663–676.
91. Baseler L, *et al.* (2017) The pathogenesis of Ebola virus disease. *Annu. Rev. Pathol.* **12**: 387–418.
92. Oldstone MBA, Rosen H (2014) Cytokine storm plays a direct role in the morbidity and mortality from influenza virus infection and is chemically treatable with a single sphingosine-1-phosphate agonist molecule. *Curr. Top. Microbiol. Immunol.* **378**: 129–147.
93. Wong JP, *et al.* (2017) Current and future developments in the treatment of virus-induced hypercytokinemia. *Future Med. Chem.* **9**: 169–178.
94. Refardt D, Kümmerli R (2013) Defying bacteriophages: Contrasting altruistic with individual-based resistance mechanisms in *Escherichia coli*. *Commun. Integr. Biol.* **6**: e25159.
95. Pavlovich SS, *et al.* (2018) The Egyptian roussette genome reveals unexpected features of bat antiviral immunity. *Cell* **173**: 1098–1110.
96. Cheng V, Tang B, Wu A, Chu C, Yuen K (2004) Medical treatment of viral pneumonia including SARS in immunocompetent adult. *J. Infect.* **49**: 262–273.
97. Wu Q, *et al.* (2017) Altered lipid metabolism in recovered SARS patients twelve years after infection. *Sci. Rep.* **7**: 9110.
98. Maestroni GJM, Conti A, Pierpaoli W (1986) Role of the pineal gland in immunity. *J. Neuroimmunol.* **13**: 19–30.
99. Maestroni GJ, Conti A, Pierpaoli W (1987) Role of the pineal gland in immunity: II. Melatonin enhances the antibody response via an opiate mechanism. *Clin. Exp. Immunol.* **68**: 384–91.
100. Cuzzocrea S, *et al.* (1997) Protective effect of melatonin in carrageenan-induced models of local inflammation: relationship to its inhibitory effect on nitric oxide production and its peroxynitrite scavenging activity. *J. Pineal Res.* **23**: 106–116.
101. Cuzzocrea S, Zingarelli B, Costantino G, Caputi AP (1998) Protective effect of melatonin in a non-septic shock model induced by zymosan in the rat. *J. Pineal Res.* **25**: 24–33.
102. Costantino G, Cuzzocrea S, Mazzon E, Caputi AP (1998) Protective effects of melatonin

- in zymosan-activated plasma-induced paw inflammation. *Eur. J. Pharmacol.* **363**: 57–63.
103. Gitto E, *et al.* (2001) Effects of melatonin treatment in septic newborns. *Pediatr. Res.* **50**: 756–760.
104. Zhao Q, *et al.* (2018) Melatonin attenuates the inflammatory response via inhibiting the C/EBP homologous protein-mediated pathway in taurocholate-induced acute pancreatitis. *Int. J. Mol. Med.* **42**: 3513–3521.
105. Mannino G, *et al.* (2019) Melatonin reduces inflammatory response in human intestinal epithelial cells stimulated by interleukin-1 $\beta$ . *J. Pineal Res.* **67**: e12598.
106. Dai W, *et al.* (2019) Melatonin prevents sepsis-induced renal injury via the PINK1/Parkin1 signaling pathway. *Int. J. Mol. Med.* **44**: 1197–1204.
107. Sung PH, *et al.* (2018) Melatonin attenuated brain death tissue extract-induced cardiac damage by suppressing DAMP signaling. *Oncotarget* **9**: 3531–3548.
108. Srinivasan V, Mohamed M, Kato H (2012) Melatonin in bacterial and viral infections with focus on sepsis: A Review. *Recent Pat. Endocr. Metab. Immune Drug Discov.* **6**: 30–39.
109. Luo J, *et al.* (2018) Melatonin mediated Foxp3-downregulation decreases cytokines production via the TLR2 and TLR4 pathways in H. pylori infected mice. *Int. Immunopharmacol.* **64**: 116–122.
110. Hu Y, *et al.* (2017) Melatonin protects against blood-brain barrier damage by inhibiting the TLR4/ NF- $\kappa$ B signaling pathway after LPS treatment in neonatal rats. *Oncotarget* **8**: 31638–31654.
111. Ge W, *et al.* (2019) Melatonin protects against lipopolysaccharide-induced epididymitis in sheep epididymal epithelial cells in vitro. *Immunol. Lett.* **214**: 45–51.
112. Chamanara M, *et al.* (2019) Melatonin ameliorates TNBS-induced colitis in rats through the melatonin receptors: involvement of TLR4/MyD88/NF- $\kappa$ B signalling pathway. *Inflammopharmacology* **27**: 361–371.
113. Xu X, *et al.* (2018) Melatonin suppresses TLR9-triggered proinflammatory cytokine production in macrophages by inhibiting ERK1/2 and AKT activation. *Sci. Rep.* **8**: 15579.
114. Martinon F (2008) Detection of immune danger signals by NALP3. *J. Leukoc. Biol.* **83**: 507–511.
115. García JA, *et al.* (2015) Disruption of the NF- $\kappa$ B/NLRP3 connection by melatonin requires retinoid-related orphan receptor- $\alpha$  and blocks the septic response in mice. *FASEB J.* **29**: 3863–3875.
116. Slominski AT, *et al.* (2014) ROR $\alpha$  and ROR $\gamma$  are expressed in human skin and serve as receptors for endogenously produced noncalcemic 20-hydroxy- and 20,23-dihydroxyvitamin D. *FASEB J.* **28**: 2775–2789.
117. Slominski AT, Zmijewski MA, Jetten AM (2016) ROR $\alpha$  is not a receptor for melatonin (response to DOI 10.1002/bies.201600018). *Bioessays* **38**: 1193–1194.
118. Rahim I, *et al.* (2017) Melatonin administration to wild-type mice and nontreated NLRP3 mutant mice share similar inhibition of the inflammatory response during sepsis. *J. Pineal Res.* **63**: e12410.
119. Hu X, *et al.* (2018) Melatonin inhibits endoplasmic reticulum stress-associated TXNIP/NLRP3 inflammasome activation in lipopolysaccharide-induced endometritis in mice. *Int. Immunopharmacol.* **64**: 101–109.
120. Ariozi BI, *et al.* (2019) Melatonin attenuates LPS-induced acute depressive-like behaviors and microglial NLRP3 inflammasome activation through the SIRT1/Nrf2 pathway. *Front. Immunol.* **10**: 1511.

121. Wang X, *et al.* (2019) Melatonin alleviates cigarette smoke-induced endothelial cell pyroptosis through inhibiting ROS/NLRP3 axis. *Biochem. Biophys. Res. Commun.* **519**: 402–408.
122. Fernández-Gil B, *et al.* (2017) Melatonin protects rats from radiotherapy-induced small intestine toxicity. *PLoS One* **12**: e0174474.
123. Cao Z, *et al.* (2017) Melatonin alleviates cadmium-induced liver injury by inhibiting the TXNIP-NLRP3 inflammasome. *J. Pineal Res.* **62**: e12389.
124. Wu HM, *et al.* (2019) Melatonin biosynthesis restored by CpG oligodeoxynucleotides attenuates allergic airway inflammation via regulating NLRP3 inflammasome. *Life Sci.* **239**: 117067.
125. Mohanty A, Tiwari-Pandey R, Pandey NR (2019) Mitochondria: the indispensable players in innate immunity and guardians of the inflammatory response. *J. Cell Commun. Signal.* **13**: 303–318.
126. Chen Y, Zhou Z, Min W (2018) Mitochondria, oxidative stress and innate immunity. *Front. Physiol.* **9**: 1487.
127. Rodríguez-Nuevo A, Zorzano A (2019) The sensing of mitochondrial DAMPs by non-immune cells. *Cell Stress* **3**: 195–207.
128. Tan DX, Manchester LC, Qin L, Reiter RJ (2016) Melatonin: A mitochondrial targeting molecule involving mitochondrial protection and dynamics. *Int. J. Mol. Sci.* **17**: 2124.
129. Reiter RJ, *et al.* (2017) Melatonin as a mitochondria-targeted antioxidant: one of evolution's best ideas. *Cell. Mol. Life Sci.* **74**: 3863–3881.
130. Majumdar S, Nandi D Thymic atrophy: experimental studies and therapeutic interventions. *Scand. J. Immunol.* **87**: 4–14.
131. Tian YM, Zhang GY, Dai YR (2003) Melatonin rejuvenates degenerated thymus and redresses peripheral immune functions in aged mice. *Immunol. Lett.* **88**: 101–104.
132. Oner H, *et al.* (2004) Possible effects of melatonin on thymus gland after pinealectomy in rats. *Neuro Endocrinol. Lett.* **25**: 115–118.
133. Ren W, *et al.* (2017) Melatonin signaling in T cells: Functions and applications. *J. Pineal Res.* **62**: e12394.
134. Maestroni GJ, Conti A (1990) The pineal neurohormone melatonin stimulates activated CD4+, Thy-1+ cells to release opioid agonist(s) with immunoenhancing and anti-stress properties. *J. Neuroimmunol.* **28**: 167–176.
135. Pozo D, Garcia-Maurino S, Guerrero JM, Calvo JR (2004) mRNA expression of nuclear receptor RZR/RORalpha, melatonin membrane receptor MT1, and hydroxindole-O-methyltransferase in different populations of human immune cells. *J. Pineal Res.* **37**: 48–54.
136. Hardeland R (2018) Melatonin and retinoid orphan receptors: Demand for new interpretations after their exclusion as nuclear melatonin receptors. *Melatonin Res.* **1**: 77–92.
137. Li J, Cao J, Wang Z, Dong Y, Chen Y (2015) Melatonin plays a critical role in inducing B lymphocyte proliferation of the bursa of fabricius in broilers via monochromatic lights. *J. Photochem. Photobiol. B Biol.* **142**: 29–34.
138. Lopez-Gonzalez MA, Guerrero JM, Sanchez B, Delgado F (1998) Melatonin restores and enhances the human type B tonsillar lymphocyte subset in recurrent acute tonsillitis. *Neurosci. Lett.* **247**: 131–134.
139. Yu Q, Miller SC, Osmond DG (2000) Melatonin inhibits apoptosis during early B-cell development in mouse bone marrow. *J. Pineal Res.* **29**: 86–93.
140. Ramos A, *et al.* (2010) Evolution of oxidative/nitrosative stress biomarkers during an open-

- field vaccination procedure in sheep: effect of melatonin. *Vet. Immunol. Immunopathol.* **133**: 16–24.
141. Ramos A, *et al.* (2018) Melatonin enhances responsiveness to *Dichelobacter nodosus* vaccine in sheep and increases peripheral blood CD4 T lymphocytes and IgG-expressing B lymphocytes. *Vet. Immunol. Immunopathol.* **206**: 1–8.
142. Regodón S, *et al.* (2005) The use of melatonin as a vaccine agent. *Vaccine* **23**: 5321–5327.
143. Lardone PJ, *et al.* (2011) Melatonin synthesized by T lymphocytes as a ligand of the retinoic acid-related orphan receptor. *J. Pineal Res.* **51**: 454–462.
144. Tan DX, *et al.* (1999) Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. *Biochim. Biophys. Acta* **1472**: 206–214.
145. Gómez-Corvera A, *et al.* (2009) Evidence of immune system melatonin production by two pineal melatonin deficient mice, C57BL/6 and Swiss strains. *J. Pineal Res.* **47**: 15–22.
146. Naranjo MC, *et al.* (2007) Melatonin biosynthesis in the thymus of humans and rats. *Cell. Mol. Life Sci.* **64**: 781–790.
147. Hardeland R. (2013) Melatonin and the theories of aging: a critical appraisal of melatonin's role in antiaging mechanisms. *J. Pineal Res.* **55**: 325–356.
148. Elmahallawy E, *et al.* (2015) Potential relevance of melatonin against some infectious agents: A review and assessment of recent research. *Curr. Med. Chem.* **22**, 3848–3861.
149. Bagnaresi P, Nakabashi M, Thomas AP, Reiter RJ, Garcia CRS (2012) The role of melatonin in parasite biology. *Mol. Biochem. Parasitol.* **181**: 1–6.
150. Madigan AP, Egidi E, Bedon F, Franks AE, Plummer KM (2019) Bacterial and fungal communities are differentially modified by melatonin in agricultural soils under abiotic stress. *Front. Microbiol.* **10**: 2616.
151. Tekbas OF, Ogur R, Korkmaz A, Kilic A, Reiter RJ (2008) Melatonin as an antibiotic: new insights into the actions of this ubiquitous molecule. *J. Pineal Res.* **44**: 222–226.
152. He C, *et al.* (2016) Mitochondria synthesize melatonin to ameliorate its function and improve mice oocyte's quality under in vitro conditions. *Int. J. Mol. Sci.* **17**: 939.
153. Quintela T, Gonçalves I, Silva M, *et al.* (2018) Choroid plexus is an additional source of melatonin in the brain. *J. Pineal Res.* **65**: e12528.
154. Wang L, *et al.* (2017) Plant mitochondria synthesize melatonin and enhance the tolerance of plants to drought stress. *J. Pineal Res.* **63**: e12429.
155. Jiao J, *et al.* (2016) Melatonin-producing endophytic bacteria from grapevine roots promote the abiotic stress-induced production of endogenous melatonin in their hosts. *Front. Plant Sci.* **7**: 1387.
156. Nehela Y, Killiny N (2018) Infection with phytopathogenic bacterium inhibits melatonin biosynthesis, decreases longevity of its vector, and suppresses the free radical-defense. *J. Pineal Res.* **65**: e12511.
157. Chen X, *et al.* (2018) Melatonin treatment inhibits the growth of *Xanthomonas oryzae pv. oryzae*. *Front. Microbiol.* **9**: 2280.
158. Chatel-Chaix L, *et al.* (2016) Dengue virus perturbs mitochondrial morphodynamics to dampen innate immune responses. *Cell Host Microbe* **20**: 342–356.
159. Zhang L, Qin Y, Chen M (2018) Viral strategies for triggering and manipulating mitophagy. *Autophagy* **14**: 1665–1673.
160. Ahmadi-Motamayel F, Vaziri-Amjad S, Goodarzi MT, Samie L, Poorolajal J (2017) Evaluation of salivary melatonin levels in HIV-positive patients: a historical cohort study. *Rev. Recent Clin. Trials* **12**: 168–173.



161. Nunnari G, *et al.* (2003) Reduction of serum melatonin levels in HIV-1-infected individuals' parallel disease progression: correlation with serum interleukin-12 levels. *Infection* **31**: 379–382.
162. Ma Y, *et al.* (2018) The reduced level of plasma melatonin in HFERS patients is correlated with disease severity and stage. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* **34**: 1027-1031.
163. Zhao L, *et al.* (2015) Melatonin alleviates brain injury in mice subjected to cecal ligation and puncture via attenuating inflammation, apoptosis, and oxidative stress: the role of SIRT1 signaling. *J. Pineal Res.* **59**: 230–239.
164. Wen H, Lei Y, Eun SY, Ting JPY (2010) Plexin-A4-semaphorin 3A signaling is required for Toll-like receptor- and sepsis-induced cytokine storm. *J. Exp. Med.* **207**: 2943–2957.
165. Siu KL, *et al.* (2019) Severe acute respiratory syndrome coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC. *FASEB J.* **33**: 8865-8877.
166. Xu L, *et al.* (2019) Protective effect of melatonin against polymicrobial sepsis is mediated by the anti-bacterial effect of neutrophils. *Front. Immunol.* **10**: 1371.
167. Crespo E, *et al.* (1999) Melatonin inhibits expression of the inducible NO synthase II in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction syndrome in rats. *FASEB J.* **13**: 1537-1546.
168. Escames G, *et al.* (2003) Melatonin counteracts lipopolysaccharide-induced expression and activity of mitochondrial nitric oxide synthase in rats. *FASEB J.* **17**: 932-934.
169. López LC, *et al.* (2006) Identification of an inducible nitric oxide synthase in diaphragm mitochondria from septic mice: its relation with mitochondrial dysfunction and prevention by melatonin. *Int. J. Biochem. Cell Biol.* **38**: 267–278.
170. Escames G, *et al.* (2006) Melatonin counteracts inducible mitochondrial nitric oxide synthase-dependent mitochondrial dysfunction in skeletal muscle of septic mice. *J. Pineal Res.* **40**: 71–78.
171. Escames G, *et al.* (2006) Age-dependent lipopolysaccharide-induced iNOS expression and multiorgan failure in rats: effects of melatonin treatment. *Exp. Gerontol.* **41**: 1165–1173.
172. Escames G, *et al.* (2006) Pharmacological utility of melatonin in the treatment of septic shock: experimental and clinical evidence. *J. Pharm. Pharmacol.* **58**: 1153–1165.
173. Ellis LC (1996) Melatonin reduces mortality from Aleutian disease in mink (*Mustela vison*). *J. Pineal Res.* **21**: 214–217.
174. Ben-Nathan D, Maestroni GJ, Lustig S, Conti A (1995) Protective effects of melatonin in mice infected with encephalitis viruses. *Arch. Virol.* **140**: 223–30.
175. Bonilla E, Valero-Fuenmayor N, Pons H, Chacín-Bonilla L (1997) Melatonin protects mice infected with Venezuelan equine encephalomyelitis virus. *Cell. Mol. Life Sci.* **53**: 430–434.
176. Araghi-Niknam M, Lane L, Watson RR (1998) Physical inactivity of murine retrovirus infected C57bl/6 mice is prevented by melatonin and dehydroepiandrosterone. *Exp. Biol. Med.* **219**: 144–148.
177. Huang SH, Cao XJ, Liu W, Shi XY, Wei W (2010) Inhibitory effect of melatonin on lung oxidative stress induced by respiratory syncytial virus infection in mice. *J. Pineal Res.* **48**: 109–116.
178. Tuñón MJ, *et al.* (2011) Melatonin attenuates apoptotic liver damage in fulminant hepatic failure induced by the rabbit hemorrhagic disease virus. *J. Pineal Res.* **50**: 38–45.
179. Tang X, *et al.* (2020) On the origin and continuing evolution of SARS-CoV-2. *Natl. Sci. Rev.* **2020**: nwaa036. doi.org/10.1093/nsr/nwaa036



180. Wong MC (2020) Evidence of recombination in coronaviruses implicating pangolin origins of nCoV-2019. *bioRxiv* **2020**: 02.07.939207. doi:10.1101/2020.02.07.939207
181. Zhang T, Wu Q, Zhang Z. (2020) Pangolin homology associated with 2019-nCoV. *bioRxiv* **2020**: 02.19.950253. doi: 10.1101/2020.02.19.950253
182. Virk RK, Gunalan V, Tambyah PA (2016) Influenza infection in human host: Challenges in making a better influenza vaccine. *Expert Rev. Anti Infect. Ther.* **14**: 365–375.
183. Petrova VN, Russell CA (2018) The evolution of seasonal influenza viruses. *Nat. Rev. Microbiol.* **16**: 47-60.
184. Zhou Y, *et al.* (2020) Network-based repurposing for human coronavirus. medRxiv **2020**: 02.03.2020263. doi: 10.1101/2020.02.03.2020263
185. Weishaupt JH, *et al.* (2006) Reduced oxidative damage in ALS by high-dose enteral melatonin treatment. *J. Pineal Res.* **41**: 313-321.
186. Goeijenbier M, *et al.* (2014) Ebola virus disease: a review on epidemiology, symptoms, treatment and pathogenesis. *Neth. J. Med.* **72**: 442–448.
187. Baize S, *et al.* (2002) Inflammatory responses in Ebola virus-infected patients. *Clin. Exp. Immunol.* **128**: 163–168.
188. Anderson G, Maes M, Markus RP, Rodriguez M (2015) Ebola virus: melatonin as a readily available treatment option. *J. Med. Virol.* **87**: 537–543.
189. Tan DX, Korkmaz A, Reiter RJ, Manchester LC (2014) Ebola virus disease: potential use of melatonin as a treatment. *J. Pineal Res.* **57**: 381–384.
190. Masters A, Pandi-Perumal SR, Seixas A, Girardin JL, McFarlane SI (2014) Melatonin, the Hormone of Darkness: From Sleep Promotion to Ebola Treatment. *Brain Disord. Ther.* **4**: doi: 10.4172/2168-975X.1000151
191. Junaid A, *et al.* (2020) Ebola hemorrhagic shock syndrome-on-a-chip. *iScience* **23**: 100765.
192. Voordouw BC, *et al.* (1992) Melatonin and melatonin-progestin combinations alter pituitary-ovarian function in women and can inhibit ovulation. *J. Clin. Endocrinol. Metab.* **74**: 108–117.



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

Please cite this paper as:

*Tan, D.X. and Hardeland, R. 2020. Potential utility of melatonin in deadly infectious diseases related to the overreaction of innate immune response and destructive inflammation: focus on COVID-19. Melatonin Research. 3, 1 (Mar. 2020), 120-143. DOI:https://doi.org/https://doi.org/10.32794/mr11250052.*