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Immunological Insights of Bat Coexistence with Viruses and beyond: A Holistic Review

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ABSTRACT

Bats are the reservoir host of the novel coronavirus/SARS-CoV-2. Bats are known to host hundreds of viruses, although they remain unharmed. Scientific evidence revealed that bats have various immunological specializations that enabled them to remain unaffected to coronaviruses. This manuscript highlights the aspects of bats' defense mechanism against the viral load and their unique adaptability. Its ability to serve as propagating ground for viruses is favored by its extraordinary physiological traits and unique immune responses, including constitutive active interferons (IFNs), dampened inflammasome response, reduced DNA sensing mechanisms, and unique B and T cell components. Furthermore, bats have evolved with their efficient mode of oxidative phosphorylation, loss of PYHIN gene family, and positive selection for DNA damage checkpoints. These multiple mechanisms are detrimental to the viral co-existence in bats and spillover events. We have discussed future directions to enhance knowledge and understanding of bat-human interactions and the genetic diversity of bat-borne viruses, which will play a crucial role in preventing future outbreaks.

Keywords: Bat, Bat-human Interactions, Coronaviruses, SARS-CoV-2, Co-existence, Defense mechanisms, Spillover.

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INTRODUCTION

The novel coronavirus also known as SARS-CoV-2, is spherical or pleomorphic in shape with single-stranded RNA (29.8 kilobases in size) and a diameter of 80-160nm, having club-shaped spike projections on its surface [1,2]. It is the seventh coronavirus that has infected humans [3,4]. The first coronavirus (HKU-229E) to infect humans was reported in 1966, followed by HCoV-OC43 in 1967, and the rest of the five coronaviruses was reported after 2000[4–6]. The natural host of these seven coronaviruses has been identified as either bats or rodents. SARS-CoV-2 is believed to originate from BatCoV RaTG13 (GenBank: MN996532)[7]. Its closest relative was isolated from horseshoe bats [8]. This pervasive disease caused a great loss to human lives and economic burden worldwide that has triggered exhaustive discussions on its origin, etiology, and possible treatment strategies.

The natural hosts of several viruses, including the novel coronavirus, are bats, which can host such lethal viruses without any symptomatic pathology[9]. Almost none of them is virulent to bats[10]. The metagenomic approaches have been used to identify and characterize viruses in the bats[11], which are publicly available through the PubMed database of the US National Library of Medicine. The data revealed that bats show an enormous diversity of viruses and even more viral infections per species than rodents [12]. Various classes of viruses have been identified in bats in several studies [13–15], including Japanese encephalitis virus, Nipah virus, MERS-CoV, SARS-CoV, Ebola virus, Influenza A virus, Hendra virus, Paramyxovirus, Chikungunya virus, Hantaan virus, Polyomavirus, Toscana virus, Morbillivirus and Saboya virus. A broader list of viruses present in different species of bats can be obtained from a study by Calisher et al. [14] and Hayman [16]. Various families of bats, belonging to the order Chiroptera of the class Mammalia, have several features that make them unique among mammals. Bats are present in all continents except Antarctica. Being nocturnal, bats seek shelter in natural or man-made structures such

as buildings, bridges, etc., where they may contact humans or their livestock[17]. Dense colonies of bats, along with their strong social behavior and feeding habits, may facilitate the transmission of viruses among themselves and to humans[17]. At the physiological level, bats possess abilities that stand them out from similar size mammals. Their metabolic rate is approximately three times higher than similar size mammals[18]. Their ability to control metabolism during flight is also extraordinary such as their metabolic rate enhanced by approx 35 times during the flight, compared to the basal metabolic rate[18]. As a result, their energy demand also reaches 1,200 calories per hour during flight[19]. Similarly, the heart rate ranges from 10 to more than 1,000 per minute[20]. This mammalian species also goes for hibernation. The immune responses may be suppressed due to low body temperature and slow metabolic rate, which leads to a delay in the clearance of viral load [13]. A coincidence has been found between the pregnancy in bats and the seroprevalence and seasonal spillover of several viruses[21]. Apart from that, their lifespan is around 40 years, which exceeds all mammalian species of their size. The extended lifespan of bats also suggests that they must have very good disease tolerance ability, which primarily depends on the immune system. Altogether, these adaptations may explain the ability of bats to reduce but not eliminate viral load during infection [22]. Due to these unique abilities, bats are the reservoir of various viruses. This indicates that bats are inbuilt with a vivid array of immunological specifications, enabling a potent host immunological response against these viruses[23].

Spillover events cause the viruses to move to the new host as observed with SARS-CoV-2. It is also expected that soon similar events might happen and cause the next pandemic. Therefore, it is important to understand how bat remains unaffected from viruses or virus-mediated pathogenesis. More importantly, bats do not exhibit excessive production of cytokines as observed in people suffering from COVID-19, indicating a unique mechanism that regulates viral number and pathology. Deciphering the molecular mechanism of viral tolerance in bats will also pave the path to identify chemical molecules or natural compounds as therapeutic agents to control this novel coronavirus. The manuscript compiled the current understandings of the antiviral response of bats. We focused on the immunological perspective of bats antiviral response and highlighted the major players involved in this phenomenon. We will explore the immunological perspective of bats' resistance to these viruses in the subsequent segments.

BATS INNATE IMMUNOLOGICAL SPECIALIZATIONS

Some of the important aspects of bats' immune system can be understood in terms of innate and adaptive components, which act differently in terms of cells involved and response strategies as appropriately required. Some unique anti-inflammatory responses in bats may neutralize pro-inflammatory stimuli, which might have provided longevity and tolerance to viral infection. These abilities of bats to prevent excess activation of their immune responses might have also contributed to their potential of viral tolerance, as described below.

Bats have constitutive active IFNs

The interaction of any host with the infectious agents triggers a wide array of immunological responses, including the innate response. Its major components include morphological, anatomical, physiological barriers and different types of leukocytes and cytokines, which sense and respond to infections at the earliest. Interferons (IFNs) are produced and secreted by the host viral infected cells, which leads to suppression of viral propagation inside the host. Several antiviral factors have been identified among different bat species, including the black flying fox (*Pteropus Alecto*), which are highly conserved in vertebrates, including pattern recognition receptors (PRRs), IFNs, their receptors, as well as the interferon-stimulated genes (ISGs) [24]. IFNα and IFNβ (Type I), and IFN λ(Type III) have been detected in cells of *P. Alecto* bats [25]. Bats like *M. Lucifugus* and *P. Vampyrus* have 61 ORFs for type I IFNs, which have been further split into sub-groups including IFN-α, IFN-β, IFN-k, IFN-ω and IFN-δ[26]. Studies show that the Hendra and Palau virus infection of kidney cells (PaKiT03) in *P. Alecto* fails to induce the expression of IFNα, suggesting that the basal level of IFNα is already high in bat cells [27]. Such a phenomenon is not observed in other species, indicating the unique immune response of bats to the viruses that enable their coexistence with viruses [27]. Another study revealed that IFN-ϒ in *P. Alecto* has been conserved and showed functional similarity with other mammals [28]. One more determinant of the IFN-dependent immune response to viruses is interferon regulatory factor 7 (IRF7), considered a master regulator [29]. The IRF7 is distributed in many cells, including immune cells of bats [29], assuming that broader distribution of IRF7 will facilitate antiviral response by activating IFN. Coronaviruses are restricted to gastrointestinal tracts in bats, whereas these viruses invade respiratory tracts in humans and others [30]. Due to this, coronaviruses might be unable to stimulate broad immune responses in bats. Furthermore,

the TNFα promoter of big brown bat (*Eptesicus fuscus*) has two NFkB binding sites, whereas the human counterpart has one more, which may cause low TNFα promoter activity [31]. Altogether, the constitutively active IFNs provide a unique perspective to the bat's immune system.

Toll-like receptors (TLRs) of bats have altered ligand binding affinities

Upon infection with RNA viruses, pattern recognition receptors (PRRs) of the host cell recognize pathogen-associated molecular patterns (PAMPS). Numerous types of PRRs have been observed, including the Toll-like receptor (TLR) family, which activates IFN pathways [32][33]. The intracellular TLRs can recognize several ligands, including double-stranded RNA and single-stranded RNA [34]. These TLRs are localized in several intracellular organelles, and they recognize PAMPs. Subsequently, upon activation, the TLRs transduce signals with their Toll/interleukin-1 receptor (TIR) domain. High expression of TLR3 in bat liver instead of dendritic cells is a notable point [35,36], and TLR13 can be considered a virus-sensing TLR [35,37]. Also, the number and location of leucine-rich repeats vary among bat species, possibly affecting the ligand-binding affinities [32]. It may indicate toward co-evolution of viruses with bats [32]. In bats, TLRs have unique mutations in ligand-binding sites, altering their function and properties [38].

Reduced inflammasome and DNA sensing mechanisms

Viral infections in mammals cause several responses, including inflammation induced by the inflammasome sensor, the NLR family pyrin domain with 3 (NLRP3). NLRP3 recognizes infections caused by bacteria or viruses and senses the different stress forms, including mitochondrial or oxidative stress. One of the studies revealed the role of NLRP3 in activating immune response upon infection with rabies and influenza viruses [39]. Furthermore, the comparative study between human, mouse, and bat counterparts show that the bat exhibits dampened activation of NLRP3 [39], primarily due to the reduced functional capacity of bat NLRP3. Consequently, the bats tolerate high viral doses inside them because of reduced inflammasome response, and they remain unharmed. Hence, the bat remains tolerant to these viruses; however, upon spillover to other host species, the pathogenesis can be observed, and they are often lethal.

Intracellular and foreign DNA is sensed by immune sensors that include PYHIN gene family members and subsequently trigger the immune responses [40,41]. It includes five proteins – AIM2, IFI16, MNDA, PYHIN1, and POP3 [42]. A study conducted on ten different bats species revealed a complete loss of PHYIN locus in all of them [43]. Consequently, bats can limit excessive inflammatory activation by sensing self DNA from DNA damage and thereby attenuate type I interferon induction. [43]. The stimulator of interferon genes (STING) is a well-established molecule involved in the inflammasome response [44,45]. STING is an ER-resident transmembrane protein with cytosolic C-terminal domain (CTD) [44,46]. STING also stimulates a response to nuclei acid ligands, including dsDNA [45][47,48]. After interaction with MAVS and RIG-I, STING acts as a potential antiviral effector by producing type I IFNs without affecting the TLR pathway [49]. Translating viral RNAs and B-form DNAs may be detected by STING, and then antiviral immunity is induced via TBK1 [49]. An amino acid substitution (serine at S538) has been reported in the STING protein of bats, and studies suggest that this mutation might dampen STING-dependent IFN activation [25]. It can inhibit viral pathogens, possibly by restricting its transcription, independent of viral immune sensing and induction of IFN [50]. Being a sensor of viral dsRNA motifs, RIG-I induces the expression of type I IFNs [51]. The SARS-CoV encodes a protease that inhibits IRF3 activation through deubiquitinating RIG-I and other proteins [52]. The M protein also interacts with RIG-I to prevent the induction of IFN [53]. Bat RIG-1 has altered sensing mechanisms [54]. Altogether, the loss of the PYHIN gene family substantially reduces the DNA sensing mechanism, which had provided an adaptation to minimize the over expression of immune responses like inflammation [55,56].

Unique role of IRF and Bat Mx genes

IFN regulatory factors(IRFs) have been recognized as an important modulator of antiviral responses and cytokines like IFNs and others[57]. In mammals, cells respond earliest to viral infections through transcription of IFN genes. After that, IFN induces proteins including IRF7 through phosphorylation, causing its activation that subsequently induces delayed IFNs. IFNs, after being synthesized in cells infected by viruses, bind to their receptors and induce target gene expression leading to antiviral responses of cells[58]. In mammals, IRF7 is considered a master regulator of IFNs and part of the innate defense mechanism [29]. It has been preferentially expressed in lymphoid cells. Still, it has been broadly distributed in the tissues of bats, and this might be helpful in rapidly activating the IFN response in these tissues compared to other mammals[29,59,60]. This may be a reason for the coexistence of viruses with bats without any clinical signs of disease[29].

Myxovirus resistance(Mx) proteins are found in most vertebrates and have been correlated with antiviral activity[61]. They inhibit negative-stranded RNA and other viruses by recognizing their nucleoproteins or nucleocapsid proteins[61]. The expression of Mx genes is controlled by Type-I and type-III IFNs[62,63]. Several pieces of evidence of co-evolution between viruses and Mx proteins with antiviral activities[61]. A study has demonstrated that bat Mx1 can efficiently inhibit several viruses. It has been speculated that IFN-induced Mx proteins in bats may be an antiviral factor coevolved with bat-borne viruses playing a significant role in restricting viral responses by suppressing viral polymerase activity[64].
Overall, the inherent defense mechanism of a bat, described above, has been summarized in figure 1. Based on well-established studies, scientists have characterized the innate immune response of bats against viruses [65–67]. One major component of the innate response of bats is triggered by the overexpression of oxidative phosphorylation (OXPHOS), which generates excessive reactive oxygen species (ROS). ROS is extremely harmful to cells because it causes oxidative DNA damage. However, the dampened STING in bats reduces the sensing of damaged DNA, leading to suppressed inflammasome pathways that contribute to weaker immune responses or more tolerance to viruses. More importantly, this damage is counterbalanced by overexpression of proteins involved in the DNA damage checkpoint, as shown in figure 1. The viral nucleic acids are detected by various classes of host pattern-recognition receptors (PRRs), including RIG-I-like receptors (RLRs), TLRs, cyclic GMP-AMP synthase (cGAS), etc. The RIG-1 and Melanoma Differentiation-Associated protein 5 (MDA-5) are cytosolic PRRs that recognize viral RNA, with RIG-I recognizing short dsRNA and MDA5 recognizing long dsRNA and inducing the expression of type I interferons (IFNs). The intracellular TLRs can recognize RNA and DNA and induce downstream signaling via the TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway. These molecules activate several signaling, which leads to the upregulation of interferons, as shown in figure 1. Furthermore, the higher-constraints of interferon-stimulated genes (ISGs) and downstream effectors keep the cells prepared to cope with the viral load.

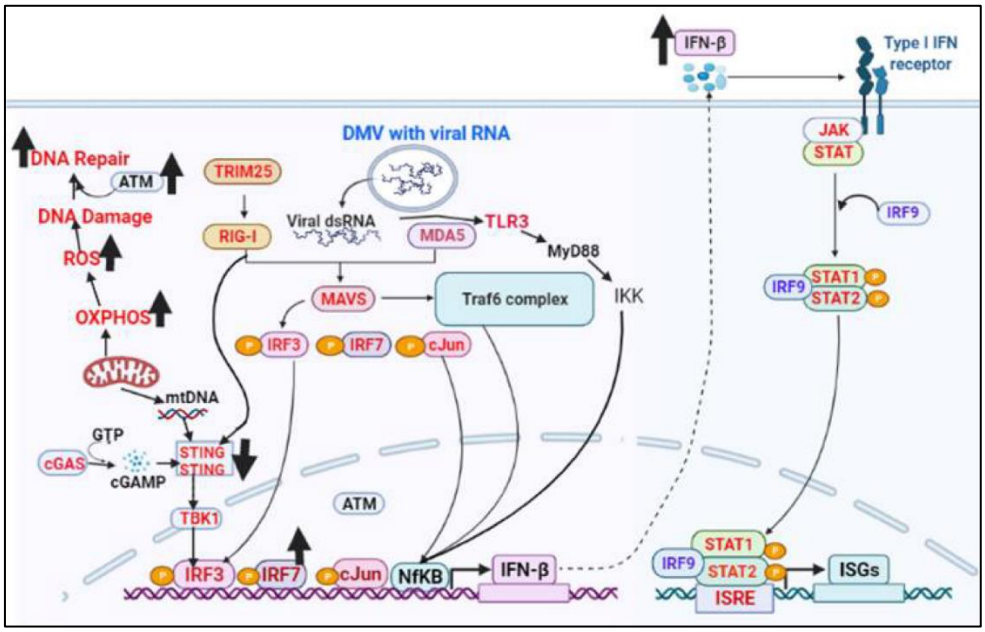

Figure 1: Proposed innate immune response of bat against viruses. The ROS generated by overexpression of OXPHOS leads to oxidative DNA damage, which is counterbalanced by overexpression of DNA damage checkpoint proteins, like ATM, in bats. Various host PRRs, such as RIG-I, TLRs, etc., detect viral nucleic acids. The Stimulator of interferon genes (STING) interacts with mitochondrial antiviral signaling (MAVS) and RIG-I. It exerts a significant immune response against viruses by producing type I IFNs without affecting TLR.TLR3 activates Interferon regulatory factors 7 (IRF7) via the adaptor molecule TRIF, which forms a complex with TANK-binding kinase 1 (TBK1), an inhibitor of the nuclear factor-κB kinase(IKK), and IRF7. Prior to nuclear translocation and induction of Type-I or Type-III IFNs, phosphorylated IRF7 forms a homodimer or a heterodimer with IRF3. The Janus Kinase (JAK) family members JAK-1 and tyrosine-protein kinase 2 (TYK-2) phosphorylate two signal transducer and activator of transcription (STAT) proteins, STAT-1 and STAT-2. They form a heterodimer that recruits IRF-9 to form the IFN stimulated gene factor 3 (ISGF-3) complex, which moves inside the nucleus and interacts with IFN-stimulated response elements (ISRE). ISRE has been

observed in the promoter of IFN-stimulated genes (ISG). Furthermore, IRF7 is a master regulator and is constitutively expressed in excess in bats. Upward and downward arrows indicate upregulation and downregulation of the molecules in bats. Different sensors and mediators are shown in ovals.

BATS ADAPTIVE/HUMORAL IMMUNE SYSTEM COMPONENTS

Persistent infection or incomplete eradication of microbial load triggers the adaptive immune response. This response depends mainly on the activities of β and T lymphocytes, which have variable strategies to respond and reduce viral loads. The bats' blood, spleen, and bone marrow have proportions of β T and NK cells different from other mammals [68]. The major components of the adaptive immune response of bats are explained below.

Bats β cell components

Bats exhibit adaptive responses to viral infections, including IgE, IgG, IgM, IgA, cytokines, etc. [69]. Hollow bones of bats do not have bone marrow, affecting the production of β cells, due to which they may be able to carry a heavy viral load without illness [70]. But the formation of βcells may be increased in other places, like bone marrow of legs and pelvis of bats [70]. Antibodies constitute a major part of the humoral immune response, which helps capture and neutralize viruses and other pathogenic particles [71]. Antibodies bind to viral particles and block infections to the host cell, whereas T cells recognize and destroy the cells infected with viruses [72]. It has been observed that the bats have a rich repertoire of IgG isotypes along with copy numbers [73,74]. For example, Carolliaper Spicillata has a single IgG isotype, while *Eptesicus fuscus* has two, and *Myotis lucifugus* has five isotypes [75]. In *M. Lucifugus*, the VDJH locus shows high diversity, but little evidence of somatic hypermutation has been reported. Furthermore, the researchers have also detected the transcripts of IgM, IgE, and IgA in bats [68]. Although the number of white blood cells (WBCs) decreases with age in greater sac-winged bats (*Saccopteryx bilineata*), IgG antibody levels are found to be higher in older bats [28].

Bats T cell components

Among T cells, both CD4 and CD8 types are involved in antiviral immunity, but each of them recognize peptides derived from viral antigens bound to different MHC proteins [72]. Since viruses replicate within and spread directly between cells, T cell functioning is more vital for the resolution of the infection [72]. The presence of leucocytes like macrophages, β, and T cells has been confirmed in the spleen and lymph nodes of Indian fruit bats Pteropus giganteus. The number of T cells has been reported to be more than β cells in *P. Alecto* and *E. spelaea* [76] [68]. This highlights that bats may have a strong T-cell response compared to humoral response or the steady immune state, or the presence of viruses [77]. Bats have a CD4 to CD8 T cell ratio of 2:1 as opposed to 1:2 in humans and mouse bone marrow [78], which may be due to infection, inflammation, and/or autoimmunity [79–81]. The T cell coreceptor, CD4, and CD3+ have been identified in bats [69] [77]. The production of T lymphocyte-derived cytokine in bats is delayed compared to mice [28], indicating a unique humoral immune response of bats. A study on *R. aegyptiacus* CD4 cDNA sequencing revealed similarities of CD4 with cats and dogs compared to that of humans and mice. Unique changes have been observed in bat CD4, including the addition of 18-amino acid and lack of a cysteine compared to humans, suggesting structural differences in bat CD4 [82].

Contracted MHC genes and diverse APOBEC proteins

Class I and II major histocompatibility complex (MHC) proteins constitute the fundamental components of acquired immunity. [83]. MHCs are involved in the presentation of the peptide from the processing of foreign agents, which are subsequently recognized by T cells. Cytotoxic CD8+ T cells recognize class I MHC peptides on the surface of nucleated cells, while the MHC II peptides, presented by antigen-presenting cells (APCs), like dendritic cells (DCs), macrophages, or β cells, lead to activation of CD4+ T cells [83]. Apolipoprotein B editing complex (APOBEC) restricts the replication of retroviruses by deamination of cytosine residues of viral cDNA[84][85,86]. In comparison to other mammals, Pteropid bats contain contracted MHC I region, fewer IFN genes but a huge quantity of diversified group of APOBEC proteins, indicating its vast antiviral activity[87,88]. It is assumed that APOBEC diversification might have occurred in bats, possibly to counteract the effect of different viruses [86].

Overall, the adaptive immune response of bats for viruses is summarized in figure 2. Upon encounter with viral antigens, the β cells are activated and differentiate into two distinct cell types, plasma and memory cells. The β cell produces antibodies that bind to viral particles, block further infections, and neutralize the virus. Another component of adaptive immunity is the response of T cells. They specifically recognize and destroy cells infected with viruses. The antigen-presenting cells (APCs) process viral proteins and present them via their MHC

class I/II peptides, which are recognized by specific CD8 cytotoxic T lymphocyte (CTLs) and dendritic cells (DCS), respectively. Finally, activated CTLs and DCs induce apoptosis and phagocytosis of viral-infected cells.

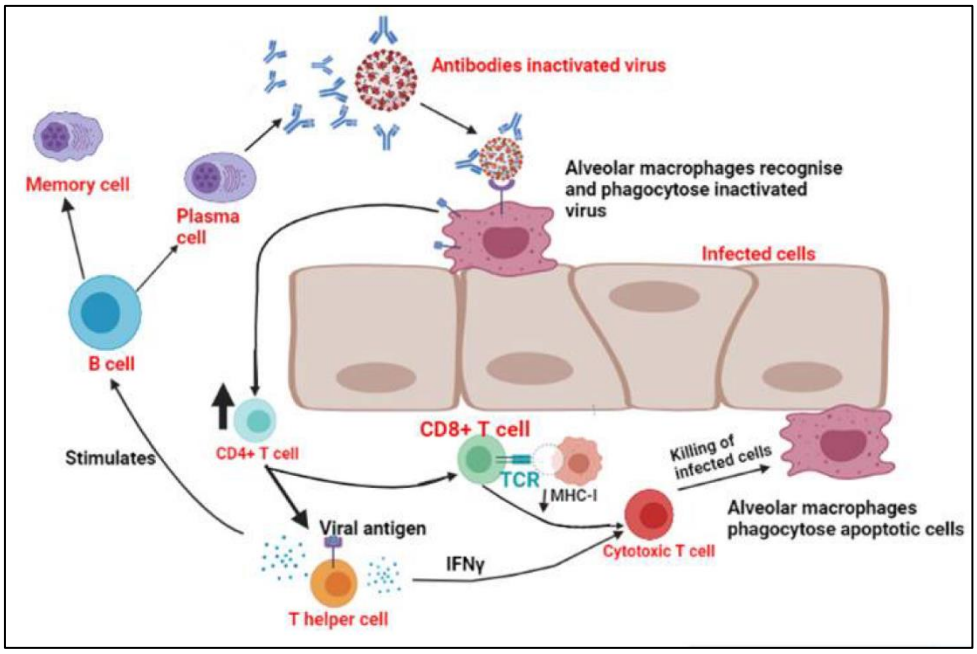

Figure 2: Adaptive immune response in bats. *Encountering antigens* is required for activation of βcells, which differentiate into plasma and memory cells after being activated.Antibodies bind to viral particles and block infections to the host cell. T cells recognize and destroy the cells infected with viruses. Specific CD8 CTLs get activated after recognizing antigens bound to MHC class I on professional antigen-presenting cells (APCs) and then proliferate and differentiate into effectors, which enter the efferent lymph and bloodstream reach other locations. Different immune cells are shown in ovals.

OXIDATIVE PHOSPHORYLATION (OXPHOS) RATE ARE HIGHER IN BATS

Amongst mammals, only bats are equipped with the ability of powered flight. It consumes a lot of energy to fly, so their metabolic rates have been immensely increased. A comparative study has confirmed that a relatively higher rate of positive selection has been observed in mitogenome encoded OXPHOS genes; however, five-fold fewer values were obtained for nuclear-encoded non-respiratory genes [89]. This natural selection of OXPHOS genes led to an increased rate of oxidative phosphorylation to generate ATP, but this oxidative process comes at a price. OXPHOS also generates precarious free radicals like ROS. These ROS cause DNA damage that can lead to cancer and contribute to aging; however, bats are long-lived. In bats, the OXPHOS-related genes of the mitochondrial genome evolved particularly rapidly, faster than genes in the nuclear genome. It has been found that the genes associated with the OXPHOS pathway in micro-and megabat species have been enriched [25].

BATS ARE BETTER AT DEALING WITH DNA DAMAGE

The bat has a unique ability to enhance its basal metabolic rate during its flight. The detailed analysis of the OXPHOS of bats revealed that their overall energy metabolism is unregulated, supporting ATP production during flight adaptation. Although the positive selection of OXPHOS helps bats survive longer flights by supplying a sufficient amount of ATP, it is also responsible for generating damaging molecules like ROS. These ROS are responsible for intracellular damages, including oxidative DNA damage. To cope with such damages, bats have evolved so that they have enhanced the ability of DNA repair. Several bat species, including *Pteropus electo* and *M. davidii*, have shown a rapid evolution of genes involved in DNA repair [2,25]. In these bats, a positive selection of DNA damage checkpoint pathway has been observed along with the required components of the innate immune system, suggesting that flight adaptations regulate bat immunity [25]. Bats can repair excessive DNA damages and prevent their body from further damages.

INSIGHTS ON VIRUS SPILLOVER EVENTS

The spillover of viruses from bats to humans is directly linked to an increase in the frequency of their interactions among them. These interactions are caused due to a reduction in the bat habitats due to anthropogenic activities leading to an alteration in the host-pathogen

relationships [90,91]. Furthermore, it has been demonstrated that viral transmission from bats is directly associated with their eating behavior. For example, the fruit bats contaminated partially eaten fruits are consumed by other animals, which get easily infected [92]. Spillover events are rare phenomena in nature and comprise sequential processes of contributing factors as reviewed by Subudhi et al. [67]. One such example has been elegantly shown by Plowright et al. [10] about the series of events leading to spillover of Hendra virus from bat to horses. These hierarchical events include the reservoir hosts, infection of reservoir hosts; shedding of viruses from reservoir hosts; survival of virus outside reservoir host; accessibility of virus to the recipient host; and the susceptibility of recipient hosts to the virus infection. Apart from these, several additional factors are needed to prevent the successful infection of the recipient host. The health status of the recipient host, previous exposure to the virus, environmental conditions, immune system of the host, and genetics plays a crucial role in establishing infection during spillover event[93]. The hypothesis proposed by Plowright et al. [10] highlights that the virus reactivates inside bats from time to time in response to different stimuli in bats, which probably increases the potential of viral shedding responsible for spillover. Furthermore, various factors contribute to the co-existence of bats and viruses, including- metabolic adaptations, constitutively active IFN, dampened inflammasomes responses, and several others, as shown in figure 3.

Moreover, the genetics of the bat allows the suppression of its immune response that allows the virus to significantly increase the viral titer leading to continuous and recurrent virus shedding. Such observations have been reported in pteropid bats, in which the bat's unique immune system causes henipavirus shedding [94]. Similarly, Sohayati et al. [94]reported that waning antibody levels and different stresses led to a recrudescence of NiV infection in bats. Gerow et al. [95] stated that the reactivation of viruses in brown bats occurred even after their arousal from hibernation.

Altogether, viral shedding is also a contributor to successful spillover events, which is highly dependent on the reactivation of the virus [96]. With the recent advances in our understanding of bat immunology, it is conceivable that such information will be useful for preventing viral shedding and subsequent infections in humans.

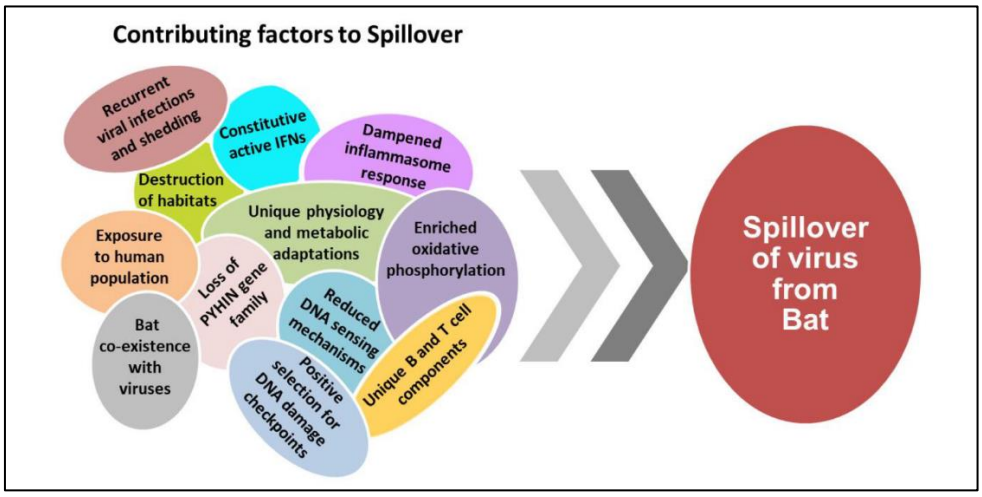

Figure 3: Summary of contributing factors that might lead to viral spillover from bat to recipient host.

CONCLUSIONS AND FUTURE PERSPECTIVE

We have summarized the mounting evidence suggesting the mechanisms by which bats limit viral infected diseases, rather enabling them to co-exist. Due to delayed /reduced immune response in humans, extensive immunopathogenesis has been observed due to viral infection; however, the same virus does not cause any problem in bats. Therefore, to tackle the spillover of viruses from bats to humans, it is necessary to understand the biology of host-pathogen interactions in context with bat viruses. One of the interesting progress undertaken is the ongoing genome sequencing of all species of bats as Bat1K project[97]. It is expected that upon completion of the Bat1K project, the researchers will understand the bat biology in detail and help them elucidate bat-pathogen and host/ recipient species interactions. Furthermore, the available data indicates that the viruses present in the bats are not geographically even [16], indicating the possible role of phylogeographic processes, which require validation of studies in the future. The stable bat cell lines should be developed to accelerate in vitro studies on bat cells. Such cell lines could be used to perform CRISPR-mediated screening experiments genome-wide studies, including transcriptomics and proteomics. Few cell lines have been obtained from the pteropid bat, *P. alecto*[98], and few

assays have been conducted [99]. Recently, a genome-wide transcriptome study on *R. aegyptiacus* bat has been conducted to identify several unique features [100]. Similar studies and screens might be performed in other bats to better understand the host-pathogen interactions, making the cell lines study more relevant. Such studies can be translated to organoids derived from bat cell lines to understand immune system adaptations' molecular basis. The nature of the antibody response to viral infection by a bat is relatively unknown. The mechanism of dampened antiviral response in a bat is another interesting direction for researchers to work soon. Altogether, several key mysteries remain unresolved. Therefore, humankind must enhance knowledge and understanding of bat and virus coexistence to prevent future outbreaks.

Several strategies have been suggested to control this pandemic. The identification and successful clinical trial of oral therapeutics are helping clinicians manage people suffering from coronavirus. Highly collaborative and intensive efforts are obligatory to further strengthen our doctors with better medicines and treatment regimes. Furthermore, the implementation of non-pharmacological interventions, including "proning" and respiratory exercises, positively impacts the survivability of COVID-19 patients. The use of booster vaccines to recharge waning immunity is also required, and many countries have already started it.

Furthermore, it seems mandatory to intensify global vaccination efforts in low and middle-income countries because it will substantially decrease the emanation of new coronavirus divergence. Vaccine equity in all countries is required to reduce the spread and possible elimination. The regular surveillance of a new cluster of cases and quickly identifying the causative variant is needed to control the spread of the virus. It is also necessary to create multivalent vaccines that can be quickly modified to incorporate changes that occurred in the variants to maintain overall vaccine efficacy. Another important area of concern is to develop the healthcare infrastructure to handle a large surge in cases. More importantly, the general public has to maintain proper hand hygiene, social distancing norms, use of masks, and avoid unnecessary gathering/ super-spreader events to reduce the spread of this virus. The experience gained from the previous waves of COVID-19 indicates that without international coordination, it is difficult to control the emergence of new variants and spread this virus. Altogether, the information gained from previous waves of COVID-19 suggests that a coherent collaboration is of utmost necessity between the governments, scientists, clinicians, epidemiologists, economists, and sociologists to control this virus with minimal economic and social impact on human society.

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