Chapter

Learning from Bats to Escape from Potent or Severe Viral Infections

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Abstract

The COVID-19 pandemic that started in December 2019 in Wuhan city, China has created chaos all over the world with over 185 million infection cases and 4 million deaths world-wide. The pathogen behind COVID-19 has been identified as severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) that is more close to the previous SARS-CoV responsible for SARS epidemic 2002–2003. Although, SARS-CoV-2 also differs from SARS-CoV in many aspects as indicated by genetic studies. For example, SARS-CoV does not have a furin binding domain or site, whereas its presence in SARS-CoV-2 spike (S) protein increases its potential for infectivity. The horseshoe bats (Rhinolphus species) from China are considered as primary animal reservoirs for SARS-CoV and SARS-CoV-2. However, along with CoVs, bats also harbor many other viral pathogens (Ebola, Nipah, and Hendra viruses) without having serious infections. The bat physiology plays a crucial role in harboring these viruses along with adaptations to longevity and slow aging process. The immune system plays a crucial role in the clearance or establishment of the infection. Present chapter discusses different immunological aspects (innate immune response comprising the virus recognizing pattern recognition receptors (PRRs), type 1 interferon production, pro- and anti-inflammatory immune response, and adaptive immune response) that help bats to control viral infection without getting a severe infection as compared to other mammals, including humans.

Keywords: Bats, innate immunity, autophagy, infection, IFNs, adaptive immunity

1. Introduction

Bats and flying foxes, including large flying foxes (*Pteropus vampyrus*) and variable flying foxes (*P. hypomelanus*) are the mammals belonging to the order *Chiroptera* (hand wing). This order contains 1232 species of bats and flying foxes constituting a more diverse and important order of mammals after rodents. They evolved approximately 52 million years ago [1, 2]. Taxonomically, bats represent approximately 20% of mammalian diversity [3]. They are the real flying mammals and come out for prey in the night time (nocturnal aerial predators). Many species of bats are frugivorous (fruit eating), insectivorous (insect eating), and some feed on blood of other animals (hematophagous). Some species of bats fly long distances during seasonal migration with a speed of 100 miles per hour, making them the fastest mammal (free-flying Brazilian free-tailed bats or *Tadarida brasiliensis*) on earth [4]. Some species of bats fly during night and some are diurnal or crepuscular. Bats are found in all continents, except Antarctica. They live in caves or in other

dark spaces in large groups or colonies and some are solitary in nature. Besides playing a crucial role in maintaining biodiversity or ecological balance through their different roles (insects eating, pollination, and seed dispersal etc.), they remain crucial to researchers due to their strange characteristics and reservoir for different pathogens [2]. For example, the advancing knowledge in bat biology has implicated them (the tropical frugivorous Honduran white bat *Ectophylla alba*) to be studied as a mammalian model for skin carotenoid metabolism [5].

Bats are crucial primary reservoirs for emerging viral infections that can be transferred to humans or cross the species barrier to infect other wild or domesticated animals through spill over [6]. Studies have indicated that they harbor higher numbers of zoonotic viruses per species than rodents [7]. Even they have higher (3.9 times stronger) sympatry than bats and sympatry within a taxonomic order serves as a most crucial host trait for zoonotic virus enrichment [7]. Of note, despite harboring more zoonotic viruses per species than rodents, the total number of zoonotic viruses found in bats (61) are lower than rodents (68) due to double the number of rodent species than bat species. However, bats are the primary host for more virulent viruses than other mammals, including rodents [8]. Before, the emergence of recent virus infections, including severe acute respiratory syndrome (SARS), middle-eastern respiratory syndrome (MERS), Ebola virus infection, and most recent Coronavirus disease 19 (COVID-19) pandemic caused by SARS-CoV-2, MERS-CoV, Ebola virus or *Zaire Ebolavirus* (three different species of Ebola viruses have been found in greater long-fingered bat (*Miniopterus inflatus* or *M. inflatus*) in Liberia's Sanniquellie-Mahn District that borders to Guinea and insect-eating bat, *M. schreibersii*), and SARS-CoV-2, the studies of natural histories of bats, their importance as primary reservoirs for different zoonotic viral diseases have been largely underappreciated, underrated, and underfunded [9–12]. Although, they (vampire bats or *Desmodus rotundus murinua* found only in the Latin America) were considered for their role in the rabies transmission called vampire bat rabies as suggested first in 1959 [13–16].

Fruit bats, including *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquate* have also been suggested as potential reservoirs for *Zaire Ebolavirus* [12, 17]. In addition to these zoonotic viral infections, bats also serve as potential reservoirs for other viruses responsible for infections in humans that include Nipah, Hendra, Marburg, Hepadna (able to infect human hepatocytes), and Lyssa viruses etc. Thus, different viruses of 23 virus families have been detected in different bat species (196) in 69 countries all over the world [3, 18]. The mortality among bats due to bacterial or viral infection has been the least observed cause of death [19]. In comparison to humans, where 7% of the genome encodes for the immune or related genes (1562 immune genes recorded in humans as of 1st October 2004 by the immunogenetic related information source or IRIS), only less than 4% of the bat (Australian flying fox or *Pteropus alecto*) genome encodes from immune related genes (about 500) [20, 21]. For example, Jamaican fruit bat or *Artibeus jamaicensis* has 466 immune-related genes (IRGs) and the Egyptian Rousette bat (*Rousettus aegyptiacus*), a common fruit bat species has 407 or 2.75% IRGs of their total genome [22, 23]. Thus, either bats have lower numbers of IRGs as compared to humans or we need further studies in other potential bat species harboring potent virus pathogens that can infect humans directly or indirectly through secondary reservoir hosts.

Also, Panamanian Seba's short-tailed bats (*Carollia perspicillata*), a widely distributed neotropical species shows individual and population-specific diversity in their major-histocompatibility complex 1 or MHC-1 genes with an unique genotype in each individual comparable to passerine or perching or singing birds [24]. The MHC-II diversity is also correlated with the geographic origin and population

admixture in *Carollia perspicillata* and *Molossus molossus*, and in *Desmodus rotundus* MHC-II DRB gene diversity depends on the environment only [25]. The MHC diversity in bats may impact their defense against different reservoir viruses inducing resistance against them and providing an opportunity or a perfect animal niche for the virus evolution that may infect other hosts, including humans severely [24]. The Egyptian Rousette or fruit bat does not support the productive growth or replication of the Nipah virus [26]. No seroconversion against Nipah virus glycoprotein has been reported in these bats. Hence, only specific bat species serve as potential reservoirs for Nipah viruses. This may be true for other viruses too. The *in vitro* study based on bat cells (RoNi/7.1 (*Rousettus aegyptiacus*) and PaKiT01 (*P. alecto*) cells) lines has indicated the enhanced interferon (IFN)-mediated antiviral immune response generation of either constitutive or induced form that allows a rapid cell to cell virus transmission rate (β) within the host [27]. The IFN-induced antiviral state protects live cells from apoptotic or other forms of cell death *in vitro* that (the *in vitro* epidemic or extended life of the cells) enhances the probability of developing and establishing a long-term persistent infection [27]. This phenotype of infection and associated host-pathogen interaction response is absent in Vero cells (a cell line derived from the kidneys of African green monkeys) due to the genetic defect in the IFN production [27, 28]. Hence, viruses evolved in bats as reservoirs have an increased IFN capabilities that helps to achieve a rapid within-host transmission rates without inducing clinical symptoms of the disease. Thus these rapidly reproducing viruses in bats may become more virulence upon spillover to hosts, including humans lacking similar immune capabilities like bats. Hence, understanding the bat immune function or response becomes crucial to understand. The present chapter describes the immunological aspects or features of bats preparing them to harbor a wide range of viruses without severe disease causing mortality.

2. Innate immune adaptation of bats as preventing to develop severe infections

The innate immune system is primary or first line of the defense against invading pathogens. The pattern recognition receptors (PRRs), including toll-like receptors (TLRs), Nod-like receptors (NLRs), absent in melanoma-2 (AIM2)-like receptors (ALRs), retinoic acid-inducible gene-1 (RIG-1)-like receptors (RLRs, RIG-1 and melanoma differentiation-associated protein 5 or MDA5), C-type lectin receptors (CLRs), and cyclic GMP (guanosine monophosphate)–AMP (adenosine monophosphate) synthase (cGAS) and stimulator of interferon genes (STING) signaling pathways play a crucial role in the host defense and the generation of pro-inflammatory immune response (cytokine, chemokines, reactive oxygen and nitrogen species (ROS and RNS), and type 1 interferon (IFN) production) [29–34]. TLR4 is a crucial PRR to recognize Gram-negative bacterial lipopolysaccharide (LPS) as a potent microbial or pathogen-associated molecular pattern (MAMP or PAMP) to induce a potent pro-inflammatory immune response to clear the infection. However, its overactivation may cause severe inflammation. Pallas's mastiff bats (*Molossus molossus*) upon exposure to the *Escherichia coli* (*E. coli*)-derived LPS do not develop leucocytosis and hyperthermia or fever independent of their sex (**Figure 1**) [35]. However, they show weight loss upon exposure to the LPS. This study indicates the presence of defective TLR4 signaling responsible for the NF-κB-dependent pyrogenic cytokines (IL-1 and IL-6) (**Figure 1**). This defect may also prevent the further activation of cytosolic NLRP3-dependent inflammasomes responsible for generating IL-1β and IL-18. Bat (little brown bat or *Myotis lucifugus*) mitochondria produce lesser ROS (a potent inducer of NLRP3 activation) [36].

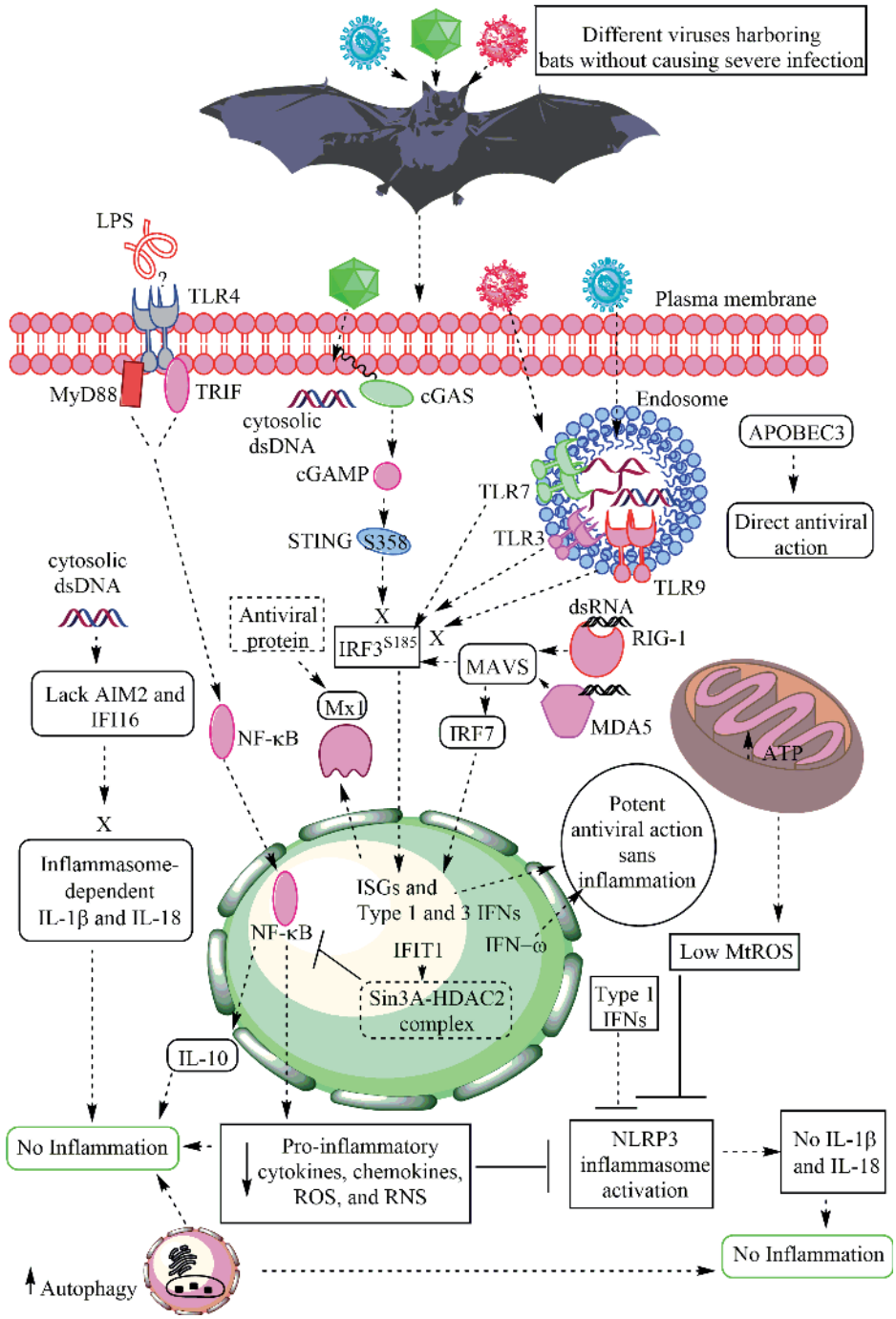

Figure 1.
Schematic representation of immune response in bats preventing development of severe infection and inflammation. The gram-negative bacteria or its PAMP (LPS) recognition in bats do not stimulate pro-inflammatory cytokine production through NF-κB activation and increase in body temperature. The increase in autophagy further increases cellular longevity, acts as an antiviral mechanism to clear or control the infection, decreases or suppresses inflammation. The PYHIN domain containing AIM2 and IFI16 inflammasomes are absent and hence, do not take part in cytosolic DNA recognition as DAMP to inflammasome activation-based maturation of IL-1β and IL-18. This further decreases the incidence of inflammation and associated tissue damage. The cGAS-STING-based signaling mechanism recognizing cytosolic dsDNA as DAMP also does not work in bats due to the presence of serine at 358 AA position in STING that is unable to activate IRF3 and type 1 IFN production. Hence, this further prevents inflammatory events in response to the self-DNA. Only the cytosolic RNAs activate different PRRs (RIG-1, MDA5, and TLR3) that via IRF3 and IRF7 activation synthesize type 1 and 3 IFNs, which exert antiviral action, but damp pro-inflammatory action of NRLP3 and NLRP1 inflammasomes. Mx1 is an IFN-inducible antiviral protein with a GTPase activity. APOBEC3 also directly acts as an antiviral host factor without inducing inflammation. Hence, only protective antiviral immune response works in bats to control their number without inducing severe inflammation.

The reduced mitochondrial ROS (mtROS) production in Seba's short-tailed bats involves a mild depolarization of the inner mitochondrial membrane that decreases the membrane potential to a level sufficient to produce ATP molecules but insufficient to synthesize mtROS (**Figure 1**) [37]. This mechanism decreases with age in mice but remains intact in these bats. For example, in 2.5 years old mice this mechanism of mild mitochondrial depolarization disappears in different organs (lungs, liver, spleen, skeletal muscles, heart, brain, and kidneys). Hence, mtROS-mediated DNA and protein damage is seen in mice or other mammals but not in bats.

The immune challenge among bats does not alter their oxidative stress irrespective of their pre-migration and migration seasons [38]. However, bats have higher baseline leukocytes but lower neutrophil numbers during their migratory seasons as compared to their pre-migratory season. Their plasma haptoglobin (a humoral innate immune component) levels also remain same during both seasons [38]. However, plasma haptoglobin level of migratory bats increases upon an immune (LPS) challenge that remains unchanged in non-migratory or pre-migratory bats under the same immunogenic stimulation. Of note, bats do not upregulate genes associated with chronic inflammation with the advancement of age that is seen in other mammals, including humans [39]. Hence, this protects them from age related inflammatory diseases and predisposes them towards healthy aging and longevity along with tolerance to infections, including Ebola, Nipah, and many more. Also, the bat microbiota (Firmicutes and Proteobacteria are dominant bacteria) differs from other terrestrial mammals (strict anaerobic phylum Bacteroidetes in mice and humans), and remains intact throughout their life that further protects them from age-associated inflammation and inflammatory diseases [40, 41]. On the other hand in mice and humans gut microbiota changes with time and aging that predispose them to age-associated inflammatory diseases associated with gut bacteria dysbiosis [42–44].

A study has shown the TLR3, TLR7, and TLR9 expression at mRNA levels in different organs of Leschenault's Rousette bats (*Rousettus leschenaulti*) [45]. Another study has shown the expression of full length mRNA transcripts of TLR1-TLR10 in the Australian flying fox or *P. alecto* [46]. This bat species also expresses the pseudogene for TLR13. However, their functional protein level expression in different bat species needs further investigation. The evolutionary studies have shown that the bats evolved under the influence of positive selection for TLR7, TLR8, and TLR9 that is highest for TLR9 and lowest for TLR7 [47]. The TLR3 in bats has evolved under a negative selection process. This study indicates the adaptation of host-pathogen interaction in bats, particularly in bat TLR9. The bat TLR8 has an extensive sequence variation within them that separates them from other mammals, including humans [48]. Bat TLRs are evolving slowly under purifying selection in response to the functional constraints with a divergence process that is overall congruent with the species tree [49]. The bat TLRs show unique mutations in their ligand-binding domains even involving their non-conservative amino acid (AA) change and/or targets of positive selection. These changes can modify the binding of the corresponding TLR ligands. Hence, bat TLRs may vary in recognizing the same ligand recognized by other mammalian or human TLRs.

Flying fox bats (*P. alecto*) have other cytosolic dsRNA recognizing receptors called RLRs, including RIG-1, MDA5, and laboratory of genetics and physiology 2 (LGP2), like humans that upon recognizing cytosolic dsRNA induce the type 1 IFN production [50]. LGP2 synergy with MDA5 to generate antiviral immune response during RLR-dependent dsRNA recognition [51]. LGP2 interacts with the IFN-inducible, dsRNA binding protein PACT (a cofactor of DICER in the processing of microRNAs) through its regulatory C-terminal domain that inhibits RIG-1-dependent signaling but promotes MDA5-dependent antiviral

immune response [52]. TLR3, RLRs (RIG-1), and MDA5 serve as potent antiviral immune response inducers in bats to protect them from severe infection caused by Encephalomyocarditis virus (EMCV) and Japanese encephalitis virus (JEV) (**Figure 1**) [53]. The functionally conserved RLR adaptor called mitochondrial antiviral signaling (MAVS) protein has been demonstrated in the Chinese rufous horseshoe bat (*Rhinolophus sinicus*) and straw-colored fruit bat (*Eidolon helvum*) that upon RLR (RIG-1 and MDA5)-based activation transmits signals to produce type 1 IFNs (IFN-β) and interferon stimulated gene (ISG) called IFN-induced protein with tetratricopeptide repeats 1 (IFIT1) that further enhances IFN gene program (IFN-β, IRF7, and OAS1 or 2′-5'oligadenylate synthase 1), which activates ISGs, immune homeostasis, and cell's internal antiviral immune response (**Figure 1**) [54–56].

The activation of MAVS involves the RIG-1 and MDA5 dimer formation [57]. Also, the IFIT1 generated exerts an anti-inflammatory action via suppressing TLR-dependent NF-κB-mediated pro-inflammatory cytokines (TNF-α, IL-1β) and chemokines (CCL3) through activating Sin3A-histone deacetylase 2 (HDAC2) transcriptional regulatory complex containing SAP25 that has an inhibitory action (**Figure 1**) [56]. Hence, these PRRs protect bats from developing severe viral infections through increased type 1 IFN production but low tissue damaging pro-inflammatory immune response. It should be interesting to observe that viruses harboring bats as their primary reservoirs may have evolved strategies to escape this innate immune mechanism to recognize cytosolic dsRNA viruses or bats have developed other mechanisms to escape from exaggerated pro-inflammatory innate immune response upon recognizing cytosolic dsRNA viruses. The MERS-CoV replicates efficiently in Jamaican fruit bats (*Artibeus jamaicensis*) without causing a productive infection with clinical signs of the disease [58]. The interferon regulatory factor (IRF3) transcription factor activation plays a crucial role in generating the potent antiviral immune response in the bat (*Eptesicus fuscus*) against MERS-CoV (**Figure 1**) [59]. In comparison to humans or other mammals, MERS-CoV fails to subvert the IRF3 activation and dependent type 1 IFN response generation in *E. fuscus*. The IRF3 in bats differs from humans due to the presence of serine185 (S185) that provides an enhanced antiviral protection (**Figure 1**) [60]. The S185 insertion in the human IRF3 increases its antiviral action. Hence, the positive selection of S185 in the bat IRF3 increases its antiviral action. Also, the bats persistently infected with MERS-CoV have increased type 1 IFN levels than non-infected ones and its disruption increases the virus replication [61].

The bat cells repeatedly select for the mutant MERS-CoV called delta open reading frame (ΔORF5) MERS-CoV and are resistant to superinfection by wild type (WT) MERS-CoV due to deficiency of MERS-CoV binding receptor dipeptidyl peptidase 4 (DPP4) and increased type 1 IFN levels [61]. Additionally, the Australian black flying foxes in response the cytosolic TLRs and RLRs recognizing viral PAMPs (dsRNA) also activate IRF7, which also induces type 1 IFNs mediated antiviral immune response (**Figure 1**) [62]. The deficiency or the defective activation of IRF7 in bats enhances viral replication and the development of the productive infection. Of note, virus (bat paramyxovirus, Tioman virus) infection to bats also induces protective type III IFN production that further provides protection from the development of productive infection (**Figure 1**) [63]. Egyptian rousette bats (*Rousettus aegyptiacus*) are the naturally harbor Marburg virus (MARV) and do not develop clinical symptoms of the disease as compared to humans due to generation of IFN-based immune response by DCs and suppressing pro-inflammatory immune response [64, 65]. This is because these bats secrete IFN-ω, which have antiviral action against RNA viruses (**Figure 1**). Also, the 13% of genes induced by IFN-ω in bats are not found in the interferome and other ISG databases, indicating their uniqueness to bats [64].

Bat immune cells exert protective type 1 (IFN-α, β, and ω) type II (IFN-γ) IFNs against Filoviruses (Marburg and Ebola viruses) but human immune cells fail to do so (**Figure 1**) [66]. Myxovirus resistance 1 (Mx1, a GTPase) is another antiviral protein induced in response to the IFNs is evolutionary conserved in vertebrates and can restrict a wide range of viruses in host cells (**Figure 1**) [67]. In bats these Mx1 proteins protect against Ebola and Influenza viruses through reducing the polymerase activity of these viruses along with other circulating viruses [68]. However, bat Mx1 does not inhibit Thogoto virus (enveloped negative sense ssRNA virus of *Orthomyxoviridae* family) as it does not infect them. On the other hand, mice Mx1 in hematopoietic cells inhibits Thogoto virus infection [67]. Hence, Mx1 is another IFN-induced antiviral protein in bats to protect against severe viral infections (**Figure 1**). Also, the production of type 1 IFN inhibits the NLRP1 and NLRP3 inflammasome-induced IL-1β and IL-18 production and induces IL-10 synthesis via STAT1 transcription factor (**Figure 1**) [69]. The IL-10 further activates STAT3 to reduce the IL-1β and IL-1α levels. IFNs also inhibit inflammasome-mediated Caspase 11 (CASP11) to inhibit the pro-inflammatory IL-1β and IL-18 release via activating immunity-related GTPases M clade 2 (Irgm2) and Gate16 (an ATG8 family member), which inhibit CASP11 maturation or activation [70]. Hence, IFN levels control exaggerated inflammation through different mechanisms.

The cGAS-STING signaling-mediated type 1 IFN production against DNA viruses is lost in bats due to the loss of serine AA at 358 (S358) position of the STING (**Figure 1**) [71, 72]. The S358 AA of the STING from other non-bat mammals is conserved and its phosphorylation is crucial for STING-dependent IRF3 activation and type 1 IFN release. For example, in human STING the S3666 and S358 phosphorylation is crucial for IRF3 binding and activation, but not for TBK1 [73]. Also, the TLR9-dependent cytosolic DNA recognition in bats is not as functional as in other mammals, including humans as result to adapt its high metabolic rate that increases the body temperature over 41°C during migratory flight that can induce DNA damage and its migration to the cytosol (**Figure 1**) [49]. Along with, defective cGAS-STING and TLR9 signaling for cytosolic DNA recognition, absent in melanoma 2 (AIM2) and gamma-interferon-inducible protein Ifi-16 (IFI16 or p204 in mouse) or interferon-inducible myeloid differentiation transcriptional activator are the PYRIN and HIN domain containing (PYHIN) proteins also recognizing cytosolic DNA are absent the genome of most bats, including *P. alecto* and *M. davidii* [74–76]. Both, AIM2 and IFI16 are involved in the cytosolic DNA recognition-induced inflammasome activation, and the maturation and release of pro-inflammatory cytokines (IL-1β and IL-18) (**Figure 1**) [75]. Only, a bat called *Pteronotus parnellii* has a truncated AIM2. Hence, the removal of cytosolic DNA sensors or PRRs adds to escape from the inflammatory immune response generated due to DNA damage observed high metabolic rate-induced rise in temperature during long migratory flights and helps in the coexistence of host and pathogens. Also, the killer immunoglobulin-like receptors (KIRs) encoded by genes in the leukocyte receptor complex (LRC), and killer cell lectin-like receptors (KLRs, also called Ly49 receptors), encoded within the natural killer gene complex (NKC) are required for potent antiviral function of NK cells. However, *P. alecto* lacks both KLRs and KIRs and *M. davidii* has only one Ly49 pseudogene [76].

The pteropodidae or cave nectar bat (*Eonycteris spelae*) monocytes, macrophages and granulocytes resemble human counterparts depending on the immune parameters that are divergent between mice and humans [77]. However, mast cells, eosinophils, basophils, platelets or thrombocytes have not been identified and characterized in different bat species [54]. Further studies are required in this direction. Also, the genome-wide comparison of immune-related genes have indicated their much closer phylogenetic relationship with humans than rodents. Also, bats express

largest and most diverse array of apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3 (APOBEC3) genes (encode antiviral DNA cytosine deaminases), which are potent antiviral proteins and act as antiviral restriction factors for viruses, including hepadnaviruses (hepatitis DNA virus), and parvoviruses [78, 79]. The potent antiviral immune response of APOBEC3 involves its cytosine deaminase activity that deaminates cytosine residues in the nascent retroviral DNA to block retrovirus replication via hypermutation (**Figure 1**) [80]. This hyper-mutated retroviral DNA, then gets degraded or becomes non-functional [81]. In other mammals, including humans and laboratory mice the expression and action of APOBEC3 might threaten the integrity of the host genome triggering the incidence of cancer [82]. For example, a common APOBEC3 overexpression in humans is associated with the incidence of breast cancer in humans and the overexpression of APOBEC1 (A1) in mice is associated with hepatocellular carcinoma [83–85]. However, bats are more resistant to developing cancer despite expressing APOBEC3 as they express a higher quantity of ABC transporter called ABCB1 than humans and efficiently removes cytotoxic agents (doxorubicin) and damaged DNA [86]. Hence, in bats APOBEC exerts its only antiviral action and remains sans to increase susceptibility to cancer. However, further studies are warranted. Of note, even minor levels of IFNs are able to induce APOBEC3 family of proteins (A3A, A3G, and A3F) expression and their antiviral action [87].

Lower NLRP3 inflammasome activation in the cytosol prevents exaggerated inflammatory immune response in immune cells bats due to lower ROS production (crucial for NLRP3 activation) and apoptosis-associated speck-like protein containing a CARD (ASC) speck formation and secretion of interleukin-1β (**Figure 1**) [88]. Also, bats produce less TNF-α due to the interaction of c-Rel (a member NF-κB family) with the promoter sequence of TNF-α [89]. The antiviral innate immune response in bat macrophages in response to the virus-derived PAMPs is also accompanied by sustained production of an increased amount of anti-inflammatory cytokine (IL-10) (**Figure 1**) [90]. These unique anti-inflammatory mechanisms in bats, including greater mouse-eared bats, *Myotis myotis* may have evolved due to their high metabolic rate (but produce low ROS that regulates NLRP3 inflammasome activation) and long distance flights [90]. For example, this bat species along with other long-distance traveling bats exhibit a delayed aging process as indicated by the absence of shortened telomerase and due to strategies to check induction of severe inflammation, but the induction of potent anti-inflammatory mechanisms [91, 92]. Also, the expression of high basal levels of heat shock proteins (HSP70 and HSP90) in bats protects them from increased metabolic stress that further contributes to their longevity and healthy aging [93]. Hence, these processes may contribute to longevity and healthy aging among bats.

Autophagy is an essential cellular process through which cells maintain homeostasis, including immune homeostasis [94–96]. Autophagy involves the breakdown of cellular components and the sequestration of the portion of cytoplasm into the double or multi-membraned vesicle called autophagosomes, which then fuse with cellular suicide or waste bags or lysosomes (contain hydrolases in their lumen and their membranes have permeases) to form autophagolysosomes or autolysosomes [96–98]. Autolysosomes are the junk crashers of the cell, in which luminal materials, including internal membrane, are degraded and exported out of the cell through permeases to recycle in the cytosol [96]. Hence, autophagy is the renewal process for cytosolic components through which cytoplasmic macromolecules mobilize to generate energy-rich compounds to meet cellular energy requirements during conditions with decreased internal and external energy resources. The impaired autophagy predisposes the host towards premature aging and inflammatory and degenerative diseases. Hence, autophagy helps the host to escape from

premature aging and different diseases (cancer, neurodegeneration, and other chronic inflammatory conditions) through cellular self-digestion [99].

Autophagy also plays a crucial role in immune response to infections and inflammation that works downstream to different PRRs (TLRs, NLRs, RLRs, and cGAS-STING signaling) discussed earlier (**Figure 1**) [100–102]. The increased autophagy in Australian black fly foxes also dampens the severity of the lyssavirus infection through suppressing the virus replication and increases the tolerance to the prolonged infection with lesser cell death than humans (**Figure 1**) [103]. Autophagy increases with the increases in the viral load in bats. The pharmacological activation of the autophagy decreases the virus replication that shows its antiviral action. Another virus called Nelson Bay Orthoreovirus (NBV that in humans causes severe respiratory tract infection) isolated from the Australian fruit bat increases autophagy in host cells depending on the viral replication without causing severe infection [104]. Hence, increased autophagy along with increasing longevity and suppressing aging mechanisms among bats also increases their antiviral immune response to protect them from severe productive infection.

3. Adaptive immune response in bats to make them resistant severe viral infections

We do not have greater immunological data for adaptive immunity in bats as compared to humans due to lack of experimental reagents specific for bats and corresponding appropriate animal models. The genes [MHC-I and II, TCR (TCR-α and –β) and co-receptors, including CD3, CD4, CD8, and CD28 along with B cell-specific markers (CD22, CD19, CD20, CD27, and Igs)] involved in adaptive immunity in other species are conserved in bats [21–23]. The transcripts of both pro- and anti-inflammatory cytokines (IL-2, IL-4, IL-5, IL-6, IL-12a, IL-12b, IL-17a, IL-23, IL-10, TGF β, TNF, IFN γ, IL-1 β, CCL2, CCL5, and CXCL10) are also present [23]. The alpha1 (α1) domain of the H chain of MHC-I of *P. alecto* have three sequential AAs (Met, Asp, and Leu), which are absent in other mammals, including humans [105]. These 3 extra AAs in bat MHC-I help to form an extra salt-bridge chain between the H chain and the N-terminal of aspartic acid (Asp) of the antigenic peptide that promotes peptide presentation to the MHC I with high affinity during antigen presentation process. This study indicates the induction of stronger MHC-1-dependent T cells ($CD8^+$ cytotoxic T cells) immune response against viruses that helps them to survive otherwise lethal viral infections as seen in other mammals.

P. alecto has a predominant population of $CD8^+$T cells in their spleen and $CD4^+$T cells are predominantly present in blood, lymph nodes (LNs), and bone marrow [106]. Forty percent of these splenic T cells constitutively express IL-17, IL-22, and TGF-β mRNA, indicating the polarization of these T cells towards, Th17 and regulatory T cells (T_{regs}) [106]. Recent identification and development of bat-specific cross-reactive Abs and establishment of captive experimental bat colonies have advanced the field. Immunoglobulins or Abs, including IgG, IgA, IgM, and IgE have been detected in bats (*P. alecto*) [107, 108]. However, IgA in secretion is lesser than expected but that is compensated by increased presence of IgG in the mucosal surfaces [108]. IgM is the second most abundant Ab in the serum after IgG in *P. alecto*. Of note, bats have a bigger repertoire of germline genes encoding Ig variable (V), diversity (D), and joining (J) segments than humans, indicating a provision of a larger number of antigen (Ag) specificities in their naïve B cell receptor (BCR) repertoire [54]. For example, little brown bats (*Myotis lucifugus*) rely more on the germline encoded repertoire to fight against infections than somatic hypermutation (SHM) [109]. On the other hand, SHM in humans increase the affinities

of Abs for diverse antigens [110]. Thus, human Ab response generates more diverse Abs in humans than bats.

The maternal Abs transferred to Egyptian Rousette bats against the Marburg virus last for their first five months after birth and Abs last for approximately 1 year in these bats infected naturally [111]. However, the reinfection of bats with the same virus induces anamnestic immune or Ab response within 5 days of the post viral infection clearing the virus systemically as well as from major organs (salivary glands, intestine, urinary bladder, and the reproductive tract). Hence, reinfection with the virus to bats in the natural environment is not sufficient to induce the productive infection. Another study indicates that the maternally-derived Abs (MDAs) in seasonally breeding bats (African fruit bats) do not last long for other viruses, including Lagos bat lyssavirus (LBV, a member of genus lyssavirus and gamily *rhadoviridae*) [112]. Also, the Abs developed in captive bats decay more slowly than these MDAs, indicating the fast decay of these MDAs. However, Abs produced in captive bats decay faster than seasonally breeding bats living in their natural environment, indicating the Ab may persist for life in natural environment harboring bats.

The Abs-mediated virus neutralization is not a universal mechanism for protection against Ebola, Marburg, and Sosuga (a recently discovered pathogenic Paramyxovirus in Uganda) viruses in the Egyptian Rousette bats [113, 114]. Similarly, maternal Abs to the Henipavirus become undetectable between 4 and 12 months after birth [115]. The seasonal horizontal transmission of the virus makes seronegative bats seropositive for Abs and seasons of late pregnancy/lactation in bats may increase the risk of zoonotic diseases. Further studies have shown that in the straw colored fruit bats (*Eidolon helvum*) fruit bats maternal Abs provide protection against Lagos bat lyssavirus and African Henipavirus for 6 months and acquired immunity in developed adult bats against them lasts for 12 years (Lagos bat virus) and 4 years (Henipavirus) [116]. However, the disturbed pregnancy and lactation (seasonal birth pulse) impacts the maternal Ab-based immunity on persisting virus that depends on the transmission characteristics (prolonged infection period or within host latency). It is interesting to note that despite the diminished Abs level the Egyptian Rousette bats exert a protective immune response against severe Marburg infection that may be due to the anamnestic response generating Abs and type 1 IFNs [117].

Abs specific to the glycoprotein GP2 to another *Filoviridae* family member called Lloviu virus (LLOV) have been detected in insectivorous Schreiber's Bent-winged bats in the caves of Northern Spain [118]. A study has shown that the reinfection with the particular virus is essential to explain the shortness (hours to days) of acute infections and development of immunity lasting for another 1–2 years [119]. Hence, recurring latent infections are warranted for immunoprotection in bats to severe viral infections. The migrating status of the bats or other migratory animals//birds also determine the reactivation or suppression of the latent infection depending on the immune status [120, 121]. For example, the relapse at either the start or end of migration may increase the prevalence across the year and may maintain pathogens with low transmissibility and short infectious periods in the migratory population [120]. For example, relapse at the beginning of the migration may reduce the prevalence of highly virulent or infectious viruses by amplifying death of infected hosts during migration, especially for highly transmissible viruses and those transmitted during migration or breeding season. The long-distance migratory Nathusius' pipistrelles (*Pipistrellus nathusii*) show difference in the immune status, for example, during migration they have increased number of lymphocytes with decreased neutrophils as compared to the non or pre-migratory period [38]. The oxidative stress is higher during migration period without any association between blood oxidative status and immunological impact. Of note, the

immune challenge does not induce any changes in the oxidative stress irrespective of the migratory or pre-migratory season.

4. Future perspectives and conclusion

Bats always remain the source of attraction and fascinate humans. Even in Hollywood movies the character of the Dracula has been inspired from bats living on blood and coming out for the prey in night time. However, they became important to the medical community upon the first recognition of transfer of rabies virus to the animals serving as their prey for blood in 1959 in Trinidad. Since, then different have been suggested as the career for many viral pathogens that are responsible for different endemics, epidemics, and pandemics, including Nipah virus infection, Hendra virus infection, Ebola virus infection, SARS, MERS, and the current COVID-19 or SARS-CoV-2 infection. However, the direct causal virus for COVID-19, called SARS-CoV-2 has not been directly isolated from them, but genetically related or more close viruses have been identified in them [9, 10]. Hence, understanding the factors responsible for no severe pathogenic outcomes in the bats as compared to other mammals, including humans becomes crucial by keeping in mind the damages (both, life and economical) associated with current COVID-19 pandemic. The bat immune system has evolved in such a way to guard itself through the damages associated with high speed flight for long migration. For example, low ROS production to protect from DNA damage and inflammation. However, to keep a check on invading pathogens, especially viruses it has evolved the potent IFN-dependent antiviral immune response without inducing severe pro-inflammatory immune response as seen in other mammals, including humans during Ebola virus and severe COVID-19 infection. A recent study has shown that the Ebola virus in humans and fruit bats (*Epomops buettikoferi*) evolves differently by undergoing short term evolution as studied through circular sequencing [122]. For example, the Ebola virus (EBOV) passaged in fruit bat (*E. buettikoferi*) cells shows a sequence markers specific for host RNA editing enzyme activity, including evidence for adenosine deaminase acting on RNA (ADAR) editing of the EBOV glycoprotein (GP), show increased G to A transitions depending on the EBOV genome strand, and increased average genomic Shannon entropy compared to Ebola virus passaged in human 293 T cells. The bat EpoNi/22.1 cells express approximately 12-fold more ADAR1 mRNA than 293 T cells due to unique features of bat cells or bats. Hence, host-specific factors, including ADAR impact mutation/evolution of the virus. Of note, the mutation rate for Ebola virus is same for both bat and human cell lines. Hence, studying and identifying bat-specific factors have a potential to answer the unknowns associated with mild or no infection with the same pathogen that proves lethal to humans. For example, the evolution of the pathogen in the reservoir host is drift-driven, but in the incidental host it favors positive selection to adapt and reduces the tropism for primary host (bats) [123]. Hence, the pathogen becomes severe in the incidental host and transmits among human hosts as seen in Ebola virus infection and COVID-19. Also, the virus related to the Rubella called Ruhugu virus (RuhV shares identical genomic structure with the Rubella virus) has also been isolated from cyclops leaf-nosed bats (*Hipposideros cyclops*) sampled in Uganda [124]. This indicates that Rubella virus may have evolved from bat virus or in future Rubella-like infection may affect humans and other mammals as zoonotic disease from bats. Thus the future zoonotic (bats-specific) infections-associated endemics, epidemics, and pandemics, including vampire bat (*D. rotundus*) rabies caused by vampire bat rabies virus (VBRV, Lyssavirus of Rhabdoviridae family) will depend on the host-pathogen evolutionary signatures or relationships [125].

5. Conclusion

Bats are unique mammals with a potential to have true flight, harboring different viral pathogens that have caused or may cause severe infections to humans and other mammals. Understanding their immune system associated uniqueness may open avenues to deal effectively with zoonotic diseases coming from them.

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