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Current Opinion in Virology

Fruit bats as natural reservoir of highly pathogenic henipaviruses: balance between antiviral defense and viral tolerance

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Bats are the natural reservoir host for a number of zoonotic viruses, including Hendra and Nipah viruses of *Henipavirus* genus, which are highly pathogenic in humans and numerous other mammalian species. Despite being infected, bats present limited signs of disease but still retain the ability to transmit the infection to other susceptible hosts, presenting thus a permanent source of new viral outbreaks. Different mechanisms have evolved in fruit bats permitting them to efficiently control the *Henipavirus* infection. These mechanisms likely allow bats to establish an adequate equilibrium between viral tolerance and antiviral defense, enabling them thus to avoid both uncontrollable virus expansion as well as immunopathology linked to excessive antiviral responses.

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Introduction

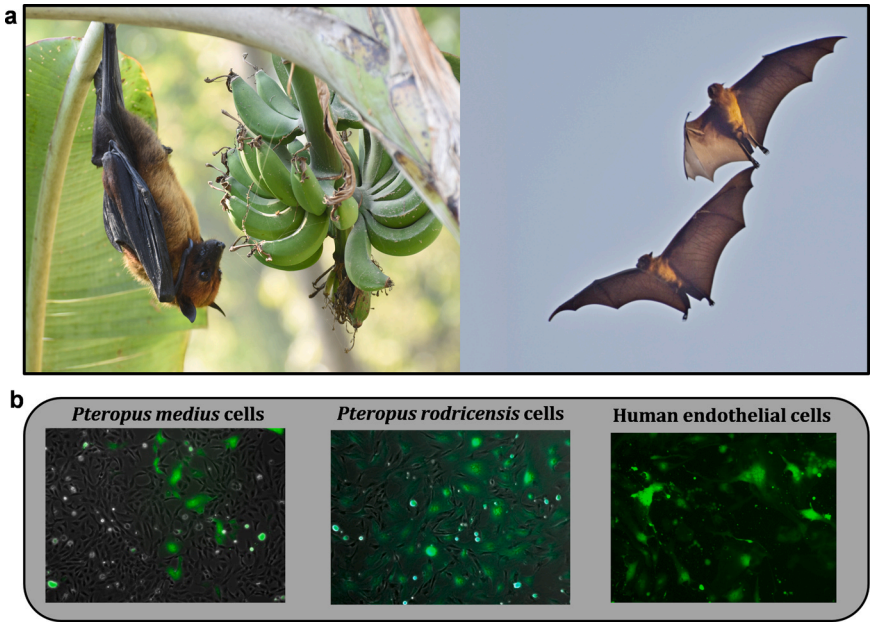
The ongoing SARS-CoV-2 pandemic emphasizes a relentless trend of spillover of zoonotic viruses likely to emerge from bats [1,2]. Bats are one of the most abundant and widespread vertebrates on the earth and they are known to host more zoonotic viruses per species than any other mammalian taxon [3,4]. In addition to coronaviruses, the other highly pathogenic RNA viruses have emerged from bats, including filoviruses, henipaviruses and lyssaviruses, which attract particular attention as they cause serious and often lethal human diseases [5]. The mechanism of bat coexistence with viruses is complex and requires understanding a long path of host–pathogen coevolutionary relationship, which is likely to be unique to bats, known to be one of the most ancient mammals [6]. Sequencing of the numerous bat genomes during the last decade has provided important information in understanding bat biology and evolution [7–10]. The nature of virus–bat relationship has been intensively analyzed during the last years for different bat species and several bat-borne viral families [11–13]. Here, we focus to bat interactions with henipaviruses, which include two highly lethal emerging paramyxoviruses, Hendra and Nipah, to delineate and discuss the mechanisms allowing bats to coexist with these viruses without inducing clinical signs of disease.

Henipavirus and their natural host, fruit bats

Henipavirus genus belongs to the *Paramyxoviridae* family of enveloped negative-sense single-stranded and non-segmented RNA viruses, involving five viral species known so far [14]. Among them, Hendra virus (HeV) and Nipah virus (NiV) cause respiratory disease and encephalitis with high case-fatality rates (up-to 90%) in humans and are classified as biosafety level 4 pathogens [15]. HeV was identified in the 1990s in Australia, after the severe disease in horses and equine workers, while NiV first emerged in Malaysia in 1998, following the spillover from infected pigs [16]. However, not all henipaviruses are pathogenic and another bat-borne Cedar virus is not capable of counteracting the host type I interferon (IFN) response and does not induce disease in hamsters [17].

Fruits bats from the *Pteropodidae* family (order *Chiroptera*, suborder *Yinpterochiroptera*), also known as the flying foxes, are the natural reservoir hosts of henipaviruses (Figure 1). In Australia, fruit bats *Pteropus alecto* and *P. conspicillatus* were recognized as reservoir hosts of HeV [18], while subsequent work in Malaysia, identified the large and small flying foxes, *P. vampyrus* and *P. hypomenalus*, respectively, as the natural reservoir of NiV [19]. The Indian flying fox (*P. medius*) was determined to be the natural host of NiV in Bangladesh and India, based initially on serologic evidence and detection of viral RNAs, and then on the isolation and full

Figure 1



Pteropus fruit bats and *Henipavirus* infection. **(a)** Fruit bat *Pteropus medius (*also known as *P. giganteus and* Indian flying fox*)* in its natural habitat. This bat species is widely distributed in South East Asia, particularly in Bangladesh and India. It is one of the largest bats in the world, reaching a wingspan range of 1.5 m [73], and life span up to 30 years. It is known to be a natural host of NiV. Photos are courtesy of Ausraful Islam, icddr,b, Dhaka, Bangladesh. **(b)** Permissiveness of cells to NiV infection: *P. medius* and *P. rodricensis* bat cells infected with recombinant NiV expressing eGFP (MOI=0.5, 48 H post infection), in comparison to the human endothelial cell line HPMEC. Infection and imaging were done in BSL4 laboratory Jean Mérieux in Lyon, France.

genome characterization of the live virus from this species in Bangladesh [20]. Furthermore, *P. lylei* in Cambodia was found to host highly pathogenic NiV of Malaysia genotype, although this virus has not caused any outbreak so far [21].

Henipavirus genome encodes for nine proteins governed by six transcription units. N, P, M, F, G and L proteins are structural, while C, V, and W represent the *Henipavirus* nonstructural (accessory) proteins and are encoded from P gene (Figure 2a). C protein is translated from an alternative codon start. V and W proteins result, respectively, from +1 to +2 frameshifts, caused by a transcriptional editing of P mRNA by the viral polymerase L, through adding one or two guanosine (G) residues at the single P editing site [22].

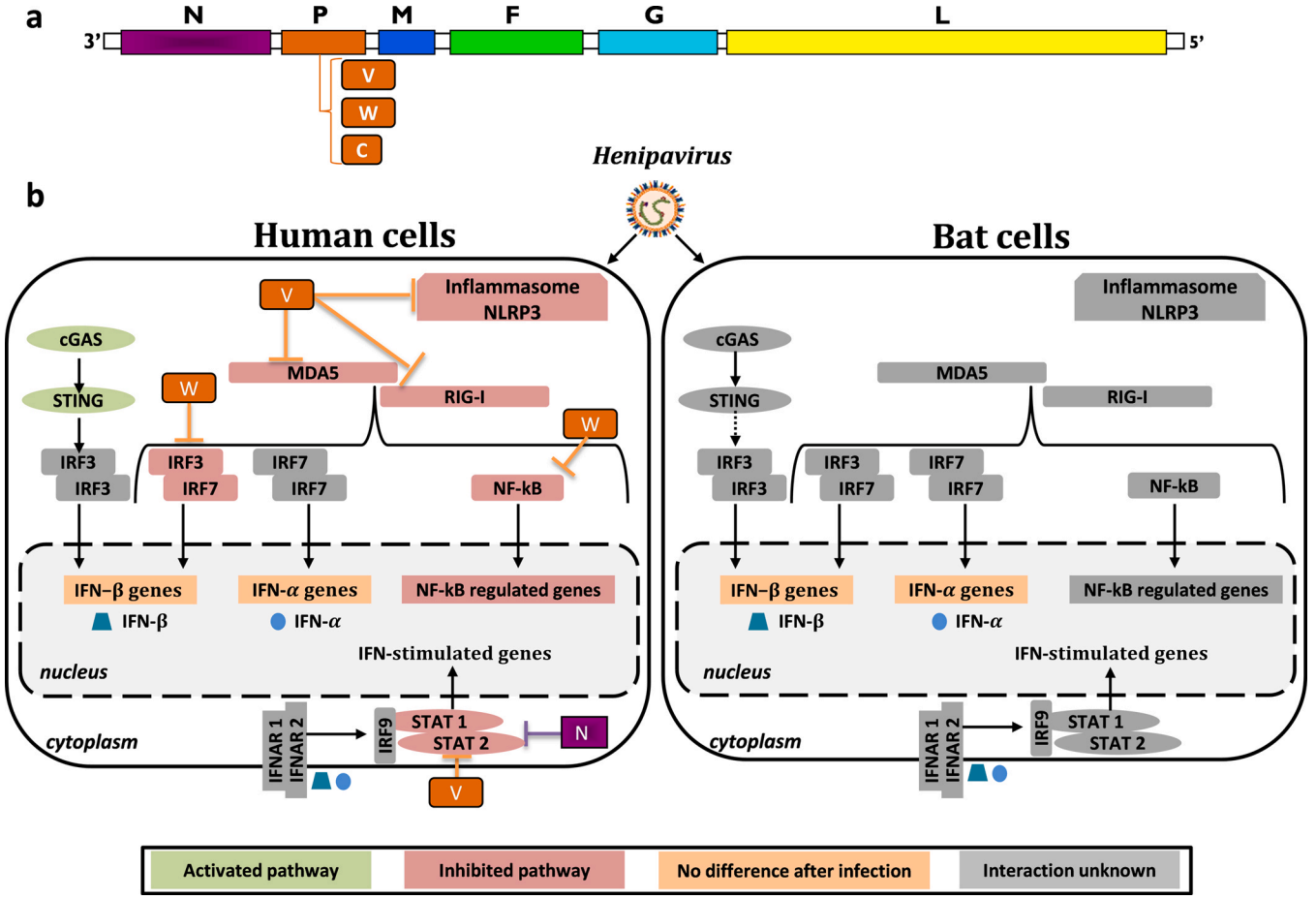
Both NiV and HeV use the highly conserved Ephrin-B2 and Ephrin-B3 as receptors, conferring them a broad host range. They are thus able to infect and cause disease in diverse species including hamsters, ferrets, cats, horses, pigs, and non-human primates [23,24], inducing in some of them a clinical disease similar to humans [15,25]. NiV proteins, especially the nonstructural proteins C, V and W, are major actors in the *Henipavirus*-induced immunopathogenesis and present currently a target of intensive research studies [26–29].

Bat physiology

Bats are the only mammals capable of powered flight. It has been proposed that the higher metabolic rates in bats (approximatively 7–8 higher than in birds) and increased body temperature during flight mimic the effects of an intermittent fever, boosting the activation of their immune system on a daily cycle [30,31]. A subset of underlying mechanisms has also evolved in bat metabolism and immune system to mitigate oxidative stress incurred during flight. Approximately, 23% of the mitochondrial-encoded and 4.9% of nuclear-encoded oxidative phosphorylation genes have undergone active positive selection in bat genomes [32]. In addition, genome analysis of *P. alecto* gave evidence for positive selection of genes involved in the DNA damage checkpoint and repair pathways such as ATM, DNA-PKc, RAD50, KU80 and MDM2 [7].

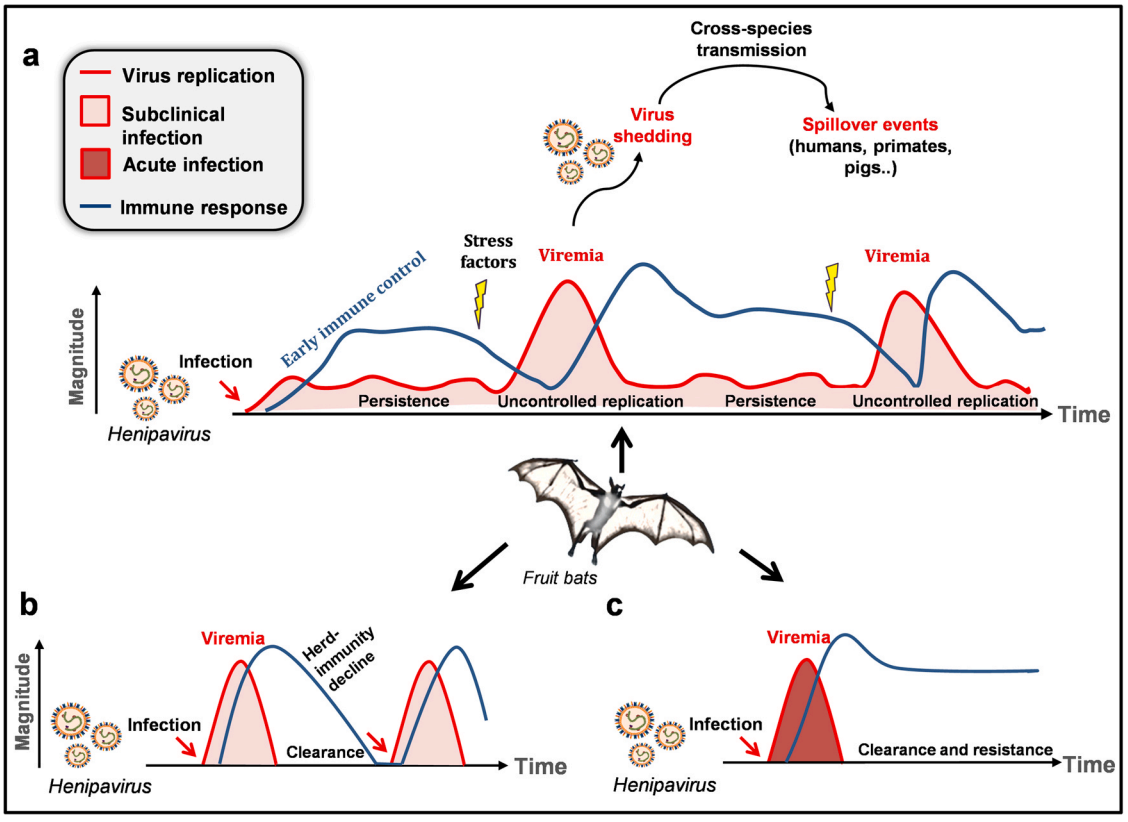
Elevation of the body temperature and the increase in oxidative stress during flight has resulted in the high basal heat-shock protein (HSP) expression in bats [33]. In addition to numerous physiological functions in the cells, these proteins, particularly HSP90, could chaperon the activity of polymerase from *Mononegaviridaes*, including the NiV, allowing its maturation, necessary for the viral replication in the cell [34], and possibly facilitating the initial phases of viral replication in bats.

Figure 2



Innate immune response in human and bat cells after *Henipavirus* infection. **(a)** Schematic representation of the NiV genome, which comprises six genes coding for the structural proteins nucleoprotein (N), phosphoprotein (P), matrix (M), fusion protein (F), attachment protein (G) and viral polymerase (L). In addition, the P gene encodes for three additional non-structural proteins: V, W and C. **(b)** Comparison of innate immune response after *Henipavirus* infection in human (left) and bat cells (right) and impact of non-structural viral proteins. . The *Henipavirus* N and non-structural proteins have been described to be immunomodulators in human cells and the role of V and W was suggested in bat cells following transcriptomic profiling [66] but their precise level of action within the bat cells remains to be determined. The N protein inhibits STAT1/STAT2 complex [74] while V protein interacts only with STAT2 [28]. V protein is also an inhibitor of MDA5, RIG-I [75] and the inflammasome NLRP3 [58]. NiV W can interact with IRF3/7 [76] and NF-kB pathway [29]. Finally, pathways remaining to be studied in bats are marked in gray, highlighting the current gaps in understanding bat–virus interaction.

Figure 3



Dynamics of *Henipavirus* circulation within their natural hosts, fruit bats. **(a)** The episodic shedding hypothesis, according to which, bats carry persistent infections controlled by their immune systems, and virus replication and shedding occur as temporal pulses upon weakening of the immune system, caused by intrinsic or extrinsic stressors such as pregnancy. For example, during a one-year study, captive bats seroconverted and even shed NiV after being seronegative against the virus over a period [77]. Seasonal peaks in NiV seroprevalence have also been shown to coincide with pregnancy season of the fruit bat *Eidolon dupreanum* in Madagascar [78]. **(b)** The transient epidemics hypothesis proposes that bats experience oscillating cycles of infections and shedding — transient immunization — virus clearance – loss of immunity and then reinfection upon new encounter with the virus. This hypothesis may explain the spatiotemporal dynamic of excretion of some henipaviruses like HeV [79]. Periodic shedding of NiV from *P. lylei* were also observed in Thailand, suggesting a role of bat oscillating herd immunity in the recrudesce of the virus [80]. **(c)** The scenario of susceptible-infectious-recovered hosts suggests that within a bat population only naïve individual could be infected (acute infection). Infection is then followed by establishment of a long-lasting immunity that clears the virus and the bat remains resistant to new infections. According to this scenario, virus infection is maintained in bat populations by the colony turnovers via migration and births.

Lifespan in mammals is allometrically linked to body mass; however, bats break the rule and live significantly longer than non-flying placental mammals of comparable body size. Molecular basis of this extended longevity has recently been associated with telomerase maintenance, although functional studies are still required to validate it [35]. Furthermore, the recent analysis of the DNA methylation profile of 26 bat species allowed us to predict their chronological age and suggested the association of DNA methylation change with innate immunity and tumorigenesis genes [36]. This long bats' lifespan undoubtedly allows long-term carriage of viruses within bat colonies and their transmission to offspring, thus promoting virus persistence across generations.

Antiviral defense strategies in bats

In the context of their evolution, vertebrates have evolved three different strategies to protect themselves against pathogens: avoidance, resistance and tolerance [37]. Avoidance involves reducing the risk of exposure to the pathogen, working at the level of host behavior, escaping the contact with the pathogen. Once the infection is established, resistance confers to the ability of the host to develop efficient innate and adaptive immune response, ultimately leading to pathogen clearance. On the other hand, tolerance refers to the capacity of a host to limit the impact of damage caused by both specific pathogen-associated pathology and the immune response raised against it, which may be associated with different types of immunopathology [38,39]. While it has been suggested that Egyptian rousette bat, natural

reservoir of Marburg virus, relies on immune tolerance mechanisms to manage viral infections, [8] it remains unclear which mechanism may be employed by fruit bats to host henipaviruses.

Although several cell lines have been established from *Pteropus* bats, most of them present immortalized primary cells [40,41] (Figure 1b) and immortalization process induces changes in the cell programmes that may alter the interactions between virus and cell host, questioning the relevance of observed mechanisms. Different primary cell lines have been established as well [42]; however, the nature of cell types replicating henipaviruses in bats is still not clear. Recent generation of reprogrammed *Pteropus* bat stem cells [43] and their possible further differentiation into other bat cell types may present an important experimental tool for further studies.

Study of antiviral responses in bats *in vivo* is indispensable for the further advances in the field; however, the bat husbandry constraints and absence of commercial bat suppliers make it very difficult. Several studies performed so far suggest that bat susceptibility to *Henipavirus* infection does not seem to be too high. Experimental infections of *P. poliocephalus* and *P. vampyrus* with NiV via subcutaneous and oronasal routes, respectively, result only on subclinical manifestations, brief viremia, low virus replication in different organs, low shedding and an inconspicuous seroconversion [44,45]. Comparative experimental infection of Australian black flying foxes and ferrets, as a susceptible host, with HeV demonstrated viral infection in both species [46]. However, while HeV RNA was widespread in ferrets, leading to the fatal infection, HeV RNA was found only in bats' lungs, associated with the upregulation of numerous immune regulation pathways. These data suggest the efficient control of *Henipavirus* infection in bats preventing the development of a clinical disease in this species.

Low level of *Henipavirus* infection in bats raises questions regarding how the virus may persist in bat populations and spill over to other species. Indeed, the carriage of henipaviruses in bat colonies seems relatively high as evidenced by serological surveys [47,48]. Moreover, high-level viremia and peaks of shedding of henipaviruses were observed in nature and have been shown to coincide with spillover events to other species [49]. Different hypotheses have been proposed in literature [50,51] and are presented and discussed in Figure 3.

Dampened inflammatory response

Metabolic stress and intracellular DNA release upon mitochondrial damages during the flight in bats have probably led during the evolution to the positive selection of the mutations in the Stimulator of interferon genes (STING) protein, an important pattern recognition receptor that mediates cytosolic DNA-induced signaling. Conserved phosphorylation site (S358) was found to be mutated in STING of diverse bat genomes including *Pteropus*. This has been shown to dampen STING-dependent IFN activation and may be responsible for rendering bats more tolerant to flight-induced release of cytosolic DNA [52]. Interestingly, NiV was shown to induce STING activation in human cells [53]; however, little is known about NiV-induced STING activation in bat cells (Figure 2b). STING controls the induction of IFN-β and potential subsequent cell death through TNF-related apoptosis-inducing ligand (TRAIL) expression [52,54]. However, both HeV and Cedar virus were shown to induce higher TRAIL-induced death in *in vitro* cultured bat cells compared to human cells, indicating that *Henipavirus* replication stimulates pro-apoptotic pathways in a currently unknown manner [55,56].

Among different mechanisms, cell death can occur through pyroptosis, a pathway under the control of NLRP3 inflammasome. Bats have acquired during their evolution a triple mechanism to decrease efficiency of NLRP3, including low transcription priming, low activity and over-representation of a deficient splice variant [57]. This pathway seems of importance for henipaviruses as *Paramyxovirus* V proteins have the ability to prevent inflammasome formation and subsequent IL-1β release [58]; however, whether V protein has any effect on bat NLRP3 is currently unknown.

Analyses of bat genomes conducted during the Bat1k project revealed multiple genes that have undergone positive selection in the bat ancestor, including genes involved in activating the NF-κB pathway, IL17D and IL1B. Two immunostimulating genes were identified to be inactivated in bats during evolution, namely the LRRC70 and IL36G, which both act through the NF-kB pathway [9]. Furthermore, positive selection in bats has also been shown to affect the member of the NF-κB pathway, c-REL, which is involved in DNA damage response but also in the regulation of proinflammatory cytokine expression [7]. It is tempting to link this finding to the study of *Eptesicus fuscus* kidney cells, which do not respond to the stimulation with synthetic dsRNA (poly(I:C)) by the production of proinflammatory cytokine TNF-α, in contrast to the human cells where a robust expression was observed [59]. This controlled inflammatory response has been linked to the presence of a repressor c-Rel-binding motif in the TNF-α promoter of this insectivore bat. Therefore, investigating the effect of NF-kB c-Rel selection in fruit bats could give insights into the mechanism of their extraordinary capacity to control *Henipavirus*-induced systemic inflammation. An important transcriptional upregulation of NF-kB has also been observed 8 h after HeV infection in *P. Alecto* cells, but not in human cells, raising questions

regarding the role of this early NF-kB activation in bat tolerance to henipaviruses [56]. Finally, analysis of the genomes from 37 bat species confirmed the lineage-specific expansion of the APOBEC3 and MHC-I gene families and loss of the proinflammatory the pyrin and HIN domain (PYHIN) gene family and the natural-killer gene complex [60]. These data suggested that bats have evolved fundamental functional differences in both innate and adaptive immune system, compared to other mammals, with the potential to enhance the antiviral response while dampening the inflammatory signaling [60].

Role of bats' Interferon system in the control of *Henipavirus* infection

During evolution, tandem duplication events have resulted with an expansion of IFN type I loci in mammals. In contrast to the other mammals, IFN locus in *Pteropus* bat genomes has undergone contraction, giving rise to fewer IFN-α genes. Moreover, type I IFN system [46,61] and several non-inflammatory interferon-stimulated genes (ISGs) [62] seem to be constitutively activated in *Pteropus* bats [46,61]. Evidence for constitutive STAT1 phosphorylation has also been reported for the Jamaican fruit bats *Artibeus jamaicensis* [63]. In addition, basal expression of IFN immune-related genes (IFIT2 and IFIT3) is higher in *Eptesicus* bat cells compared with human cells [64]. This permanent activation has been proposed to hamper virus replication in bats at the early stages of infection and a part of these IFN-α transcripts remain in an untranslated state, serving as sources for rapid translation upon virus invasion [63]. A serine residue in the transcription factor IRF3 has also been demonstrated to be positively selected in multiple bat species including *P. alecto* and *P. vampyrus*, and has been linked to an enhanced antiviral protection in bat cells [65]. IFN constitutive expression of diverse cell types and different bat species is not homogenous, although it seems that the presence of basal expression is detected in most of primary cell lines and tissues but not in immortalized cell lines (Table 1), which may present the consequence of cell immortalization.

It is thus not astonishing to expect that *Henipavirus* IFN antagonist mechanisms remain active in bat cells in order to maintain a minimal level of virus replication in their natural hosts. Indeed, both NiV and HeV have been shown to inhibit both type I IFN production and signaling in different cell types derived from *P. vampyrus* and *P. alecto* [41,66]. Stimulation with poly(I:C) resulted in fall of IFN-α and IFN-β transcripts as well in IL-29, IL-28B, ISG54 and ISG56 mRNA levels when cells were infected with henipaviruses [67]. In human cells, this inhibition was only detected for IFN production but not for the IFN signaling pathway [68]. *Henipavirus* V and W proteins have been described to be the main antagonists of IFN production and signaling, namely via preventing STAT1 and STAT2 activation [69,70]

Table 1

Constitutive expression of IFN in bat cells and tissues varies with cell types and bat species.

Bat species	Cell and tissue type analyzed	Type of IFN expression observed	Ref
Pteropus alecto	• Different tissues including lung and brain • Primary cell lines	• Constitutive expression of IFNα mRNA expression but not IFNβ	[61]
	• Lung and spleen tissue	• Higher constitutive expression of IFNα and IFNλ mRNA expression in comparison with ferrets	[46]
Pteropus rodricensis *Pteropus lylei* *R. aegyptiacus* *Eidolon helvum*	• Plasma	• Constitutive expression of IFNα protein	[81]
Pteropus rodricensis *Pteropus lylei* *R. aegyptiacus* *Eidolon helvum*	• Leukocytes	• Constitutive expression IFNα mRNA except for *R. aegyptiacus*. IFNα2 mRNA subtype was the most highly expressed	[81]
Cynopterus brachyotis	• Different tissues	• Constitutive expression of IFNα mRNA expression but no for IFNβ	[61]
Rousettus aegyptiacus	• $CD14^+$ monocytes/macrophages	• Absence of type I IFN constitutive expression	[82]
Pteropus giganteus *Pteropus vampyrus*	• Primary cells and bat reprogrammed stem cells	• Absence of type I and III IFN constitutive expression	[43]
Rousettus aegyptiacus	• Immortalized fibroblast from kidney tissue (RoNi)	• Absence of IFN constitutive expression	[8]
Eidolon helvum	• Immortalized Lung (EidLu/20.2) • Immortalized kidney cell line (EidNi/41.3)	• Absence of elevated constitutive expression of IFNB1 mRNAs	[83]
Eptesicus fuscus	• Immortalized kidney epithelial cells (Efk3B) cells (Efk3B)	• Absence of IFNB constitutive expression	[64]
Eptesicus nilssonii	• Immortalized kidney-derived cells (EnK)		
Myotis daubentoniid	• Immortalized kidney cell line (MyDauNi/2c)	• Absence of elevated constitutive expression of IFNs	[84]
Desmodus rotundus	• Immortalized fetal lung (FluDero)	• Absence of IFNα or IFNβ constitutive expression	[85]

following the infection (Figure 2b). Interestingly, infection of bat cells with Cedar virus, which lacks the V and W nonstructural proteins, elicits an upregulation of type I and II IFN transcripts, in addition to RIG-I, MDA5, LGP2, TLR3, NLRC5 mRNAs [55].

The bat IFN-stimulated antiviral effector protein Tetherin (BST-2) has been shown to have an important role in controlling NiV infection in *Epomops buettikoferi* fruit bat cells [71], most likely by blocking the release of viral particles, as evidenced in human cells using NiV virus-like particles [72]. Furthermore, transcriptome analyses of *P. alecto* identified ribonuclease L (RNASEL) as an atypical induction profile ISG [62]. RNASEL is an antiviral protein that is constitutively expressed in human cells and acts by degrading viral RNAs, in concert with IFN-inducible oligoadenylate synthase (OAS) family of enzymes. Basal level of RNASEL is similar between human and bat cells; however, only bats are able to upregulate both parts of the OAS/RNASEL pathway in response to viruses, which may allow them to be more reactive to viral infections than humans. In addition, knocking-out RNASEL increased *Pteropus* cells susceptibility to RNA viruses. The authors also reported a distinctive temporal profile of ISGs expression between humans and bats, suggesting that ISG kinetic in bats is finely tuned compared to humans, providing a well-balanced and adapted response in bats to viruses [62].

Conclusions

A high permissiveness of bat cell lines to the infection and low susceptibility at the level of animal is the hallmark of *Henipavirus* infection in *Pteropus* fruit bats. Current evidence suggests numerous immune-mediated mechanisms which could induce resistance to the infection, associated with a well-balanced inflammatory response, limiting early viral replication while avoiding self-damage. Bat physiology and metabolism may play a determinant role in the establishment of the balance between immune response and tolerance to *Henipavirus* infection during evolution. Further understanding the mechanisms allowing bats to coexist with RNA viruses known to be highly lethal in many other species may provide critical fundamental insights into how to achieve better resilience in humans.

Conflict of interest statement

Nothing declared.

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