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(TMAO) in Foxp3^{GFPΔCNS1} mice. Interestingly, in other studies, TMAO upregulation was positively correlated with upregulation of Zonulin—a modulator of intercellular tight junctions and hence a biomarker for intestinal integrity. The authors excluded the alteration of intestinal barrier integrity in Foxp3^{GFPΔCNS1} by measuring serum endotoxin levels; however, it would be interesting to specifically measure the levels of Zonulin to directly assess gut permeability and correlate to both TMAO and the loss in border-dwelling bacteria observed in Foxp3^{GFPΔCNS1} pTreg-depleted mice. Such a correlation will shed light on the mechanism of this bacterial loss. As a finale, [Campbell et al. \(2018\)](#) correlated the microbial phenotype to the host's energy harvest capability and body weight. Although the mechanism is yet to be determined, this correlation is extremely intriguing.

As the field is moving beyond the microbiome and into the metabolome, more questions are awaiting to be answered. This study, with solid supportive data, adds an important strategic thinking to the interplay between gut microbiota and host—a reciprocal and functional metabolite-driven communication loop, where

gut bacteria control pTreg cell numbers via their metabolites, and pTreg cells affect both the gut bacteria composition and consequently the host metabolome. This loop raises the question of what came first—the microbiota or the Treg cells? With their findings, [Campbell et al.](#) pave a new ground for research with crucial therapeutic relevance.

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Holy Immune Tolerance, Batman!

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<https://doi.org/10.1016/j.immuni.2018.05.016>

Bats are reservoir hosts of numerous viruses that cause severe pathology in humans. How bats cope with such pathogens remains elusive. In a recent issue of *Cell*, [Pavlovich et al. \(2018\)](#) describe several key adaptations in innate immune-related genes that suggest that the Egyptian rousette fruit bat relies on immune tolerance mechanisms to manage viral infections.

Throughout folklore, literature, and films, bats signify darkness and mystery, are often associated with the undead, and are portrayed as night creatures able to strike primal fear in the hearts of those that encounter them. Although the threat that bats pose to humans à la Count

Dracula is an imaginary one, bats serve as asymptomatic carriers of many viruses known to cause highly virulent, often lethal human diseases, including but not limited to the Ebola and Marburg filoviruses, rabies virus, and SARS coronavirus ([Smith and Wang, 2013](#)). The immunolog-

ical means through which bats are able to harbor pathogens seemingly without any significant impact on host fitness remain elusive. However, two broad mechanisms have been proposed: immune tolerance and host resistance. Tolerance refers to the capacity of a host to limit the impact



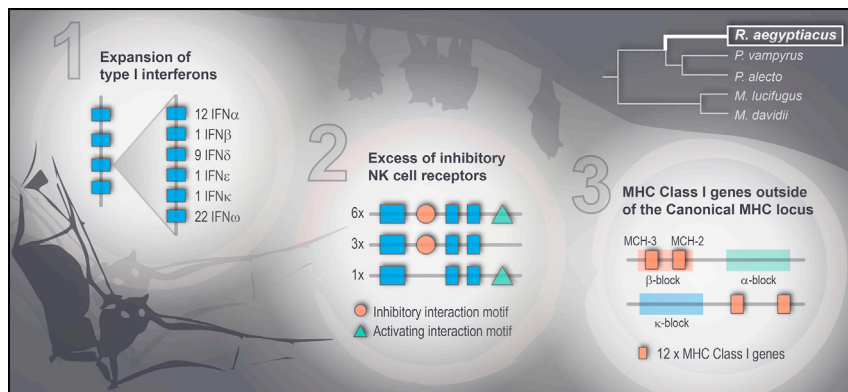


Figure 1. Genomic Signatures of Viral Infection Tolerance Mechanisms in *R. aegyptiacus*
Multiple adaptations in innate immune-related genes suggest that *R. aegyptiacus* employs immune tolerance strategies to control viral infections. Such strategies include (1) the expansion of the type I interferon family, (2) an abundance of NK cell receptors with inhibitory signaling capacities, and (3) the dispersion of MHC class I genes to non-canonical regions of the genome. (Design credit to M. Gutiérrez.)

of damage caused by both infection-associated pathology, or the specific pathogen itself, and immune-related pathology, or the immune responses raised against it. On the other hand, resistance describes the ability of a host to mount especially potent innate and adaptive immune responses capable of efficiently controlling pathogen load, ultimately leading to infection clearance.

A recent study from Pavlovich et al. proposes a mechanism of disease tolerance to explain why the Egyptian rousette fruit bat (*Rousettus aegyptiacus*), a natural host of the Marburg virus, is able to asymptotically harbor a pathogen known to devastate humans (Pavlovich et al., 2018). The authors utilize a comparative immunogenomic approach to gain insight into the evolution of gene families involved in innate immune responses and antiviral defense in *R. aegyptiacus*. The authors used a combination of both short- and long-read next-generation sequencing technology followed by whole-genome annotation to construct a highly contiguous and complete *R. aegyptiacus* genome, containing a total of 19,668 protein-coding genes. After genome assembly, the authors performed comparative analyses of homologous protein families among *R. aegyptiacus* and additional mammals to elucidate evolutionary relationships across various species. They found that many gene families were significantly expanded in the Egyptian rousette as compared to the ancestral megabat. Interestingly, the importance of natural selection in

shaping the evolution of immune genes in *R. aegyptiacus* is evident in that multiple signatures of positive selection were detected among several interferon (IFN)-related genes.

Three major findings are consistent with the hypothesis that the Egyptian rousette evolved toward a state of immune tolerance to successfully manage viral infections, as opposed to a reliance on mechanisms of host resistance to clear infections: (1) the expansion of the type I IFN gene family, (2) the discovery of natural killer (NK) cell receptors with distinct signaling components, and (3) the presence of MHC class I genes dispersed throughout the genome (Figure 1).

During early stages of viral infections, type I IFN responses are triggered and subsequently initiate an antiviral immune cascade that leads to the activation of hundreds of interferon-stimulated genes (ISGs). Strikingly, *R. aegyptiacus* harbors 46 type I IFN genes, more than twofold the number found in the megabat ancestor. The marked expansion of genes in the IFN- ω subfamily (22 in total) contributes the most to this increase. Although the type I IFN gene family is found to be expanded, only minimal basal expression of these genes is detected, whereas a previous study describes constitutive activation of IFN genes in the bat *Pteropus alecto* (Zhou et al., 2016). This suggests that *R. aegyptiacus* harbors a baseline expression profile with reduced inflammatory capacity compared to that of other bats. Further, in an *in vitro* antiviral assay in which immortalized

R. aegyptiacus cells were infected with vesicular stomatitis virus (VSV), Pavlovich et al. showed that IFN- ω 4—one of the IFN- ω subfamily members—hindered VSV infection, although IFN- β induced a stronger antiviral effect. This indicates that certain Egyptian rousette IFNs are capable of inducing a less potent, more restrained response that might confer reduced immune-related pathology.

Being able to quickly recognize and eliminate virus-infected cells, NK cells are a critical part of the innate immune system. NK cell responses are mediated by a balance of signals provided from activating receptors, which promote NK cell killing, and inhibitory receptors, which suppress NK cell responses. Although previous reports described an absence of NK cell receptors in numerous bat genomes (Zhang et al., 2013), Pavlovich et al. showed that this is not the case. They identified several putative NK cell receptor genes, namely those belonging to the killer lectin-like receptor (KLR) NKG2/CD94 family, in the *R. aegyptiacus* genome. Furthermore, upon re-examination of previously sequenced bat genomes, they were able to identify multiple NKG2-like genes in other bats. Interestingly, in *R. aegyptiacus*, all NKG2 genes except one encode receptors containing inhibitory interaction motifs. Notably, two of the inhibitory receptor genes identified, *NKG2-13* and *NKG2-14*, are expressed at high levels in peripheral lymphoid tissue and blood mononuclear cells of uninfected bats, indicating that *R. aegyptiacus* might be primed to activate inhibitory signaling pathways. Of the receptors with activating domains, most seem to favor preferential recruitment of the adaptor molecule DAP10 instead of DAP12. Compared to DAP12 activation, activation of DAP10 is associated with less robust cytokine production (Lanier, 2009), suggesting that *R. aegyptiacus* activating receptors might elicit dampened proinflammatory responses. Surprisingly, the majority of NK receptors discovered concurrently encode both activating and inhibitory motifs. Such dual-function receptors are atypical among mammals, and it is unclear what would determine which function is engaged or whether one function is repeatedly utilized to a greater extent. The sole dual-function receptor in humans, KIR2DL4, has been shown

to induce strong IFN- γ responses and limited cytotoxicity (Kikuchi-Maki et al., 2005), although it is unknown if *R. aegyptiacus* receptors would follow this pattern.

Finally, Pavlovich et al. reported the expansion of MHC class I genes in the Egyptian roussette genome. Many of the identified MHC-I genes mapped to non-classical genomic contexts, suggesting that MHC-I genes were the subject of genomic dispersion as well as expansion. It is possible that these expanded MHC-I genes can act as ligands for the similarly expanded KLRs and that specific NKG2 receptor/MHC-I ligand interactions exist in *R. aegyptiacus*. The authors hypothesize that the spread of MHC-I genes outside of the typical MHC locus serves to generate ligand redundancy which in turn might result in increased activation thresholds for NK cells, ultimately rendering them less reactive.

The findings of Pavlovich et al. support other findings that suggest that certain bat species forego mounting highly proinflammatory immune responses in the face of viral infection, and instead rely on mechanisms of tolerance. Reports of prolonged incubation periods and viremia in bats along with the observation of only marginal inflammation in highly infected bat tissues suggests that augmented antiviral defenses are not the key mechanism driving the uniquely avirulent state characterized by these reservoir hosts (Jones et al., 2015). Yet, there is still a long road ahead to uncover the functional relevance of the genomic expansions described by Pavlovich et al. and to what extent they contribute to the basis of increased viral

resilience in bats. The role of IFNs, especially the significantly expanded IFN- ω subfamily, is intriguing because different patterns of ISG activity have been shown to trigger responses of varying magnitude as well as antiviral effectiveness and thus lead to differential host immune outcomes (Hoffmann et al., 2015). For example, SARS patients who exhibit constitutive type I IFN production coupled with high ISG expression frequently manifest poor outcomes, suggesting that heightened inflammation can be deleterious in certain viral-infection contexts (Totura and Baric, 2012). Furthermore, KLR signaling in murine T cells has been shown to restrict T cell activation and attenuate immunopathology during viral infection (Rapaport et al., 2015), raising the question of whether KLR-dependent immune tolerance mechanisms significantly impact adaptive immune-cell responses, in addition to those of innate cells, by potentially repressing T cell activity in bats. The availability of high-quality genomes is the first step toward performing future comparative studies of immune responses to define the immunological parameters that are specific to bats. Other mammals, including mice, baboons, and rhesus macaques, are also particularly resilient to infection. It will be of great interest to assess whether other species share similar mechanisms of immune tolerance.

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