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Forum

Bat Influenza Viruses: Making a Double Agent of MHC Class II

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MHC class II (MHCII) has recently been identified as a cellular receptor for bat influenza viruses. Here, we discuss the possible implications of viral exploitation of this critical host defense molecule and highlight the need for more intense study of bat–influenza virus interactions.

Influenza A viruses (IAVs) circulate globally and, based on WHO estimates, annual epidemics of influenza result in ~1 billion infections, 3–5 million cases of severe illness, and 300 000–500 000 deaths. The severity of pandemic influenza depends on multiple factors, such as the virulence of the pandemic virus strain and the level of pre-existing immunity in the population. The discovery of two novel bat influenza viruses, H17N10 and H18N11 (Table 1) [1–3] has raised several questions about the evolution, host range, tropism, and transmission potential of these viruses.

The hemagglutinin (HA) of avian IAVs has a preference for sialic acids that display an α2,3 linkage to the penultimate sugar, whereas human IAVs preferentially bind to the α2,6 linkage [4,5]. In two striking studies, Karakus *et al.* and Giotis *et al.* identified MHCII as a receptor for bat H17N10 and H18N11 viruses [6,7]. Karakus *et al.* used transcriptomic profiling and genome-wide CRISPR-Cas9 screening of susceptible and nonsusceptible cells to identify and characterize MHCII as an entry determinant for bat H17N10 and H18N11 viruses [6]. The authors also observed that bat H17N10 and H18N11 viruses could initiate virus entry via MHCII homologs encoded by multiple bat species, pigs, humans, and chickens [6]. In a subsequent study, Giotis *et al.* confirmed human HLA-DR as the receptor for H17 using pseudotyped vesicular stomatitis viruses expressing H17 as the surface glycoprotein [7].

The use of MHCII as a receptor is unique to bat H17N10 and H18N11 influenza viruses, which might suggest altered cellular tropism relative to other known IAVs. In bats, H17N10 RNA was detected in liver, kidney, lung, and intestinal tissues, whereas H18N11 RNA was found exclusively in the intestine [1]. Ciminski *et al.* demonstrated that, in bats experimentally infected with H18N11, virus could be detected in the feces and transmitted to co-housed naïve bats, presumably via the fecal–oral route [8]. This is akin to the behavior of avian influenza viruses in waterfowl. Going forward, it will be important to determine with greater resolution the specific cell types that are permissive for replication of H17N10 and H18N11 viruses in bats. This will be essential for understanding the coevolutionary relationship of the virus with these hosts. The discovery of MHCII as a receptor for bat influenza viruses also raises several important questions about the consequences of immune cell susceptibility in terms of both viral pathogenicity and host range.

The fact that viruses pseudotyped with bat influenza virus HAs could infect avian and human cells raised concerns regarding the potential for epizootic and zoonotic transmissions. The studies of Giotis *et al.* and Karakus *et al.* using viruses pseudotyped with bat H17 and H18 demonstrated that bat IAVs have broad tropism. In humans, certain cells of the immune system, as well as epithelial cells in human lung and intestine, constitutively express MHCII. However, it remained unclear whether wild-type (WT) bat IAVs were able to replicate and transmit in species other than bats. A recent study by Ciminski *et al.* has provided important insight into this critical question. Using WT and cell-culture-adapted H18N11, the authors performed *in vivo* replication and transmission studies in mice, ferrets, and bats. These viruses were produced from infectious clones since, as yet, there are no natural isolates. Serial passage of WT H18N11 in canine RIE1495 cells selected for adaptive mutations in the viral HA (S235Y or V254F) and deletions of the neuraminidase (NA) ectodomain [8]. Experimental infection of mice with H18N11 also selected for adaptive mutations in HA and deletion mutations in NA. Virus replication was limited to the upper respiratory tract and infected mice were unable to transmit the virus to naïve contact mice. Ferrets are considered the 'gold-standard' model for studies of influenza virus pathogenesis and transmission. In these animals, a recombinant cell-culture-adapted bat virus (rP11), which contains adaptive mutations in HA and a stop codon in the stalk of NA that results in deletion of the ectodomain, replicated in multiple organs, including

Table 1. Serological and Genomic Detection of Bat Influenza Viruses

Bat species	Subtype detected	Location	Genome positive (no. of bats)	Antibody positive (no. of bats)	Refs
Artibeus lituratus	H18N11	Brazil	2/129	NT[b]	[3]
	H18N11[a]	Peru	0/3	3/3	[2]
Artibeus obscurus	H18N11[a]	Peru	0/10	9/10	[2]
Artibeus planirostris	H18N11[a]	Peru	1/15	13/15	[2]
Carollia brevicauda	H18N11[a]	Peru	0/2	1/2	[2]
Carollia castanea	H18N11[a]	Peru	0/2	0/2	[2]
Carollia perspicillata	H18N11[a]	Peru	0/29	11/29	[2]
Desmodus glaucus	H18N11[a]	Peru	0/1	0/1	[2]
Desmodus rotundus	H18N11[a]	Peru	0/8	7/18	[2]
Diphylla ecaudata	H18N11[a]	Peru	0/1	0/1	[2]
Glossophaga soricina	H18N11[a]	Peru	0/2	0/2	[2]
Molossus	H18N11[a]	Peru	0/10	3/10	[2]
Myotis sp.	H18N11[a]	Peru	0/6	1/6	[2]
Phyllostomus discolor	H18N11[a]	Peru	0/2	2/2	[2]
Phyllostomus hastatus	H18N11[a]	Peru	0/2	2/2	[2]
Platyrrhinus recifinus	H18N11[a]	Peru	0/1	1/1	[2]
Rhinophylla pumilio	H18N11[a]	Peru	0/2	1/2	[2]
Sturnira lilium	H17N10	Guatemala	3/29	NT	[2]
Sturnira sp.	H18N11[a]	Peru	0/2	0/2	[2]
Vampyressa bidens	H18N11[a]	Peru	0/2	1/2	[2]

[a]Based on serological data.
[b]NT, not tested.

trachea, nasal conchae, lung, cerebrum, and cerebellum. However, the virus was not shed or transmitted to naïve contact ferrets. Interestingly, on passaging of rP11 in ferrets, a single point mutation restored the N11 open reading frame, suggesting that it may have functional relevance. However, viral transcripts could not be detected in nasal lavage of ferrets infected with WT H18N11 [8].

The rP11 virus also contained K170R and N250S mutations in HA, which were shown to enhance infectivity in mammalian cells *in vitro*. These mutations were maintained in rP11 after passage in ferrets when the mutation restoring NA expression emerged. NA expression was also required for efficient transmission in bats. Infection of *Artibeus jamaicensis* bats (a close relative of *Artibeus planirostris*, the species from which H18N11 was initially characterized) with rP11 also resulted in restoration of the full-length N11 coding sequence collected from both index and contact animals. Therefore, while full-length N11 does not appear to be required for replication and shedding, it is likely to be necessary to facilitate efficient transmission in bats. Ciminski *et al.* demonstrated that ectopically expressed N11 reduced surface expression of MHCII. They speculated that this might facilitate progeny virus egress, just as cleavage of sialic acid facilitates the egress of other IAVs [8]. However, the ability to downregulate MHCII has implications for adaptive immune responses against bat influenza viruses and may contribute to viral pathogenesis.

MHCII is responsible for presenting antigen to $CD4^+$ T cells, which, along with co-stimulatory factors, promotes their activation [9]. $CD4^+$ T cells are then essential for the promotion of high-quality antibody responses by providing B cells with the signals required to induce somatic hypermutation/affinity maturation. Ciminski *et al.* reported seroconversion in bats after experimental infection with WT H18N11, demonstrating that antibody responses are generated in response to infection [8]. Nevertheless, it will be interesting to determine whether H18N11 infection impairs T cell activation and, as a result, the breadth and quality of the antibody response (Figure 1).

Emerging data have suggested that broadly neutralizing antibodies are capable of neutralizing H17N10 viruses [10]. However, the titers and prevalence of antibodies capable of neutralizing bat H17 and H18 viruses in humans have not yet been studied. It will be interesting to determine whether humans and other species in areas where bat influenza viruses are endemic (i.e., South America) have already been exposed to these viruses and have mounted specific adaptive immune responses. Sero-epidemiological studies could shed important light on this question.

The observed inability of bat influenza viruses to efficiently transmit within mice and ferrets, alongside their limited reassortment potential with conventional IAVs (reviewed in [11]) reduces the probability that these viruses pose an imminent zoonotic threat. However, that does not rule out the potential for epizootic transmission, and gene segments of bat H17N10 and H18N11 viruses are compatible for reassortment [12]. Important questions therefore remain regarding the true diversity of bat influenza viruses and their evolutionary relationship to conventional IAVs. Frugivorous and insectivorous bats diverged over 50 million years ago and there are over 1400 known species

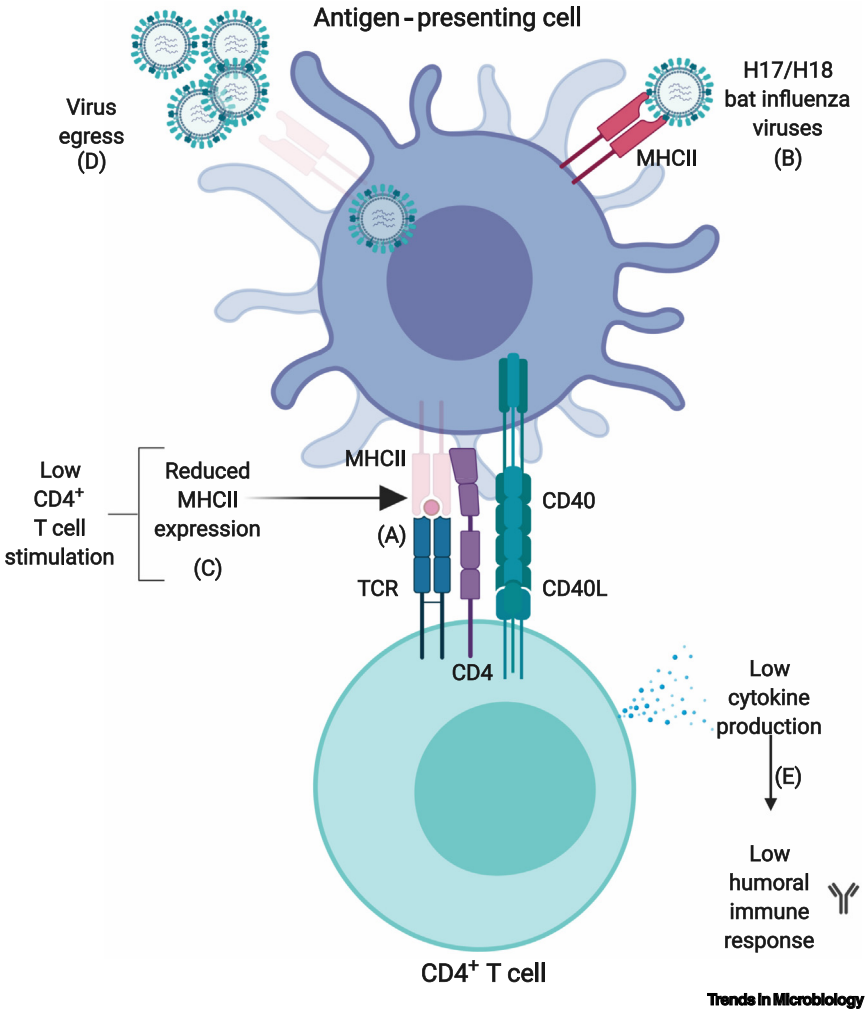

Figure 1. The Ability of Bat Influenza Viruses to Downregulate MHC Class II (MHCII) Expression May Have Immunological Consequences for the Host. (A) MHCII-mediated antigen presentation in infected cells, along with other co-stimulatory signals, plays a critical role in CD4$^+$ T cell activation. Activated CD4$^+$ T cells produce cytokines that prime a strong humoral response against invading viruses. (B) Bat influenza viruses use MHCII as a cellular receptor. These viruses are known to infect epithelial cells. However, it is unclear whether they infect MHCII-expressing antigen-presenting cells. (C) Ciminski *et al.* [8] demonstrated that N11 reduced surface expression of MHCII. (D) While this is likely to facilitate virus egress from infected cells, it might in turn reduce CD4$^+$ T cell activation – especially if it occurs in antigen-presenting cells. This could have deleterious effects on the host adaptive immune response (E), including the priming of humoral antiviral response. Further studies are required to fully understand the role of N11 in downregulating host MHCII and to discern the potential implications for the adaptive immune response, viral pathogenesis, and disease manifestation. Abbreviations: TCR, T cell receptor.

of bats. Although H17N10 and H18N11 viruses have only been detected in New World fruit bats (Table 1), *in vitro* studies with pseudotyped viruses demonstrated that ectopically expressed MHCII from the insectivorous bats *Eptesicus fuscus* and *Myotis lucifugus* was sufficient to serve as a cellular receptor for entry of these viruses [6]. Thus, there is a pressing need to perform more detailed and systematic surveys of multiple bat species in different geographical locations to better understand the prevalence and diversity of bat influenza viruses.

The growing frequency of outbreaks caused by emerging viruses reinforces the critical need to adopt a 'One Health' approach to effectively control infectious diseases. Bats have proved to be an especially important reservoir for many pathogens with zoonotic potential, including SARS-CoV, SARS-CoV-2, and Ebola virus. The discovery and ongoing characterization of bat influenza viruses highlights the unpredictable nature of IAVs and the need for more intense study of their evolutionary diversity and pathobiology, especially in non-human hosts.

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Forum

Bacterial Cell Mechanics Beyond Peptidoglycan

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The bacterial cell envelope plays essential roles in controlling cell shape, division, pathogenicity, and resistance against external stresses. In *Escherichia coli*, peptidoglycan (PG) has long been thought to be the primary component that conveys mechanical strength to the envelope. But a recent publication demonstrates the key contribution of the lipoprotein Lpp in defining the stiffness of the cell envelope and its sensitivity to drugs.

Bacterial Cell Mechanics

The mechanical properties of bacteria are important for cellular function, physiology and disease [1,2]. Importantly, the stiff cell surface also provides a protection against antibiotics, which, together with the continuous rise in drug resistance, makes bacterial pathogens more and more difficult to eradicate. A grand challenge in mechanomicrobiology is to identify the molecular players defining bacterial cell mechanics, and to understand how the cells sense and respond to mechanical stress [2,3]. Today, newly developed nanotechniques offer a wealth of opportunities to tackle these problems (Figure 1; see later for details).

Gram-negative bacteria harbor a three-layered cell envelope (Figure 2A) [4]. The inner layer is a fluid inner membrane (IM) composed of a phospholipid bilayer. The outermost layer is an asymmetrical outer membrane (OM) made of phospholipids in its inner leaflet and lipopolysaccharides in its outer leaflet. Both membranes are separated by an aqueous space, the periplasm, which contains a PG mesh with covalently cross-linked stem peptides. Besides playing a major role as a barrier between the inner side of the cell and its environment, the Gram-negative cell envelope provides mechanical strength for defining cell shape, stiffness, and permeability. For years, PG has been assumed to be the main actor controlling cell shape and mechanical rigidity, the IM and OM functioning essentially as chemical barriers [5]. However, evidence is accumulating that the OM and its connections to PG are also involved in cellular mechanics [6]. In particular, the lipoprotein Lpp, also known as Braun's lipoprotein, the numerically most abundant protein in *E. coli*, provides the only covalent crosslink between the OM and the PG (Figure 2A). The Lpp bridge defines the size of the periplasm, allowing the efficient transfer of stress signals across the envelope [7] and indirectly controlling the length of the flagellum rod. Yet, how exactly Lpp contributes to cell mechanics has remained mysterious.

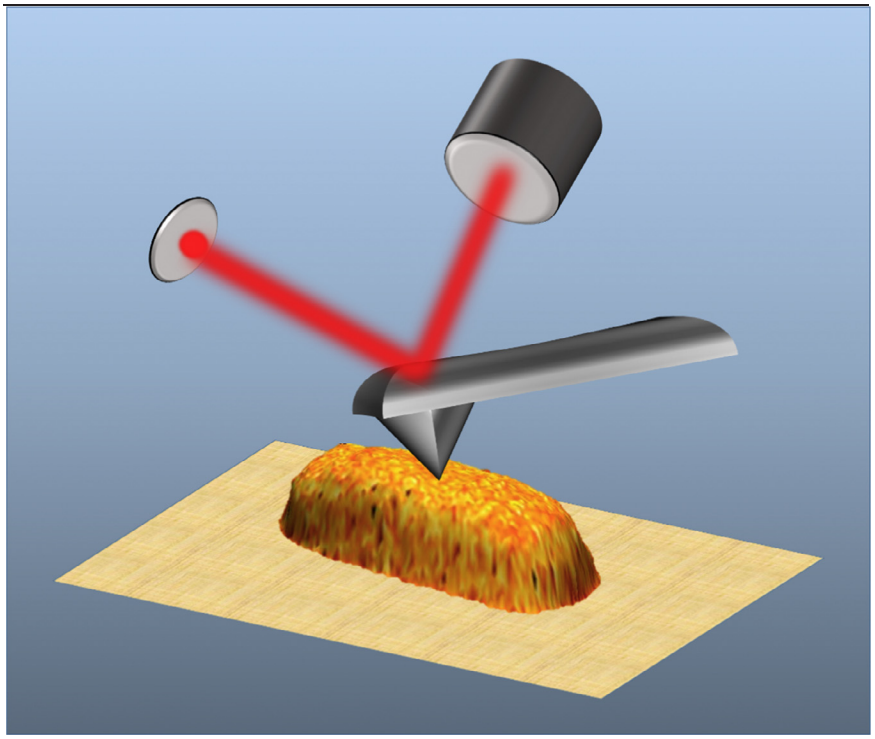

Figure 1. Atomic Force Microcopy (AFM), a Unique Window into Bacterial Cell Mechanics. AFM setup showing a nanoscale tip simultaneously probing the 3D topography and mechanical properties of a single living *Escherichia coli* cell.