News & views

Virology

Holding avian influenza A viruses at bay

Laura Graf & Peter Staeheli

Avian influenza A viruses that can cross the species barrier could cause the next pandemic. Mutations in the viral genome have now been found that can overcome a newly discovered antiviral protein, BTN3A3, in human cells. See p.338

The natural reservoir of influenza A viruses (IAVs) is aquatic birds, but these viruses can occasionally also move into humans or other animals. There, they might evolve or acquire genes from other viral strains to generate variants that have the potential to cause pandemics. Fortunately, several mechanisms make it difficult for IAVs to spill over the species barrier from birds into humans. On page 338, Pinto *et al.*[1] reveal another such mechanism, in the form of a human protein, BTN3A3, which inhibits a crucial step needed for viral replication.

To vault the species barrier, viruses need to acquire adaptive mutations that enhance their fitness in their new host. Adaptive mutations might allow virus particles to bind more efficiently to the surface of human cells, co-opt proviral host factors that enable the intracellular trafficking of viral components, or enhance the activity of the viral polymerase enzyme that replicates the virus's RNA genome in human cells[2]. The host, by contrast, needs to try to maintain a formidable and multi-pronged barrier.

A 2021 study found that a component of the human innate immune system — the protein MX1, which is induced by the immune-signalling protein interferon — prevents the transmission of the H7N9 IAV from birds to humans[3]. Now, Pinto *et al.* provide further evidence to support the idea that the innate immune system should be regarded as part of the species barrier that lowers the risk of IAV transmission from birds to humans.

The authors screened a library of interferon-induced proteins for antiviral activity in cultured human cells. They identified BTN3A3 as a factor that selectively inhibits the replication of IAV strains of avian, but not human, origin. The authors also expressed human BTN3A3 in the lungs of mice and found that it confers resistance to infection with avian IAVs, suggesting that the authors' cell-culture experiments faithfully reflect the *in vivo* situation.

Pinto *et al.* next showed that BTN3A3 targets a viral protein (the nucleoprotein) that envelops the virus's RNA genome, and in doing so interferes with viral-genome replication (Fig. 1). A comparison of amino-acid sequences from various viruses revealed that the rare successful transmissions of IAVs from birds to humans are typically accompanied by the acquisition of mutations at amino-acid positions 313 and 52, or both, of the nucleoprotein, which are located near each other on the nucleoprotein's surface. This means that BTN3A3 targets the same surface-exposed region as does MX1.

However, the antiviral activity of BTN3A3 does not depend on the presence of MX1. Nevertheless, both proteins interfere with an early step of the viral replication cycle that is essential for viral-genome amplification in infected cells. MX1 is located in the cell cytoplasm, and is thought to block the trafficking of viral ribonucleoprotein complexes in the cell[4]. BTN3A3 is mainly present in the nucleus, but it is unclear exactly where and how it interferes with viral-genome replication.

The authors went on to show that bona fide human IAV strains — viruses that circulate in the human population — all possess nucleoprotein variants that have mutations at amino-acid positions 313 and 52, or both. These positions have also been described as being involved in escape from inhibition by MX1. To fully escape MX1, avian IAVs must acquire further adaptive mutations, including those that compensate for the fitness costs of the resistance-conferring changes[5–7]. Interestingly,

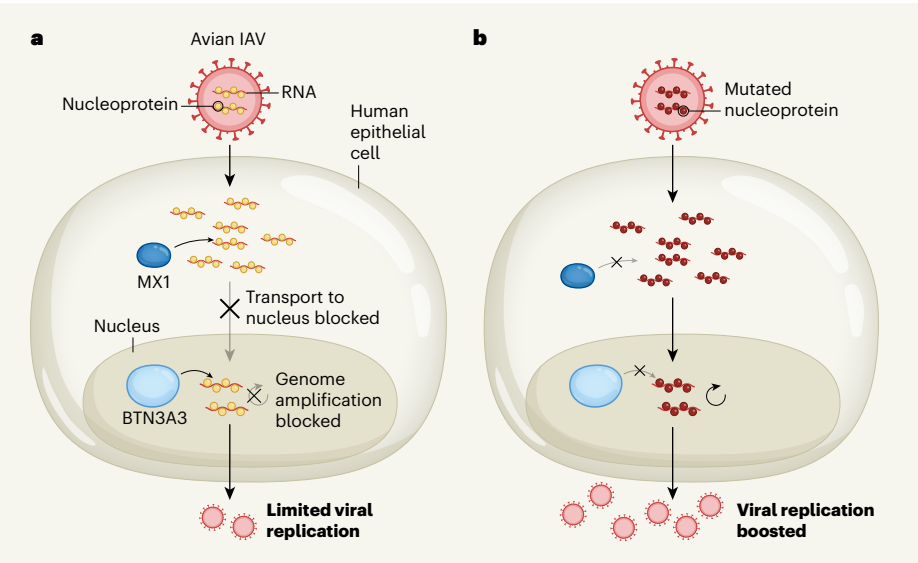

Figure 1 | Avian influenza A viruses (IAVs) break down a protective barrier. a, The RNA genome of avian IAVs is packaged in the virus by the nucleoprotein. In the epithelial cells that line human lungs, the cytoplasmic protein MX1 targets the nucleoprotein to prevent transport of avian IAVs into the nucleus. Pinto *et al.*[1] report that the BTN3A3 protein selectively blocks genome amplification of avian IAVs by targeting the same surface-exposed region of the nucleoprotein that MX1 targets. **b**, Most avian IAVs that successfully spill over into humans have gained mutations in the viral nucleoprotein at positions 313 and 52, or both, allowing the viruses to escape inhibition by BTN3A3 (and, to some extent, by MX1). Such mutations provide the virus with a growth advantage in human epithelial cells.

Pinto and colleagues' data indicate that viruses with a single nucleoprotein mutation sufficient to cause escape from BTN3A3 show no substantial fitness loss.

Curiously, Pinto *et al.* found that amino-acid changes in the viral nucleoprotein that confer resistance to BTN3A3 were present in some avian IAV lineages before those viruses spilt over into humans. But BTN3A3-family members with anti-IAV activity are found exclusively in Old World monkeys and apes, and not in birds, suggesting that, when viral resistance to BTN3A3 first evolved in birds, it did so without any selection pressure. The authors' database searches also revealed fluctuations in the frequency of BTN3A3-resistant IAVs over time, correlating with recorded 'zoonotic' spillover events from birds to humans.

At present, there are no real clues to why resistance to BTN3A3 emerges in avian IAVs. But the co-occurrence with zoonotic infections of humans points to the importance of BTN3A3 resistance for the zoonotic potential of avian IAVs. That said, some disease-associated variants, termed highly pathogenic avian H5N1 IAVs, seem to cause human infections despite lacking the 'escape' mutations documented by Pinto and colleagues; this would indicate that resistance to this protein is not an absolute requirement for infecting humans. Presumably, a collection of genetic variants act together to allow these H5N1 viruses to override BTN3A3-mediated restriction and to replicate efficiently in human tissues.

Analyses of IAV sequences obtained from archival samples of lungs of people infected with the 1918 pandemic H1N1 virus have suggested that mutations conferring escape from MX1 arose sequentially in humans[8]. What's more, isolates from the beginning of that pandemic already possessed a nucleoprotein variant with changes at position 313 that confer BTN3A3 resistance[8]. It is tempting to speculate that this early adaptation drove the subsequent escape from MX1. Further studies are needed, however, to understand the possible synergistic interplay between BTN3A3 and MX1 resistance during zoonotic virus transmissions.

Overall, the identification of BTN3A3 as a component of the species barrier highlights the role of immune-related antiviral factors in preventing the spillover of avian IAVs to humans. This discovery has consequences for assessing the zoonotic potential of these IAVs. Only by understanding the diversity of adaptive mutations that viruses must acquire for successful transmission into the human population will we be able to identify, in a timely manner, zoonotic IAVs that have pandemic potential.

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Environmental science

Collaborations uncover extent of plastic pollution

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Ambitious campaigns to sample plastic pollution in coral-reef and freshwater ecosystems demonstrate the value of international cooperation in assessing contamination to identify drivers and inform management. **See p.311 & p.317**

Plastic pollution evokes powerful images of injured marine life tangled in plastic ropes, or starving seabirds whose guts are filled with broken plastics. But clear evidence is emerging that plastic contamination extends beyond marine ecosystems to affect wildlife around the planet[1], as well as Earth's atmosphere[2]. In this issue, researchers report global evidence that plastics contaminate coral reefs and freshwater lakes — some of which are far away from the human populations that create such pollution. On page 317, Nava *et al.*[3] quantify microplastics in surface waters of lakes and reservoirs in 23 countries, and on page 311, Pinheiro *et al.*[4] measure larger plastic debris contaminating coral-reef ecosystems in 25 locations across the Pacific, Atlantic and Indian oceans.

As environmental contaminants, plastics are remarkably diverse in size — spanning nanometres to kilometres — and also vary in their chemistry, shape and other physico-chemical characteristics. This diversity complicates analyses of the abundance and distribution of plastics in the environment, because each type of plastic requires a tailored approach to

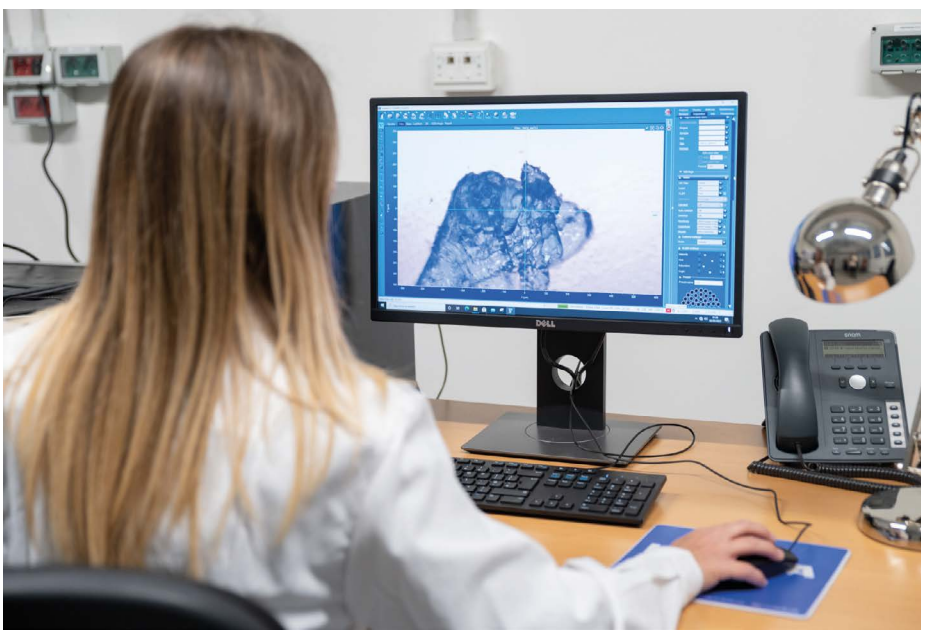

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Figure 1 | Analysing microplastics in freshwater systems. Nava *et al.*[3] sampled microplastics from 38 lakes and reservoirs, analysed them in a single laboratory and obtained a consistent data set showing that contamination is widespread, yet variable.