

detected within the olfactory mucosa. In contrast, highly pathogenic avian influenza H5N1 virus predominantly replicates in the olfactory mucosa and only in a few cells in the respiratory mucosa [3, 4].

Our study suggests that virus replication within the olfactory mucosa was efficiently reduced or blocked by vaccine-induced IgG antibodies, thereby preventing CNS invasion via the olfactory nerve. However, this does not necessarily mean that the transport of IgG from the circulation to the olfactory mucosa is efficient. First of all, we used a homologous vaccination and challenge approach—a perfect match. We did not quantify the concentration of neutralizing IgG in the olfactory mucosa, so it might be that low concentrations were sufficient for virus neutralization. In addition, even though this vaccination approach generates cross-reactivity to other H5 clades [5], the ability to prevent virus spread to the CNS via the olfactory nerve of other H5 viruses remains to be determined. Second, in the same study, oseltamivir was not able to reduce or block virus replication enough to prevent virus spread to the CNS. Although virus-specific IgG and oseltamivir have different mechanisms to block virus replication, both IgG and oseltamivir have to enter the olfactory mucosa via the circulation. This might indicate that the fenestrated phenotype of blood vessels within the olfactory mucosa does not support transport of all components from the circulation. Third, influenza virus vaccination studies that induce IgG often lack pathological analysis and specific immunohistochemical staining to visualize virus replication in different cell types of the nasal cavity. Since the majority of vaccination studies target influenza virus strains that replicate predominantly in the respiratory mucosa, it can be assumed that reduction of virus replication within the nasal cavity reflects less virus replication in the respiratory mucosa. The effect of vaccination on replication within the olfactory mucosa is often not included.

The ability of vaccine-induced neutralizing antibodies to reduce or block influenza virus replication within the olfactory

mucosa is largely unknown. Different vaccination strategies, including inactivated or live-attenuated vaccines, can induce either circulating IgG, mucosal immunoglobulin A (IgA), or both [6]. Mucosal IgA is better than IgG in neutralizing influenza viruses in the nasal cavity [7]; however, the ability of mucosal IgA to reduce or block influenza virus replication within the olfactory mucosa is not known.

Taken together, our knowledge of the capacity of vaccine-induced neutralizing antibodies to reduce or block virus replication within the olfactory mucosa is limited. Since the endothelium within the olfactory mucosa has a unique phenotype [1], it might be that neutralizing IgG and IgA are not equally present in the respiratory and olfactory mucosa. To get more insight into the role of neutralizing antibodies after vaccination in both the respiratory and olfactory mucosa, these tissues should be included in future vaccination studies for influenza viruses, especially those with a known neurotropic potential.

## Notes

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## Reply to Mori

TO THE EDITOR—We thank Mori for his correspondence [1]; we agree that circulating neutralizing immunoglobulin G (IgG) antibodies are most likely responsible for the prevention of abundant influenza A(H5N1) virus replication in the olfactory mucosa of vaccinated ferrets. This lack of virus replication prevented central nervous system (CNS) invasion via the olfactory nerve in our ferret model [2].

Within the nasal cavity, influenza viruses can infect and replicate in the respiratory mucosa, olfactory mucosa, or both. Ferret studies have shown that both seasonal H3N2 and 2009 pandemic H1N1 viruses replicate predominantly in the respiratory mucosa, although replication is also

- of seasonal H3N2, pandemic H1N1 and highly pathogenic avian influenza H5N1 virus infections in ferrets. *PLoS One* **2012**; 7:e42343.
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