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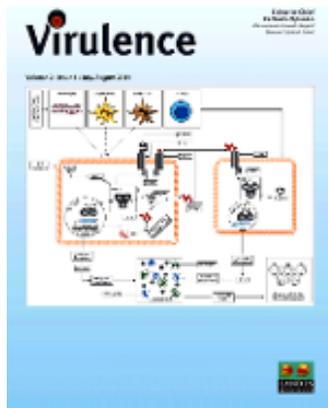
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### Host strategies against virus entry via the olfactory system

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# Host strategies against virus entry via the olfactory system

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**I**n mammals, odorants are inhaled through the nose and inside the nasal cavity they trigger olfactory sensory neurons (OSN) that are located within the olfactory epithelium. OSN project their axons into glomerular structures of the olfactory bulb. There they synapse with dendrites of second-order neurons that project their axons to the olfactory cortex. Thus, olfaction is based on direct interaction of environmental matters with OSN. This poses the question of how neurotropic viruses are prevented from infecting OSN and entering the central nervous system. Recent evidence indicates that upon instillation of neurotropic virus OSN are readily infected. By axonal transport virus reaches the glomerular layer of the olfactory bulb where it is efficiently curbed by a type I IFN-dependent mechanism. In this review local mechanisms limiting virus entry via the olfactory system and inhibiting virus spread within the CNS are recapitulated in the context of anatomical properties of the olfactory system.

**Key words:** olfactory bulb, olfactory sensory neuron, virus infection, interferon

**Abbreviations:** AL, axonal layer; BBB, blood brain barrier; CNS, central nervous system; EPL, external plexiform layer; GCL, glomerular cell layer; GL, glomerular layer; IFN, interferon; ML, mitral cell layer; OEC, olfactory ensheathing cell; OR, olfactory receptor; OSN, olfactory sensory neuron; PG, periglomerular layer; TLR, toll-like receptor; VSV, vesicular stomatitis virus

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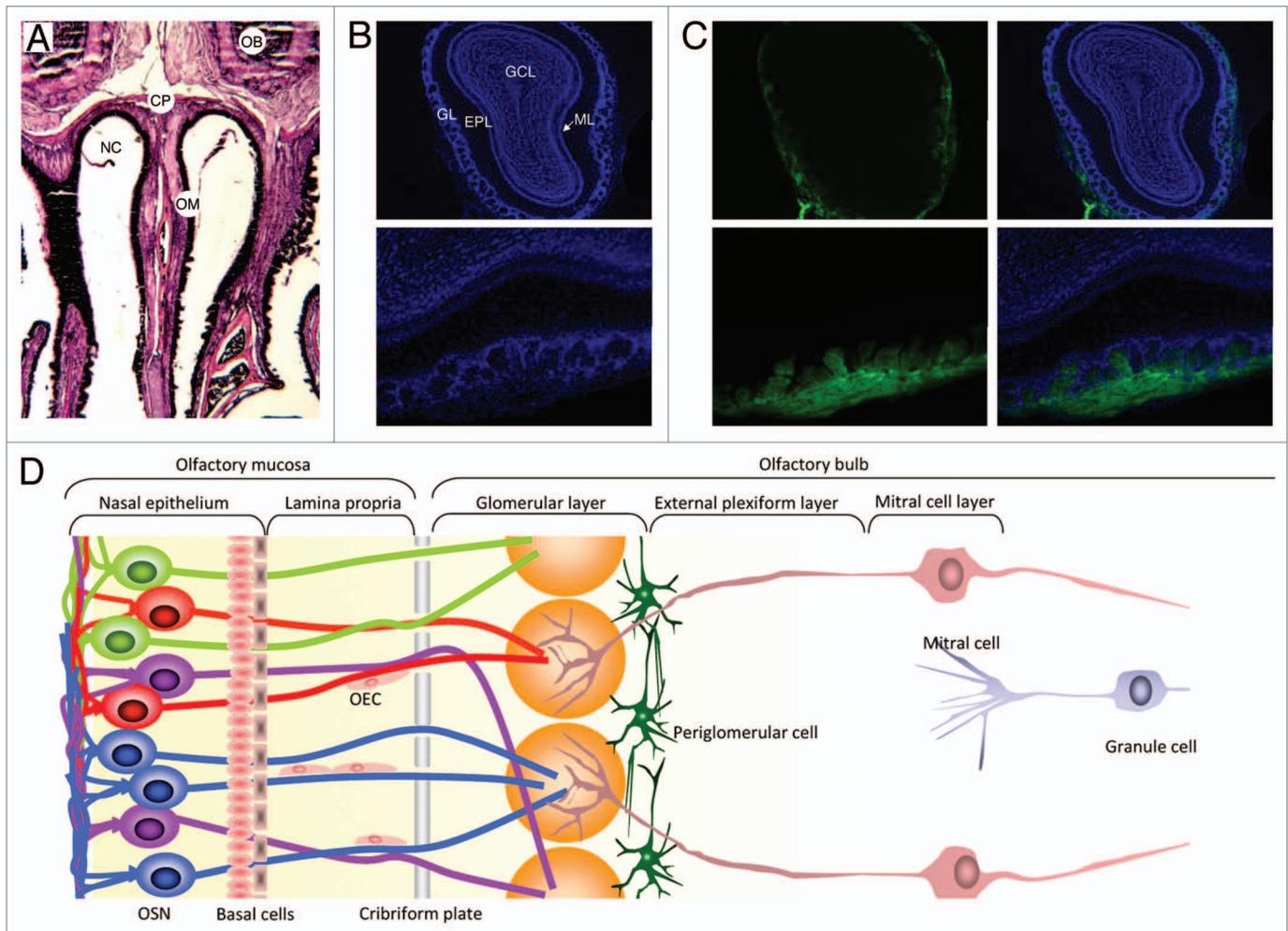
and the spinal cord, eventually leading to paralysis and death of the host. Current knowledge about the olfactory system as an entry port for neurotropic infections is primarily grounded on experiments carried out under conditions resulting in high mortality. Of note, classical experiments performed in the 90s already indicated that neurotropic viruses may readily enter the CNS and that efficient mechanisms must be in place to eradicate the virus.<sup>2-4</sup> In these publications, at early time points after intranasal infection with 10<sup>5</sup> pfu VSV of 5–7-week-old BALB/c mice virus could be re-isolated from brains of all analyzed mice, whereas approximately 50% of the infected mice survived. These experiments suggested that in approximately 50% of the infected mice virus was cleared from the CNS. More recent experiments conducted under non-lethal infection conditions indicated that upon intranasal VSV instillation virus readily spread to the olfactory bulb and was efficiently prevented from infecting periglomerular cells by a type I IFN-dependent mechanism.<sup>5</sup> How can this be explained?

## Intranasal Infection with Neurotropic Viruses

More than 15 years ago it was recognized that upon intranasal vesicular stomatitis virus (VSV) inoculation, virus spreads to the glomerular layer of the olfactory bulb and to anterior olfactory nuclei.<sup>1</sup> From these sites virus may expand trans-neuronally to other parts of the CNS. Furthermore, VSV can infect cells lining the ventricular system where it is released into the cerebrospinal fluid and then spreads to other regions of the brain

## Anatomy of the Olfactory System

The olfactory mucosa consists of the olfactory epithelium and the underlying lamina propria. OSN are located within the olfactory epithelium. Each OSN is capped by approximately 10 hair-like cilia that protrude into the mucus layer lining the nasal cavity (see **Fig. 1A and D**). The cilia express odorant receptors (OR) that are triggered by odorant molecules. Of note, one OSN expresses only one kind of OR.<sup>6,7</sup> Each OSN carries one axon that



**Figure 1.** Anatomical characteristics of the olfactory system and of intranasal VSV infection. (A) Histochemical analysis of the fore part of a mouse skull (NC, nasal cavity; OB, olfactory bulb; OM, olfactory mucosa; CP, cribriform plate). (B) Hoechst stain of the olfactory bulb (GL, glomerular layer; EPL, external plexiform layer; GCL, granule cell layer; ML, mitral cell layer). (C) In WT mice intranasally infected with a sublethal dose of VSV-eGFP virus is found in the olfactory glomeruli. A histological section of day 4 after infection is shown. (D) Schematic model of the olfactory system and of olfactory sensory neuron axonal wiring within the olfactory bulb (OSN, olfactory sensory neuron; OEC, olfactory ensheathing cell).

projects into one glomerulus of the olfactory bulb. It was found that one OR can be triggered by multiple odorants and that one odorant can be recognized by multiple ORs. Nevertheless, for appropriate perception of a single odorant a defined combination of ORs has to be triggered.<sup>8</sup> OSNs are compassed by non-neuronal supporting cells, termed sustentacular cells.<sup>9-11</sup> The olfactory epithelium is bordered by globose basal cells, which presumably carry a regenerative potential and that are followed by a layer of horizontal basal cells.<sup>20</sup> It has to be emphasized that epithelial cells express several pathogen recognition receptors and thus potentially can be triggered by viruses to directly mount antiviral responses. However, in context of

VSV infection very little is known about antiviral responses of the olfactory epithelium. Within the olfactory epithelium Bowman's gland and ducts are found. The lamina propria consists of loose connective tissue made up of various different cell types and olfactory ensheathing cells (OEC) that ensheath bundles of olfactory receptor axons projecting into the olfactory bulb.<sup>12-14</sup> The bundles of OSN axons course through the cribriform plate and enter the axonal layer (AL) of the olfactory bulb, the most rostral part of the mouse brain. There, single axons synapse with second order neurons in the glomerular layer (GL). Nucleic Hoechst-staining reveals important anatomical structures of the olfactory bulb: the axonal layer

(AL) is located at the surface of the bulb, whereas the following glomerular layer (GL) contains glomerular structures (G) surrounded by periglomerular cells (PG). Then an unstained and thus cell body-depleted and axonal rich area follows the external plexiform layer (EPL). The subsequent mitral cell layer (ML) shows a distinct nuclear staining, whereas the granule cell layer (GCL) located in the central part of the olfactory bulb is more loosely stained (reviewed in ref. 15 and Fig. 1B).

### Axonal Wiring within the Olfactory System

Amongst known senses, olfaction plays a phylogenetically important role as

demonstrated in mice by 3.5% of the total brain being dedicated to olfaction compared to less than 1% in humans. The current view is that OSN specifically detect a large variety of different odor molecules and send information through their axons to the olfactory bulb, the first site of olfactory information processing within the brain.<sup>16</sup> Specific recognition of single odor molecules is conferred by odorant receptors (ORs) of which 1,000–1,200 single members are known in the mouse. ORs are comprised in a multigene family of seven-transmembrane domain G-protein-coupled receptors showing an overall amino acid sequence similarity of approximately 37%.<sup>17</sup> A single olfactory sensory neuron expresses only one OR. Each OSN projects a single unbranched axon to a single glomerulus. One glomerular module is formed by several coalescing OSN axons expressing the same odorant receptor. This is astonishing because OSNs expressing the same OR seem to be scattered throughout the olfactory epithelium. Thus, individual glomerular modules process information of several OSN expressing the same type of OR.<sup>18</sup> In the murine olfactory bulb approximately 1,800 glomeruli are found that are cortically located within the glomerular layer of the olfactory bulb. Within each hemisphere of the olfactory bulb there are typically found two glomeruli processing information provided by one type of OR. Within the glomeruli, OSN axons synapse with the dendrites of mitral (or tufted) cells, which are second-order neurons that project their axons to the olfactory cortex (Fig. 1D), as well as with the dendrites of periglomerular cells (PG). PG cells presumably regulate the fine tuning of the output level of each glomerulus which is assumed to be relevant for information processing within the olfactory system.<sup>19</sup>

The olfactory epithelium regenerates itself throughout life.<sup>20</sup> OSN have an average lifespan of approximately 90 days and then die. It is currently unknown whether this lifespan is due to an intrinsic program or follows the statistical risk of infection and subsequent elimination. Nevertheless, there is a continuous production of new OSNs by the olfactory epithelium. Axons of new OSN do not project to pre-existing structures but they dynamically

form glomeruli by coming together and forming synapses with other OSNs expressing the same OR. Thus, the glomerular layer is a dynamic anatomical structure and shows continuous regeneration and reorganization. Axonal wiring in the mouse olfactory system is remarkably precise and the underlying mechanism is as yet not fully understood.<sup>16</sup> It is possible that the exposure of the olfactory system to infection provided a high evolutionary pressure to create such a life-long dynamic system.

### Physical Barriers and/or Anti-Viral Mechanisms within the CNS

Many viral pathogens show a tropism for neurons. Upon peripheral infection such viruses may enter the central nervous system (CNS) and cause massive damage, either by direct virus-conferred effects or by immunopathology. Although many viruses are neurotropic, viral infection of the CNS is a comparably rare condition. Thus, efficient mechanisms must be in place protecting the CNS from infection: either the CNS is secluded from pathogens by physical barriers, and/or efficient mechanisms are in place that curb viral spread and eradicate the pathogen. The blood-brain barrier (BBB) has often been seen as a major physical barrier against virus infection that, nevertheless, easily can be overcome by viruses: in a recent study it was shown that virus-induced TNF $\alpha$  transiently changed the permeability of the BBB thus allowing West Nile virus to infect the CNS.<sup>21</sup> Interestingly mice deficient in innate TLR-3 signaling showed reduced virus burden within the CNS and prolonged survival compared with wild-type controls. More recent studies describe the BBB as a more physiological barrier that is readily traveled by immune cells.<sup>22</sup> In case infected cells pass the BBB, pathogens such as *Toxoplasma* may use immune cells as Trojan horses to move into the CNS.<sup>23</sup>

To further address whether neurotropic viruses primarily have to be cleared in the periphery or whether mechanisms within the CNS are in place that inhibit virus spread, we performed experiments with mice showing a selective type I

interferon receptor depletion only in neuroectodermal cells, including neurons, oligodendrocytes and astrocytes of the CNS (NesCre<sup>+/+</sup>-IFNAR<sup>fllox/fllox</sup> mice). Upon intranasal instillation of VSV, an infection mode that models aspects of airborne infections, in NesCre<sup>+/+</sup>-IFNAR<sup>fllox/fllox</sup> mice the virus moved via the olfactory nerves to the olfactory bulb and further spread over the whole CNS.<sup>5</sup> As a consequence of broad CNS infection mice died around day 6. On the contrary, control mice infected with the same virus dose also showed infection of olfactory nerves. However, within the olfactory bulb the virus was arrested in the glomerular layer. Experiments with VSV-eGFP verified that the virus was present in olfactory neurons and that it did not move beyond glomeruli.<sup>5</sup> Histochemical analysis of the olfactory bulb of wild-type mice infected with wild-type virus further revealed expression of the viral G protein in glomerular structures. Thus, under conditions of non-lethal infection in wild-type mice virus was arrested at the interface between glomerular structures and periglomerular cells (Fig. 1C). These experiments indicated that within the olfactory bulb a type I IFN-dependent mechanism efficiently inhibited virus spread.<sup>5</sup> When mice were infected with higher virus doses VSV was also found in periglomerular cells and later in mitral and granule cells.<sup>24</sup> These observations indicated that the glomerular layer serves as a barrier that, nevertheless, can be overcome by an overload of virus.

### Retrograde and Anterograde Transneuronal Virus Spread

VSV seems to similarly spread from an infected presynaptic to a postsynaptic cell (anterograde spread) as well as from a postsynaptic to a presynaptic cell (retrograde spread). As the OSN cell body is located in the olfactory epithelium, whereas OSN axons project into the olfactory bulb, virus can readily spread within the olfactory bulb in an anterograde manner.<sup>25</sup> To further move to other brain regions the virus can spread transsynaptically, using both retrograde and anterograde transport. Although VSV can enter the CNS also via non-olfactory paths, it shows such a strong

OSN tropism that upon intranasal VSV instillation OSN are preferentially used for CNS entry. Only if OSN are experimentally destroyed, alternative entry paths such as via the cerebrospinal fluid or the trigeminal nerve can be observed.<sup>26</sup> For other viruses like HSV it was shown, that upon intranasal instillation the vomeronasal system is used as a route for neuroinvasion. The vomeronasal organ is another peripheral chemosensory module in the nasal cavity that is the primary sensor for the detection of pheromones.<sup>27-29</sup> Whether the vomeronasal organ also plays a role in the CNS entry of VSV is so far unknown.

### Future Perspectives

It will be a matter of future research to determine whether locally active type I IFN is also induced locally, and if so, which cell type is triggered upon virus infection by which mechanism in order to produce IFN. Alternatively, peripherally produced type I IFN might cross the BBB to become locally active within the olfactory bulb.<sup>30</sup> If this was the case, the mechanism enhancing type I IFN transit via the BBB would be of interest. Furthermore it is not known whether type I IFN stimulation of OSN has an impact on axonal virus spread and infection of the CNS. Still it is not fully understood whether olfactory ensheathing cells and/or macrophages in the connective tissue play a role in viral pathogenesis. Finally, it would be of interest to know whether under conditions of sublethal VSV infection, where basically all OSN seem to be infected, olfaction is perturbed and whether the regenerative potential of olfactory epithelia fully reconstitutes olfaction. One of the central dogmas of neuroscience, i.e., brains of adult mammals cannot regenerate nerve cells, was shaken when it became evident that in the olfactory bulb as well as in the dentate gyrus of the hippocampus neurogenesis takes place.<sup>31</sup> As for the latter, the "rostral stream" provides a unique avenue for new neurons from the ventricular ependyma to the olfactory

bulbs<sup>32</sup> it is attractive to speculate whether the regenerative potential of the olfactory epithelium developed because the olfactory system is a complex, vitally important, intimately exposed and particularly vulnerable sensory system and also because it plays a role in pathogen defense. Future research will presumably solve this question.

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