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The Olfactory Bulb: An Immunosensory Effector Organ during Neurotropic Viral Infections

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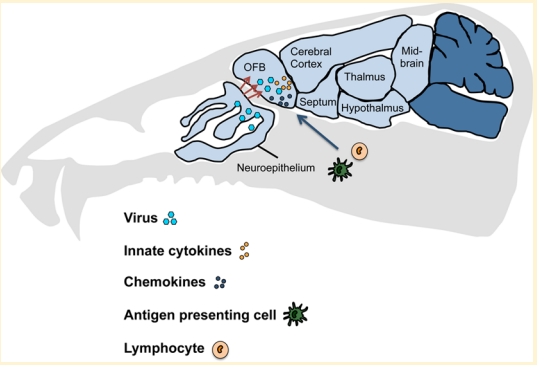
ABSTRACT: In 1935, the olfactory route was hypothesized to be a portal for virus entry into the central nervous system (CNS). This hypothesis was based on experiments in which nasophayngeal infection with poliovirus in monkeys was prevented from spreading to their CNS via transection of olfactory tracts between the olfactory neuroepithelium (ONE) of the nasal cavity and the olfactory bulb (OB). Since then, numerous neurotropic viruses have been observed to enter the CNS via retrograde transport along axons of olfactory sensory neurons whose cell bodies reside in the ONE. Importantly, this route of infection can occur even after subcutaneous inoculation of arboviruses that can cause encephalitis in humans. While the olfactory route is now accepted as an important pathway for viral entry into the CNS, it is unclear whether it provides a way for infection to spread to other brain regions. More recently, studies of antiviral innate and adaptive immune responses within the olfactory bulb suggest it provides early virologic control. Here we will review the data demonstrating that neurotropic viruses gain access to the CNS initially via the olfactory route with emphasis on findings that suggest the OB is a critical immunosensory effector organ that effectively clears virus.



KEYWORDS: *Olfactory bulb, virus, encephalitis, olfactory sensory neurons, neuroinvasion*

Viral infections of the central nervous system (CNS) are rare and often devastating, leading to death or permanent neurologic damage. Neurotropic viruses may gain access to the CNS via several routes including anterograde neuronal spread through sensory nerves,[1] across the blood-brain barrier (BBB) as free virions, or via the entry of infected immune cells.[2] However, studies examining the kinetics of neurotropic viral invasion after peripheral routes of inoculation have identified the olfactory bulb (OB) as the earliest site of CNS infection.[3] Indeed, the most direct conduit from the periphery to the brain occurs at the level of the olfactory neuroepithelium (ONE) within the nasal cavity, where cell bodies of olfactory sensory neurons (OSNs) reside and send their axons into the CNS to synapse with dendrites of mitral neurons within the olfactory bulb (OB). This route of entry was first investigated in the early 1900s in the context of poliovirus infection. Faber and Gebhardt first demonstrated that virus establishes its initial focus in the OB.[4] In 1936, Flexner reported that instillation of poliovirus into the nasal cavity, but not the stomach, leads to CNS manifestations of disease.[5] Faber and others later demonstrated that ablation of the ONE with zinc sulfate, which induces selective and rapid OSN necrosis,[6] prevents CNS infection.[5b] Evidence from a variety of animal models and human cases has since indicated that many DNA and RNA viruses, including herpesviruses,[7,7a] rhabdoviruses including vesicular stomatitis and rabies viruses (VSV, RABV),[8] neurotropic flaviviruses West Nile and Japanese encephalitis viruses (WNV, JEV),[9] paramyxoviruses parainfluenza and measles viruses (PIV, MV),[3f,10] alphaviruses Venezuelan Equine Encephalitis and chikungunya viruses (VEEV, CHIKV),[11] Bunyavirus LaCrosse virus (LACV),[12] and influenza A[13] are detected first within the OB during neuroinvasive infection. Several authors have also shown that virus within the OB is quickly cleared.[8a,14] This and the overall rarity of viral encephalitis suggests effective, neuroprotective immunity within the OB may quickly eliminate virus entering via this route, protecting the rest of the brain from infection. While the complete mechanisms of virologic control within the OB are unknown, studies demonstrate that innate immune mechanisms are specialized at this site, involving interactions between immune and neural cells and recruited leukocytes that influence viral infection and clearance at more distant brain regions. This Review will discuss the olfactory route of viral access to the CNS with emphasis on evidence that OB innate immune response to viral infection of the CNS is an early event that controls viral entry and replication throughout the CNS.

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ANATOMY AND VIRAL INFECTIONS OF THE OB

Viruses that utilize the olfactory nerve as entry into the CNS encounter many cell types progressing from the nasal cavity into the central olfactory nervous system. Cells of the ONE, which is located within the nasal cavity, include olfactory receptor neurons (ORN), supporting (sustentacular) cells, basal cells, microvillar cells, and Bowman's glands.[3j,14d,15] ORNs are unique among these cells since they establish the connective conduit between the nasal cavity and the CNS. These specialized bipolar neurons extend a single dendrite from their neuronal cell body into the ONE and an axon that crosses the basement membrane of the ONE and passes through the cribriform plate, which separates the nasal and cranial cavities. These axons terminate in the OB where they converge to form glomeruli and form synaptic contacts with neurons resident in the OB. ORN axons are supported by olfactory ensheathing cells (OECs) (i.e., Schwann cell-like glial cells) and are surrounded by mucus-secreting Bowman's glands, connective tissue, and blood and lymphatic vessels.[3j] Within the olfactory glomeruli, ORN axon terminals convey information to projected neurons such as tufted cells and mitral cells, which transmit information deeper into the CNS primarily the ipsilateral primary olfactory cortex.

Evidence of viral transmission along the olfactory route is based on studies in experimental animals and a few human cases. Entry into the CNS has been documented through detection of viral antigen within the olfactory mucosa and within the glomerular and mitral cell layers of the OB for many viruses including, influenza virus, HSV, poliovirus, paramyxoviruses, including canine distemper virus (CDV), Hendra virus, and Nipah virus, VSV, RABV, parainfluenza virus, adenoviruses, JEV, WNV, chikungunya virus, La Crosse virus, mouse hepatitis virus, and bunyaviruses which have been extensively reviewed previously[3j,16] (Table 1, Figure 1). Although rare, viral antigen has also been directly detected in ORNs within the olfactory mucosa following infection as is the case with influenza virus,[1i,3b,17] several herpesviruses, including HSV-1, bovine herpesvirus (BHV)-5, and equine herpesvirus (EHV)-1 and -9,[16] CDV,[18] VSV,[3h] and RABV,[19] suggesting that these viruses are transported through the axons of ORNs to access the OB.

Several studies have concluded that the initial infection of influenza A occurs at the olfactory bulb (OB).[17d,20] H5N1 is the most common form of influenza A virus detected in the olfactory bulb of patients and animal models.[20a,21] More recently, studies of HPAI H5N1 in animal models reported the entry of H5N1 virus primarily through the olfactory nerve with viral antigen detectable in the olfactory mucosa and olfactory receptor neuron.[17b,20b] Studies in H7N9-infected ferrets similarly detected viral antigen in the OB by 3 days postinfection.[22] Additional studies have demonstrated that influenza A virus infection of the OB is not strain specific.[3j,20c,21,22] Postmortem study of an immunocompromised human infant infected with H3N2 virus depicted the presence of viral load in the olfactory bulb with viral antigen detected in both neurons and glial cells.[3j] These studies strongly suggest that olfactory route is the primary route for CNS invasion in Influenza A mediated infection.

Previous studies have demonstrated that HSV, RABV, VSV, and influenza viruses are capable of transaxonal transport.[20a,23] Potentially viruses may also access the OB though direct infection of OECs or via channels in the cribriform plate. OECs are unique cells that form a continuous channel surrounding the axons of ORNs from the ONE as it passes through the cribriform plate and ends the OB. All together, numerous studies have shown that a variety of viruses are able to use the olfactory nerve as a shortcut into the CNS, however more comprehensive studies

Table 1. Neurotropic Virus Detection in the Olfactory Neuroepithelium (ONE), Olfactory Sensory Nerve (OSN), Olfactory Bulb (OB), and Other Regions of the CNS Following Infection in Various Animal Models via Isolation or by Immunohistochemistry (IHC)[a]

		virus detection				
virus (strain)	route of infection	ONE	OSN	OB	other CNS regions	ref
Influenza A (PR8)	i.n.		+	+	+	13b, 20c
Influenza A (R404BP)	i.n.		+			17e
Influenza A (H5N1)	i.n.		+	+	+	1i, 13a, 17a, d, 36
Influenza A (WSN/33)	i.n.	+	+	+	+	3b
Herpesvirus	i.n.	+	+	+	+	7b, 37
Parainfluenza (Sendai)	i.n.		+	+	n.d.	3f, 38
Nipah virus	i.n.	+	+	+	+	3g, 39
Hendra virus	i.n.		+	+	+	1d
Western Equine encephalitis virus	i.n.	+		+	+	40
Venezuelan Equine encephalitis virus	f.p.	+	+	+	+	3c
Eastern Equine encephalitis virus	i.n.	+	+	+	+	41
Rabies virus (CVS)	i.n.	+		+		19
Vesicular stomatitis virus	i.n.	+	+	+		42
Poliovirus	i.n.	+	+	+	+	4, 5b, 43
Japanese encephalitis virus	i.n.		+	+	n.d.	44
St. Louis encephalitis virus	i.p.	+		+	+	45
West Nile virus	i.p., f.p., i.n.	+	+	+	+	46
Murray Valley encephalitis virus	f.p.			+	+	47

[a]n.d.: none detected.

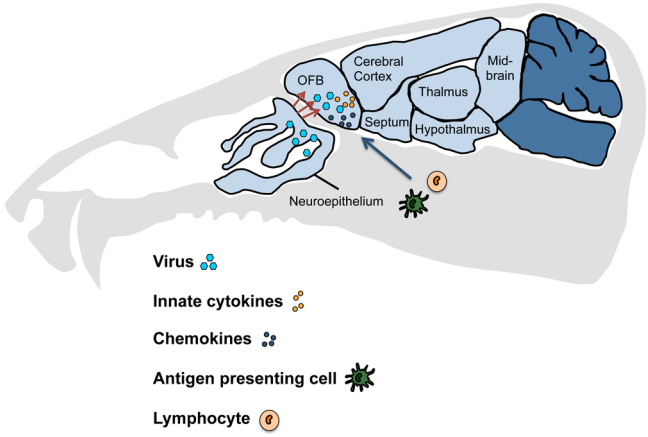

Figure 1. Viral entry via the olfactory neuroepithelium induces antiviral responses in the olfactory bulb. Depicted is a cartoon of a mouse brain in which viral particles enter the CNS via axons of olfactory receptor neurons within the neuroepithelium of the nasal cavity. Infection of neurons within the olfactory bulb (OB) leads to expression of innate cytokines and chemokines, which recruit lymphocytes and antigen presenting cells.

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are necessary to define the mechanisms by which viruses use the olfactory nerve as a route of entry into the CNS.

INNATE IMMUNE RESPONSES OF THE OB DURING VIRAL INFECTION

Early studies examining transneuronal spread of viruses from the ONE to the OB reported that virus could no longer be detected at the latter site several days after infection. Investigators interpreted these findings as evidence that this brain region was unsatisfactory for growth, rather than postulating that it had specialized immune responses that efficiently cleared virus. In an early study, innate immune responses within the OB after application of VSV to the ONE included expression of nitric oxide and up-regulation of major histocompatibility antigens (MHC) I and II by infected astrocytes, microglia and endothelial cells.[14a] Additional studies utilizing viruses or pathogen associated molecular patterns (PAMPs) demonstrated OB expression of innate cytokines including interleukin 12,[3h] tumor necrosis factor (TNF)-α, TNFR1, interleukin (IL)-1β,[14f] and IkappaB.[24] For instance, recent studies with the flavivirus, tick-borne encephalitis virus (TBEV), and the alphavirus, Sindbis virus (SINV), confirm that pattern recognition receptor (PRR) signaling within the OB results in the upregulation of the innate cytokine interferon (IFN), which restricts viral replication in the CNS. This upregulation of IFN leads to increased expression of interferon regulatory factors (IRFs), which enhance the ability of IFN to control viral replication.[14e,25] Indeed, the expression of innate immune molecules within the OB results in rapid antiviral responses and improved survival. Similarly, intranasal inoculation of H1N1 virus, leads to upregulation of cytokines within 5−7 h post infection.[20c]

The source of some of these innate immune molecules has been traced to the OECs that envelope the olfactory nerves throughout their trajectory from the ONE to the OB. OECs, which have significant roles in OB development and repair,[26] are postulated to provide immunological protection against neutrotropic pathogens. Treatment of OECs with PAMPs or agonists of PRRs leads to production of iNOS,[27] nuclear translocation of nuclear factor kB (NK-kB) with cytokine expression.[28] Other studies implicate OB microglia in innate immune responses to PAMPs or damage associated molecular patterns (DAMPs) at this site.[29] The role of these innate immune molecules in the OB during viral infections is unclear. Studies using intranasal infection with lab adapted influenza A did not impact survival in mice deficient for iNOS, type I or II interferon (IFN) receptors, or transporter associated with antigen processing (TAP)1.[3b] However, persistent infection could be detected in 80% of surviving animals. These mice also had limited CNS recruitment of infiltrating lymphocytes suggesting that innate immunity in the OB limits viral persistence and induction of adaptive immunity within the CNS. The role of OB innate immune responses by neural and microglial cells in leukocyte trafficking and function is an active area of research.

LEUKOCYTE TRAFFICKING INTO THE OB DURING VIRAL ENCEPHALITIS

Although most viruses that invade the CNS via the olfactory nerve cause an inflammatory response characterized by an influx of neutrophils and mononuclear cells, there are few in-depth studies on their specific role. While it has been shown that type I IFN is critical for survival following intranasal infection with VSV it is also necessary for the induction of IL-12 by astrocytes and inflammatory monocytes.[8a,30] Multiple studies have demonstrated that the expression of IL-12 decreases viral titer within the OB and is strongly correlated with the rapid infiltration of both CD4+ and CD8+ T cells as well as NK cells.[8a,14a,b,30] Lymphocyte infiltration into the OB has been shown to be instrumental in limiting viral replication and spread beyond the OB as has been shown following T lymphocyte depletion during VSV[31] and MHV[32] infection. In addition, TAP-1 deficient mice were used to demonstrate that the ability to present antigens within the context of MHCI was crucial for T lymphocytes to maintain viral control within the OB following MHV infection of mice.[10] Interestingly, a recent study demonstrated that dendritic cells infiltrate into the OB during VSV infection[33] suggesting that these cells may play a role in the activation of recruited lymphocytes. In addition to T lymphocytes we recently observed that NK cell infiltration into the OB during WNV infection is crucial for viral control specifically within the hindbrain regions of the CNS (under review). Together these studies demonstrate the lymphocytic infiltration is instrumental in limiting viral replication and spread and that in their absence or inability to be full activated, viruses are able to spread from the OB into other regions of the CNS increasing damage.

CONCLUDING REMARKS

Many viruses are able to invade the CNS via the olfactory route. In general, if a viral infection is not contained locally (due to inefficient intrinsic and innate immune responses), it can spread to vital organs, causing severe pathologies. Viral spread within the CNS can be severe as well as deadly not only due to the fact that infected neurons may die, but also because of the immune-mediated pathology in the brain. The OB, although commonly recognized as a sensory organ for olfaction, also serves as an immunoeffector organ within the CNS. The CNS encounters an unknown number of pathogens primarily through the nasal cavity. Since this sensory organ is intimately exposed and particularly vulnerable it is likely there was high evolutionary pressure for neuroprotective mechanisms within the olfactory system. Use of genetic approaches to deplete OSNs[34] via temporally controlled diphtheria toxin A expression or conditional deletion of innate immune signaling in response to type I or II IFNs[35] will elegantly address the role of these neurons and innate immune responses in virologic control within the OB. In addition, the role of supporting cells, such as the OECs, during CNS viral infection is an area not well explored. It is unclear whether OECs are susceptible to certain viral infections or whether they have a definitive role in immunoprotection and spread of viruses from the OB to the rest of the CNS. As further studies are accomplished focusing on this vital yet vulnerable organ, it will become more clear that the OB is a complex sentinel immune organ that is instrumental in preventing passage of pathogens to other vital regions of the CNS preventing injury of neural cells and/or immunopathology.

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