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Pathogens Penetrating the Central Nervous System: Infection Pathways and the Cellular and Molecular Mechanisms of Invasion

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Pathogens penetrating the central nervous system: infection pathways and the cellular and molecular mechanisms of invasion

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Running title: Bacterial mechanisms of brain invasion

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SUMMARY

The brain is well protected against microbial invasion by cellular barriers, such as the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). In addition, cells within the central nervous system (CNS) are capable of producing an immune response against invading pathogens. Nonetheless, a range of pathogenic microbes make their way to the CNS and the resulting infections can cause significant morbidity and mortality. Bacteria, amoebae, fungi and viruses are capable of CNS invasion, with the latter using axonal transport as a common route of infection. In this review we compare the mechanisms by which bacterial pathogens reach the CNS and infect the brain. In particular, we focus on recent data regarding mechanisms of bacterial translocation from the nasal mucosa to the brain, which represents a little explored pathway of bacterial invasion but has been proposed as being particularly important in explaining how infection with *Burkholderia pseudomallei* can result in melioidosis encephalomyelitis.

INTRODUCTION

Invasion of the CNS by bacteria: a clinical perspective

Microbial infection involving the CNS is an important and relatively common presentation. CNS infections are frequently caused by viruses, such as the enteroviruses, which cause the majority of cases of aseptic meningitis and meningoencephalitis (1-3). Other neurotropic viruses such as human cytomegalovirus, herpes simplex viruses, varicella zoster virus and the emerging viruses West Nile virus, henipaviruses, Japanese encephalitis virus, chikungunya virus, Ebola virus and rabies virus may also cause CNS infections (4-7). There are many bacterial pathogens that are associated with CNS invasion. Rapid detection of a bacterial agent in such presentations and the initiation of appropriate antibiotic therapy influences morbidity and mortality (8-10). The clinical presentations of bacterial CNS infection range from meningitis and meningoencephalitis to focal CNS syndromes.

Meningitis

Meningitis, inflammation of the meninges, is usually acute but can also be subacute and most frequently presents with headache, fever and neck stiffness (11). Meningitis can be pyogenic (pus-forming) - associated with common bacterial etiologies (described below), or aseptic, in which pyogenic bacteria are not isolated from the cerebrospinal fluid (CSF) and the causative agent may be viral or mycobacterial or there is a non-infective etiology. Features of CSF, which would generally exclude a bacterial etiology for meningitis, include a protein concentration of < 600 mg/L and a white cell count of < 90 $x10^6$/L (2). The classic triad of fever, neck stiffness and a change in mental state is observed in 44% of patients with bacterial meningitis (11).

A prospective, laboratory-based surveillance study in 1986 defined the epidemiology of

24 bacterial meningitis in a population of 34 million in the United States (12). This study
25 reported an average of 6.7 cases of bacterial meningitis per 100, 000 population across six
26 states, and demonstrated that *Haemophilus influenzae*, *Neisseria meningitidis* and
27 *Streptococcus pneumoniae* caused 77% of cases, which was consistent with a previous report
28 by the National Surveillance of Bacterial Meningitis (13). More recently, it was reported that
29 the incidence of bacterial meningitis in the United States declined by 31% during the
30 surveillance period of 1998 to 2007, most likely due to the success of vaccine programs (14).
31 *S. pneumoniae* was the most common cause of bacterial meningitis (58.0%), followed by
32 *Streptococcus agalactiae* (group B streptococcus, 18.1%), *N. meningitidis* (13.9%), *H.*
33 *influenzae* (6.7%) and *Listeria monocytogenes* (3.4%). *Staphylococcus aureus* and
34 *Escherichia coli* are also important causes of bacterial meningitis (15) and *Streptococcus suis*
35 is emerging as a zoonotic etiology of meningitis (16); however, it must be noted that
36 meningitis can be caused by a plethora of different bacteria (17). The most common
37 alternative bacterial cause of meningitis is *Mycobacterium tuberculosis*, which is recognized
38 particularly in pediatric populations of the developing world (18, 19). These infections
39 involve a clinical onset that is usually insidious with hydrocephalus being a prominent
40 feature.

41 Bacterial meningitis is usually preceded by nasopharyngeal or middle ear colonization,
42 followed by: (i) microbial invasion of the tissue and intravascular space, and (ii) bacteremia.
43 Meningeal invasion occurs following penetration of the cellular barriers of the CNS. High-
44 level bacteremia was shown to be necessary for the development of meningitis in
45 experimental animal models (20, 21), which is consistent with clinical observations in
46 humans (22-24). Bacteria that commonly cause meningitis express anti-phagocytic capsular
47 polysaccharide, which enables survival/multiplication within the blood. Several species-
48 specific factors that promote bacterial survival in blood, resistance to complement and

49 survival within phagocytes have been identified (25-30). Bacterial meningitis resulting from
50 non-hematogenous spread is less common and is a consequence of infection pre-existing in
51 an adjacent intracranial site. This would include mastoiditis, frontal sinusitis or rupture of an
52 intracerebral abscess into the ventricles of the brain. Such infections may occur following
53 head trauma or neurosurgical procedures.

54 **Pneumococcal meningitis**

55 There are no specific distinguishing clinical features of pneumococcal meningitis. While a
56 rash is uncommon, it is occasionally seen in splenectomised patients with overwhelming
57 sepsis. There is a close correlation between bacteremic and meningitic serotypes of *S.*
58 *pneumoniae* implicating hematogenous spread as the commonest mode of acquisition (31). It
59 is the most frequent cause of bacterial meningitis in the United States and has a case fatality
60 rate of 14.7% (14). Since the introduction of the 7-valent pneumococcal conjugate vaccine
61 (which contains serotype antigens 4, 6B, 9V, 14, 18C, 19F and 23F) in the United States, the
62 incidence of pneumococcal meningitis declined by 30.1% from 1.13 cases per 100, 000
63 population in 1998 – 1999 to 0.79 cases per 100, 000 population in 2004 – 2005 (32). In
64 patients < 2 years and > 65 years of age, the incidence of pneumococcal meningitis
65 throughout the surveillance period declined by 64.0% and 54.0% respectively. Across all age
66 groups, the number of meningitis cases caused by *S. pneumoniae* serotypes covered by the 7-
67 valent vaccine dropped by 73.3% (32). Similar reductions in invasive pneumococcal disease
68 caused by 7-valent serotypes have also been reported in Australia, England and Wales (33-
69 35); however, this has led to a replacement phenomenon in which the rates of disease caused
70 by non-7-valent *S. pneumoniae* serotypes have significantly increased (32, 34, 35).

71 **Meningococcal meningitis**

72 *N. meningitidis* is most likely to cause meningitis in children and adolescents, and has a

mortality rate of 10.1% in the United States (14). The serotypes most commonly implicated are A, B, C, W135 and Y (14, 36-38) and meningitis without shock is the most common presentation (38). The predominant clinical feature, which can distinguish *N. meningitidis* from other causes of bacterial meningitis, is the presence of a petechial rash. It can rapidly become purpuric, which in the presence of meningitis or sepsis, would strongly implicate *N. meningitidis* as the etiologic agent. These lesions are a consequence of meningococci adhering to the endothelial cells of the capillaries and small veins in the skin, thereby altering the antithrombotic surface of the endothelium. This results in the formation of clots and the extravasion of erythrocytes, which appears as skin hemorrhages. Lesions > 1.0 cm usually occur in patients developing shock, with high levels of circulating endotoxin (38).

Immunization against *N. meningitides* serotypes A and C has resulted in a significant decrease in meningococcal disease. The United Kingdom was the first country to introduce routine immunization with a monovalent serotype C conjugate vaccine in 1999. During the first follow-up surveillance period (2000 - 2001), the incidence of serotype C meningococcal disease fell by 80% in target age groups (39). Meningococcal serotype C vaccination programs in Australia have also led to a marked decrease in disease due to *N. meningitidis* serotype C. Of the 90% of meningococcal cases in Australia in 2010 that had serotype data available, *N. meningitidis* serotype B caused 84.0% of cases (40). In 2013, a recombinant meningococcus serotype B vaccine (Bexsero®) was added to the Australian Register of Therapeutic Goods for use in individuals > 2 months of age, and was also approved for use in Europe. In New Zealand, the introduction of an outer membrane vesicle vaccine against an epidemic *N. meningitidis* serotype B clone in New Zealand was reported to be 80.0% effective in fully immunized children aged 6 months to < 5 years (41).

H. influenzae, L. monocytogenes and other bacterial causes of meningitis

97 *H. influenzae* as a cause of meningitis is now uncommon in most western countries due to
98 widespread immunization against *H. influenzae* capsular type B, which was previously
99 responsible for the majority of cases of *H. influenzae* meningitis. As a cause of meningitis, *H.*
100 *influenzae* has fallen from 45.0% in 1986 (12) to 6.7% in 2003 – 2007 (14). *L.*
101 *monocytogenes* is also an infrequent cause of meningitis; however, it is associated with a high
102 mortality rate (17.0% - 28.0%) (14, 42, 43). *L. monocytogenes* meningitis occurs primarily in
103 children < 2 months of age and in immunocompromised and elderly individuals (14, 42, 43).
104 The signs and symptoms of *L. monocytogenes* meningitis are not different from those
105 associated with other causes of community-acquired meningitis, but CSF findings may be
106 atypical (43, 44). Listeriosis is associated with the consumption of contaminated food,
107 implicating the gastrointestinal tract as the portal of entry into the blood stream.

108 Group B streptococcus and *E. coli* are common causes of meningitis in neonates (45, 46),
109 with group B streptococcus accounting for 86.1% of bacterial meningitis cases in patients < 2
110 months of age (14). Gram negative aerobic bacteria such as *Klebsiella pneumoniae, Serratia*
111 *marcescens, Salmonella* spp. and *Pseudomonas aeruginosa* can occasionally be responsible
112 for meningitis in certain patient groups, including infants and patients with head injuries, or
113 those undergoing post-neurosurgical procedures or immunosuppression (31, 47, 48). *M.*
114 *tuberculosis* is a common cause of chronic or subacute meningitis (49) and is associated with
115 non-specific symptoms and an absence of neck stiffness during early disease (50).
116 Tuberculosis meningitis occurs following hematogenous spread of bacilli from the lungs and
117 the establishment of a caseating Rich focus (tuberculosis granuloma) in the brain cortex,
118 meninges or choroid plexus. Bacteria are subsequently released from the foci and enter the
119 subarachnoid space (51).

120 **Encephalitis**

121 Acute meningitis can, in certain circumstances, be clinically indistinguishable from acute
122 encephalitis, which refers to inflammation of the brain parenchyma in association with
123 neurologic dysfunction (52). Encephalitis is typically characterized by headache with an
124 acute confusional state, with or without seizures. The California Encephalitis Program
125 identified the clinical profiles and etiologies of encephalitis in a large cohort of
126 immunocompetent patients from 1998 – 2005 (7). A confirmed or probable etiology was
127 identified only in 15.8% of cases; of these, viruses were the causative agent in 68.5% of
128 cases. Herpes simplex virus is the most common cause of encephalitis in western countries
129 (6, 53, 54). Enteroviruses, varicella zoster virus, Epstein-Barr virus, measles virus and
130 arboviruses such as Japanese encephalitis virus, West Nile virus and Murray Valley
131 encephalitis virus have also been implicated as causes of encephalitis (6, 7, 54, 55). Beckham
132 and Tyler provide a comprehensive list of other important and emerging viral causes of
133 encephalitis (52). *L. monocytogenes* may cause meningoencephalitis, a syndrome where
134 features of both meningitis and encephalitis are present.

135 *Burkholderia pseudomallei*, the causal agent of melioidosis, has a relatively uncommon
136 association with CNS involvement, accounting for around 4% of cases in northern Australia
137 (56, 57), with distinctive clinical features being brain stem encephalitis, often with cranial
138 nerve palsies, together with peripheral motor weakness, or occasionally just flaccid
139 paraparesis alone (myelitis) and presenting with urinary retention (56-58). Fevers are usually
140 a prominent feature, and headaches are common but neck stiffness is usually mild or absent.
141 The CT scan is often normal, but dramatic changes are seen on magnetic resonance imaging,
142 most notably a diffusely increased T_2-weighted signal in the midbrain, brain stem, and spinal
143 cord, with variable symmetry and enhancement with gadolinium. CSF shows high protein,
144 glucose normal or slightly decreased and most notably a pleocytosis with usually
145 lymphocytes predominant. Bulbar palsy and decreased respiratory drive often necessitate

146 ventilation, which may be required for many weeks (56, 59-62). In northern Australia patients
147 infected with the flavivirus Murray Valley encephalitis virus can present with clinical
148 features indistinguishable from those seen with melioidosis encephalomyelitis.

149 Neurologic melioidosis is occasionally seen outside Australia, although it appears to be less
150 common in melioidosis patients in Southeast Asia than in Australia. Rather than the
151 distinctive brainstem encephalitis and/or myelitis seen in Australia, patients with neurological
152 melioidosis in Southeast Asia usually present with macroscopic cortical brain abscesses (63),
153 suggesting that in these cases there has been bacteremic spread to the brain. Frank meningitis
154 is rarely seen in melioidosis. A recent report described the first case of neurological
155 melioidosis in the United States, in a 58 year old male who presented with low-grade fever,
156 nausea, vomiting, headaches, diplopia, left-sided ptosis and transient episodes of marked
157 somnolence, after recent travel to Cambodia (64). CSF demonstrated increased levels of
158 glucose, protein and lymphocytes; however, microbiological investigation consistently
159 showed no organisms by Gram stain and failed to detect any organisms on culture medium.
160 *B. pseudomallei* was only diagnosed following the culture of biopsy material collected during
161 a suboccipital craniectomy. The mortality of neurological melioidosis is up to 30%, with
162 ongoing residual neurological deficit in up to 50% overall (56-58).

163 **Focal infections**

164 The range of focal CNS infections includes brain abscesses, subdural empyema and epidural
165 abscesses. Brain abscess refers to an intracerebral infection that usually begins as localized
166 cerebritis, which develops into a collection of pus that is surrounded by a well-vascularized
167 capsule (65). The clinical presentations of brain abscesses are variable and depend on the
168 anatomical area of the brain that is compressed or affected. Brain abscesses commonly
169 originate from a contiguous focus of infection, such as the middle ear, mastoid cells or

170 paranasal sinuses. Brain abscesses may also occur following: (i) hematogenous spread of 171 bacteria from a distant focus of infection, or (ii) brain trauma or neurosurgery (66). A recent 172 systematic review and meta-analysis of 123 brain abscess studies reported between 1935 and 173 2012 demonstrated that *Streptococcus* and *Staphylococcus* spp. were the most frequently 174 isolated microorganisms, causing 34.9% and 18.9% of infections respectively (67). Gram-175 negative enteric bacteria were responsible for 15.0% of cases of brain abscess and were 176 frequently associated with polymicrobial infections. Other less common causative agents 177 include anaerobic bacteria (*Bacteroides* spp., *Peptostreptococcus* spp., *Fusobacterium* spp.), 178 Actinomycetales, *Haemophilus* spp. and *Pseudomonas* spp. (66-68).

179 Subdural empyema refers to a collection of pus in the subdural space, which is located 180 between the dura mater and arachnoid mater layers of the meninges (see 'barriers of the 181 CNS' below). In contrast, epidural abscesses are defined as a collection of pus between the 182 dura mater and the skull or vertebral column (69). The etiologies of subdural empyema and 183 epidural abscesses are similar to those of brain abscesses; however, due to the anatomical 184 location of these infections bacteria do not need to penetrate the BBB to establish disease.

185 BARRIERS OF THE CNS

186 The CNS is physically protected from injury by bony structures such as vertebrae and the 187 cranium, and is also enveloped by the meninges and surrounded by CSF. The meninges 188 comprise three layers: the dura mater, arachnoid mater and pia mater. The arachnoid mater 189 and pia mater are separated by the subarachnoid space, which is filled with CSF that is 190 produced by the choroid plexus in the ventricles of the brain (Figure 1) (70). The CSF 191 provides mechanical support, regulates ion composition and maintains chemical stability. 192 Once it has circulated through the ventricles of the brain, the CSF enters the subarachnoid

193 space and drains into the blood through arachnoid villi and granulations. CSF also drains
194 through the cribriform plate into the lymphatics within the nasal mucosa (71-73).

195 The complex functions of the brain are dependent on homeostatic mechanisms that regulate
196 the ionic composition of the interstitial fluid that surrounds neurons in the parenchyma (74).
197 This homeostasis is maintained by two cellular barriers, which separate the CNS from the
198 systemic circulation: the BBB and the BCSFB (Figure 1). These barriers protect the brain
199 from abrupt changes in blood biochemistry while at the same time allowing delivery of
200 nutrients and removal of metabolites. Importantly, these barriers also protect the CNS from
201 invasion by microbes that may be present within the blood.

202 **The blood-brain barrier**

203 The BBB (Figure 1) exists at the level of cerebral microvessels and represents the largest
204 interface for blood-brain exchange (75). The BBB is composed of endothelial cells that line
205 cerebral microvessels in association with pericytes, astrocytes and the basement membrane
206 (collectively referred to as a neurovascular unit in association with neurons, microglia and
207 peripheral immune cells). Pericytes are attached to the abluminal surface of the endothelium
208 and together with the astrocytic endfeet form a contiguous membrane barrier. A physical
209 barrier is formed at the interendothelial cleft by complex junctions that regulate the flux of
210 ions, polar molecules and macromolecules from the systemic circulation (74, 75).

211 At the interendothelial cleft, two types of junctions comprise the BBB: adherens junctions
212 and tight junctions. These junctions block the transport of a wide range of molecules,
213 restricting paracellular permeability across the BBB (75). In addition, endothelial cells
214 forming the BBB lack fenestrations in their plasma membranes and have a reduced number of
215 pinocytotic vesicles compared to endothelial cells in other tissues, which restricts
216 transcellular flux (76). Despite this, certain solutes and macromolecules may be transported

217 across the BBB *via* specific and non-specific mechanisms, such as passive diffusion, receptor
218 and/or adsorptive-mediated transcytosis, or *via* ATP-binding cassette transporters and solute
219 carrier transporters, which are expressed on cerebral microvessels (75, 77-79). The
220 transcellular and paracellular routes of entry into the CNS are highly relevant for microbial
221 pathogens.

222 **The blood-cerebrospinal fluid barrier**

223 The BCSFB (Figure 1) is formed by tight junctions between: (i) epithelial cells located at the
224 choroid plexus in the lateral, third and fourth ventricles of the brain and (ii) endothelial cells
225 of the veins and venules within the subarachnoid space. Tight junctions between the epithelial
226 cells of the arachnoid mater also contribute to the BCSFB, by forming a barrier between the
227 subarachnoid space and the leaky fenestrated blood vessels of the dura mater. Of these
228 BCSFB locations, the choroid plexus has a large vascular surface area and represents the
229 major interface between fenestrated blood vessels and CSF. Similar to the BBB, the BCSFB
230 functions to protect the CNS and helps to maintain homeostasis; however, the choroidal
231 epithelial cells of the BCSFB also secrete CSF from the choroid plexus into the brain
232 ventricular system, and the tight junctions between the epithelial cells have a lower electrical
233 resistance compared to BBB endothelial cells (80). Thus, the choroid plexus BCSFB may be
234 more vulnerable to microbial penetration *via* paracellular mechanisms. The BCSFB at the
235 arachnoid mater has a smaller total surface area compared to other barriers of the CNS, and is
236 not thought to be an important route for the entry of solutes into the brain (74). However, the
237 arachnoid BCSFB may represent an important site of microbial entry.

238 **Immunosurveillance of the CNS**

239 The brain parenchyma and spinal cord are populated throughout by resident immune cells,
240 the microglia, which are highly specialized tissue macrophages that are maintained through *in*

241 *situ* self-renewal without reconstitution from the bone marrow (81-83). Microglia
242 continuously survey the brain parenchyma by sampling their microenvironment with highly
243 motile processes and protrusions (84). Recent data has also revealed cells expressing CD11c
244 are localized within the juxtavascular parenchyma of the healthy mouse brain, and extend
245 processes to the glia limitans (astrocytic foot processes at the parenchymal basement
246 membrane, surrounding the brain under the pia mater) (85). In agreement with a previous
247 study (86), this raises the possibility that dendritic cells, which are characterized by high-
248 level CD11c expression, may reside within the brain parenchyma. However, a range of cells
249 of the myeloid lineage (including activated microglia) also express CD11c, albeit at lower
250 levels. Thus the CD11c-expressing cells described within the brain parenchyma may also
251 represent a subpopulation of microglia (85).

252 Other resident immune cells of the CNS include perivascular macrophages in addition to
253 small populations of blood-derived dendritic cells and macrophages within the choroid plexus
254 and meninges. Dendritic cells constitute approximately 1% of immune cells within the
255 choroid plexus and meninges (87, 88), but unlike the putative dendritic cells of the brain
256 parenchyma, choroid plexus and meningeal dendritic cells are distinct from microglia and
257 closely resemble classical dendritic cells that require FMS-like receptor tyrosine kinase 3
258 ligand for development (88). The CSF also contains a trafficking population of mononuclear
259 cells, comprising T cells (90%), B cells (5%), monocytes (5%) and dendritic cells (less than
260 1%) (89).

261 Normal CNS immune surveillance occurs when central memory T cells migrate from the
262 blood across the choroid plexus epithelium, enter the CSF and scan the subarachnoid space.
263 In the absence of recognizing ligands presented by local antigen presenting cells, T cells exit
264 the subarachnoid space and drain to the secondary lymphoid organs *via* the nasal mucosa (90,
265 91). In contrast, recognition of presented antigens results in the formation of immunological

266 synapses and is accompanied by cellular signalling that results in the recruitment of
267 peripheral inflammatory cells to the CNS *via* paracellular or transcellular diapedesis (92).

268 The production of cytokines, free radicals (such as reactive oxygen species) and matrix
269 metalloproteinases by inflammatory cells within the CSF spaces can subsequently result in
270 degradation of the glia limitans, BBB/BCSFB dysfunction and cellular death (93-95). In
271 bacterial meningitis, neuronal injury also occurs due to the direct effects of bacterial toxins
272 and virulence factors (96-98). A recent study of human bacterial meningitis cases
273 demonstrated that the profile of pro- and anti-inflammatory cytokines and matrix
274 metalloproteinases within the CSF is dependent upon the causative pathogen (99). The
275 sequelae of bacterial meningitis and the associated neuroinflammation include vasculitis and
276 cerebral venous thrombosis, which can lead to cerebral ischemia. An increase in CSF outflow
277 resistance in combination with interstitial edema results in raised intracranial pressure, which
278 causes hypoxic brain damage and may lead to death (100).

279 **MICROBIAL ENTRY TO THE CNS**

280 Microbial pathogens may access the CNS (i) by penetrating the BBB or the BCSFB; or (ii)
281 *via* the olfactory and/or trigeminal nerves (101). Peripheral nerves other than the olfactory
282 and trigeminal cranial nerves have also been implicated as routes of entry for some viruses,
283 but these will not be reviewed here. Penetration of the BBB and BCSFB represent common
284 routes of CNS invasion, resulting in encephalitis or brain abscesses, and meningitis,
285 respectively. In contrast to a diverse group of neurotropic viruses for which mechanisms are
286 described for invasion of the brain *via* the olfactory sensory nerves, bacterial infection *via* the
287 olfactory epithelium and the olfactory nerve bundles has been infrequently described. Even
288 less well understood is that, in addition to the olfactory nerve, the trigeminal nerve may also
289 present a potential pathway to the brain.

290 ***In vivo* and *in vitro* models of CNS invasion**

291 Much of our understanding of the mechanisms of microbial CNS invasion has been obtained
292 from *in vivo* and *in vitro* studies. Mice and rats are the most common animals used to model
293 CNS infections. These animal models have been used to investigate the sites of microbial
294 entry (20, 102-106) and the roles of virulence determinants in CNS invasion and pathogenesis
295 (107-111). Despite clear differences in the size and complexity of mouse and human brains,
296 comparative transcriptomic studies between mice and humans have demonstrated that gene
297 expression profiles of the healthy brain and choroid epithelium are highly conserved between
298 the species (112-114).

299 There are, however, several important differences between the mouse and human CNS that
300 are relevant to microbial invasion studies. In mice, the leptomeninges (comprising the
301 arachnoid mater and pia mater) are significantly thinner and less vascular than in humans
302 (115). Additionally, the human choroid plexus epithelium has more abundant fibrovascular
303 stroma along the vascular channels and has a relatively larger surface area compared to
304 mouse choroid epithelium. Mice also possess very large olfactory bulbs compared to humans
305 (115), which may result in a higher incidence of CNS invasion *via* the olfactory route
306 compared to humans. Furthermore, mice lack several anatomically defined subclasses of
307 astrocytes that are present in humans (116). These combined anatomical differences may
308 influence the initial site of microbial attachment and subsequent CNS invasion.

309 *In vitro* cell culture systems have been used to model the BBB and elucidate the cellular and
310 molecular mechanisms of microbial CNS invasion. Primary human, rodent and bovine brain
311 microvascular endothelial cells have been used in some studies (97, 117-119); however, the
312 isolation of these cells is technically demanding and *in vitro* culture dramatically alters the
313 transcriptome leading to downregulation of genes involved in BBB function (120, 121).

314 Human umbilical vein endothelial cells (HUVECs) (122-124), caco-2 cells and Mading-
315 Darby canine kidney cells (125) have been used as surrogate models of the BBB, although
316 the non-cerebral origin of these cells may limit their relevance. The availability of
317 immortalized brain endothelial cell lines (for example: human brain microvascular
318 endothelial cells [HBMECs], hCMEC/D3, TY10 and BB19) has enabled the human BBB to
319 be studied. When grown on Transwell membranes, HBMECs: (i) form tight junctions, as
320 measured by transendothelial electrical resistance; (ii) express the adherens junction and tight
321 junction proteins zonula occludens-1, junctional adhesion molecule A, claudin-5, occludin
322 and vascular endothelial-cadherin; and (iii) demonstrate negligible paracellular permeability
323 (126-129).

324 It must be noted that brain microvascular endothelial cells grown in static monocultures
325 represent a surrogate model for the human BBB, as they lack other components of the
326 neurovascular unit that are required for the development of true BBB properties. Studies
327 using pericyte deficient mice have shown that pericytes (see Figure 1) are required for BBB
328 integrity. Compared to wild type mice, pericyte deficient mice demonstrated an accumulation
329 of water and tracer dyes within the brain, structural abnormalities in cerebral endothelial tight
330 junctions and increased expression of genes known to increase vascular permeability,
331 endothelial vesicle trafficking and immune cell recruitment (130-132). Astrocytes also play a
332 key role in regulating the BBB (133-136), and as such, a range of co-culture systems have
333 been developed to more closely model the BBB properties of the neurovascular unit (137-
334 139). Choroid plexus epithelial cell lines that display the characteristic properties of the
335 BCSFB have been described (140, 141); however, the majority of *in vitro* microbial invasion
336 studies have been performed using monocultures of brain microvascular endothelial cells.

337 BACTERIAL INVASION THROUGH THE BLOOD-BRAIN AND BLOOD-
338 CEREBROSPINAL FLUID BARRIERS

339 **Sites of entry**

340 Classical bacterial meningeal pathogens may potentially enter the CSF by penetrating the
341 BBB of cerebral microvessels and entering the extracellular fluid of the brain, which is
342 continuous with the CSF. However, a more likely route involves direct entry into the CSF *via*
343 penetration of the BCSFB at either the arachnoid, or the choroid plexus (142). The highly
344 vascularized choroid plexus has been favored as a major site of bacterial entry to the CSF due
345 to lower electrical resistances between choroid epithelial cells. Autopsies of neonates who
346 died from bacterial meningitis demonstrated that meningitis was frequently (84.0%)
347 associated with an accumulation of a purulent exudate within the ventricles (ventriculitis) and
348 the choroid plexus (plexitis) (143). Ventriculitis preceding meningitis would support the role
349 of the choroid plexus as the initial site of bacterial invasion; however, ventriculitis frequently
350 occurs as a complication of meningitis, and the site of bacterial CNS invasion remains
351 incompletely understood.

352 In experimentally-infected rhesus macaques, the choroid plexus was the site at which the
353 earliest histopathologic lesions were observed in *H. influenzae* meningitis (144). Following
354 intra-nasal inoculation, *H. influenzae* was isolated from the CSF within the lateral cerebral
355 ventricle and this was associated with intravascular inflammatory infiltration and mild
356 choroid plexitis (145). In 5 – 7 week old pigs, *S. suis* was localized within the choroid plexus
357 and caused disruption of the brush border of the choroid epithelium and loss of the apical
358 cytoplasm (146). Interestingly, autopsy of a 2 month old infant who died of fulminant
359 meningococcemia prior to the onset of meningitis revealed adherent bacteria within the
360 capillaries of the choroid plexus and, to a much lesser extent, the capillaries within the
361 meninges (147). This would appear to support the choroid plexus as the preferential site of *N.*
362 *meningitidis* entry to the CSF; however, no bacteria were found within or between choroid
363 epithelial cells. In contrast, a study of autopsy material from symptomatic patients with

364 meningococcal disease revealed the presence of *N. meningitidis* within the choroid plexus
365 epithelium, interstitium and blood vessels (148).

366 Neonatal rat models of *E. coli* meningitis have been used to identify the potential site of entry
367 to the CSF. Parkkinen *et al*. demonstrated that *E. coli* binds to the luminal surface of choroid
368 epithelial cells, vascular endothelial cells and ependymal cells lining the brain ventricles
369 (102). Similar findings were reported by Zelmer *et al*., who suggested that *E. coli* K1 enters
370 the CSF *via* the choroid plexus, circulates to the subarachnoid space and then adheres to the
371 meninges (103). In contrast, Kim *et al*. reported that in the brains of 5 day old rats, *E. coli*
372 was localized within the perivascular areas of the subarachnoid space, and not within the
373 choroid plexus (20). Recently, the spatiotemporal events of CNS invasion were investigated
374 in a BALB/c mouse model of pneumococcal meningitis (104). *S. pneumoniae* preferentially
375 adhered to vessels within the subarachnoid space at 1 hour post-infection and formed clusters
376 around the endothelium at this location at later time points. Remarkably, pneumococci could
377 only be detected in the choroid plexus at 8 hours post-infection, suggesting that this is not the
378 site of initial entry to the CSF.

379 **Mechanisms of entry**

380 Bacteria may cross the BBB and BCSFB to access the CNS by transcellular penetration,
381 paracellular entry or *via* infected leukocytes from the peripheral circulation (the "Trojan
382 horse" mechanism; Figure 2). Transcellular penetration occurs following bacterial adhesion
383 to endothelial or epithelial cells. Bacteria are subsequently translocated across these barriers
384 by pinocytosis or receptor-mediated mechanisms. Alternatively, bacteria may invade the CNS
385 paracellularly following disruption of the tight junctions between cells forming the BBB
386 and/or BCSFB, resulting in increased permeability. Microorganisms may exploit more than
387 one of these mechanisms to access the CNS.

388 Bacterial attachment to HBMECs is a prerequisite for transcellular and paracellular
389 penetration. There has been significant research into the host-pathogen interactions that occur
390 prior to bacterial invasion of brain microvascular endothelial cells (Table 1), including the
391 identification of host receptors that mediate these interactions. *E. coli*, *H. influenzae*, *N.*
392 *meningitidis* and *S. pneumoniae* bind to HBMECs *via* the 37/67 kDa laminin receptor (149-
393 152). The 37 kDa laminin receptor is a precursor for the mature 67 kDa laminin receptor,
394 which binds laminin-1 within the basement membrane on the abluminal surface of eukaryotic
395 cells (153). Kim *et al*. (150) demonstrated that the 67 kDa laminin receptor is expressed on
396 the basolateral side of HBMECs (i.e. the abluminal surface); however, the cytoxic necrotizing
397 factor-1 (CNF-1) of *E. coli* triggered clustering of the 67 kDa laminin receptor on the apical
398 side (i.e. the luminal surface), where it could potentially interact with bacteria within the
399 cerebral microvasculature. In an *in vivo* model, Orihuela *et al*. reported that fluorescent
400 microspheres coated with laminin receptor-binding adhesins (such as the choline binding
401 protein A (CbpA) of *S. pneumoniae*) adhered to the cerebral endothelium of mice *via* the
402 37/67 kDa laminin receptor following intravascular injection, as demonstrated by cranial
403 window imaging (151). Several bacterial meningeal pathogens have been shown to bind host
404 glycoproteins such as fibrinogen, vitronectin, fibronectin, laminin and collagen, which may
405 act as bridging molecules between the bacterium and HBMECs (109, 154-159). In addition,
406 the heat shock protein gp96 and a gp96 homologue act as receptors for the surface-expressed
407 virulence protein (Vip) of *L. monocytogenes* (160) and the outer membrane protein A
408 (OmpA) of *E. coli* (161, 162) respectively.

409 **Transcellular penetration of brain microvascular endothelial cells**

410 Transcellular penetration of brain microvascular endothelial cells, mainly *via* receptor-
411 mediated mechanisms (Figure 2), has been demonstrated for several bacteria, including *E.*

412 *coli*, *N. meningitidis, S. pneumoniae*, group B streptococcus, *S. suis*, *L. monocytogenes* and
413 *M. tuberculosis*.

414 ***E. coli***

415 *E. coli* translocates from the upper compartment to the lower compartment in a HBMEC
416 Transwell model without increasing cellular permeability, thus supporting a transcellular
417 mechanism of penetration (163). Electron microscopy studies have demonstrated that *E. coli*
418 invades HBMECs through a zipper-like, receptor-mediated endocytosis mechanism (164).
419 Intracellular *E. coli* E44 was observed in membrane-bound vesicles within HBMECs 30
420 minutes post-infection; by 45 minutes the bacteria had translocated to the basolateral side
421 (164). Both early (early endosome antigen 1 and transferrin receptor) and late (ras-related
422 protein-7 and lysosomal-associated membrane protein-1) endosomal markers were recruited
423 to vacuoles containing *E. coli* K1. However, the lysosomal hydrolytic enzyme cathespin D
424 did not accumulate within the vacuoles, demonstrating that lysosomal fusion was prevented.
425 This was found to occur in a capsule-dependent manner (165).

426 Several *E. coli* proteins contribute to the adhesion to, and invasion of, HBMECs (Table 1).
427 Attachment of *E. coli* to HBMECs induces host cytoskeletal rearrangements and actin
428 condensation below adherent bacteria (164). This is associated with a CNF-1-mediated
429 recruitment of focal adhesion kinase and the cytoskeletal protein paxillin to the 67 kDa
430 laminin receptor, which forms clusters that co-localize with adherent *E. coli* (150).
431 Interactions between *E. coli* and HBMECs result in tyrosine phosphorylation of focal
432 adhesion kinase and paxillin. The activity of focal adhesion kinase and its
433 autophosphorylation site tyrosine 397 was shown to be essential for *E. coli* invasion of
434 HBMECs (166). Furthermore, the Src kinase-dependent activation of phosphatidylinositol 3-
435 kinase and its interaction with focal adhesion kinase were required for *E. coli* K1 invasion

436 and host cytoskeletal rearrangement (167, 168). Sukumaran *et al*. demonstrated that the
437 association of phosphatidylinositol 3-kinase with focal adhesion kinase resulted in the
438 downstream activation of protein kinase Cα in an Omp-A-dependent manner (169). Activated
439 protein kinase Cα was recruited to the *E. coli* entry site, where it interacted with its substrate
440 (myristoylated alanine-rich C kinase substrate), leading to an accumulation of actin (169).
441 The activated protein kinase Cα also interacted with caveolin-1 co-localized with condensed
442 actin underneath the bacterial entry site to form caveolae, plasma membrane invaginations
443 that are involved in endocytosis and signal transduction (170). CNF-1 (*via* the 37/67 kDa
444 laminin receptor) and type 1 fimbrial adhesin (FimH, *via* CD48) have also been shown to
445 induce cytoskeletal rearrangements through the activation of the GTPase RhoA (149, 171,
446 172).

447 In an independent mechanism, *E. coli* outer membrane protein A (OmpA) and new
448 lipoprotein-1 (Nlp1) promote the activation of host cytosolic phospholipase A_2 (173, 174).
449 The activation of cytosolic phospholipase A_2 generated arachidonic acid metabolites and
450 induced host actin cytoskeletal rearrangements, and was essential for *E. coli* K1 invasion of
451 HBMECs (173, 175-177). OmpA and invasion of brain endothelial cell protein A (IbeA) also
452 trigger the phosphorylation of STAT3, which results in the activation of Rac1, a Rho family
453 GTPase that regulates host cytoskeletal rearrangements (178). Notably, OmpA is highly
454 conserved between Gram negative bacteria and may play a role in CNS invasion by those in
455 which the mechanisms of entry are unknown. Similarly, a *B. pseudomallei* toxin,
456 *Burkholderia* lethal factor-1 (BLF-1), is structurally similar to *E. coli* CNF-1 and possesses a
457 number of conserved residues that, in CNF-1, promote deamidation of glutamine and result in
458 the activation of RhoA and subsequent host cytoskeletal rearrangements (179). Thus, OmpA
459 and BLF-1 may be attractive targets for future studies into the mechanisms of *B.*
460 *pseudomallei* CNS invasion.

461 ***N. meningitidis***

462 The attachment of *N. meningitidis* to HBMECs is mediated by type IV pili. This initial
463 attachment is inhibited by high cerebral microcirculation shear stress levels; however, once
464 attached, *N. meningitidis* proliferates and is able to resist high blood velocities by forming
465 microcolonies with strong bacteria-bacteria interactions (124) and bacteria-host interactions
466 (122). CD46 was previously identified as the receptor for type IV pili (180); however, this
467 was not corroborated by additional studies and it is likely that the type IV pilus attaches to
468 host cells independently of CD46 (181). The type IV pilus protein PilQ, in addition to the
469 outer membrane porin PorA, have more recently been demonstrated to bind to the 37/67 kDa
470 laminin receptor on HBMECs (151). Furthermore, studies with human bronchial epithelial
471 cells show that the meningococcal type IV pilus binds to the platelet activating factor (PAF)
472 receptor, and synergy between a pilin-linked glycan and phosphorylcholine decorating
473 moieties is required for pili to efficiently engage the receptor (182).

474 *N. meningitidis* is thought to primarily invade HBMECs by paracellular mechanisms (see
475 below); however, *in vitro* studies have demonstrated that small numbers of both encapsulated
476 and non-encapsulated meningococci are internalized by HUVECs and HBMECs (183-185).
477 Following initial bacterial attachment, the meningococcal minor pilin protein, PilV, triggers a
478 cellular response in which endothelial cells form protrusions around *N. meningitidis* (122).
479 These cellular projections further protect the bacterial microcolony from the mechanical
480 stresses associated with high velocity blood flow (122), and also lead to the engulfment and
481 internalization of *N. meningitidis* (186). The host signalling events that contribute to these
482 interactions have been elucidated. Following type IV pilus-mediated attachment, *N.*
483 *meningitidis* activates the host cell β2-adrenoceptor, leading to the translocation of β-arrestin
484 to the site of bacterial attachment at the plasma membrane (187). Several host proteins,
485 including ezrin and moesin, which regulate the cortical cytoskeleton through F-actin binding

486 sites, are then recruited to the site of bacterial attachment and accumulate in honeycombed
487 shaped molecular complexes, referred to as cortical plaques, underneath the meningococcal
488 microcolonies (186, 188, 189). The translocation of β-arrestin also results in the docking and
489 activation of the protein tyrosine kinase Src, which phosphorylates cortactin (187, 188).
490 Cortical actin is then polymerized in a Rho GTPase- and Cdc42-dependent manner, which
491 leads to the formation of cell membrane protrusions (186). In human bone marrow
492 endothelial cells, the recruitment of cortactin and formation of membrane protrusions was
493 also shown to be dependent on the activation of a phosphoinositide-3-kinase/Rac1 signalling
494 pathway by lipooligosaccharide (190).

495 Once internalized, the membrane-bound vesicles containing meningococci associate with
496 transferrin receptor and lysosomal-associated membrane protein-1 endosomal markers (185).
497 It is likely that lysosomal fusion is subverted, as live meningococci have been shown to
498 translocate from the basolateral side to the apical side in a Transwell BCSFB invasion model
499 (140). *In vitro* studies have demonstrated that capsular polysaccharide inhibits the invasion of
500 *N. meningitidis* into HBMECs (191) and human choroid plexus epithelial cells (140), but is
501 essential for survival and replication within the intracellular niche (185).

502 In non-encapsulated meningococcal strains, the invasion of HBMECs is dependent upon
503 outer membrane protein C (Opc). Opc binds to the human serum factor fibronectin, which
504 acts as a bridging molecule and anchors the bacterium to the $\alpha_5\beta_1$ integrin receptor of
505 HBMECs (191). This interaction results in the activation of mitogen-activated protein kinase
506 (c-Jun N-terminal kinase (JNK)1, JNK2 and p38) and protein tyrosine kinase signal
507 transduction pathways (192). Pre-treatment of cells with specific JNK1, JNK2 and protein
508 tyrosine kinase inhibitors significantly reduced the internalization of *N. meningitidis* by
509 HBMECs, without affecting bacterial adherence. Blocking of the $\alpha_5\beta_1$ integrin receptor of
510 HBMECs also decreased JNK activation in the presence of *N. meningitidis*. Furthermore, the

511 use of Opc-deficient mutant strains demonstrated that JNK signalling, but not p38 signalling,
512 was mediated by Opc expression (192). A more recent study identified that Opc preferentially
513 binds to activated vitronectin within human serum and this interaction promotes *N.*
514 *meningitidis* invasion of HBMECs (155). *In vitro*, the enhanced invasion of non-encapsulated
515 *N. meningitidis* strains is thought to be due to the unmasking of Opc; however, the role of
516 Opc *in vivo* is yet to be determined.

517 ***S. pneumoniae***

518 *S. pneumoniae* is internalized by human and rat brain microvascular endothelial cells *via*
519 receptor-mediated endocytosis. This uptake does not involve the formation of cellular
520 membrane protrusions, as discussed for *N. meningitidis* (193). The adhesion of pneumococci
521 to HBMECs is mediated by interactions between CbpA and the 37/67 kDa laminin receptor
522 (151), and neuraminidase A (NanA) and an unknown cellular receptor(s) (194). Uchiyama *et*
523 *al*. demonstrated that NanA, a surface-anchored sialidase, was necessary and sufficient to
524 promote pneumococcal adhesion to, and invasion of, HBMECs (195). Following initial
525 attachment, the laminin G-like domain of NanA initiates chemokine signalling and
526 inflammatory activation of endothelial cells (194). The activation of endothelial cells results
527 in the increased expression of PAF receptor - the receptor for pneumococcal
528 phosphorylcholine, which is a component of the cell wall and acts as a molecular mimic of
529 the chemokine PAF (196).

530 In HBMECs, *S. pneumoniae* within vacuoles may: (i) transit through the cell to the
531 basolateral surface; (ii) recycle back to the apical surface; or (iii) be killed within the vacuole
532 (193). Opaque *S. pneumoniae* variants, which express more capsular polysaccharide and less
533 phosphorylcholine than transparent variants, were efficiently killed, suggesting that vacuoles
534 containing these strains were targeted for lysosomal fusion. In contrast, transparent

535 pneumococci may undergo some recycling back to the apical surface; however, by 5 hours
536 post-infection in a Transwell system, the majority of bacteria had translocated to the
537 basolateral chamber (193). The invasion of HBMECs by transparent *S. pneumoniae* depends
538 on phosphorylcholine, the PAF receptor and β-arrestin, which targets G-protein coupled
539 receptors (such as PAF receptor) to clathrin-coated vesicles (193, 197). *S. pneumoniae*
540 induces the translocation of β-arrestin from the cytosol to the plasma membrane, where it co-
541 localizes with the PAF receptor. This interaction also stimulates mitogen-activated protein
542 kinase signalling, which is required for pneumococcal uptake (197). Vacuoles containing *S.*
543 *pneumoniae* co-localized with early and late endosomal markers; however increased
544 expression of β-arrestin subverted these vacuoles from lysosomal trafficking and promoted
545 the transcytosis of viable bacteria. The small number of pneumococci-containing vacuoles
546 that underwent recycling to the apical surface demonstrated co-localisation with ras-related
547 protein-11, which regulates endosome recycling. These findings suggest that the
548 pneumococcal-induced interactions between PAF receptor and β-arrestin contribute to the
549 transcellular penetration of HBMECs by *S. pneumoniae* (197).

550 **Group B streptococcus**

551 Several group B streptococcal proteins promote the adhesion to, and invasion of, HBMECs
552 (Table 1). The hypervirulent group B streptococcus adhesin (HvgA) was identified as a
553 sequence type-17 (ST-17)-specific virulence factor, which is anchored to the group B
554 streptococcal cell wall by sortase A (198). ST-17 is a clonal complex belonging to the group
555 B streptococcus capsular serotype III, and is responsible for > 80% of neonatal meningitis
556 cases. Therefore, it was proposed that HvgA expression may contribute to the hypervirulence
557 of ST-17. *In vitro* studies using an isogenic *hvgA* mutant demonstrated that HvgA was
558 required for efficient group B streptococcal adhesion to HUVECs, primary rodent choroid
559 plexus epithelial and brain microvascular endothelial cells, and immortalized HBMECs.

560 Furthermore, in a murine model of hematogenous meningitis, HvgA was required for CNS
561 invasion, although had no effect on the levels of bacteremia (198).

562 A common characteristic of several other group B streptococcal adhesins/invasins is the
563 ability to bind components of the HBMEC extracellular matrix, such as laminin, collagen and
564 fibronectin (109, 157, 159, 199). The group B streptococcal pilus tip adhesin (PilA) and the
565 recently identified streptococcal fibronectin binding protein A (SfbA) bind immobilised
566 collagen and fibronectin respectively. The collagen and fibronectin then associate with
567 HBMECs *via* integrins, and these interactions facilitate the entry of group B streptococcus
568 (109, 199). Similarly, the group B streptococcal serine-rich repeat (Srr-1) glycoprotein binds
569 the fibrinogen Aα chain within human blood through a dock, lock and latch mechanism (200,
570 201). During this interaction, fibronectin docks between two IgG-like folds (N2 and N3
571 domains) of the binding region of the adhesin, which initiates a conformational change
572 whereby a flexible region of the N3 domain latches to form a β-strand and completes a β-
573 sheet within the N2 domain, effectively locking the ligand in place (200). Seo *et al.*
574 demonstrated that deletion of the latch-like domain of the C-terminal end of the Srr-1
575 fibrinogen binding region significantly reduced group B streptococcal adhesion to HBMECs
576 (201). The fibrinogen-binding protein (FbsA) also mediates group B streptococcal adherence
577 to HBMECs by binding immobilised fibrinogen (156), although it is unknown if this
578 interaction also occurs *via* a dock, latch and lock mechanism.

579 Electron microscopy studies have demonstrated that *in vitro*, group B streptococcus
580 associates closely with the cell membrane of HBMECs, and is enveloped by microvillus
581 structures (202). Group B streptococci are internalized within membrane-bound vesicles and
582 translocate from the apical side to the basolateral side of HBMECs without a marked change
583 in transendothelial electrical resistance. Serotype III group B streptococci were shown to
584 invade HBMECs more efficiently than representative strains of serotypes Ia, Ib, II and V;

585 however, the serotype III capsule itself did not facilitate group B streptococcal invasion
586 (202). Internalized group B streptococci did not undergo significant replication and survived
587 within HBMECs for up to 20 hours (202). Intracellular survival of group B streptococcus
588 may be promoted by the CiaR/CiaH two-component regulatory system, which regulates
589 several genes associated with stress tolerance and the subversion of host defences (203). A
590 serotype III strain that was deficient in the CiaR response regulator demonstrated similar
591 adhesion and invasion levels to wild type group B streptococcus in HBMECs, but was
592 associated with a significant decrease in intracellular survival. Broth inhibition assays
593 demonstrated that CiaR conferred resistance to antimicrobial peptides, lysozyme and reactive
594 oxygen species (203).

595 The host signal transduction pathways that are involved in the uptake of group B
596 streptococcus by HBMECs are similar to those discussed for *E. coli*. *In vitro*, the tri-
597 molecular interactions between PilA, collagen and $\alpha_2\beta_1$ integrins on HBMECs stimulate the
598 phosphorylation of focal adhesion kinase at tyrosine 397, which then induces host
599 cytoskeletal rearrangements and the uptake of group B streptococci *via* the recruitment and
600 activation of phosphatidylinositol 3-kinase and paxillin (109, 204). It has also been
601 demonstrated that group B streptococcus invasion occurs *via* cytoskeletal rearrangements that
602 are induced following the activation of RhoA and Rac1 (205). Furthermore, group B
603 streptococcus induces serine 505 phosphorylation of host cytosolic phospholipase A_2, which
604 leads to the release of arachidonic acid metabolites including cysteinyl leukotrines (206). *In*
605 *vitro* pharmacological inhibition studies demonstrated that the activation of cytosolic
606 phospholipase A_2 was required for efficient group B streptococcal invasion of HBMECs. In
607 addition, brain colonization by group B streptococcus was significantly reduced in mice
608 deficient in cytosolic phospholipase A_2 compared to wild type animals in a model of
609 hematogenous meningitis. Following cytosolic phospholipase A_2 activation and the release of

610 cysteinyl leukotrines, downstream signalling activates protein kinase Cα, which is involved in
611 the regulation of actin cytoskeletal rearrangements (206).

612 ***S. suis***

613 The zoonotic pathogen *S. suis* adheres to, but does not invade, HBMECs *in vitro* (207). In
614 contrast, invasion of porcine brain microvascular endothelial cells has been observed for this
615 swine pathogen. Inhibition studies demonstrated that *S. suis* invasion of porcine brain
616 microvascular endothelial cells required actin filaments, but not microtubular cytoskeletal
617 elements, or active bacterial RNA or protein synthesis (208). Electron microscopy studies
618 have shown that *S. suis* adheres to porcine brain microvascular endothelial cells 5 minutes
619 post-infection, and at 2 hours post-infection the streptococci were in close contact with the
620 cells, within invaginated cell membrane structures and underneath the cell surface behind the
621 cell membrane. Unlike group B streptococci, following internalization within membrane-
622 bound vacuoles, the number of viable intracellular *S. suis* steadily decreased over time, and
623 viable cocci were not detected after 7 hours (208). However, similar to *S. pneumoniae* and
624 group B streptococcus, the capsular polysaccharide of *S. suis partially* inhibited interactions
625 with brain microvascular endothelial cells (208).

626 The *S. suis* proteins and virulence factors that mediate invasion of porcine brain
627 microvascular endothelial cells remain largely unknown. Pre-treatment of porcine brain
628 microvascular endothelial cells with *S. suis* lipoteichoic acid reduced, but did not abolish, the
629 adhesion and invasion of *S. suis* in a dose-dependent manner, suggesting that lipoteichoic
630 acid may play a role in *S. suis* interactions with porcine brain microvascular endothelial cells
631 (209). Vanier *et al*. screened a transposon mutagenesis library of *S. suis* and identified several
632 genes that may contribute to the invasion of porcine brain microvascular endothelial cells
633 (210). These genes were characterized as belonging to the following groups: surface proteins,

634 transport/binding proteins, regulatory functions, metabolism, amino acid synthesis, protein
635 synthesis and hypothetical proteins. However, the role of these genes in *S. suis* invasion of
636 porcine brain microvascular endothelial cells has not been confirmed using isogenic mutants.

637 As discussed above, the choroid plexus may be the site of entry into the CNS for *S. suis*. In
638 order to study the interactions between *S. suis* and choroid plexus epithelial cells, Tenenbaum
639 *et al*. developed an inverted Transwell model, in which the translocation of *S. suis* from the
640 basolateral 'blood' side to the apical 'CSF' side could be investigated (211). Following *S.*
641 *suis* infection of the basolateral side, the transepithelial electrical resistance and paracellular
642 permeability remained constant for up to 4 hours. *S. suis* invaded porcine choroid plexus
643 epithelial cells from the basolateral side in the inverted Transwell system; however, invasion
644 was rare when *S. suis* was inoculated into the apical chamber in the standard Transwell
645 model. These findings were also replicated in a human choroid plexus epithelial cell inverted
646 Transwell model (140). This suggested that *S. suis* may interact with components of the
647 extracellular matrix that are accessible on the basolateral side. The translocation of *S. suis*
648 through porcine brain microvascular endothelial cells was inhibited when cells were treated
649 with a phosphatidylinositol 3-kinase inhibitor, demonstrating that phosphatidylinositol 3-
650 kinase may be involved in the uptake of *S. suis* (211).

651 ***L. monocytogenes* and *B. pseudomallei***

652 *L. monocytogenes* can enter the CNS by the Trojan horse mechanism (see below) or by
653 transcellular penetration of HBMECs. Using HUVECs and HBMECs, it was demonstrated
654 that *L. monocytogenes* can invade endothelial cells directly, or *via* cell-to-cell spread from
655 adherent, infected mononuclear phagocytes (212, 213). In HUVECs, *L. monocytogenes*
656 attaches to the cellular surface and induces membrane ruffling, which leads to internalization
657 (214). In HBMECs, *L. monocytogenes* invasion is preceded by intimate interactions between

658 the bacterium and microvilli on the cell surface (215). The invasion of *L. monocytogenes* into
659 HBMECs requires actin microfilaments (213), but unlike other meningeal pathogens, does
660 not involve the activation of phosphatidylinositol 3-kinase (213) or cytosolic phospholipase
661 A_2 (175).

662 Once internalized, *L. monocytogenes* degrades the phagosome *via* the activity of a pore-
663 forming toxin (listeriolysin) and two phospholipases (PlcA and PlcB), and escapes into the
664 cytoplasm using actin-based motility, which is driven by the actin assembly-inducing protein,
665 ActA. Following escape into the cytoplasm, *L. monocytogenes* can replicate within HUVECs
666 and HBMECs and use actin-based motility to spread to adjacent cells (212, 213), thus
667 avoiding an extracellular lifestyle. However, *L. monocytogenes* can be isolated from the CSF
668 (216), suggesting that translocation through the cellular barriers of the CNS also occurs.

669 *B. pseudomallei* has a similar intracellular lifestyle to *L. monocytogenes*: the type III
670 secretion system of *B. pseudomallei* (particularly the BopE and BopA effector proteins, and
671 the Bsa translocation apparatus) is required for escape from the vacuole and the subversion of
672 autophagy (217-222). In an epithelial cell line or a murine macrophage-like cell line, cell-to-
673 cell spread then occurs by cell fusion, with the formation of multinucleated giant cells, in a
674 type VI secretion-dependent process; BimA, necessary for actin-mediated motility, or Fla2,
675 which is thought to mediate intracellular motility are also required and are considered to
676 mediate prior cell-to-cell contact prior to cell fusion (223-227). To our knowledge, the ability
677 of *B. pseudomallei* to directly invade HBMECs has not been investigated, but it would be
678 reasonable to hypothesize that the intracellular lifestyle of *B. pseudomallei* may contribute to
679 the pathogenesis of CNS invasion. Interestingly, a recent study investigated the variable
680 virulence factors of *B. pseudomallei* associated with melioidosis in Australia, and reported
681 that *B. pseudomallei* strains harboring a *bimA* allele that shares 95% homology with *B. mallei*
682 *BimA* (*bimA*$_{\mathrm{Bm}}$) (228) were significantly associated with neurological melioidosis (229).

683 Patients that were infected with *B. pseudomallei* harboring the *bimA*$_{\mathrm{Bm}}$ allele were found to be
684 14 times more likely to present with neurological involvement compared to patients infected
685 with strains harboring the *bimA*$_{\mathrm{Bp}}$ variant (229), indicating a role for actin-mediated motility
686 in either transgression of the BBB/BCSFB or transit to the brain *via* the olfactory or
687 trigeminal nerve pathways (see below).

688 Several studies have shown that internalin B (InlB) is required for *L. monocytogenes* invasion
689 of HUVECs (212-214) and HBMECs (213, 215). Greiffenberg *et al*. demonstrated that
690 although *L. monocytogenes* deficient in InlB could adhere to HBMECs at comparable levels
691 to the wild type strain, the invasive capability of the InlB mutant was reduced by > 100-fold
692 (215). In contrast, one study reported that neither InlA nor InlB were required for *L.*
693 *monocytogenes* invasion into HUVECs (230). It was suggested that these inconsistent
694 findings may be attributable to differences in experimental conditions, especially involving
695 the addition of normal human serum, which markedly affects InlB-mediated invasion due to
696 the presence of anti-*Listeria* antibodies (231). In an inverted Transwell model of human
697 choroid plexus epithelial cells, the cellular receptors for InlA and InlB (E-cadherin and met
698 receptor tyrosine kinase respectively) were expressed on the basolateral 'blood' side, but not
699 the apical 'CSF' side (232). In this model, *L. monocytogenes* invaded human choroid plexus
700 epithelial cells exclusively from the basolateral side, and both InlA and InlB were required
701 for efficient invasion.

702 Other *L. monocytogenes* virulence factors that may play a role in the attachment to, and
703 invasion of, HBMECs include Vip and the autolysin IspC. Vip is a surface-expressed protein
704 that is absent from non-pathogenic *Listeria* and binds to gp96 on host cells (160). A *vip*
705 allelic replacement mutant was shown to be significantly less invasive than wild type *L.*
706 *monocytogenes* in Caco-2 cells and L2071 mouse fibroblasts, but was not investigated in
707 HBMECs. However, in intravenously inoculated mice, Δ*vip* was attenuated for virulence and

708 associated with a significant decrease in bacterial loads within the brain (160). Similarly,
709 IspC was also shown to contribute to *L. monocytogenes* invasion of the brain in a mouse
710 model of hematogenous meningitis (233). Interestingly, the deletion of IspC did not affect the
711 ability of *L. monocytogenes* to attach to, and invade HBMECs *in vitro*. In contrast, Δ*ispC*
712 demonstrated a significant reduction in attachment and invasion in sheep choroid plexus
713 epithelial cells (compared to wild type *L. monocytogenes*), and purified IspC was capable of
714 binding to these cells. Proteomic analyses revealed that IspC regulates the expression of other
715 virulence factors, including ActA, InlC2 and a flagellin homologue (FlaA); therefore the
716 phenotype observed by the Δ*ispC* strain may be a result of the deletion of IspC in
717 combination with a reduction in the expression of these factors (233).

718 ***M. tuberculosis***

719 *M. tuberculosis* translocates from the apical chamber to the basolateral chamber in a
720 Transwell model of HBMECs (118). *In vitro*, *M. tuberculosis* interacts with microvillus-like
721 protrusions on HBMECs and is observed intracellularly, but not paracellularly. Antibiotic
722 protection assays demonstrated that *M. tuberculosis* invaded HBMECs. Furthermore, *M.*
723 *tuberculosis* co-localized with actin on the cell surface. Treatment of HBMECs with
724 cytochalasin D significantly decreased *M. tuberculosis* invasion, suggesting that actin
725 polymerization is required for internalization (118).

726 The mechanisms and host-pathogen interactions involved in CNS invasion by *M. tuberculosis*
727 remain incompletely understood. In an *in vitro* BBB model consisting of bovine brain
728 microvascular endothelial cells (BBMECs) co-cultured with rat astrocytes, the addition of
729 purified recombinant heparin-binding haemagglutinin adhesin (rHBHA) induced actin
730 filament rearrangement (234). This was shown to be dependent on the ability of rHBHA to
731 bind to heparan sulfate glycosaminoglycans on the surface of BBMECs. The role of HBHA

in brain microvascular endothelial cell invasion by *M. tuberculosis* has not been investigated in the context of live bacteria. Furthermore, gene expression profiling of *M. tuberculosis* associated with HBMECs *in vitro* (versus non-adherent bacteria), did not identify HBHA as a gene that was significantly upregulated during infection (118). Jain *et al*. demonstrated that 33 genes were upregulated > 8 fold in *M. tuberculosis* associated with HBMECs. Transposon mutants were created to determine if these upregulated genes were associated with HBMEC invasion; of the 33 tested mutants, 4 genes (*Rv0980c*, *Rv0987*, *Rv0989c* and *Rv1801*) were required for efficient invasion and/or intracellular survival (118).

In vivo screening of transposon mutant libraries has also been employed to identify potential *M. tuberculosis* virulence factors that are associated with CNS invasion. Be *et al.* inoculated pools of *M. tuberculosis* mutants intravenously into BALB/c mice and determined that mutants deficient in the expression of 5 genes (*Rv0311*, *Rv0805*, *Rv0931c*, *Rv0986* and *MT3280*) were significantly attenuated in the brain, but not the lungs. *In vitro* studies confirmed that these mutants invaded HBMECs at significantly reduced levels compared to a negative control transposon mutant (235). *Rv0931c* (also known as *pknD*) encodes a serine-threonine protein kinase, and was also identified as a key microbial factor required for brain infection in a guinea pig model of hematogenous meningitis, from a library of 398 mutants (158). When studied independently in a mouse model of infection, the *pknD* mutant was associated with a significant reduction in bacterial loads within the brain, but not the lungs. *In vitro*, *pknD* was required for efficient invasion and intracellular survival within HBMECs, this phenotype was not observed in HUVECs, A549 lung epithelial cells or J447 macrophages. Microspheres coated with *pknD* adhered to HBMECs at significantly higher levels that microspheres coated with bovine serum albumin, and it was proposed that *pknD* binds to HBMECs *via* interactions with laminin within the extracellular matrix (158).

Paracellular penetration of brain microvascular endothelial cells

757 The breakdown of tight junctions between cells of the BBB and/or BCSFB may occur due to
758 the activity of microbial virulence factors/toxins, and/or inflammatory-mediated processes.
759 Early studies in intracisternally infected rats demonstrated that *E. coli* K1, *S. pneumoniae* and
760 *H. influenzae* type B caused an increase in pinocytotic vesicle formation in the cerebral
761 capillary endothelium, in addition to the complete separation of intercellular junctions (236).
762 Further experiments with *H. influenzae* showed that these cerebral capillary morphological
763 changes were associated with a significant increase in BBB permeability to circulating ^{125}I-
764 albumin (236). This suggests that bacterial meningeal pathogens may open a paracellular
765 route of CNS entry, although these data should be interpreted cautiously as intracisternal
766 inoculation cannot be used to model the physiological events that occur during hematogenous
767 meningitis, as the inoculum is injected directly into the cisternal spaces between the
768 arachnoid and pia mater. The development of *in vitro* models of the human BBB/BCSFB and
769 *in vivo* models of hematogenous meningitis has enabled microbial invasion *via* the
770 paracellular route to be studied in some detail. From these studies (reviewed below for
771 specific pathogens), it appears that microbes may initially attach to and transcellularly invade
772 brain microvascular endothelial cells, and that these host-pathogen interactions may lead to a
773 subsequent increase in barrier permeability and a loss of tight junctions. Thus it is likely that
774 both transcellular and paracellular mechanisms of CNS entry are exploited by bacterial
775 pathogens. It is of considerable clinical importance that the breakdown of the BBB/BCSFB in
776 bacterial meningitis also results in generally higher levels of antibiotics in the CSF.

777 ***E. coli***

778 Several *E. coli* virulence factors promote the destruction of tight junctions between brain
779 microvascular endothelial cells. In addition to triggering the internalization of *E. coli* K1 into
780 HBMECs, the binding of OmpA to its gp96 homologue receptor stimulates inducible nitric
781 oxide synthase and nitric oxide production, which enhances the generation of cyclic GMP

782 (237). This increase in cyclic GMP activates protein kinase Cα (237), which leads to a ras
783 GTPase-activating-like protein (IQGAP1)-mediated dissociation of β-catenin from vascular
784 endothelial-cadherin at adherens junctions (238). Immunofluorescence experiments
785 demonstrated that vascular endothelial-cadherin was redistributed from the intercellular
786 junctions to the sites of bacterial attachment (239). This resulted in a significant increase in
787 HBMEC permeability to horseradish peroxidase and a parallel decrease in transendothelial
788 electrical resistance (239). Furthermore, the activation of host cytosolic phospholipase A_2 by
789 *E. coli* OmpA and Nlp1 may play a role in reducing the integrity of the BBB. Using co-
790 cultures of primary BBMECs and primary bovine retinal pericytes seeded onto Transwell
791 membranes, Salmeri *et al*. demonstrated that the arachidonic acid released following
792 cytosolic phospholipase A_2 activation acts as a substrate for cyclooxygenase to produce
793 prostaglandins, which trigger the synthesis of vascular endothelial growth factor by brain
794 endothelial cells (119). This was associated with a significant decrease in transendothelial
795 electrical resistance and a dramatic detachment of pericytes from the Transwell membrane.
796 These effects were not observed when the pericyte vascular endothelial growth factor
797 receptor-1 was blocked using a polyclonal antibody. These findings suggest that the vascular
798 endothelial growth factor released by brain endothelial cells may bind to the vascular
799 endothelial growth factor receptor-1 on adjacent pericytes, and trigger ablation of the
800 pericytes from the basal membrane, potentially opening a paracellular route (119).

801 *In vitro* studies using the mouse brain microvascular endothelial cell line, Bend.3, have
802 demonstrated a role for lipopolysaccharide (LPS, *E. coli* 055:B5) in inducing BBB
803 permeability. Similar to OmpA, LPS was shown to activate protein kinase C isoforms, which
804 led to the activation of RhoA, and the stimulation of downstream nuclear factor-κB signalling
805 and myosin light chain phosphorylation (240, 241). These signalling events were associated
806 with a decrease in the expression of the tight junction proteins claudin-5 and zonula

807 occludens-1, and a reduction in transendothelial electrical resistance. LPS also induced
808 changes in F actin organization, leading to paracellular gaps and stress fiber formation (240).
809 In Shiga toxin-producing *E. coli* associated with hemolytic uremic syndrome and CNS
810 involvement, both Shiga toxin-1 and Shiga toxin-2 induce cytotoxic effects that lead to a
811 decrease in BBB integrity and cellular injury *in vivo* and *in vitro* (242-245). LPS may mediate
812 these effects (242, 245).

813 ***H. influenzae***

814 Quagliarello *et al*. first demonstrated that *H. influenzae* caused an increase in BBB
815 permeability and tight junction disruption in adult Wistar rats following intracisternal
816 inoculation of approximately 10^6 colony forming units (236). Using this intracisternal
817 inoculation model, the role of *H. influenzae* type B lipooligosaccharide (LOS) was
818 investigated. Purified LOS and outer membrane vesicles containing non-cell associated LOS
819 induced a dose-dependent increase in BBB permeability to ^{125}I-bovine serum albumin and
820 CSF pleocytosis (246, 247). In leukopenic rats, no increases in BBB permeability and CSF
821 leukocyte counts were observed following LOS or outer membrane vesicle challenge,
822 suggesting that the decrease in BBB integrity is mediated by the intact host leukocyte
823 response (246, 247). In contrast, Tunkel *et al*. demonstrated that *H. influenzae* type B LOS
824 significantly increased the permeability of rat brain microvascular endothelial cells to ^{125}I-
825 albumin in the absence of host inflammatory cells *in vitro* (248). Furthermore, in the absence
826 of monocytic cells, purified LOS was cytotoxic to BBMECs. However, *in vivo* studies have
827 shown that nitric oxide production within the CSF is required for LOS-mediated pleocytosis
828 and increased BBB permeability (249), and that host PAF synergizes with *H. influenzae* LOS
829 to augment these events (250).

830 Peptidoglycan within the *H. influenzae* cell wall may also contribute to BBB/BCSFB
831 dysfunction *in vivo*. Intracisternally inoculated peptidoglycan induced an increase in BBB
832 permeability to ^{125}I-albumin (251) and CSF leukocytosis (252), although the CSF
833 leukocytosis was attenuated compared to animals challenged with LOS (252). It was
834 subsequently hypothesized that *H. influenzae* cell wall components, such as peptidoglycan,
835 activate brain microvascular endothelial cells and induce the separation of intercellular
836 junctions. LOS may then augment this response by activating the recruited leukocytes to
837 produce pro-inflammatory cytokines that further contribute to BBB breakdown (252);
838 however, the mechanisms by which this may occur have not been elucidated. The *H.*
839 *influenzae* type B porin may also contribute to these events. The inoculation of purified porin
840 into the fourth cerebral ventricle of rats induced the expression of interleukin (IL)-1α, tumor
841 necrosis factor-α (TNF-α) and macrophage inflammatory protein-2, which preceded an
842 increase in BBB permeability to serum proteins and leukocytes, and increased brain water
843 content (108).

844 ***N. meningitidis***

845 As discussed above, the *N. meningitidis*-induced β2-adrenoceptor/β-arrestin signalling in
846 HBMECs results in the formation of cortical plaques and membrane protrusions. In parallel,
847 the type IV pilus-mediated translocation of β-arrestin to the cortical plaque also acts as a
848 molecular scaffold for additional *N. meningitidis*-induced signalling events that open the
849 paracellular route (187). These events include the recruitment of the Par3/Par6/PKCζ polarity
850 complex to the cortical plaque in a Cdc42-dependent manner, and the accumulation of
851 adherens junction (vascular endothelial-cadherin, p120-catenin, β-catenin) and tight junction
852 (zonula occludens-1, zonula occludens-2, claudin-5) proteins underneath the site of bacterial
853 adhesion (253). Labelling of vascular endothelial-cadherin demonstated that *N. meningitidis*
854 delocalized this protein from the adherens junctions between HBMECs and caused it to be

855 redistributed to the cortical plaque. This depletion of junctional proteins was associated with
856 an increase in barrier permeability to Lucifer yellow and the formation of gaps between cells,
857 thus opening a paracellular route of entry (253). Interestingly, the recruitment of junctional
858 components by *N. meningitidis* occurs only in endothelial cells, as it was demonstrated that
859 meningococci do not activate the β2-adrenoceptor/β-arrestin signalling pathway in epithelial
860 cells (254). Therefore, different host-pathogen interactions may occur at the BBB and
861 BCSFB.

862 In an inflammatory-mediated process, *N. meningitidis* stimulates HBMECs to produce matrix
863 metalloproteinase-8, which was shown to cleave occludin and cause it to dissociate from tight
864 junctions *in vitro* (255). The cleavage of occludin was prevented in the presence of matrix
865 metalloproteinase-8 inhibitors and in cells transfected with matrix metalloproteinase-8
866 siRNA. Inhibition of matrix metalloproteinase-8 activity also significantly decreased the *N.*
867 *meningitidis*-induced permeability of HBMECs to 40 kDa FITC-dextran at 24 hours.
868 Furthermore, the inhibition of matrix metalloproteinase-8 prevented the caspase-independent
869 detachment of HBMECs from a matrigel matrix on a solid support following *N. meningitidis*
870 infection (255).

871 ***Streptococcus* spp.**

872 Group B streptococcus, *S. pneumoniae* and *S. suis* invade brain microvascular endothelial
873 cells *via* transcellular mechanisms; however, the associated production of pro-inflammatory
874 cytokines, the activity of pore-forming toxins and the induction of plasmin activity may also
875 lead to a concurrent weakening of the BBB, potentially opening a paracellular route of entry.
876 *In vivo* and *in vitro* studies have shown that group B streptococci, pneumococci and *S. suis*
877 induce the production of a range of cytokines and chemokines, including IL-1β, IL-6, IL-8,
878 IL-10, TNF-α, MCP-1, GM-CSF, Gro-α and Gro-β in brain microvascular endothelial cells

879 (109, 256-258) and brain tissue (94, 259, 260). The stimulation of HBMECs with cytokines
880 (including TNF-α, IL-1β, IL-6 and IL-17F) leads to cytoskeletal rearrangements and a
881 redistribution of tight junction and adherens junction proteins, resulting in a decrease in
882 barrier integrity (261-267). Therefore, the host inflammatory response to group B
883 streptococcus, *S. pneumoniae* and *S. suis* meningitis may contribute to the disruption of tight
884 junctions. In HBMECs, the group B streptococcal PilA-mediated activation of focal adhesion
885 kinase induces bacterial uptake, but also triggers a parallel mitogen-activated protein
886 kinase/extracellular signal-regulated kinase pathway, which leads to the secretion of IL-8
887 (109). Furthermore, the production of the IL-8 homologue (KC) in mice intravenously
888 inoculated with group B streptococci resulted in the recruitment of neutrophils and an
889 increase in BBB permeability to Evans blue and FITC-albumin (109). It is likely that
890 disruption of the BBB/BCSFB by host inflammatory factors represents a common
891 physiological event that occurs during bacterial CNS invasion.

892 Using a separate mechanism, group B streptococcus, *S. pneumoniae* and *S. suis* may hijack
893 the host plasminogen system to cause cellular injury and disrupt tight junctions between
894 HBMECs. Plasminogen is found in plasma and extracellular fluids, and upon activation is
895 converted to plasmin, which degrades the extracellular matrix and may upregulate matrix
896 metalloproteinases that degrade tight junctions (268). *In vitro*, plasminogen binds to
897 streptococcal glyceraldehyde-3-phosphate dehydrogenase (269-271). Enolase and choline-
898 binding protein E have also been identified as additional pneumococcal plasminogen binding
899 proteins (272, 273). Once plasminogen is bound to its binding protein, it becomes activated
900 and is subsequently converted to plasmin by exogenous tissue plasminogen activator and
901 urokinase (269, 270, 274). Plasmin bound to the surface of group B streptococci enhanced the
902 ability of bacteria to adhere to, and invade HBMECs, and induced a significant decrease in
903 transendothelial electrical resistance (275). Furthermore, streptococcal-bound plasmin

904 contributed to cellular injury, characterized by human brain microvascular cell detachment
905 and lactate dehydrogenase release (275). In the human vascular endothelial cell line EaHy,
906 plasmin bound to the pneumococcal cell surface cleaved vascular endothelial-cadherin from
907 adherens junctions and increased bacterial translocation across endothelial barriers (276).
908 Taken together, group B streptococcus, *S. pneumoniae* and *S. suis* may bind host
909 plasminogen and hijack the proteolytic activity of plasmin to potentially open a paracellular
910 route between cells. Plasminogen has also been shown to bind to *N. meningitidis* (277, 278),
911 suggesting that this mechanism may be exploited by several common meningeal pathogens.

912 Group B streptococcus, *S. pneumoniae* and *S. suis* produce pore-forming hemolysins named
913 β-hemolysin/cytolysin, pneumolysin and suilysin respectively. In HBMECs, these
914 hemolysins are cytotoxic and promote lactate dehydrogenase release, the loss of cytoplasmic
915 density, discontinuity of cytoplasmic membranes, clumping of nuclear chromatin, dislation of
916 the endoplasmic reticulum, cell rounding and detachment (97, 202, 207). These
917 morphological changes most likely result in the formation of paracellular gaps. The effects of
918 pneumolysin have also been investigated in rat astrocytes; the addition of pneumolysin to
919 monolayers of primary astrocytes resulted in cell shrinkage and the subsequent separation of
920 cells from each other (107). In rat brain slices, exposure to pneumolysin led to astrocytic
921 process retraction and reorganization of astrocytes within the glia limitans. These
922 pneumolysin-induced changes were associated with increased interstitial fluid retention and
923 BBB permeability, which facilitated the penetration of macromolecules and bacteria into
924 brain slices (107). These data suggest that in addition to mediating cytotoxic effects in brain
925 microvascular endothelial cells, bacterial hemolysins may also induce morphological changes
926 in other components of the neurovascular unit.

927 **Trojan horse penetration of brain microvascular endothelial cells**

Bacteria that are capable of surviving within host peripheral immune cells have the ability to invade the CNS *via* the Trojan horse route. This route of entry has been suggested for *L. monocytogenes* (279), *B. pseudomallei* (280), *S. suis* (146) and *M. tuberculosis* (281). In C57Bl/6 x DBA/2 mice, *L. monocytogenes*-infected peripheral blood leukocytes disseminated to the CNS and were shown to induce brain colonization more efficiently than extracellular *L. monocytogenes* (279). Furthermore, CNS infection was not reduced following the elimination of extracellular *L. monocytogenes* with gentamicin delivered by surgically implanted osmotic pumps (282), suggesting that intracellular bacteria were responsible for neuroinvasion. Drevets *et al*. demonstrated that in C57Bl/6 mice, *L. monocytogenes* associated with CD11b+ monocytes, and further analysis revealed that most of these infected monocytes belonged to the Ly-6C^{high} subset (283). Following systemic *L. monocytogenes* infection, 90% of CD11b+ leukocytes isolated from the brain were Ly-6C^{high} and the influx of these cells correlated with an increase in brain colonization, suggesting that Ly-6C^{high} monocytes may act as Trojan horses *in vivo* (283). In BALB/c mice, bone marrow myelomonocytic cells (CD31+: Ly-6C+: CD11b+: Ly-6G^{low}) may also transport *L. monocytogenes* to the CNS (284).

In a hematogenous model of melioidosis meningitis, *B. pseudomallei* established a primary focus of infection in the spleen, followed by migration to the bone marrow and subsequent spread to the brain (280). *B. pseudomallei* associated with CD11b+ cells within the bone marrow and spleen, and it was hypothesized that these cells may serve as Trojan horses that facilitate bacterial spread to the brain (280). In adoptive transfer experiments, splenic CD11b+ cells and bone marrow CD11b+ cells harboring *B. pseudomallei* induced brain colonization. In contrast, bacterial burdens within the brain were attenuated following the adoptive transfer of *B. pseudomallei*-infected CD11b- cells, and equivalent numbers of extracellular *B. pseudomallei* were unable to colonize the brain. These data suggest that *B. pseudomallei*-infected CD11b+ cells within the spleen and bone marrow may disseminate to

953 the CNS and contribute to the pathogenesis of neurological melioidosis with meningitis.
954 Further experiments demonstrated that the expression of selectin on *B. pseudomallei*-infected
955 CD11b+ cells was required for the development of meningitis in recipient mice (280).

956 **THE OLFACTORY NERVE AS A PORTAL TO THE BRAIN**

957 The olfactory system (Figure 3A) comprises the odor detecting sensory system that exists in
958 the peripheral nervous system within the nasal cavity and, within the CNS, the olfactory bulb
959 and higher processing centers of the brain. Within the nasal cavity, the olfactory epithelium
960 lines the more dorsal and caudal regions of the cavity. Olfactory sensory neurons reside
961 within the olfactory epithelium and project a dendritic knob-like swelling with 20-30 cilia
962 into the mucous layer lining the nasal cavity. The olfactory sensory neuron cilia possess
963 odorant receptors (285), which bind a large spectrum of ligands (286). Binding of an inhaled
964 odorant to its receptor results in signal amplification and the activation of a signal
965 transduction pathway, leading to the generation of an action potential (287). The olfactory
966 epithelium also comprises sustentacular cells, which are glia-like cells whose apical surfaces
967 form the epithelial surface lining the nasal cavity. These supporting cells form tight junctions
968 with each other and with the dendrites of the olfactory sensory neurons, forming the primary
969 barrier from the environment (288). The olfactory epithelium also contains basal cells, which
970 are stem and multipotent progenitors from which new olfactory sensory neurons are
971 generated; and developing neurons, which are newly generated from the basal cells (289,
972 290). The axons of olfactory sensory neurons penetrate the basement membrane beneath the
973 epithelium entering the lamina propria where they are met by specialized glial cells, olfactory
974 ensheathing cells. Olfactory ensheathing cells surround multiple axons and bundle them
975 together in larger fascicles that comprise the olfactory nerve (291, 292). Within the lamina
976 propria are characteristic Bowman's glands of the olfactory mucosa, whose ducts penetrate
977 the epithelium above to supply specialized mucus to the epithelium.

978 The axons of the olfactory nerve course through the lamina propria towards the brain,
979 penetrating the skull through the cribriform plate and enter the brain at the olfactory bulb
980 (Figure 3A-C). Within the olfactory bulb the sensory axons form specialized structures,
981 glomeruli, where they synapse with mitral cells, which carry the sensory signal to higher
982 brain structures (293, 294). Olfactory sensory neurons are directly exposed to the external
983 environment *via* the nasal cavity; therefore microbes within the nasal cavity may potentially
984 exploit the olfactory pathway and access the subarachnoid space and the olfactory bulb. From
985 the olfactory bulb, viruses have been shown to migrate to higher brain regions including the
986 basal nuclei, thalamus, hypothalamus, cerebrum and cerebellum in animal models of
987 infection (295-297).

988 **THE TRIGEMINAL NERVE AS A PORTAL TO THE BRAIN**

989 The trigeminal nerve is the largest cranial nerve whose afferent branches carry touch, pain
990 and noxious stimuli from the face, the corneas of the eyes, and the oral and nasal cavities.
991 The trigeminal nerve innervates the olfactory and respiratory epithelia of the nasal cavity *via*
992 branches of the ophthalmic (V1) and maxillary (V2) nerves (Figure 4A) (298). The trigeminal
993 nerve endings are separated from the lumen of the nasal cavity *via* an apical tight junction
994 complex at the epithelial surface (299, 300). The same trigeminal nerves that innervate the
995 olfactory epithelium also branch to innervate the olfactory bulb, providing an alternative
996 route for entry of pathogens (301). Lipid soluble trigeminal irritants within the nasal cavity
997 may reach their receptors located on trigeminal nerve endings by diffusing across tight
998 junctions, potentially using paracellular mechanisms (302), or by interacting with specialized
999 solitary chemosensory cells within the nasal and respiratory epithelium, which form synaptic
1000 contacts with trigeminal nerve fibers (303). Solitary chemosensory cells isolated from mice
1001 were shown to detect bacterial signals such as acyl-homoserine lactones and trigger
1002 downstream signalling pathways associated with bitter irritant transduction (304).

The trigeminal nerve fibers course to the brain forming the trigeminal ganglion outside the brainstem but beneath the dura mater (298). From the trigeminal ganglion the sensory axons innervate the trigeminal nucleus in the brainstem. Therefore the trigeminal nerve, like the olfactory nerve, could provide a direct pathway by which bacteria within the nasal mucosa may access the brain independently of the blood. It should be noted that bacterial entry to the brain *via* the trigeminal nerve might not be limited to intranasal infection. For example, viruses such as herpes simplex virus type 1 access the trigeminal nerve through infection of the cornea (305) and potentially any epithelial infection of the facial skin or oral cavity could allow access to the trigeminal nerve.

PROTECTING THE CNS FROM MICROBIAL INVASION VIA THE NASAL CAVITY

The nasal cavity is constantly exposed to inhaled microbes, allergens and particulate material. The epithelium lining the nasal nares (306, 307) and the nasal cavity (308) harbor normal bacterial flora; and CNS pathogens such as *S. aureus*, *S. pneumoniae, N. meningitidis* and *H. influenzae* may asymptomatically colonize the nasopharynx in some individuals (306, 309, 310). Therefore, the nasal cavity features innate defense mechanisms to filter inhaled air and prevent microbes from invading deeper tissues.

Goblet cells and submucousal glands secrete mucins (primarily MUC5AC and MUC5B) into the lumen of the airway and nasal cavity. MUC5AC and MUC5B cross-link to form the structural framework of a mucous barrier, which floats on top of a periciliary layer of membrane-bound mucins (including MUC1 and MUC4) and glycoproteins (311). Through a mucociliary clearance process, inhaled bacteria and particulate matter become trapped in the mucous layer, and are swept towards the pharynx by the coordinated beating of cilia located on the surface of epithelial cells. A recent study demonstrated that MUC5B, but not

1027 MUC5AC, is essential for mucociliary clearance activity and the prevention of bacterial
1028 spread from the nasal cavity to the lower respiratory tract (312). Mucous secretion and
1029 mucociliary clearance are enhanced in the presence of inflammatory mediators (313) and
1030 microbial pathogens (314-316), and following exposure to cigarette smoke (317).
1031 Antimicrobial substances, including enzymes (lysozyme), protease inhibitors (secretory
1032 leukoprotease inhibitor, elastase inhibitor, α1-antiprotease, antichymotrypsin), antimicrobial
1033 peptides (β-defensins, L37) and oxidants (nitric oxide and hydrogen peroxide) are secreted by
1034 epithelial cells into the airway surface liquid, and provide a further line of defense against
1035 microbes (318). Finally, tight junctions and adherens junctions between the epithelial cells
1036 lining the nasal cavity and airway create a cellular barrier that prevents microbial spread.
1037 Dendritic cells localized beyond the epithelium extend processes through these tight junctions
1038 to interact with ligands and collaborate with resident macrophages to remove foreign antigens
1039 (319).

1040 Despite the host defences that exist within the nasal cavity and upper airways, bacteria
1041 colonizing the epithelium may invade deeper tissues and cause disease. In the case of
1042 asymptomatic colonizers, the transition from a commensal to a pathogenic phenotype may be
1043 due to within-host evolution that results in genetic changes to the regulation of virulence
1044 factors (320, 321). Alterations in susceptibility to disease in immunocompromised individuals
1045 may also promote the spread of bacteria from the nasal cavity (318). Respiratory pathogens
1046 such as *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* have been demonstrated to
1047 trigger the disassembly of tight junctions between epithelial cells to migrate to the lower
1048 respiratory tract (322, 323), whereas *N. meningitidis* redistributes junctional proteins at the
1049 olfactory epithelium (324). Harris *et al*. (325) demonstrated that the intact olfactory
1050 epithelium usually represents an effective barrier against infiltration of *S. aureus* into the
1051 nasal lamina propria. However, following mild detergent damage to the nasal epithelium of

1052 C57Bl/6 mice, intranasally delivered *S. aureus* was detected in the olfactory epithelium,
1053 olfactory nerve and the olfactory bulb as early as 6 hours post infection (325). Indeed,
1054 damage to the olfactory epithelium within the nasal cavity appears to be an important and
1055 common event in bacterial spread to the CNS *via* the olfactory nerve (324-327).

1056 PATHOGENS THAT ENTER THE BRAIN THROUGH THE NOSE

1057 Bacteria

1058 Bacteria within the nasal cavity could potentially penetrate the olfactory epithelium by
1059 directly infecting olfactory sensory neurons; they could enter *via* the ducts of the Bowman's
1060 glands; or they could enter after reactive inflammatory processes compromise the epithelial
1061 tissue leading to extravasion and subsequent tissue damage after exposure to bacterial
1062 virulence factors. Bacterial signalling molecules, such as acyl-homoserine lactones, are
1063 detected by solitary chemosensory cells within the nasal mucosa that synapse with the
1064 trigeminal nerve, triggering trigeminally-mediated reflex reactions (304). Mouse solitary
1065 chemosensory cells exposed to *P. aeruginosa* acyl-homoserine lactones responded with an
1066 increase in intracellular Ca^{2+}. Furthermore, *in vivo* studies demonstrated that mice exposed to
1067 acyl-homoserine lactones *via* the retronasal stream experienced significant reductions in
1068 respiratory rate, suggesting that interactions between bacterial acyl-homoserine lactones and
1069 chemosensory cells are capable of inducing responses indicative of nasal trigeminal irritants
1070 (304). The activation of nasal solitary chemosensory cells by acyl-homoserine lactones
1071 triggers a pro-inflammatory response (328); this response could damage the integrity of the
1072 epithelium and enable bacteria to access the trigeminal nerve endings. Once bacteria have
1073 penetrated the epithelium, they could potentially travel along the olfactory and/or trigeminal
1074 nerves to the brain within axons, between the axons, within the surrounding glia (olfactory

1075 ensheathing cells or Schwann cells), or between the glia and the connective tissue sheath
1076 (perineurium) that contains the nerve bundle.

1077 Meningeal pathogens such as *S. pneumoniae* and *N. meningitidis* unequivocally enter the CSF
1078 *via* the hematogenous route. Animal models of infection have also highlighted an alternative
1079 route of CNS entry, where these bacteria may directly invade the olfactory bulb within the
1080 brain from the nasal mucosa. *B. pseudomallei* has also been shown to invade the brain *via* the
1081 olfactory and trigeminal nerves, whereas *L. monocytogenes* demonstrates a predilection for
1082 the trigeminal nerve and brainstem. The following section reviews the evidence for CNS
1083 invasion from the nasal cavity for these pathogens.

1084 ***S. pneumoniae***

1085 Early studies by Rake demonstrated that *S. pneumoniae* rapidly enters the olfactory bulb in
1086 Swiss mice following intranasal inoculation (329). Remarkably, pneumococci were isolated
1087 from the olfactory bulbs of the brain 1 minute post-infection. At this time point, bacteria were
1088 not isolated from the blood, effectively excluding a hematogenous route of CNS invasion. At
1089 2 minutes post-infection, pneumococci were observed between the sustentacular cells of the
1090 olfactory epithelium, within the perineural space of the olfactory nerve and within the
1091 subarachnoid space (329). Similar findings were reported by van Ginkel *et al.*, who
1092 demonstrated that intranasally delivered *S. pneumoniae* (strain EF3030) could be recovered
1093 from nasal washes, the olfactory nerve and epithelium, and the olfactory bulb 24 hours post-
1094 infection in CBA/CAHN/*xid* mice (330). These sites remained colonized throughout the
1095 duration of the experiment, until day 39 post-infection; however, this did not lead to
1096 extensive brain infection. In contrast, non-encapsulated pneumococci were unable to persist
1097 within the nasal cavity and olfactory epithelium, and did not invade the olfactory bulb within
1098 the brain. Briles *et al.* confirmed that the pneumococcal capsular polysaccharide is an

important mediator of olfactory bulb infection in CBA/CAHN/*xid* mice, as only opaque variants were isolated from the olfactory bulb following intranasal inoculation (331). In these studies, intranasally delivered *S. pneumoniae* did not lead to bacteremia, suggesting a role for direct brain invasion from the nasal cavity. *S. pneumoniae* was also recovered from the trigeminal ganglia, further suggesting that the trigeminal nerve bundles may also be a potential route to the brain for this bacterial pathogen (330). However, it must be noted that these studies utilised CBA/CAHN/*xid* mice, which possess a mutation in the Bruton's tyrosine kinase gene and thus do not respond to thymus-independent type II antigens (332). These mice also fail to respond to capsular polysaccharide; therefore pneumococcal infection in this mouse strain is unlikely to accurately model human disease.

Incubation of *S. pneumoniae* with gangliosides prior to intranasal infection resulted in reduced colonization of the nasal mucosa, olfactory system and brain (330). Phosphorylcholine residues on the pneumococcal cell wall have been shown to bind to gangliosides (333), suggesting that gangliosides could be an important target for *S. pneumoniae* attachment in the neuroepithelium. *In vitro* studies have also demonstrated that *S. pneumoniae* can invade olfactory ensheathing cells *via* mannose receptor-mediated endocytosis (334); however, the role of olfactory ensheathing cells in pneumococcal infection and the exact mode of transport of *S. pneumoniae* along the olfactory and/or trigeminal nerves has not yet been studied.

N. meningitidis

In meningococcal meningitis, >60% of patients develop meningitis without septic shock (335), suggesting the possibility that *N. meningitidis* may also penetrate the CNS without prior bacteremia. In a mouse model of intranasal *N. meningitidis* infection, 20% of mice developed lethal meningitis; however, no bacteria were isolated from the blood (324). At 3

1123 days post-infection, lesions and polymorphonuclear cells were observed within the olfactory
1124 epithelium. Furthermore, *N. meningitidis* infection was associated with significant damage to
1125 the olfactory epithelium, and reduced its thickness in discrete, non-continuous sections of
1126 epithelium by more than 50% compared to controls. Immunofluorescence studies
1127 demonstrated *N. meningitidis* within the olfactory epithelium, basement membrane, lamina
1128 propria, along the olfactory nerves in the cribriform plate and within the nerve fiber layer of
1129 the olfactory bulb. Meningococci were also isolated from the CSF. Due to the absence of
1130 bacteremia, it was suggested that *N. meningitidis* may travel from the nasal cavity to the
1131 meninges and subarachnoid space *via* the olfactory nerves (324). In rhesus macaques,
1132 commensal neisseriae (RM *Neisseria*) were shown to be transmitted between animals, and
1133 naturally colonized the epithelium covering the cribriform plate (336), suggesting that
1134 migration of neisseriae along the olfactory pathway is not a phenomenon that is observed
1135 only in animals experimentally-inoculated with pathogenic *N. meningitides*. In the African
1136 "meningitis belt" the onset of meningococcal meningitis epidemics coincides with harsh, dry
1137 harmattan winds. It has been hypothesized that these harsh environmental conditions may
1138 irritate the mucous membranes (such as the olfactory mucosa), and enable *N. meningitidis* to
1139 penetrate the epithelium and invade the CNS (337), although this has not yet been
1140 investigated.

1141 Sjölinder and Jonsson demonstrated that the expression of the junctional protein N-cadherin
1142 in the olfactory epithelium and lamina propria was significantly reduced in mice intranasally
1143 infected with *N. meningitidis* (324). These data suggest that *N. meningitidis* may invade the
1144 olfactory epithelium *via* the destruction or rearrangement of cellular junctions between
1145 sustentacular cells and olfactory sensory neurons, although additional studies are required to
1146 confirm these findings. The mechanism by which *N. meningitidis* may travel along the
1147 olfactory nerve pathway to invade the nerve fiber layer of the olfactory bulb, the meninges

1148 and CSF are also unknown. The meninges and the subarachnoid space extend over the
1149 cribriform plate and further into the olfactory foramen where the olfactory nerves pass
1150 through the cribriform plate (338, 339). Bacteria travelling along the olfactory pathway could
1151 potentially invade the meninges after transversing the cribriform plate, and subsequently
1152 enter the CSF. However, there is a positive pressure from the brain to the nasal lymphatics
1153 due to the drainage of CSF through the cribriform plate, and it is unknown how *N.*
1154 *meningitidis* could travel against this pressure gradient.

1155 ***B. pseudomallei***

1156 The olfactory pathway has been identified as a route of CNS invasion by *B. pseudomallei*.
1157 Owen *et al.* used a capsule-deficient strain, which fails to survive within the blood, to
1158 demonstrate that bacteria were present in the olfactory epithelium and olfactory bulb of
1159 BALB/c mice 24 hours post-infection, in the absence of bacteremia. It was also shown that
1160 when colonization occurred only in one side of the nasal cavity, wild-type *B. pseudomallei*
1161 was detected in the olfactory epithelium and olfactory bulb of the infected side only.
1162 Combined, these data suggest that *B. pseudomallei* is able to directly invade the brain *via* the
1163 olfactory pathway without blood-borne infection (105). These findings also demonstrate that
1164 the presence of the polysaccharide capsule is not a prerequisite for CNS invasion *via* the
1165 olfactory pathway, presumably in contrast to systemic invasion *via* the BBB/BCSFB.
1166 However, it might be the case that an absence of capsule does diminish translocation *via* the
1167 olfactory or trigeminal nerves, since it is not possible to definitively compare with a presence
1168 of capsule due to hematogenous spread in the latter case. Figure 3 (D-E) illustrates *B.*
1169 *pseudomallei* invasion of the nasal cavity, olfactory epithelium and ventral nerve fiber layer
1170 of the olfactory bulb in a mouse model of acute melioidosis. It may be noted that mice, like
1171 most mammals, are naturally susceptible to melioidosis.

1172 The mechanisms by which *B. pseudomallei* migrates along the olfactory nerves have recently
1173 been investigated (326). In BALB/c mice, we demonstrated that intranasally-delivered *B.*
1174 *pseudomallei* was associated with widespread crenellation of the olfactory epithelium and
1175 loss of the neuron cell bodies. This neuronal loss led to the degeneration of olfactory axons
1176 within 24 – 48 hours, and the axon-devoid, hollow olfactory nerve fascicles that are
1177 surrounded by olfactory ensheathing cells provided an open conduit for bacterial passage
1178 from the nasal cavity through the cribriform plate and into the nerve fiber layer of the
1179 olfactory bulb. No bacteria were observed within the supporting sustentacular cells of the
1180 olfactory epithelium, nor within the Bowman's glands (326). By migrating within the nerve
1181 sheath, *B. pseudomallei* would be shielded from the flow of CSF out of the brain. This may
1182 also explain why *B. pseudomallei* is not often isolated from the CSF in neurological
1183 melioidosis (56). During our initial studies in mice, we also demonstrated that *B.*
1184 *pseudomallei* was present in the brain stem, increasing in number to 48 hours post infection,
1185 indicating, though not definitively demonstrating, that bacteria may enter *via* the trigeminal
1186 nerve after intranasal inoculation (105). We have now confirmed that *B. pseudomallei*
1187 penetrates the intact respiratory epithelium in mice and migrates along Schwann cell-encased
1188 trigeminal nerve bundles to the cranial cavity (326). Figure 4 (B-D) demonstrates *B.*
1189 *pseudomallei* localized within the branches of the trigeminal nerve. As noted above, clinical
1190 evidence points to a role for BimA, and hence actin-mediated motility, in neurological
1191 melioidosis (229); assuming inhalational acquisition to be associated with such cases, it
1192 remains to be determined where in the olfactory/trigeminal pathways such motility is utilized.

1193 Although there is currently no direct evidence of *B. pseudomallei* penetration of the brain *via*
1194 the olfactory and/or trigeminal pathways in humans, two important considerations provide
1195 support for CNS invasion from the nasal mucosa. First, *B. pseudomallei* can colonize the
1196 nasal cavity in humans (340), and this may represent a potential reservoir of infection. Our

unpublished data also demonstrate that following the inhalation of aerosolized *B. pseudomallei*, considered a major route of infection in humans, bacteria can be recovered in high numbers from the mouse olfactory epithelium. Second, in human cases of primary neurological melioidosis, Currie *et al*. reported that bacteremia was only observed in 3 out of 14 patients (21%) (57). In addition to our animal studies, these data provide evidence that *B. pseudomallei* can invade the CNS by a non-hematogenous route. Furthermore, the trigeminal route of CNS invasion may explain the brainstem-related neurological presentations of melioidosis patients, without requiring frank encephalitis.

L. monocytogenes

In ruminants naturally infected with L. *monocytogenes*, bacteria migrate along several cranial nerves (including the trigeminal nerve) to the brain stem and cause encephalitis (341). Similar findings are observed in human cases of *L. monocytogenes* brain stem encephalitis, where inflammatory lesions may be observed in the nuclei and tracts of the Vth, VIIth, IXth, Xth and XIIth cranial nerves innervating the oropharynx (342). Of the cranial nerves, the trigeminal nerve pathway is thought to represent a primary route by which *L. monocytogenes* may invade the brain stem from the oropharynx (343, 344). It has been proposed that *L. monocytogenes* may bind to E-cadherin on Schwann cells surrounding trigeminal nerves and subsequently invade the trigeminal axons by receptor-independent cell-to-cell spread (345). However, binding of *L. monocytogenes* to E-cadherin-expressing Schwann cells has not yet been demonstrated.

In animals spontaneously infected and experimentally infected with *L. monocytogenes*, bacteria were observed within the axons of the trigeminal nerve, suggesting that these bacteria may use intra-axonal spread to migrate to the CNS (343, 344, 346). In a mouse model of brain stem encephalitis, *L. monocytogenes* was inoculated into the snout of

1221 immunodeficient mice and was observed within the cytoplasm of trigeminal nerve cell bodies
1222 at 7 days post-infection (344). Following this, bacteria were then observed within the brain
1223 stem, but not other regions of the brain. In this model, IFN-γ release by natural killer cells
1224 was required for efficient control of *L. monocytogenes* neuroinvasion *via* the trigeminal route
1225 (344). *In vivo* and *in vitro* studies have demonstrated that colony-stimulating factor 1-
1226 dependent cells (including macrophages and dendritic cells) facilitate the neuronal spread of
1227 *L. monocytogenes* to the CNS (347, 348). Following replication and escape from the
1228 phagosome in colony-stimulating factor 1-dependent cells, *L. monocytogenes* may use actin-
1229 based motility to propel itself into adjacent non-phagocytic cells such as axon terminals.
1230 Indeed, the presence of *L. monocytogenes* with actin tails has been demonstrated *in vivo* in
1231 the axons of sheep trigeminal nerves (346), and *in vitro* in mouse hippocampal neurons and
1232 hypothalamic neurons (349). Dramsi *et al*. showed that *L. monocytogenes* spreads from J774
1233 macrophages to neurons *in vitro*, in a process that was dependent on actin polymerization by
1234 the bacterium (348), suggesting that the formation of actin tails is critical for neuronal spread
1235 in listeriosis.

1236 **Viruses**

1237 Data from animal studies and human cases have demonstrated that the olfactory and/or
1238 trigeminal nerve pathway represents a major route of CNS entry for several groups of viruses.
1239 *In vivo*, herpes viruses demonstrate a tropism for the olfactory epithelium, but not the
1240 respiratory epithelium (350-353). Expression of the herpes virus receptors heparan sulfate
1241 and nectin-1 on the apical side of the olfactory epithelium may facilitate binding to the
1242 neuroepithelium (350, 351). In the respiratory epithelium, these receptors are either expressed
1243 on the basal side of the epithelium (and are thus inaccessible), or are not highly expressed
1244 (350, 351). Herpes simplex virus type 1 (354), bovine herpesvirus-5 (355) and equine
1245 herpesvirus-9 (352, 356) spread from the nasal mucosa to the CNS *via* the olfactory nerves in

1246 animal models of infection. In bovine herpesvirus-5 CNS invasion, the viral protein Us9 and
1247 the glycine-rich epitope-coding region of the glycoprotein E are required for transport from
1248 the olfactory sensory neurons to the olfactory bulb (353, 357). In suckling hamsters, equine
1249 herpesvirus-9 antigen was detected within olfactory sensory neurons 12 hours after intranasal
1250 infection (352). At 48 hours post-infection, viral antigen was detected within the olfactory
1251 nerve and olfactory bulb, and at 60 hours post-infection virus was observed within the frontal
1252 and temporal lobes of the cerebral cortex. Some positive staining occurred within the
1253 trigeminal nerve, trigeminal ganglia and the region where the trigeminal sensory nerve root
1254 connects to the brain stem, although this was observed at the later time points, suggesting that
1255 the olfactory nerve is likely to be the primary route of infection (352). Interestingly,
1256 Shivkumar *et al*. demonstrated that intranasally delivered herpes simplex virus type 1
1257 targeted the olfactory epithelium of mice and subsequently travelled along the trigeminal
1258 nerve branches and re-emerged peripherally within the facial skin after 5 days (350). In this
1259 model, the virus rarely reached the olfactory bulbs within the brain. However, in another
1260 study, herpes simplex virus type 1 was isolated from the olfactory bulbs and higher brain
1261 regions of mice 3 days after intranasal inoculation (354).

1262 In a study of human autopsy material, Harberts *et al*. reported that herpesvirus-6 was detected
1263 at high frequencies within the olfactory bulb and tract (358). Herpesvirus-6B was the variant
1264 present in most positive samples, and quantitative real-time TaqMan PCR of selected samples
1265 detected viral loads of 1 x 10^3 and 1 x 10^4 copies per million cells. In the same study, the
1266 prevalence of herpesvirus-6 within the nasal mucosa was determined in 3 cohorts of patients:
1267 (i) healthy controls; (ii) multiple sclerosis patients; and (iii) patients with loss of smell.
1268 Overall, herpesvirus-6 DNA was detected in 41.3% of nasal mucous samples, and the
1269 prevalence did not differ between the patient cohorts. These findings suggest that the nasal
1270 cavity may be a reservoir for herpesvirus-6, and that virus within the nasal cavity may travel

1271 to the olfactory bulbs and tract *via* the olfactory pathway (358). Immunohistolgical evidence
1272 from fatal cases of herpes simplex encephalitis demonstrated that herpes simplex virus type 1
1273 antigen was detected within the olfactory tract, the olfactory cortex, and regions of the limbic
1274 system that are connected by the olfactory pathway. In contrast, viral antigen was not
1275 detected within the trigeminal pathway (359). Combined, these studies suggest that the
1276 olfactory route of CNS entry is highly relevant in human cases of symptomatic and
1277 asymptomatic herpes virus infection. The role of the trigeminal nerve as a portal of entry for
1278 herpes viruses in humans is less clear, although the sensory neurons of the trigeminal ganglia
1279 are the principal site of herpes simplex virus type 1 latent infection in humans (360, 361).

1280 Several subtypes of influenza virus A, including H5N1 (362), WSN/33 (363) and H1N1
1281 (364), invade the CNS *via* the olfactory nerve pathway in animal models of infection. In
1282 intranasally infected C57Bl/6 mice, H1N1 (strain PR8) mRNA was detected within the
1283 olfactory bulb as early as 4 hours post-infection, and viral antigen was visualized within the
1284 olfactory nerves and the glomerular layer of the olfactory bulb at 15 hours (364). Iwasaki *et*
1285 *al*. demonstrated that hematogenous dissemination of H5N1 from the lungs to the CNS may
1286 not be an important entry mechanism, as viral antigen was detected within the brain prior to
1287 the lungs in a murine model (362). In ferrets intranasally-infected with different H5N1
1288 strains, 3D imaging demonstrated that brain lesions were distributed: (i) along the olfactory
1289 pathway; (ii) along the olfactory pathway and within the brain stem; or (iii) surrounding the
1290 brain vasculature (365). These data suggest that there may be different routes of entry used by
1291 H5N1 strains; however, the olfactory pathway was identified as the most common route used
1292 by the small number of strains that were investigated (365). Further studies in ferrets have
1293 demonstrated that the Vietnam/1203/2004 H5N1 strain spread along the olfactory nerve
1294 filaments passing through the cribriform plate and into the olfactory bulb (366). From the
1295 olfactory bulb, the virus migrated through the olfactory tract and into the anterior olfactory

1296 nucleus and anterior commissure, and subsequently to the pyriform lobe, cerebral cortex and
1297 Ammon's horn (366). Schauwen *et al*. demonstrated that the multibasic cleavage site in the
1298 haemagglutinin of H5N1 was required for virus spread from the nasal cavity to the olfactory
1299 bulb and the rest of the CNS (296).

1300 The neurovirulence of influenza A subtypes may be influenced by the ability of the virus to
1301 disseminate from the olfactory bulb into other regions of the brain (367), which in turn may
1302 be controlled by the host immune response. *In vivo*, mouse olfactory sensory neurons infected
1303 with influenza A R404BP virus displayed apoptotic neurodegradation and were subsequently
1304 phagocytosed by Iba1-expressing microglia/macrophages (368). The infection was therefore
1305 restricted to the neuroepithelium, and did not spread to the olfactory bulb. This suggested that
1306 apoptosis of olfactory sensory neurons might be a mechanism by which the host is protected
1307 from microbial invasion from the nasal cavity (368). Influenza A virus also stimulates a host
1308 pro-inflammatory cytokine response within the olfactory bulb (369), which may also act to
1309 protect the host from further CNS invasion (370). Autopsy of a severely
1310 immunocompromised 11 month old infant revealed influenza virus A antigen within the
1311 olfactory bulb, olfactory tract and gyrus rectus, which is located inferolaterally to the
1312 olfactory bulb (371). Viral antigen was not detected within any other regions of the CNS,
1313 respiratory tract or any other organs. Viral RNA was also not detected within plasma,
1314 suggesting that viremia was not present. These findings provide evidence for influenza A
1315 entry into the CNS *via* the olfactory route in a severely immunocompromised infant (371).

1316 Paramyxoviruses including Nipah virus, Hendra virus and parainfluenza virus may enter the
1317 CNS directly from the nasal mucosa. *In vivo*, the Sendai strain of parainfluenza virus infected
1318 mouse olfactory sensory neurons, but not sustentacular cells, and travelled to the glomeruli of
1319 the olfactory bulb (372, 373). Infection of second order neurons and virus spread to the rest of
1320 the brain did not occur (372-374). The Sendai virus nucleoprotein gene was consistently

1321 detected within the olfactory bulb up to 168 days post-infection, indicating that persistence
1322 may occur within the olfactory bulb (373). In hamsters, Nipah virus was detected in olfactory
1323 sensory neurons as they passed through the cribriform plate into the olfactory bulb, providing
1324 evidence of direct brain infection following intranasal infection (297). Similar results were
1325 reported in a porcine model of Nipah virus infection, in which Nipah virus antigen was
1326 detected within a cross section of the olfactory nerve (375). Temporal analysis demonstrated
1327 that Nipah virus entered the olfactory bulb within 4 days in mice (297), whereas the virus
1328 spread from the olfactory nerve to the granular cells of the olfactory bulb within 7 days in
1329 pigs (375). The related Hendra virus was also shown to target the olfactory pathway and
1330 invade the brain directly from the nasal cavity (in the absence of viremia) in a mouse model
1331 of encephalitis (376). Weingartl *et al*. demonstrated that Nipah virus antigen was present
1332 within the endothelial cells and smooth muscle cells of the meningeal veins at days 5-7 days
1333 post-infection (375), which is consistent with findings from autopsies of fatal human Nipah
1334 virus cases (377). Thus, it is likely that Nipah virus exploits both the hematogenous and
1335 olfactory routes of invasion.

1336 Eastern, western and Venezuelan equine encephalitis viruses can cause encephalitis in horses
1337 and humans, and are transmitted by mosquitos or following aerosol exposure. Using a
1338 bioluminescent western equine encephalitis virus, Phillips *et al*. demonstrated the progression
1339 of CNS invasion in intranasally infected mice (295). The bioluminescent signal was initially
1340 detected in the nasal turbinates and olfactory bulb, and was amplified in the basal nuclei,
1341 thalamus and hypothalamus. The distribution of lesions within the brain and the detection of
1342 viral antigen by immunohistochemistry supported the olfactory pathway as the route of
1343 infection, and suggested that the trigeminal nerve may provide a secondary conduit to the
1344 brain (295). Venezuelan equine encephalitis virus also targeted both the olfactory (primary
1345 route) and trigeminal (secondary route) nerve pathways for CNS entry (378), whereas eastern

equine encephalitis virus appeared to infect only the olfactory nerve (379). In CD-1 mice, ablation of the olfactory epithelium and the main olfactory bulb prevented invasion of Venezuelan equine encephalitis virus into the brain *via* the olfactory nerve; however, the virus was still able to spread to the CNS along the trigeminal nerve (378). Interestingly, replication of Venezuelan equine encephalitis virus within the nasal mucosa induced the expression of pro-inflammatory cytokines, matrix metalloproteinase-9 and intracellular adhesion molecule-1 within the olfactory bulb, which led to subsequent breakdown of the BBB (380). These events enabled circulating virus to penetrate the brain, suggesting that in addition to the olfactory and trigeminal routes of entry, Venezuelan equine encephalitis virus may also enter the CNS by a hematogenous route.

Within the *Rhabdoviridae* family, rabies virus and vesicular stomatitis virus within the nasal cavity directly invade the olfactory bulbs within the brain. In a fatal human case of airborne rabies encephalitis, rabies virions were observed only within the nerve fibers of the olfactory bulb, and not any other regions of the brain (381). Data from animal studies have also demonstrated that intranasally-delivered rabies virus selectively targets the olfactory epithelium and migrates to the olfactory bulb, including the glomeruli, mitral cells and tufted cells (382). Rabies virus antigen was also detected in the mouse trigeminal nerve. The tropism of rabies virus to the olfactory epithelium may be due to the expression of neural cell adhesion molecule by olfactory sensory neurons (383), which was identified as a receptor for rabies virus *in vitro* (384). Similar to rabies virus, intranasal vesicular stomatitis virus infected the olfactory epithelium, but not the respiratory epithelium in a mouse model (385). By 6 hours post-infection viral antigen was observed within the olfactory sensory neurons; at 12 hours sustentacular cells and basal cells within the neuroepithelium were also infected, as were olfactory nerve bundles within the lamina propria; and at 24 hours the Bowman’s glands

1370 and olfactory bulb were infected. In contrast to rabies virus, the trigeminal nerve was not
1371 implicated as a portal of CNS entry for vesicular stomatitis virus (385).

1372 **Protozoa**

1373 *Naegleria fowleri* is a free-living amoeba that causes primary amoebic meningoencephalitis,
1374 a rare but almost always fatal disease in humans. *N. fowleri* is found in warm fresh water and
1375 human infection occurs following the inspiration of contaminated water, and is usually
1376 associated with swimming. Contaminated tap water used to reconstitute saline for nasal
1377 irrigation, or for ablution of the nasal cavity has also been implicated as a source of infection
1378 (386, 387). Pathological investigations of fatal human cases revealed hemolytic, necrotic
1379 encephalitis of the olfactory area, the contiguous forebrain and cerebellum (388, 389). The
1380 suspected route of CNS entry was the olfactory route, due to the presence of amoebae and
1381 acute inflammation within the nasal mucosa and the olfactory nerve bundles (388, 389).

1382 In murine models of the early stages of primary amoebic meningoencephalitis, intranasal *N.*
1383 *fowleri* invaded the cribriform plate and olfactory bulb and was observed within the olfactory
1384 nerves (390, 391). *In vivo* studies have shown that *N. fowleri* induces mucous production
1385 within the nasal cavity as early as 1 hour post-infection, and that by 6 hours the amoebae
1386 were covered by mucous and surrounded by neutrophils (392). In the mucin-producing cell
1387 line, NCI-H292, *N. fowleri* induced the release of reactive oxygen species, which led to the
1388 activation of the epidermal growth factor receptor, which in turn stimulated production of the
1389 mucin MUC5A and IL-8 secretion (314). Despite this host response, by 12 hours *N. fowleri*
1390 was observed adhering to and invading the olfactory epithelium in mice, suggesting that the
1391 amoebae may have pathogenic mechanisms to efficiently penetrate the mucous layer (392).
1392 *In vitro*, it was shown that both live trophozoites and crude total *N. fowleri* extracts
1393 demonstrated mucinolytic activity. A 37 kDa cysteine protease of *N. fowleri* was

1394 subsequently identified as being responsible for this mucinolytic activity (392); this most
1395 likely enabled the amoebae to penetrate the nasal mucosa and invade the neuroepithelium.
1396 Penetration of the olfactory epithelium in mice occurred without cellular disruption or
1397 damage (390), and microscopy studies have shown that *N. fowleri* may be ingested by
1398 sustentacular cells within the epithelium, or may migrate between the sustentacular cells
1399 (393). A recent study demonstrated that *N. fowleri* degraded the epithelial tight junction
1400 proteins claudin-1 and zonula occludens-1 *in vitro*, highlighting a potential paracellular
1401 mechanism of penetration (394).

1402 *Balamuthia mandrillaris*, an opportunistic free-living amoeba that can cause granulomatous
1403 amoebic encephalitis, was also shown to enter the CNS directly from the nasal cavity after
1404 penetrating the olfactory epithelium and cribriform plate in immunodeficient mice following
1405 intranasal infection (395). However, the implications of these findings in humans are unclear
1406 as *B. mandrillaris* is primarily thought to cause CNS infections due to hematogenous spread
1407 from the lungs, or through cuts or skin abrasions (396).

1408 **Yeasts and fungi**

1409 The encapsulated yeast *Cryptococcus neoformans* is an important cause of fungal
1410 meningoencephalitis worldwide and can enter the CNS by penetrating the BBB using
1411 transcellular, paracellular and Trojan horse mechanisms, following blood-borne
1412 dissemination from the lungs (as reviewed elsewhere (397)). Although the hematogenous
1413 route of CNS is well accepted for cryptococcal meningoencephalitis, some strains of *C.*
1414 *neoformans* isolated from the CSF of human patients were shown to be rhinotropic in mice
1415 (398, 399), and persistent *C. neoformans* colonization of the nasal cavity (90 days) was
1416 reported in one study (400). These findings prompted investigations into the possibility of an
1417 alternative direct route of CNS entry from the nose. Gomes *et al*. followed the progression of

1418 intranasal *C. neoformans* infection in immunodeficient mice, and demonstrated colonization
1419 of the olfactory mucosa, invasion along the olfactory nerve and meningeal involvement
1420 (401). In a study of the pathological features of three patients with acquired immune
1421 deficiency syndrome and cryptococcal meningitis, *C. neoformans* was observed in the
1422 subarachnoid space around the olfactory tracts and bulbs and in the olfactory nerve fascicles
1423 (402). No cyrptococci were located within the lamina propria or the olfactory epithelium, and
1424 thus the authors suggested that *C. neoformans* within the CSF may have been transported to
1425 the olfactory regions due to the drainage of CSF from the subarachnoid space through the
1426 cribriform plate (402). Using a guinea pig model, Lima and Vital provided further evidence
1427 that the olfactory route is unlikely to represent a portal of *C. neoformans* CNS entry, as
1428 intranasally instilled cryptococci were unable to penetrate the olfactory epithelium and were
1429 cleared from the olfactory mucosa (316).

1430 In contrast, the fungal infection rhinocerebral mucormycosis has been demonstrated to spread
1431 to the brain *via* the trigeminal nerve. Rhinocerebral mucormycosis refers to infection caused
1432 by fungi within the order Mucorales, and usually affects individuals with poorly controlled
1433 diabetes mellitus or the immunocompromised. These organisms display a predilection for the
1434 nasal cavity and paranasal sinuses; from these sites the organisms typically invade blood
1435 vessel walls and then spread to the cavernous sinus, internal carotid artery and the brain (403-
1436 406). However, fungal hyphae and lesions have been demonstrated within the trigeminal
1437 nerve and the pons within the brain stem in the absence of leptomeningitis, suggesting that
1438 direct invasion occurred from the sinuses to the brain along the trigeminal nerve (407, 408).
1439 The perineural route of CNS entry was thought to be atypical; however, a study of the
1440 histologic features of patients with mucormycosis demonstrated that perineural invasion,
1441 characterized by fungal hyphae within the perineurium that surrounds the nerves, was a

1442 common feature that occurred concurrently with angioinvasion (409). The mechanisms of
1443 rhinocerebral mucormycosis CNS infection have not been investigated.

1444 **CONCLUDING REMARKS**

1445 A wide range of microbes can invade the CNS, and any organisms that can enter the CSF
1446 have the potential to cause meningitis. Many bacteria and other pathogens have developed
1447 sophisticated mechanisms to penetrate the BBB and/or BCSFB by transcellular and
1448 paracellular transport, or the Trojan horse route. The olfactory and trigeminal nerves
1449 represent pathways by which microbes enter the brain, without encountering the BBB or the
1450 BCSFB. These nerves are well recognized as portals of entry for many viruses, as well as
1451 protozoa and fungi, and there is now evidence from animal models that some bacteria can
1452 infect the brain *via* the olfactory and trigeminal nerves. The purpose of this review is to
1453 highlight an alternative route of entry that has thus far received little attention and may
1454 explain some of the pathological features observed in human disease. These routes of
1455 bacterial invasion require investigation in humans, especially in cases where the etiological
1456 agent is known to colonize the nasal mucosa. In such cases, clinical assessment of olfactory
1457 function and the nasal mucosa should be considered. Furthermore, several questions remain
1458 unanswered; first, what is the route for bacteria to travel along olfactory nerves – within the
1459 axons/nerve bundles, within the glia or within the perineural space? We have demonstrated
1460 that *B. pseudomallei* caused destruction of the olfactory epithelium and axonal death, which
1461 provided an open conduit for migration to the olfactory bulb within the nerve fascicles (326).
1462 It is unknown if similar mechanisms of transport are used by other bacterial pathogens such
1463 as *S. pneumoniae* or *N. meningitidis*. Remarkably, *L. monocytogenes* appears to travel intra-
1464 axonally. Second, which bacterial virulence factors are required for penetration of the
1465 olfactory epithelium and invasion of the brain *via* the olfactory and trigeminal nerves? This
1466 review has highlighted the requirement for additional research to characterize the role of the

1467 olfactory and trigeminal nerves in bacterial penetration of the brain; and determine the
1468 molecular and cellular mechanisms by which bacterial pathogens may exploit these
1469 pathways.

1470 **REFERENCES**

1471 1. **Connolly KJ, Hammer SM.** 1990. The acute aseptic meningitis syndrome. Infect Dis
1472 Clin North Am. **4:**599-622.
1473 2. **White K, Ostrowski K, Maloney S, Norton R.** 2012. The utility of cerebrospinal
1474 fluid parameters in the early microbiological assessment of meningitis. Diagn
1475 Microbiol Infect Dis. **73:**27-30.
1476 3. **Cassady KA, Whitley RJ.** 2004. Pathogenesis and pathophysiology of viral
1477 infections of the central nervous system, p 58-60. *In* Scheld WM, Whitley RJ, Marra
1478 CM (ed.), Infections of the central nervous system, 3rd ed. Lippincott Williams &
1479 Wilkins, Philadelphia, PA.
1480 4. **Denizot M, Neal JW, Gasque P.** 2012. Encephalitis due to emerging viruses: CNS
1481 innate immunity and potential therapeutic targets. J Infect. **65:**1-16.
1482 5. **McGavern DB, Kang SS.** 2011. Illuminating viral infections in the nervous system.
1483 Nat Rev Immunol. **11:**318-329.
1484 6. **Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D,**
1485 **Cunningham R, Zuckerman M, Mutton KJ, Solomon T, Ward KN, Lunn MP,**
1486 **Irani SR, Vincent A, Brown DW, Crowcroft NS.** 2010. Causes of encephalitis and
1487 differences in their clinical presentations in England: a multicentre, population-based
1488 prospective study. Lancet Infect Dis. **10:**835-844.
1489 7. **Glaser CA, Honarmand S, Anderson LJ, Schnurr DP, Forghani B, Cossen CK,**
1490 **Schuster FL, Christie LJ, Tureen JH.** 2006. Beyond viruses: clinical profiles and
1491 etiologies associated with encephalitis. Clin Infect Dis. **43:**1565-1577.
1492 8. **Durand ML, Calderwood SB, Weber DJ, Miller SI, Southwick FS, Caviness VS,**
1493 **Jr., Swartz MN.** 1993. Acute bacterial meningitis in adults. A review of 493
1494 episodes. N Engl J Med. **328:**21-28.

1495 9. **Hussein AS, Shafran SD.** 2000. Acute bacterial meningitis in adults. A 12-year review. Medicine (Baltimore). **79:**360-368.

1497 10. **Vibha D, Bhatia R, Prasad K, Srivastava MV, Tripathi M, Singh MB.** 2010. Clinical features and independent prognostic factors for acute bacterial meningitis in adults. Neurocrit Care. **13:**199-204.

1500 11. **van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M.** 2004. Clinical features and prognostic factors in adults with bacterial meningitis. N Engl J Med. **351:**1849-1859.

1503 12. **Wenger JD, Hightower AW, Facklam RR, Gaventa S, Broome CV.** 1990. Bacterial meningitis in the United States, 1986: report of a multistate surveillance study. The Bacterial Meningitis Study Group. J Infect Dis. **162:**1316-1323.

1506 13. **Schlech WF, 3rd, Ward JI, Band JD, Hightower A, Fraser DW, Broome CV.** 1985. Bacterial meningitis in the United States, 1978 through 1981. The National Bacterial Meningitis Surveillance Study. JAMA. **253:**1749-1754.

1509 14. **Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL, Harrison LH, Farley MM, Reingold A, Bennett NM, Craig AS, Schaffner W, Thomas A, Lewis MM, Scallan E, Schuchat A.** 2011. Bacterial meningitis in the United States, 1998-2007. N Engl J Med. **364:**2016-2025.

1513 15. **Okike IO, Ribeiro S, Ramsay ME, Heath PT, Sharland M, Ladhani SN.** 2014. Trends in bacterial, mycobacterial, and fungal meningitis in England and Wales 2004-11: an observational study. Lancet Infect Dis. **14:**301-307.

1516 16. **Taylor WR, Nguyen K, Nguyen D, Nguyen H, Horby P, Nguyen HL, Lien T, Tran G, Tran N, Nguyen HM, Nguyen T, Nguyen HH, Farrar J, de Jong M, Schultsz C, Tran H, Vu B, Le H, Dao T, Wertheim H.** 2012. The spectrum of

1519 central nervous system infections in an adult referral hospital in Hanoi, Vietnam.
1520 PLoS One. **7:**e42099.
1521 17. **Christodoulides M.** 2013. A history of bacterial meningitis from antiquity to modern
1522 times, p 1 - 16. *In* Christodoulides M (ed.), Meningitis: cellular and molecular basis
1523 CAB International, Oxfordshire.
1524 18. **Ting PL, Norton R.** 2013. Central nervous system tuberculosis: a disease from Papua
1525 New Guinea in North Queensland. J Paediatr Child Health. **49:**E193-198.
1526 19. **Jain SK, Ordonez A, Kinikar A, Gupte N, Thakar M, Mave V, Jubulis J,**
1527 **Dharmshale S, Desai S, Hatolkar S, Kagal A, Lalvani A, Gupta A, Bharadwaj R.**
1528 2013. Pediatric tuberculosis in young children in India: a prospective study. Biomed
1529 Res Int. **2013:**783698.
1530 20. **Kim KS, Itabashi H, Gemski P, Sadoff J, Warren RL, Cross AS.** 1992. The K1
1531 capsule is the critical determinant in the development of *Escherichia coli* meningitis
1532 in the rat. J Clin Invest. **90:**897-905.
1533 21. **Brandt CT, Holm D, Liptrot M, Ostergaard C, Lundgren JD, Frimodt-Moller N,**
1534 **Skovsted IC, Rowland IJ.** 2008. Impact of bacteremia on the pathogenesis of
1535 experimental pneumococcal meningitis. J Infect Dis. **197:**235-244.
1536 22. **Bell LM, Alpert G, Campos JM, Plotkin SA.** 1985. Routine quantitative blood
1537 cultures in children with *Haemophilus influenzae* or *Streptococcus pneumoniae*
1538 bacteremia. Pediatrics. **76:**901-904.
1539 23. **Sullivan TD, LaScolea LJ, Jr., Neter E.** 1982. Relationship between the magnitude
1540 of bacteremia in children and the clinical disease. Pediatrics. **69:**699-702.
1541 24. **Ostergaard C, Konradsen HB, Samuelsson S.** 2005. Clinical presentation and
1542 prognostic factors of *Streptococcus pneumoniae* meningitis according to the focus of
1543 infection. BMC Infect Dis. **5:**93.

1544 25. **Seib KL, Serruto D, Oriente F, Delany I, Adu-Bobie J, Veggi D, Arico B,**
1545 **Rappuoli R, Pizza M.** 2009. Factor H-binding protein is important for
1546 meningococcal survival in human whole blood and serum and in the presence of the
1547 antimicrobial peptide LL-37. Infect Immun. **77:**292-299.

1548 26. **Echenique-Rivera H, Muzzi A, Del Tordello E, Seib KL, Francois P, Rappuoli R,**
1549 **Pizza M, Serruto D.** 2011. Transcriptome analysis of *Neisseria meningitidis* in
1550 human whole blood and mutagenesis studies identify virulence factors involved in
1551 blood survival. PLoS Pathog. **7:**e1002027.

1552 27. **Ren B, Li J, Genschmer K, Hollingshead SK, Briles DE.** 2012. The absence of
1553 PspA or presence of antibody to PspA facilitates the complement-dependent
1554 phagocytosis of pneumococci *in vitro*. Clin Vaccine Immunol. **19:**1574-1582.

1555 28. **Hyams C, Trzcinski K, Camberlein E, Weinberger DM, Chimalapati S,**
1556 **Noursadeghi M, Lipsitch M, Brown JS.** 2013. *Streptococcus pneumoniae* capsular
1557 serotype invasiveness correlates with the degree of factor H binding and opsonization
1558 with C3b/iC3b. Infect Immun. **81:**354-363.

1559 29. **Harvey RM, Ogunniyi AD, Chen AY, Paton JC.** 2011. Pneumolysin with low
1560 hemolytic activity confers an early growth advantage to *Streptococcus pneumoniae* in
1561 the blood. Infect Immun. **79:**4122-4130.

1562 30. **Clark SE, Snow J, Li J, Zola TA, Weiser JN.** 2012. Phosphorylcholine allows for
1563 evasion of bactericidal antibody by *Haemophilus influenzae*. PLoS Pathog.
1564 **8:**e1002521.

1565 31. **Tunkel AR, van de Beek D, Scheld WM.** 2010. Acute meningitis, p 1189-1229. *In*
1566 Mandell GL, Bennett JE, Dolin R (ed.), Mandell, Douglas, and Bennett's principles
1567 and practice of infectious diseases, 7th ed. Churchill Livingstone, Philadelphia.

1568 32. **Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, Craig AS, Farley MM,**
1569 **Jorgensen JH, Lexau CA, Petit S, Reingold A, Schaffner W, Thomas A, Whitney**
1570 **CG, Harrison LH.** 2009. Effect of pneumococcal conjugate vaccine on
1571 pneumococcal meningitis. N Engl J Med. **360:**244-256.
1572 33. **Milton A, Stirzaker S, Trungove M, Knuckey D, Martin N, Hastie C, Pennington**
1573 **K, Sloan-Gardner T, Fitzsimmons G, Knope K, Martinek S, Mills L, Barry C,**
1574 **Wright P, Power M.** 2012. Australia's notifiable disease status, 2010: annual report
1575 of the national notifiable diseases surveillance system. Commun Dis Intell Q Rep.
1576 **36:**1-69.
1577 34. **Oftadeh S, Gidding HF, Gilbert GL.** 2013. Laboratory surveillance of invasive
1578 pneumococcal disease in New South Wales, Australia, before and after introduction
1579 of 7-valent conjugate vaccine: reduced disease, but not antibiotic resistance rates.
1580 Epidemiol Infect. **141:**1797-1806.
1581 35. **Miller E, Andrews NJ, Waight PA, Slack MP, George RC.** 2011. Herd immunity
1582 and serotype replacement 4 years after seven-valent pneumococcal conjugate
1583 vaccination in England and Wales: an observational cohort study. Lancet Infect Dis.
1584 **11:**760-768.
1585 36. **Skoczynska A, Wasko I, Kuch A, Kadlubowski M, Golebiewska A, Forys M,**
1586 **Markowska M, Ronkiewicz P, Wasiak K, Kozinska A, Matynia B, Hryniewicz**
1587 **W.** 2013. A decade of invasive meningococcal disease surveillance in poland. PLoS
1588 One. **8:**e71943.
1589 37. **Collard JM, Issaka B, Zaneidou M, Hugonnet S, Nicolas P, Taha MK,**
1590 **Greenwood B, Jusot JF.** 2013. Epidemiological changes in meningococcal
1591 meningitis in Niger from 2008 to 2011 and the impact of vaccination. BMC Infect
1592 Dis. **13:**576.

38. **Brandtzaeg P, van Deuren M.** 2012. Classification and pathogenesis of meningococcal infections, p 21 - 35. *In* Christodoulides M (ed.), *Neisseria meningitidis*: advanced methods and protocols, vol. 799. Humana Press, New York.

39. **Trotter CL, Ramsay ME, Kaczmarski EB.** 2002. Meningococcal serogroup C conjugate vaccination in England and Wales: coverage and initial impact of the campaign. Commun Dis Public Health. **5:**220-225.

40. **Lahra MM, Enriquez RP.** 2012. Annual report of the Australian Meningococcal Surveillance Programme, 2011. Commun Dis Intell Q Rep. **36:**E251-262.

41. **Galloway Y, Stehr-Green P, McNicholas A, O'Hallahan J.** 2009. Use of an observational cohort study to estimate the effectiveness of the New Zealand group B meningococcal vaccine in children aged under 5 years. Int J Epidemiol. **38:**413-418.

42. **Amaya-Villar R, Garcia-Cabrera E, Sulleiro-Igual E, Fernandez-Viladrich P, Fontanals-Aymerich D, Catalan-Alonso P, Rodrigo-Gonzalo de Liria C, Coloma-Conde A, Grill-Diaz F, Guerrero-Espejo A, Pachon J, Prats-Pastor G.** 2010. Three-year multicenter surveillance of community-acquired *Listeria monocytogenes* meningitis in adults. BMC Infect Dis. **10:**324.

43. **Brouwer MC, van de Beek D, Heckenberg SG, Spanjaard L, de Gans J.** 2006. Community-acquired *Listeria monocytogenes* meningitis in adults. Clin Infect Dis. **43:**1233-1238.

44. **Lorber B.** 2007. Community-acquired *Listeria monocytogenes* meningitis in adults. Clin Infect Dis. **44:**765-766.

45. **Greenhow TL, Hung YY, Herz AM, Losada E, Pantell RH.** 2013. The changing epidemiology of serious bacterial infections in young infants. Pediatr Infect Dis J.

1616 46. **Gaschignard J, Levy C, Romain O, Cohen R, Bingen E, Aujard Y, Boileau P.**
1617 2011. Neonatal bacterial meningitis: 444 cases in 7 years. Pediatr Infect Dis J.
1618 **30:**212-217.
1619 47. **Costerus JM, van de Beek D, Brouwer MC.** 2012. Nosocomial meningitis caused
1620 by gas producing *Klebsiella pneumoniae*. BMJ Case Rep. **2012**.
1621 48. **Wu YM, Hsu PC, Yang CC, Chang HJ, Ye JJ, Huang CT, Lee MH.** 2013.
1622 *Serratia marcescens* meningitis: epidemiology, prognostic factors and treatment
1623 outcomes. J Microbiol Immunol Infect. **46:**259-265.
1624 49. **Bennett JE.** 2010. Chronic meningitis, p 1237 - 1241. *In* Mandell GL, Bennett JE,
1625 Dolin R (ed.), Mandell, Douglas, and Bennett's principles and practice of infectious
1626 diseases, 7th ed. Churchill Livingstone, Philadelphia.
1627 50. **Thwaites GE, van Toorn R, Schoeman J.** 2013. Tuberculous meningitis: more
1628 questions, still too few answers. Lancet Neurol. **12:**999-1010.
1629 51. **Donald PR, Schaaf HS, Schoeman JF.** 2005. Tuberculous meningitis and miliary
1630 tuberculosis: the Rich focus revisited. J Infect. **50:**193-195.
1631 52. **Beckham JD, Tyler KL.** 2010. Encephalitis, p 1243 - 1163. *In* Mandell GL, Bennett
1632 JE, Dolin R (ed.), Mandell, Douglas, and Bennett's principles and practice of
1633 infectious diseases, 7th ed. Churchill Livingstone, Philadelphia.
1634 53. **Quist-Paulsen E, Kran AM, Dunlop O, Wilson J, Ormaasen V.** 2013. Infectious
1635 encephalitis: a description of a Norwegian cohort. Scand J Infect Dis. **45:**179-185.
1636 54. **Huppatz C, Kelly PM, Levi C, Dalton C, Williams D, Durrheim DN.** 2009.
1637 Encephalitis in Australia, 1979-2006: trends and aetiologies. Commun Dis Intell Q
1638 Rep. **33:**192-197.

1639 55. **Fox A, Hung TM, Wertheim H, Hoa le NM, Vincent A, Lang B, Waters P, Ha**
1640 **NH, Trung NV, Farrar J, Van Kinh N, Horby P.** 2013. Acute measles encephalitis
1641 in partially vaccinated adults. PLoS One. **8:**e71671.
1642 56. **Currie BJ, Fisher DA, Howard DM, Burrow JN.** 2000. Neurological melioidosis.
1643 Acta Trop. **74:**145-151.
1644 57. **Currie BJ, Ward L, Cheng AC.** 2010. The epidemiology and clinical spectrum of
1645 melioidosis: 540 cases from the 20 year Darwin prospective study. PLoS Negl Trop
1646 Dis. **4:**e900.
1647 58. **Koszyca B, Currie BJ, Blumbergs PC.** 2004. The neuropathology of melioidosis:
1648 two cases and a review of the literature. Clin Neuropathol. **23:**195-203.
1649 59. **Woods ML, 2nd, Currie BJ, Howard DM, Tierney A, Watson A, Anstey NM,**
1650 **Philpott J, Asche V, Withnall K.** 1992. Neurological melioidosis: seven cases from
1651 the Northern Territory of Australia. Clin Infect Dis. **15:**163-169.
1652 60. **Bartley PP, Pender MP, Woods ML, 2nd, Walker D, Douglas JA, Allworth AM,**
1653 **Eisen DP, Currie BJ.** 1999. Spinal cord disease due to melioidosis. Trans R Soc
1654 Trop Med Hyg. **93:**175-176.
1655 61. **Maguire GP, Flavell HD, Burrow JN, Currie BJ.** 1998. Relapsing neurological
1656 melioidosis from the top end of the Northern Territory. Aust N Z J Med. **28:**219-220.
1657 62. **Howe PW, Holland HM, Burrow JC, Currie BJ.** 1997. Neurological melioidosis
1658 (*Burkholderia pseudomallei*) mimicking Guillain-Barre syndrome. Anaesth Intensive
1659 Care. **25:**166-167.
1660 63. **Chadwick DR, Ang B, Sitoh YY, Lee CC.** 2002. Cerebral melioidosis in Singapore:
1661 a review of five cases. Trans R Soc Trop Med Hyg. **96:**72-76.
1662 64. **Vestal ML, Wong EB, Milner DA, Jr., Gormley WB, Dunn IF.** 2013. Cerebral
1663 melioidosis for the first time in the western hemisphere. J Neurosurg. **119:**1591-1595.

1664 65. **Mathisen GE, Johnson JP.** 1997. Brain abscess. Clin Infect Dis. **25:**763-779; quiz
1665 780-761.
1666 66. **Tunkel AR.** 2010. Brain abscess, p 1265 - 1278. *In* Mandell GL, Bennett JE, Dolin R
1667 (ed.), Mandell, Douglas, and Bennett's principles and practice of infectious diseases,
1668 7th ed. Churchill Livingstone, Philadelphia.
1669 67. **Brouwer MC, Coutinho JM, van de Beek D.** 2014. Clinical characteristics and
1670 outcome of brain abscess: Systematic review and meta-analysis. Neurology. **82:**806-
1671 813.
1672 68. **Prasad KN, Mishra AM, Gupta D, Husain N, Husain M, Gupta RK.** 2006.
1673 Analysis of microbial etiology and mortality in patients with brain abscess. J Infect.
1674 **53:**221-227.
1675 69. **Tunkel AR.** 2010. Subdural empyema, epidural abscess, and suppurative intracranial
1676 thrombophlebitis, p 1279 - 1287. *In* Mandell GL, Bennett JE, Dolin R (ed.), Mandell,
1677 Douglas, and Bennett's principles and practice of infectious diseases, 7th ed. Churchill
1678 Livingstone, Philadelphia.
1679 70. **Weller RO.** 2013. Anatomy of the meninges: structural and functional aspects, p 17-
1680 30. *In* Christodoulides M (ed.), Meningitis: cellular and molecular basis. CAB
1681 International, Oxfordshire.
1682 71. **Walter BA, Valera VA, Takahashi S, Ushiki T.** 2006. The olfactory route for
1683 cerebrospinal fluid drainage into the peripheral lymphatic system. Neuropathol Appl
1684 Neurobiol. **32:**388-396.
1685 72. **Johnston M, Zakharov A, Papaiconomou C, Salmasi G, Armstrong D.** 2004.
1686 Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in
1687 humans, non-human primates and other mammalian species. Cerebrospinal Fluid Res.
1688 **1:**2.

1689 73. **Wagshul ME, Johnston M.** 2013. The brain and the lymphatic system, p 143-164. *In*
1690 Santambrogio L (ed.), Immunology of the lymphatic system. Springer, New York.
1691 74. **Abbott NJ.** 2013. Blood-brain barrier structure and function and the challenges for
1692 CNS drug delivery. J Inherit Metab Dis. **36:**437-449.
1693 75. **Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ.** 2010. Structure
1694 and function of the blood-brain barrier. Neurobiol Dis. **37:**13-25.
1695 76. **Engelhardt B, Sorokin L.** 2009. The blood-brain and the blood-cerebrospinal fluid
1696 barriers: function and dysfunction. Semin Immunopathol. **31:**497-511.
1697 77. **Geier EG, Chen EC, Webb A, Papp AC, Yee SW, Sadee W, Giacomini KM.**
1698 2013. Profiling solute carrier transporters in the human blood-brain barrier. Clin
1699 Pharmacol Ther. **94:**636-639.
1700 78. **Uchida Y, Ohtsuki S, Katsukura Y, Ikeda C, Suzuki T, Kamiie J, Terasaki T.**
1701 2011. Quantitative targeted absolute proteomics of human blood-brain barrier
1702 transporters and receptors. J Neurochem. **117:**333-345.
1703 79. **Shawahna R, Uchida Y, Decleves X, Ohtsuki S, Yousif S, Dauchy S, Jacob A,**
1704 **Chassoux F, Daumas-Duport C, Couraud PO, Terasaki T, Scherrmann JM.**
1705 2011. Transcriptomic and quantitative proteomic analysis of transporters and drug
1706 metabolizing enzymes in freshly isolated human brain microvessels. Mol Pharm.
1707 **8:**1332-1341.
1708 80. **Redzic Z.** 2011. Molecular biology of the blood-brain and the blood-cerebrospinal
1709 fluid barriers: similarities and differences. Fluids Barriers CNS. **8:**3.
1710 81. **Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM.** 2007. Local self-renewal
1711 can sustain CNS microglia maintenance and function throughout adult life. Nat
1712 Neurosci. **10:**1538-1543.

1713 82. **Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM.** 2011. Infiltrating
1714 monocytes trigger EAE progression, but do not contribute to the resident microglia
1715 pool. Nat Neurosci. **14:**1142-1149.
1716 83. **Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF,**
1717 **Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M.** 2010. Fate mapping
1718 analysis reveals that adult microglia derive from primitive macrophages. Science.
1719 **330:**841-845.
1720 84. **Nimmerjahn A, Kirchhoff F, Helmchen F.** 2005. Resting microglial cells are highly
1721 dynamic surveillants of brain parenchyma *in vivo*. Science. **308:**1314-1318.
1722 85. **Prodinger C, Bunse J, Kruger M, Schiefenhovel F, Brandt C, Laman JD, Greter**
1723 **M, Immig K, Heppner F, Becher B, Bechmann I.** 2011. CD11c-expressing cells
1724 reside in the juxtavascular parenchyma and extend processes into the glia limitans of
1725 the mouse nervous system. Acta Neuropathol. **121:**445-458.
1726 86. **Bulloch K, Miller MM, Gal-Toth J, Milner TA, Gottfried-Blackmore A, Waters**
1727 **EM, Kaunzner UW, Liu K, Lindquist R, Nussenzweig MC, Steinman RM,**
1728 **McEwen BS.** 2008. CD11c/EYFP transgene illuminates a discrete network of
1729 dendritic cells within the embryonic, neonatal, adult, and injured mouse brain. J Comp
1730 Neurol. **508:**687-710.
1731 87. **Matyszak MK, Perry VH.** 1996. The potential role of dendritic cells in immune-
1732 mediated inflammatory diseases in the central nervous system. Neuroscience. **74:**599-
1733 608.
1734 88. **Anandasabapathy N, Victora GD, Meredith M, Feder R, Dong B, Kluger C, Yao**
1735 **K, Dustin ML, Nussenzweig MC, Steinman RM, Liu K.** 2011. Flt3L controls the
1736 development of radiosensitive dendritic cells in the meninges and choroid plexus of
1737 the steady-state mouse brain. J Exp Med. **208:**1695-1705.

1738 89. **Ransohoff RM, Engelhardt B.** 2012. The anatomical and cellular basis of immune
1739 surveillance in the central nervous system. Nat Rev Immunol. **12:**623-635.
1740 90. **Kivisakk P, Mahad DJ, Callahan MK, Trebst C, Tucky B, Wei T, Wu L,**
1741 **Baekkevold ES, Lassmann H, Staugaitis SM, Campbell JJ, Ransohoff RM.** 2003.
1742 Human cerebrospinal fluid central memory CD4+ T cells: evidence for trafficking
1743 through choroid plexus and meninges via P-selectin. Proc Natl Acad Sci U S A.
1744 **100:**8389-8394.
1745 91. **Kivisakk P, Imitola J, Rasmussen S, Elyaman W, Zhu B, Ransohoff RM, Khoury**
1746 **SJ.** 2009. Localizing central nervous system immune surveillance: meningeal antigen-
1747 presenting cells activate T cells during experimental autoimmune encephalomyelitis.
1748 Ann Neurol. **65:**457-469.
1749 92. **Engelhardt B, Ransohoff RM.** 2012. Capture, crawl, cross: the T cell code to breach
1750 the blood-brain barriers. Trends Immunol. **33:**579-589.
1751 93. **Vandenbroucke RE, Dejonckheere E, Van Lint P, Demeestere D, Van**
1752 **Wonterghem E, Vanlaere I, Puimege L, Van Hauwermeiren F, De Rycke R, Mc**
1753 **Guire C, Campestre C, Lopez-Otin C, Matthys P, Leclercq G, Libert C.** 2012.
1754 Matrix metalloprotease 8-dependent extracellular matrix cleavage at the blood-CSF
1755 barrier contributes to lethality during systemic inflammatory diseases. J Neurosci.
1756 **32:**9805-9816.
1757 94. **Barichello T, Fagundes GD, Generoso JS, Paula Moreira A, Costa CS, Zanatta**
1758 **JR, Simoes LR, Petronilho F, Dal-Pizzol F, Carvalho Vilela M, Lucio Teixeira A.**
1759 2012. Brain-blood barrier breakdown and pro-inflammatory mediators in neonate rats
1760 submitted meningitis by *Streptococcus pneumoniae*. Brain Res. **1471:**162-168.
1761 95. **Braun J.** 2009. Inducible nitric oxide synthase mediates hippocampal caspase-3
1762 activation in pneumococcal meningitis. Int J Neurosci. **119:**455-459.

1763 96. **Schmidt H, Tlustochowska A, Stuertz K, Djukic M, Gerber J, Schutz E, Kuhnt**
1764 **U, Nau R.** 2001. Organotypic hippocampal cultures. A model of brain tissue damage
1765 in *Streptococcus pneumoniae* meningitis. J Neuroimmunol. **113:**30-39.
1766 97. **Zysk G, Schneider-Wald BK, Hwang JH, Bejo L, Kim KS, Mitchell TJ,**
1767 **Hakenbeck R, Heinz HP.** 2001. Pneumolysin is the main inducer of cytotoxicity to
1768 brain microvascular endothelial cells caused by *Streptococcus pneumoniae*. Infect
1769 Immun. **69:**845-852.
1770 98. **Braun JS, Sublett JE, Freyer D, Mitchell TJ, Cleveland JL, Tuomanen EI,**
1771 **Weber JR.** 2002. Pneumococcal pneumolysin and H(2)O(2) mediate brain cell
1772 apoptosis during meningitis. J Clin Invest. **109:**19-27.
1773 99. **Grandgirard D, Gaumann R, Coulibaly B, Dangy JP, Sie A, Junghanss T,**
1774 **Schudel H, Pluschke G, Leib SL.** 2013. The causative pathogen determines the
1775 inflammatory profile in cerebrospinal fluid and outcome in patients with bacterial
1776 meningitis. Mediators Inflamm. **2013:**312476.
1777 100. **Gerber J, Nau R.** 2010. Mechanisms of injury in bacterial meningitis. Curr Opin
1778 Neurol. **23:**312-318.
1779 101. **Kristensson K.** 2011. Microbes' roadmap to neurons. Nat Rev Neurosci. **12:**345-357.
1780 102. **Parkkinen J, Korhonen TK, Pere A, Hacker J, Soinila S.** 1988. Binding sites in
1781 the rat brain for *Escherichia coli* S fimbriae associated with neonatal meningitis. J
1782 Clin Invest. **81:**860-865.
1783 103. **Zelmer A, Bowen M, Jokilammi A, Finne J, Luzio JP, Taylor PW.** 2008.
1784 Differential expression of the polysialyl capsule during blood-to-brain transit of
1785 neuropathogenic *Escherichia coli* K1. Microbiology. **154:**2522-2532.

1786 104. **Iovino F, Orihuela CJ, Moorlag HE, Molema G, Bijlsma JJ.** 2013. Interactions
1787 between blood-borne *Streptococcus pneumoniae* and the blood-brain barrier
1788 preceding meningitis. PLoS One. **8:**e68408.
1789 105. **Owen SJ, Batzloff M, Chehrehasa F, Meedeniya A, Casart Y, Logue CA, Hirst**
1790 **RG, Peak IR, Mackay-Sim A, Beacham IR.** 2009. Nasal-associated lymphoid tissue
1791 and olfactory epithelium as portals of entry for *Burkholderia pseudomallei* in murine
1792 melioidosis. J Infect Dis. **199:**1761-1770.
1793 106. **Antal EA, Loberg EM, Bracht P, Melby KK, Maehlen J.** 2001. Evidence for
1794 intraaxonal spread of *Listeria monocytogenes* from the periphery to the central
1795 nervous system. Brain Pathol. **11:**432-438.
1796 107. **Hupp S, Heimeroth V, Wippel C, Fortsch C, Ma J, Mitchell TJ, Iliev AI.** 2012.
1797 Astrocytic tissue remodeling by the meningitis neurotoxin pneumolysin facilitates
1798 pathogen tissue penetration and produces interstitial brain edema. Glia. **60:**137-146.
1799 108. **Galdiero M, D'Amico M, Gorga F, Di Filippo C, D'Isanto M, Vitiello M,**
1800 **Longanella A, Tortora A.** 2001. *Haemophilus influenzae* porin contributes to
1801 signaling of the inflammatory cascade in rat brain. Infect Immun. **69:**221-227.
1802 109. **Banerjee A, Kim BJ, Carmona EM, Cutting AS, Gurney MA, Carlos C, Feuer R,**
1803 **Prasadarao NV, Doran KS.** 2011. Bacterial pili exploit integrin machinery to
1804 promote immune activation and efficient blood-brain barrier penetration. Nat
1805 Commun. **2:**462.
1806 110. **Mittal R, Prasadarao NV.** 2011. gp96 expression in neutrophils is critical for the
1807 onset of *Escherichia coli* K1 (RS218) meningitis. Nat Commun. **2:**552.
1808 111. **Reiss A, Braun JS, Jager K, Freyer D, Laube G, Buhrer C, Felderhoff-Muser U,**
1809 **Stadelmann C, Nizet V, Weber JR.** 2011. Bacterial pore-forming cytolysins induce
1810 neuronal damage in a rat model of neonatal meningitis. J Infect Dis. **203:**393-400.

1811 112. **Strand AD, Aragaki AK, Baquet ZC, Hodges A, Cunningham P, Holmans P,**
1812 **Jones KR, Jones L, Kooperberg C, Olson JM.** 2007. Conservation of regional gene
1813 expression in mouse and human brain. PLoS Genet. **3:**e59.
1814 113. **Miller JA, Horvath S, Geschwind DH.** 2010. Divergence of human and mouse brain
1815 transcriptome highlights Alzheimer disease pathways. Proc Natl Acad Sci U S A.
1816 **107:**12698-12703.
1817 114. **Janssen SF, van der Spek SJ, Ten Brink JB, Essing AH, Gorgels TG, van der**
1818 **Spek PJ, Jansonius NM, Bergen AA.** 2013. Gene expression and functional
1819 annotation of the human and mouse choroid plexus epithelium. PLoS One. **8:**e83345.
1820 115. **Hagan CE, Bolon B, Keene CD.** 2012. Nervous system, p 339-393. *In* Treuting PM,
1821 Dintzis S, Liggitt D, Frevert CW (ed.), Comparative anatomy and histology: a mouse
1822 and human atlas. Elsevier United States.
1823 116. **Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD,**
1824 **Pilcher W, Ojemann JG, Ransom BR, Goldman SA, Nedergaard M.** 2009.
1825 Uniquely hominid features of adult human astrocytes. J Neurosci. **29:**3276-3287.
1826 117. **Chi F, Wang L, Zheng X, Wu CH, Jong A, Sheard MA, Shi W, Huang SH.** 2011.
1827 Meningitic *Escherichia coli* K1 penetration and neutrophil transmigration across the
1828 blood-brain barrier are modulated by alpha7 nicotinic receptor. PLoS One. **6:**e25016.
1829 118. **Jain SK, Paul-Satyaseela M, Lamichhane G, Kim KS, Bishai WR.** 2006.
1830 *Mycobacterium tuberculosis* invasion and traversal across an *in vitro* human blood-
1831 brain barrier as a pathogenic mechanism for central nervous system tuberculosis. J
1832 Infect Dis. **193:**1287-1295.
1833 119. **Salmeri M, Motta C, Anfuso CD, Amodeo A, Scalia M, Toscano MA, Alberghina**
1834 **M, Lupo G.** 2013. VEGF receptor-1 involvement in pericyte loss induced by

1835 *Escherichia coli* in an *in vitro* model of blood brain barrier. Cell Microbiol. **15:**1367-
1836 1384.

1837 120. **Lyck R, Ruderisch N, Moll AG, Steiner O, Cohen CD, Engelhardt B, Makrides**
1838 **V, Verrey F.** 2009. Culture-induced changes in blood-brain barrier transcriptome:
1839 implications for amino-acid transporters *in vivo*. J Cereb Blood Flow Metab. **29:**1491-
1840 1502.

1841 121. **Urich E, Lazic SE, Molnos J, Wells I, Freskgard PO.** 2012. Transcriptional
1842 profiling of human brain endothelial cells reveals key properties crucial for predictive
1843 *in vitro* blood-brain barrier models. PLoS One. **7:**e38149.

1844 122. **Mikaty G, Soyer M, Mairey E, Henry N, Dyer D, Forest KT, Morand P,**
1845 **Guadagnini S, Prevost MC, Nassif X, Dumenil G.** 2009. Extracellular bacterial
1846 pathogen induces host cell surface reorganization to resist shear stress. PLoS Pathog.
1847 **5:**e1000314.

1848 123. **Brissac T, Mikaty G, Dumenil G, Coureuil M, Nassif X.** 2012. The meningococcal
1849 minor pilin PilX is responsible for type IV pilus conformational changes associated
1850 with signaling to endothelial cells. Infect Immun. **80:**3297-3306.

1851 124. **Mairey E, Genovesio A, Donnadieu E, Bernard C, Jaubert F, Pinard E, Seylaz J,**
1852 **Olivo-Marin JC, Nassif X, Dumenil G.** 2006. Cerebral microcirculation shear stress
1853 levels determine *Neisseria meningitidis* attachment sites along the blood-brain barrier.
1854 J Exp Med. **203:**1939-1950.

1855 125. **Hellinger E, Veszelka S, Toth AE, Walter F, Kittel A, Bakk ML, Tihanyi K,**
1856 **Hada V, Nakagawa S, Duy TD, Niwa M, Deli MA, Vastag M.** 2012. Comparison
1857 of brain capillary endothelial cell-based and epithelial (MDCK-MDR1, Caco-2, and
1858 VB-Caco-2) cell-based surrogate blood-brain barrier penetration models. Eur J Pharm
1859 Biopharm. **82:**340-351.

1860 126. **Weksler BB, Subileau EA, Perriere N, Charneau P, Holloway K, Leveque M,**
1861 **Tricoire-Leignel H, Nicotra A, Bourdoulous S, Turowski P, Male DK, Roux F,**
1862 **Greenwood J, Romero IA, Couraud PO.** 2005. Blood-brain barrier-specific
1863 properties of a human adult brain endothelial cell line. FASEB J. **19:**1872-1874.
1864 127. **Kusch-Poddar M, Drewe J, Fux I, Gutmann H.** 2005. Evaluation of the
1865 immortalized human brain capillary endothelial cell line BB19 as a human cell culture
1866 model for the blood-brain barrier. Brain Res. **1064:**21-31.
1867 128. **Sano Y, Shimizu F, Abe M, Maeda T, Kashiwamura Y, Ohtsuki S, Terasaki T,**
1868 **Obinata M, Kajiwara K, Fujii M, Suzuki M, Kanda T.** 2010. Establishment of a
1869 new conditionally immortalized human brain microvascular endothelial cell line
1870 retaining an *in vivo* blood-brain barrier function. J Cell Physiol. **225:**519-528.
1871 129. **Eigenmann DE, Xue G, Kim KS, Moses AV, Hamburger M, Oufir M.** 2013.
1872 Comparative study of four immortalized human brain capillary endothelial cell lines,
1873 hCMEC/D3, hBMEC, TY10, and BB19, and optimization of culture conditions, for
1874 an *in vitro* blood-brain barrier model for drug permeability studies. Fluids Barriers
1875 CNS. **10:**33.
1876 130. **Daneman R, Zhou L, Kebede AA, Barres BA.** 2010. Pericytes are required for
1877 blood-brain barrier integrity during embryogenesis. Nature. **468:**562-566.
1878 131. **Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R, Zlokovic BV.**
1879 2010. Pericytes control key neurovascular functions and neuronal phenotype in the
1880 adult brain and during brain aging. Neuron. **68:**409-427.
1881 132. **Armulik A, Genove G, Mae M, Nisancioglu MH, Wallgard E, Niaudet C, He L,**
1882 **Norlin J, Lindblom P, Strittmatter K, Johansson BR, Betsholtz C.** 2010. Pericytes
1883 regulate the blood-brain barrier. Nature. **468:**557-561.

1884 133. **Lee SW, Kim WJ, Choi YK, Song HS, Son MJ, Gelman IH, Kim YJ, Kim KW.**
1885 2003. SSeCKS regulates angiogenesis and tight junction formation in blood-brain
1886 barrier. Nat Med. **9:**900-906.
1887 134. **Siddharthan V, Kim YV, Liu S, Kim KS.** 2007. Human astrocytes/astrocyte-
1888 conditioned medium and shear stress enhance the barrier properties of human brain
1889 microvascular endothelial cells. Brain Res. **1147:**39-50.
1890 135. **Ezan P, Andre P, Cisternino S, Saubamea B, Boulay AC, Doutremer S, Thomas**
1891 **MA, Quenech'du N, Giaume C, Cohen-Salmon M.** 2012. Deletion of astroglial
1892 connexins weakens the blood-brain barrier. J Cereb Blood Flow Metab. **32:**1457-
1893 1467.
1894 136. **Nuriya M, Shinotsuka T, Yasui M.** 2013. Diffusion properties of molecules at the
1895 blood-brain interface: potential contributions of astrocyte endfeet to diffusion barrier
1896 functions. Cereb Cortex. **23:**2118-2126.
1897 137. **Zehendner CM, White R, Hedrich J, Luhmann HJ.** 2014. A neurovascular blood-
1898 brain barrier *in vitro* model. Methods Mol Biol. **1135:**403-413.
1899 138. **Hatherell K, Couraud PO, Romero IA, Weksler B, Pilkington GJ.** 2011.
1900 Development of a three-dimensional, all-human *in vitro* model of the blood-brain
1901 barrier using mono-, co-, and tri-cultivation Transwell models. J Neurosci Methods.
1902 **199:**223-229.
1903 139. **Nakagawa S, Deli MA, Kawaguchi H, Shimizudani T, Shimono T, Kittel A,**
1904 **Tanaka K, Niwa M.** 2009. A new blood-brain barrier model using primary rat brain
1905 endothelial cells, pericytes and astrocytes. Neurochem Int. **54:**253-263.
1906 140. **Schwerk C, Papandreou T, Schuhmann D, Nickol L, Borkowski J, Steinmann U,**
1907 **Quednau N, Stump C, Weiss C, Berger J, Wolburg H, Claus H, Vogel U,**
1908 **Ishikawa H, Tenenbaum T, Schroten H.** 2012. Polar invasion and translocation of

1909 *Neisseria meningitidis* and *Streptococcus suis* in a novel human model of the blood-
1910 cerebrospinal fluid barrier. PLoS One. **7:**e30069.
1911 141. **Schroten M, Hanisch FG, Quednau N, Stump C, Riebe R, Lenk M, Wolburg H,**
1912 **Tenenbaum T, Schwerk C.** 2012. A novel porcine *in vitro* model of the blood-
1913 cerebrospinal fluid barrier with strong barrier function. PLoS One. **7:**e39835.
1914 142. **Tuomanen E.** 1996. Entry of pathogens into the central nervous system. FEMS
1915 Microbiol Rev. **18:**289-299.
1916 143. **Gilles FH, Jammes JL, Berenberg W.** 1977. Neonatal meningitis. The ventricle as a
1917 bacterial reservoir. Arch Neurol. **34:**560-562.
1918 144. **Smith AL.** 1987. Pathogenesis of *Haemophilus influenzae* meningitis. Pediatr Infect
1919 Dis J. **6:**783-786.
1920 145. **Daum RS, Scheifele DW, Syriopoulou VP, Averill D, Smith AL.** 1978. Ventricular
1921 involvement in experimental *Hemophilus influenzae* meningitis. J Pediatr. **93:**927-
1922 930.
1923 146. **Williams AE, Blakemore WF.** 1990. Pathogenesis of meningitis caused by
1924 *Streptococcus suis* type 2. J Infect Dis. **162:**474-481.
1925 147. **Pron B, Taha MK, Rambaud C, Fournet JC, Pattey N, Monnet JP, Musilek M,**
1926 **Beretti JL, Nassif X.** 1997. Interaction of *Neisseria meningitidis* with the
1927 components of the blood-brain barrier correlates with an increased expression of PilC.
1928 J Infect Dis. **176:**1285-1292.
1929 148. **Guarner J, Greer PW, Whitney A, Shieh WJ, Fischer M, White EH, Carlone**
1930 **GM, Stephens DS, Popovic T, Zaki SR.** 2004. Pathogenesis and diagnosis of human
1931 meningococcal disease using immunohistochemical and PCR assays. Am J Clin
1932 Pathol. **122:**754-764.

1933 149. **Chung JW, Hong SJ, Kim KJ, Goti D, Stins MF, Shin S, Dawson VL, Dawson**
1934 **TM, Kim KS.** 2003. 37-kDa laminin receptor precursor modulates cytotoxic
1935 necrotizing factor 1-mediated RhoA activation and bacterial uptake. J Biol Chem.
1936 **278:**16857-16862.
1937 150. **Kim KJ, Chung JW, Kim KS.** 2005. 67-kDa laminin receptor promotes
1938 internalization of cytotoxic necrotizing factor 1-expressing *Escherichia coli* K1 into
1939 human brain microvascular endothelial cells. J Biol Chem. **280:**1360-1368.
1940 151. **Orihuela CJ, Mahdavi J, Thornton J, Mann B, Wooldridge KG, Abouseada N,**
1941 **Oldfield NJ, Self T, Ala'Aldeen DA, Tuomanen EI.** 2009. Laminin receptor
1942 initiates bacterial contact with the blood brain barrier in experimental meningitis
1943 models. J Clin Invest. **119:**1638-1646.
1944 152. **Abouseada NM, Assafi MS, Mahdavi J, Oldfield NJ, Wheldon LM, Wooldridge**
1945 **KG, Ala'Aldeen DA.** 2012. Mapping the laminin receptor binding domains of
1946 *Neisseria meningitidis* PorA and *Haemophilus influenzae* OmpP2. PLoS One.
1947 **7:**e46233.
1948 153. **Nelson J, McFerran NV, Pivato G, Chambers E, Doherty C, Steele D, Timson**
1949 **DJ.** 2008. The 67 kDa laminin receptor: structure, function and role in disease. Biosci
1950 Rep. **28:**33-48.
1951 154. **Khan NA, Kim Y, Shin S, Kim KS.** 2007. FimH-mediated *Escherichia coli* K1
1952 invasion of human brain microvascular endothelial cells. Cell Microbiol. **9:**169-178.
1953 155. **Sa ECC, Griffiths NJ, Virji M.** 2010. *Neisseria meningitidis* Opc invasin binds to
1954 the sulphated tyrosines of activated vitronectin to attach to and invade human brain
1955 endothelial cells. PLoS Pathog. **6:**e1000911.
1956 156. **Tenenbaum T, Bloier C, Adam R, Reinscheid DJ, Schroten H.** 2005. Adherence to
1957 and invasion of human brain microvascular endothelial cells are promoted by

1958 fibrinogen-binding protein FbsA of *Streptococcus agalactiae*. Infect Immun. **73:**4404-
1959 4409.
1960 157. **Chang YC, Wang Z, Flax LA, Xu D, Esko JD, Nizet V, Baron MJ.** 2011.
1961 Glycosaminoglycan binding facilitates entry of a bacterial pathogen into central
1962 nervous systems. PLoS Pathog. **7:**e1002082.
1963 158. **Be NA, Bishai WR, Jain SK.** 2012. Role of *Mycobacterium tuberculosis* pknD in the
1964 pathogenesis of central nervous system tuberculosis. BMC Microbiol. **12:**7.
1965 159. **Tenenbaum T, Spellerberg B, Adam R, Vogel M, Kim KS, Schroten H.** 2007.
1966 *Streptococcus agalactiae* invasion of human brain microvascular endothelial cells is
1967 promoted by the laminin-binding protein Lmb. Microbes Infect. **9:**714-720.
1968 160. **Cabanes D, Sousa S, Cebria A, Lecuit M, Garcia-del Portillo F, Cossart P.** 2005.
1969 Gp96 is a receptor for a novel *Listeria monocytogenes* virulence factor, Vip, a surface
1970 protein. EMBO J. **24:**2827-2838.
1971 161. **Prasadarao NV.** 2002. Identification of *Escherichia coli* outer membrane protein A
1972 receptor on human brain microvascular endothelial cells. Infect Immun. **70:**4556-
1973 4563.
1974 162. **Prasadarao NV, Srivastava PK, Rudrabhatla RS, Kim KS, Huang SH,**
1975 **Sukumaran SK.** 2003. Cloning and expression of the *Escherichia coli* K1 outer
1976 membrane protein A receptor, a gp96 homologue. Infect Immun. **71:**1680-1688.
1977 163. **Stins MF, Badger J, Sik Kim K.** 2001. Bacterial invasion and transcytosis in
1978 transfected human brain microvascular endothelial cells. Microb Pathog. **30:**19-28.
1979 164. **Prasadarao NV, Wass CA, Stins MF, Shimada H, Kim KS.** 1999. Outer membrane
1980 protein A-promoted actin condensation of brain microvascular endothelial cells is
1981 required for *Escherichia coli* invasion. Infect Immun. **67:**5775-5783.

1982 165. **Kim KJ, Elliott SJ, Di Cello F, Stins MF, Kim KS.** 2003. The K1 capsule
1983 modulates trafficking of *E. coli*-containing vacuoles and enhances intracellular
1984 bacterial survival in human brain microvascular endothelial cells. Cell Microbiol.
1985 **5:**245-252.
1986 166. **Reddy MA, Wass CA, Kim KS, Schlaepfer DD, Prasadarao NV.** 2000.
1987 Involvement of focal adhesion kinase in *Escherichia coli* invasion of human brain
1988 microvascular endothelial cells. Infect Immun. **68:**6423-6430.
1989 167. **Reddy MA, Prasadarao NV, Wass CA, Kim KS.** 2000. Phosphatidylinositol 3-
1990 kinase activation and interaction with focal adhesion kinase in *Escherichia coli* K1
1991 invasion of human brain microvascular endothelial cells. J Biol Chem. **275:**36769-
1992 36774.
1993 168. **Liu W, Zhao WD, Yan JC, Ren ZY, Fang WG, Zhu L, Shang DS, Chen YH.**
1994 2010. Involvement of Src tyrosine kinase in *Escherichia coli* invasion of human brain
1995 microvascular endothelial cells. FEBS Lett. **584:**27-32.
1996 169. **Sukumaran SK, Prasadarao NV.** 2002. Regulation of protein kinase C in
1997 *Escherichia coli* K1 invasion of human brain microvascular endothelial cells. J Biol
1998 Chem. **277:**12253-12262.
1999 170. **Sukumaran SK, Quon MJ, Prasadarao NV.** 2002. *Escherichia coli* K1
2000 internalization via caveolae requires caveolin-1 and protein kinase Calpha interaction
2001 in human brain microvascular endothelial cells. J Biol Chem. **277:**50716-50724.
2002 171. **Khan NA, Wang Y, Kim KJ, Chung JW, Wass CA, Kim KS.** 2002. Cytotoxic
2003 necrotizing factor-1 contributes to *Escherichia coli* K1 invasion of the central nervous
2004 system. J Biol Chem. **277:**15607-15612.
2005 172. **Khan NA, Shin S, Chung JW, Kim KJ, Elliott S, Wang Y, Kim KS.** 2003. Outer
2006 membrane protein A and cytotoxic necrotizing factor-1 use diverse signaling

2007 mechanisms for *Escherichia coli* K1 invasion of human brain microvascular
2008 endothelial cells. Microb Pathog. **35:**35-42.
2009 173. **Maruvada R, Kim KS.** 2011. Extracellular loops of the *Eschericia coli* outer
2010 membrane protein A contribute to the pathogenesis of meningitis. J Infect Dis.
2011 **203:**131-140.
2012 174. **Teng CH, Tseng YT, Maruvada R, Pearce D, Xie Y, Paul-Satyaseela M, Kim KS.**
2013 2010. NlpI contributes to *Escherichia coli* K1 strain RS218 interaction with human
2014 brain microvascular endothelial cells. Infect Immun. **78:**3090-3096.
2015 175. **Das A, Asatryan L, Reddy MA, Wass CA, Stins MF, Joshi S, Bonventre JV, Kim**
2016 **KS.** 2001. Differential role of cytosolic phospholipase A2 in the invasion of brain
2017 microvascular endothelial cells by *Escherichia coli* and *Listeria monocytogenes*. J
2018 Infect Dis. **184:**732-737.
2019 176. **Zhu L, Maruvada R, Sapirstein A, Malik KU, Peters-Golden M, Kim KS.** 2010.
2020 Arachidonic acid metabolism regulates *Escherichia coli* penetration of the blood-
2021 brain barrier. Infect Immun. **78:**4302-4310.
2022 177. **Zhu L, Pearce D, Kim KS.** 2010. Prevention of *Escherichia coli* K1 penetration of
2023 the blood-brain barrier by counteracting the host cell receptor and signaling molecule
2024 involved in *E. coli* invasion of human brain microvascular endothelial cells. Infect
2025 Immun. **78:**3554-3559.
2026 178. **Maruvada R, Kim KS.** 2012. IbeA and OmpA of *Escherichia coli* K1 exploit Rac1
2027 activation for invasion of human brain microvascular endothelial cells. Infect Immun.
2028 **80:**2035-2041.
2029 179. **Cruz-Migoni A, Hautbergue GM, Artymiuk PJ, Baker PJ, Bokori-Brown M,**
2030 **Chang CT, Dickman MJ, Essex-Lopresti A, Harding SV, Mahadi NM, Marshall**
2031 **LE, Mobbs GW, Mohamed R, Nathan S, Ngugi SA, Ong C, Ooi WF, Partridge**

2032 **LJ, Phillips HL, Raih MF, Ruzheinikov S, Sarkar-Tyson M, Sedelnikova SE,**
2033 **Smither SJ, Tan P, Titball RW, Wilson SA, Rice DW.** 2011. A *Burkholderia*
2034 *pseudomallei* toxin inhibits helicase activity of translation factor eIF4A. Science.
2035 **334:**821-824.

2036 180. **Kallstrom H, Liszewski MK, Atkinson JP, Jonsson AB.** 1997. Membrane cofactor
2037 protein (MCP or CD46) is a cellular pilus receptor for pathogenic *Neisseria*. Mol
2038 Microbiol. **25:**639-647.

2039 181. **Kirchner M, Meyer TF.** 2005. The PilC adhesin of the *Neisseria* type IV pilus-
2040 binding specificities and new insights into the nature of the host cell receptor. Mol
2041 Microbiol. **56:**945-957.

2042 182. **Jen FE, Warren MJ, Schulz BL, Power PM, Swords WE, Weiser JN, Apicella**
2043 **MA, Edwards JL, Jennings MP.** 2013. Dual pili post-translational modifications
2044 synergize to mediate meningococcal adherence to platelet activating factor receptor
2045 on human airway cells. PLoS Pathog. **9:**e1003377.

2046 183. **Virji M, Kayhty H, Ferguson DJ, Alexandrescu C, Heckels JE, Moxon ER.** 1991.
2047 The role of pili in the interactions of pathogenic *Neisseria* with cultured human
2048 endothelial cells. Mol Microbiol. **5:**1831-1841.

2049 184. **Virji M, Makepeace K, Ferguson DJ, Achtman M, Moxon ER.** 1993.
2050 Meningococcal Opa and Opc proteins: their role in colonization and invasion of
2051 human epithelial and endothelial cells. Mol Microbiol. **10:**499-510.

2052 185. **Nikulin J, Panzner U, Frosch M, Schubert-Unkmeir A.** 2006. Intracellular survival
2053 and replication of *Neisseria meningitidis* in human brain microvascular endothelial
2054 cells. Int J Med Microbiol. **296:**553-558.

2055 186. **Eugene E, Hoffmann I, Pujol C, Couraud PO, Bourdoulous S, Nassif X.** 2002. Microvilli-like structures are associated with the internalization of virulent capsulated *Neisseria meningitidis* into vascular endothelial cells. J Cell Sci. **115:**1231-1241.

2058 187. **Coureuil M, Lecuyer H, Scott MG, Boularan C, Enslen H, Soyer M, Mikaty G, Bourdoulous S, Nassif X, Marullo S.** 2010. Meningococcus hijacks a beta2-adrenoceptor/beta-arrestin pathway to cross brain microvasculature endothelium. Cell. **143:**1149-1160.

2062 188. **Hoffmann I, Eugene E, Nassif X, Couraud PO, Bourdoulous S.** 2001. Activation of ErbB2 receptor tyrosine kinase supports invasion of endothelial cells by *Neisseria meningitidis*. J Cell Biol. **155:**133-143.

2065 189. **Merz AJ, Enns CA, So M.** 1999. Type IV pili of pathogenic *Neisseriae* elicit cortical plaque formation in epithelial cells. Mol Microbiol. **32:**1316-1332.

2067 190. **Lambotin M, Hoffmann I, Laran-Chich MP, Nassif X, Couraud PO, Bourdoulous S.** 2005. Invasion of endothelial cells by *Neisseria meningitidis* requires cortactin recruitment by a phosphoinositide-3-kinase/Rac1 signalling pathway triggered by the lipo-oligosaccharide. J Cell Sci. **118:**3805-3816.

2071 191. **Unkmeir A, Latsch K, Dietrich G, Wintermeyer E, Schinke B, Schwender S, Kim KS, Eigenthaler M, Frosch M.** 2002. Fibronectin mediates Opc-dependent internalization of *Neisseria meningitidis* in human brain microvascular endothelial cells. Mol Microbiol. **46:**933-946.

2075 192. **Sokolova O, Heppel N, Jagerhuber R, Kim KS, Frosch M, Eigenthaler M, Schubert-Unkmeir A.** 2004. Interaction of *Neisseria meningitidis* with human brain microvascular endothelial cells: role of MAP- and tyrosine kinases in invasion and inflammatory cytokine release. Cell Microbiol. **6:**1153-1166.

2079 193. **Ring A, Weiser JN, Tuomanen EI.** 1998. Pneumococcal trafficking across the
2080 blood-brain barrier. Molecular analysis of a novel bidirectional pathway. J Clin
2081 Invest. **102:**347-360.
2082 194. **Banerjee A, Van Sorge NM, Sheen TR, Uchiyama S, Mitchell TJ, Doran KS.**
2083 2010. Activation of brain endothelium by pneumococcal neuraminidase NanA
2084 promotes bacterial internalization. Cell Microbiol. **12:**1576-1588.
2085 195. **Uchiyama S, Carlin AF, Khosravi A, Weiman S, Banerjee A, Quach D,**
2086 **Hightower G, Mitchell TJ, Doran KS, Nizet V.** 2009. The surface-anchored NanA
2087 protein promotes pneumococcal brain endothelial cell invasion. J Exp Med.
2088 **206:**1845-1852.
2089 196. **Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI.** 1995.
2090 *Streptococcus pneumoniae* anchor to activated human cells by the receptor for
2091 platelet-activating factor. Nature. **377:**435-438.
2092 197. **Radin JN, Orihuela CJ, Murti G, Guglielmo C, Murray PJ, Tuomanen EI.** 2005.
2093 beta-Arrestin 1 participates in platelet-activating factor receptor-mediated endocytosis
2094 of *Streptococcus pneumoniae*. Infect Immun. **73:**7827-7835.
2095 198. **Tazi A, Disson O, Bellais S, Bouaboud A, Dmytruk N, Dramsi S, Mistou MY,**
2096 **Khun H, Mechler C, Tardieux I, Trieu-Cuot P, Lecuit M, Poyart C.** 2010. The
2097 surface protein HvgA mediates group B streptococcus hypervirulence and meningeal
2098 tropism in neonates. J Exp Med. **207:**2313-2322.
2099 199. **Mu R, Kim BJ, Paco C, Del Rosario Y, Courtney HS, Doran KS.** 2014.
2100 Identification of a Group B streptococcal fibronectin binding protein, SfbA, that
2101 contributes to invasion of brain endothelium and the development of meningitis.
2102 Infect Immun.

2103 200. **Seo HS, Minasov G, Seepersaud R, Doran KS, Dubrovska I, Shuvalova L,**
2104 **Anderson WF, Iverson TM, Sullam PM.** 2013. Characterization of fibrinogen
2105 binding by glycoproteins Srr1 and Srr2 of *Streptococcus agalactiae*. J Biol Chem.
2106 **288:**35982-35996.
2107 201. **Seo HS, Mu R, Kim BJ, Doran KS, Sullam PM.** 2012. Binding of glycoprotein Srr1
2108 of *Streptococcus agalactiae* to fibrinogen promotes attachment to brain endothelium
2109 and the development of meningitis. PLoS Pathog. **8:**e1002947.
2110 202. **Nizet V, Kim KS, Stins M, Jonas M, Chi EY, Nguyen D, Rubens CE.** 1997.
2111 Invasion of brain microvascular endothelial cells by group B streptococci. Infect
2112 Immun. **65:**5074-5081.
2113 203. **Quach D, van Sorge NM, Kristian SA, Bryan JD, Shelver DW, Doran KS.** 2009.
2114 The CiaR response regulator in group B streptococcus promotes intracellular survival
2115 and resistance to innate immune defenses. J Bacteriol. **191:**2023-2032.
2116 204. **Shin S, Paul-Satyaseela M, Lee JS, Romer LH, Kim KS.** 2006. Focal adhesion
2117 kinase is involved in type III group B streptococcal invasion of human brain
2118 microvascular endothelial cells. Microb Pathog. **41:**168-173.
2119 205. **Shin S, Kim KS.** 2006. RhoA and Rac1 contribute to type III group B streptococcal
2120 invasion of human brain microvascular endothelial cells. Biochem Biophys Res
2121 Commun. **345:**538-542.
2122 206. **Maruvada R, Zhu L, Pearce D, Sapirstein A, Kim KS.** 2011. Host cytosolic
2123 phospholipase A(2)alpha contributes to group B streptococcus penetration of the
2124 blood-brain barrier. Infect Immun. **79:**4088-4093.
2125 207. **Charland N, Nizet V, Rubens CE, Kim KS, Lacouture S, Gottschalk M.** 2000.
2126 *Streptococcus suis* serotype 2 interactions with human brain microvascular
2127 endothelial cells. Infect Immun. **68:**637-643.

2128 208. **Vanier G, Segura M, Friedl P, Lacouture S, Gottschalk M.** 2004. Invasion of
2129 porcine brain microvascular endothelial cells by *Streptococcus suis* serotype 2. Infect
2130 Immun. **72:**1441-1449.
2131 209. **Vanier G, Segura M, Gottschalk M.** 2007. Characterization of the invasion of
2132 porcine endothelial cells by *Streptococcus suis* serotype 2. Can J Vet Res. **71:**81-89.
2133 210. **Vanier G, Fittipaldi N, Slater JD, de la Cruz Dominguez-Punaro M, Rycroft AN,**
2134 **Segura M, Maskell DJ, Gottschalk M.** 2009. New putative virulence factors of
2135 *Streptococcus suis* involved in invasion of porcine brain microvascular endothelial
2136 cells. Microb Pathog. **46:**13-20.
2137 211. **Tenenbaum T, Papandreou T, Gellrich D, Friedrichs U, Seibt A, Adam R,**
2138 **Wewer C, Galla HJ, Schwerk C, Schroten H.** 2009. Polar bacterial invasion and
2139 translocation of *Streptococcus suis* across the blood-cerebrospinal fluid barrier *in*
2140 *vitro*. Cell Microbiol. **11:**323-336.
2141 212. **Drevets DA, Sawyer RT, Potter TA, Campbell PA.** 1995. *Listeria monocytogenes*
2142 infects human endothelial cells by two distinct mechanisms. Infect Immun. **63:**4268-
2143 4276.
2144 213. **Greiffenberg L, Goebel W, Kim KS, Weiglein I, Bubert A, Engelbrecht F, Stins**
2145 **M, Kuhn M.** 1998. Interaction of *Listeria monocytogenes* with human brain
2146 microvascular endothelial cells: InlB-dependent invasion, long-term intracellular
2147 growth, and spread from macrophages to endothelial cells. Infect Immun. **66:**5260-
2148 5267.
2149 214. **Parida SK, Domann E, Rohde M, Muller S, Darji A, Hain T, Wehland J,**
2150 **Chakraborty T.** 1998. Internalin B is essential for adhesion and mediates the
2151 invasion of *Listeria monocytogenes* into human endothelial cells. Mol Microbiol.
2152 **28:**81-93.

2153 215. **Greiffenberg L, Goebel W, Kim KS, Daniels J, Kuhn M.** 2000. Interaction of
2154 *Listeria monocytogenes* with human brain microvascular endothelial cells: an electron
2155 microscopic study. Infect Immun. **68:**3275-3279.
2156 216. **Koopmans MM, Brouwer MC, Bijlsma MW, Bovenkerk S, Keijzers W, van der**
2157 **Ende A, van de Beek D.** 2013. *Listeria monocytogenes* sequence type 6 and
2158 increased rate of unfavorable outcome in meningitis: epidemiologic cohort study. Clin
2159 Infect Dis. **57:**247-253.
2160 217. **Pilatz S, Breitbach K, Hein N, Fehlhaber B, Schulze J, Brenneke B, Eberl L,**
2161 **Steinmetz I.** 2006. Identification of *Burkholderia pseudomallei* genes required for the
2162 intracellular life cycle and *in vivo* virulence. Infect Immun. **74:**3576-3586.
2163 218. **Stevens MP, Wood MW, Taylor LA, Monaghan P, Hawes P, Jones PW, Wallis**
2164 **TS, Galyov EE.** 2002. An Inv/Mxi-Spa-like type III protein secretion system in
2165 *Burkholderia pseudomallei* modulates intracellular behaviour of the pathogen. Mol
2166 Microbiol. **46:**649-659.
2167 219. **Muangsombut V, Suparak S, Pumirat P, Damnin S, Vattanaviboon P,**
2168 **Thongboonkerd V, Korbsrisate S.** 2008. Inactivation of *Burkholderia pseudomallei*
2169 bsaQ results in decreased invasion efficiency and delayed escape of bacteria from
2170 endocytic vesicles. Arch Microbiol. **190:**623-631.
2171 220. **Suparak S, Kespichayawattana W, Haque A, Easton A, Damnin S,**
2172 **Lertmemongkolchai G, Bancroft GJ, Korbsrisate S.** 2005. Multinucleated giant
2173 cell formation and apoptosis in infected host cells is mediated by *Burkholderia*
2174 *pseudomallei* type III secretion protein BipB. J Bacteriol. **187:**6556-6560.
2175 221. **Stevens MP, Friebel A, Taylor LA, Wood MW, Brown PJ, Hardt WD, Galyov**
2176 **EE.** 2003. A *Burkholderia pseudomallei* type III secreted protein, BopE, facilitates

2177 bacterial invasion of epithelial cells and exhibits guanine nucleotide exchange factor
2178 activity. J Bacteriol. **185:**4992-4996.
2179 222. **Cullinane M, Gong L, Li X, Lazar-Adler N, Tra T, Wolvetang E, Prescott M,**
2180 **Boyce JD, Devenish RJ, Adler B.** 2008. Stimulation of autophagy suppresses the
2181 intracellular survival of *Burkholderia pseudomallei* in mammalian cell lines.
2182 Autophagy. **4:**744-753.
2183 223. **Stevens MP, Stevens JM, Jeng RL, Taylor LA, Wood MW, Hawes P, Monaghan**
2184 **P, Welch MD, Galyov EE.** 2005. Identification of a bacterial factor required for
2185 actin-based motility of *Burkholderia pseudomallei*. Mol Microbiol. **56:**40-53.
2186 224. **Sitthidet C, Korbsrisate S, Layton AN, Field TR, Stevens MP, Stevens JM.** 2011.
2187 Identification of motifs of *Burkholderia pseudomallei* BimA required for intracellular
2188 motility, actin binding, and actin polymerization. J Bacteriol. **193:**1901-1910.
2189 225. **Horton RE, Grant GD, Matthews B, Batzloff M, Owen SJ, Kyan S, Flegg CP,**
2190 **Clark AM, Ulett GC, Morrison N, Peak IR, Beacham IR.** 2013. Quorum sensing
2191 negatively regulates multinucleate cell formation during intracellular growth of
2192 *Burkholderia pseudomallei* in macrophage-like cells. PLoS One. **8:**e63394.
2193 226. **French CT, Toesca IJ, Wu TH, Teslaa T, Beaty SM, Wong W, Liu M, Schroder**
2194 **I, Chiou PY, Teitell MA, Miller JF.** 2011. Dissection of the *Burkholderia*
2195 intracellular life cycle using a photothermal nanoblade. Proc Natl Acad Sci U S A.
2196 **108:**12095-12100.
2197 227. **Toesca IJ, French CT, Miller JF.** 2014. The Type VI secretion system spike protein
2198 VgrG5 mediates membrane fusion during intercellular spread by pseudomallei group
2199 *Burkholderia* species. Infect Immun. **82:**1436-1444.
2200 228. **Sitthidet C, Stevens JM, Chantratita N, Currie BJ, Peacock SJ, Korbsrisate S,**
2201 **Stevens MP.** 2008. Prevalence and sequence diversity of a factor required for actin-

2202 based motility in natural populations of *Burkholderia* species. J Clin Microbiol.
2203 **46:**2418-2422.
2204 229. **Sarovich DS, Price EP, Webb JR, Ward LM, Voutsinos MY, Tuanyok A, Mayo**
2205 **M, Kaestli M, Currie BJ.** 2014. Variable virulence factors in *Burkholderia*
2206 *pseudomallei* (melioidosis) associated with human disease. PLoS One. **9:**e91682.
2207 230. **Greiffenberg L, Sokolovic Z, Schnittler HJ, Spory A, Bockmann R, Goebel W,**
2208 **Kuhn M.** 1997. *Listeria monocytogenes*-infected human umbilical vein endothelial
2209 cells: internalin-independent invasion, intracellular growth, movement, and host cell
2210 responses. FEMS Microbiol Lett. **157:**163-170.
2211 231. **Hertzig T, Weber M, Greiffenberg L, Holthausen BS, Goebel W, Kim KS, Kuhn**
2212 **M.** 2003. Antibodies present in normal human serum inhibit invasion of human brain
2213 microvascular endothelial cells by *Listeria monocytogenes*. Infect Immun. **71:**95-100.
2214 232. **Grundler T, Quednau N, Stump C, Orian-Rousseau V, Ishikawa H, Wolburg H,**
2215 **Schroten H, Tenenbaum T, Schwerk C.** 2013. The surface proteins InlA and InlB
2216 are interdependently required for polar basolateral invasion by *Listeria*
2217 *monocytogenes* in a human model of the blood-cerebrospinal fluid barrier. Microbes
2218 Infect. **15:**291-301.
2219 233. **Wang L, Lin M.** 2008. A novel cell wall-anchored peptidoglycan hydrolase
2220 (autolysin), IspC, essential for *Listeria monocytogenes* virulence: genetic and
2221 proteomic analysis. Microbiology. **154:**1900-1913.
2222 234. **Menozzi FD, Reddy VM, Cayet D, Raze D, Debrie AS, Dehouck MP, Cecchelli R,**
2223 **Locht C.** 2006. *Mycobacterium tuberculosis* heparin-binding haemagglutinin adhesin
2224 (HBHA) triggers receptor-mediated transcytosis without altering the integrity of tight
2225 junctions. Microbes Infect. **8:**1-9.

2226 235. **Be NA, Lamichhane G, Grosset J, Tyagi S, Cheng QJ, Kim KS, Bishai WR, Jain**
2227 **SK.** 2008. Murine model to study the invasion and survival of *Mycobacterium*
2228 *tuberculosis* in the central nervous system. J Infect Dis. **198:**1520-1528.
2229 236. **Quagliarello VJ, Long WJ, Scheld WM.** 1986. Morphologic alterations of the
2230 blood-brain barrier with experimental meningitis in the rat. Temporal sequence and
2231 role of encapsulation. J Clin Invest. **77:**1084-1095.
2232 237. **Mittal R, Prasadarao NV.** 2010. Nitric oxide/cGMP signalling induces *Escherichia*
2233 *coli* K1 receptor expression and modulates the permeability in human brain
2234 endothelial cell monolayers during invasion. Cell Microbiol. **12:**67-83.
2235 238. **Krishnan S, Fernandez GE, Sacks DB, Prasadarao NV.** 2012. IQGAP1 mediates
2236 the disruption of adherens junctions to promote *Escherichia coli* K1 invasion of brain
2237 endothelial cells. Cell Microbiol. **14:**1415-1433.
2238 239. **Sukumaran SK, Prasadarao NV.** 2003. *Escherichia coli* K1 invasion increases
2239 human brain microvascular endothelial cell monolayer permeability by disassembling
2240 vascular-endothelial cadherins at tight junctions. J Infect Dis. **188:**1295-1309.
2241 240. **He F, Peng J, Deng XL, Yang LF, Wu LW, Zhang CL, Yin F.** 2011. RhoA and
2242 NF-kappaB are involved in lipopolysaccharide-induced brain microvascular cell line
2243 hyperpermeability. Neuroscience. **188:**35-47.
2244 241. **He F, Yin F, Omran A, Yang LF, Xiang QL, Peng J.** 2012. PKC and RhoA signals
2245 cross-talk in *Escherichia coli* endotoxin induced alterations in brain endothelial
2246 permeability. Biochem Biophys Res Commun. **425:**182-188.
2247 242. **Landoni VI, Schierloh P, de Campos Nebel M, Fernandez GC, Calatayud C,**
2248 **Lapponi MJ, Isturiz MA.** 2012. Shiga toxin 1 induces on lipopolysaccharide-treated
2249 astrocytes the release of tumor necrosis factor-alpha that alter brain-like endothelium
2250 integrity. PLoS Pathog. **8:**e1002632.

2251 243. **Tironi-Farinati C, Geoghegan PA, Cangelosi A, Pinto A, Loidl CF, Goldstein J.**
2252 2013. A translational murine model of sub-lethal intoxication with Shiga toxin 2
2253 reveals novel ultrastructural findings in the brain striatum. PLoS One. **8:**e55812.
2254 244. **Meisen I, Rosenbruck R, Galla HJ, Huwel S, Kouzel IU, Mormann M, Karch H,**
2255 **Muthing J.** 2013. Expression of Shiga toxin 2e glycosphingolipid receptors of
2256 primary porcine brain endothelial cells and toxin-mediated breakdown of the blood-
2257 brain barrier. Glycobiology. **23:**745-759.
2258 245. **Pinto A, Jacobsen M, Geoghegan PA, Cangelosi A, Cejudo ML, Tironi-Farinati**
2259 **C, Goldstein J.** 2013. Dexamethasone rescues neurovascular unit integrity from cell
2260 damage caused by systemic administration of shiga toxin 2 and lipopolysaccharide in
2261 mice motor cortex. PLoS One. **8:**e70020.
2262 246. **Wispelwey B, Lesse AJ, Hansen EJ, Scheld WM.** 1988. *Haemophilus influenzae*
2263 lipopolysaccharide-induced blood brain barrier permeability during experimental
2264 meningitis in the rat. J Clin Invest. **82:**1339-1346.
2265 247. **Wispelwey B, Hansen EJ, Scheld WM.** 1989. *Haemophilus influenzae* outer
2266 membrane vesicle-induced blood-brain barrier permeability during experimental
2267 meningitis. Infect Immun. **57:**2559-2562.
2268 248. **Tunkel AR, Rosser SW, Hansen EJ, Scheld WM.** 1991. Blood-brain barrier
2269 alterations in bacterial meningitis: development of an *in vitro* model and observations
2270 on the effects of lipopolysaccharide. In Vitro Cell Dev Biol. **27A:**113-120.
2271 249. **Buster BL, Weintrob AC, Townsend GC, Scheld WM.** 1995. Potential role of
2272 nitric oxide in the pathophysiology of experimental bacterial meningitis in rats. Infect
2273 Immun. **63:**3835-3839.

2274 250. **Townsend GC, Scheld WM.** 1994. Platelet-activating factor augments meningeal
2275 inflammation elicited by *Haemophilus influenzae* lipooligosaccharide in an animal
2276 model of meningitis. Infect Immun. **62:**3739-3744.
2277 251. **Roord JJ, Apicella M, Scheld WM.** 1994. The induction of meningeal inflammation
2278 and blood-brain barrier permeability by *Haemophilus influenzae* type b
2279 peptidoglycan. J Infect Dis. **170:**254-256.
2280 252. **Burroughs M, Cabellos C, Prasad S, Tuomanen E.** 1992. Bacterial components
2281 and the pathophysiology of injury to the blood-brain barrier: does cell wall add to the
2282 effects of endotoxin in gram-negative meningitis? J Infect Dis. **165 Suppl 1:**S82-85.
2283 253. **Coureuil M, Mikaty G, Miller F, Lecuyer H, Bernard C, Bourdoulous S,**
2284 **Dumenil G, Mege RM, Weksler BB, Romero IA, Couraud PO, Nassif X.** 2009.
2285 Meningococcal type IV pili recruit the polarity complex to cross the brain
2286 endothelium. Science. **325:**83-87.
2287 254. **Lecuyer H, Nassif X, Coureuil M.** 2012. Two strikingly different signaling
2288 pathways are induced by meningococcal type IV pili on endothelial and epithelial
2289 cells. Infect Immun. **80:**175-186.
2290 255. **Schubert-Unkmeir A, Konrad C, Slanina H, Czapek F, Hebling S, Frosch M.**
2291 2010. *Neisseria meningitidis* induces brain microvascular endothelial cell detachment
2292 from the matrix and cleavage of occludin: a role for MMP-8. PLoS Pathog.
2293 **6:**e1000874.
2294 256. **Doran KS, Liu GY, Nizet V.** 2003. Group B streptococcal beta-hemolysin/cytolysin
2295 activates neutrophil signaling pathways in brain endothelium and contributes to
2296 development of meningitis. J Clin Invest. **112:**736-744.

2297 257. **Vanier G, Segura M, Lecours MP, Grenier D, Gottschalk M.** 2009. Porcine brain
2298 microvascular endothelial cell-derived interleukin-8 is first induced and then degraded
2299 by *Streptococcus suis*. Microb Pathog. **46:**135-143.

2300 258. **Vadeboncoeur N, Segura M, Al-Numani D, Vanier G, Gottschalk M.** 2003. Pro-
2301 inflammatory cytokine and chemokine release by human brain microvascular
2302 endothelial cells stimulated by *Streptococcus suis* serotype 2. FEMS Immunol Med
2303 Microbiol. **35:**49-58.

2304 259. **Barichello T, Pereira JS, Savi GD, Generoso JS, Cipriano AL, Silvestre C,**
2305 **Petronilho F, Dal-Pizzol F, Vilela MC, Teixeira AL.** 2011. A kinetic study of the
2306 cytokine/chemokines levels and disruption of blood-brain barrier in infant rats after
2307 pneumococcal meningitis. J Neuroimmunol. **233:**12-17.

2308 260. **Barichello T, Lemos JC, Generoso JS, Cipriano AL, Milioli GL, Marcelino DM,**
2309 **Vuolo F, Petronilho F, Dal-Pizzol F, Vilela MC, Teixeira AL.** 2011. Oxidative
2310 stress, cytokine/chemokine and disruption of blood-brain barrier in neonate rats after
2311 meningitis by *Streptococcus agalactiae*. Neurochem Res. **36:**1922-1930.

2312 261. **Blum MS, Toninelli E, Anderson JM, Balda MS, Zhou J, O'Donnell L, Pardi R,**
2313 **Bender JR.** 1997. Cytoskeletal rearrangement mediates human microvascular
2314 endothelial tight junction modulation by cytokines. Am J Physiol. **273:**H286-294.

2315 262. **Lopez-Ramirez MA, Fischer R, Torres-Badillo CC, Davies HA, Logan K,**
2316 **Pfizenmaier K, Male DK, Sharrack B, Romero IA.** 2012. Role of caspases in
2317 cytokine-induced barrier breakdown in human brain endothelial cells. J Immunol.
2318 **189:**3130-3139.

2319 263. **Peng J, He F, Zhang C, Deng X, Yin F.** 2011. Protein kinase C-alpha signals
2320 P115RhoGEF phosphorylation and RhoA activation in TNF-alpha-induced mouse
2321 brain microvascular endothelial cell barrier dysfunction. J Neuroinflammation. **8:**28.

2322 264. **You QH, Sun GY, Wang N, Shen JL, Wang Y.** 2010. Interleukin-17F-induced
2323 pulmonary microvascular endothelial monolayer hyperpermeability via the protein
2324 kinase C pathway. J Surg Res. **162:**110-121.
2325 265. **Desai TR, Leeper NJ, Hynes KL, Gewertz BL.** 2002. Interleukin-6 causes
2326 endothelial barrier dysfunction via the protein kinase C pathway. J Surg Res.
2327 **104:**118-123.
2328 266. **Rigor RR, Beard RS, Jr., Litovka OP, Yuan SY.** 2012. Interleukin-1beta-induced
2329 barrier dysfunction is signaled through PKC-theta in human brain microvascular
2330 endothelium. Am J Physiol Cell Physiol. **302:**C1513-1522.
2331 267. **Labus J, Hackel S, Lucka L, Danker K.** 2014. Interleukin-1beta induces an
2332 inflammatory response and the breakdown of the endothelial cell layer in an improved
2333 human THBMEC-based *in vitro* blood-brain barrier model. J Neurosci Methods.
2334 **228C:**35-45.
2335 268. **Sanderson-Smith ML, De Oliveira DM, Ranson M, McArthur JD.** 2012. Bacterial
2336 plasminogen receptors: mediators of a multifaceted relationship. J Biomed
2337 Biotechnol. **2012:**272148.
2338 269. **Jobin MC, Brassard J, Quessy S, Gottschalk M, Grenier D.** 2004. Acquisition of
2339 host plasmin activity by the swine pathogen *Streptococcus suis* serotype 2. Infect
2340 Immun. **72:**606-610.
2341 270. **Magalhaes V, Veiga-Malta I, Almeida MR, Baptista M, Ribeiro A, Trieu-Cuot P,**
2342 **Ferreira P.** 2007. Interaction with human plasminogen system turns on proteolytic
2343 activity in *Streptococcus agalactiae* and enhances its virulence in a mouse model.
2344 Microbes Infect. **9:**1276-1284.

2345 271. **Bergmann S, Rohde M, Hammerschmidt S.** 2004. Glyceraldehyde-3-phosphate
2346 dehydrogenase of *Streptococcus pneumoniae* is a surface-displayed plasminogen-
2347 binding protein. Infect Immun. **72:**2416-2419.
2348 272. **Bergmann S, Rohde M, Chhatwal GS, Hammerschmidt S.** 2001. alpha-Enolase of
2349 *Streptococcus pneumoniae* is a plasmin(ogen)-binding protein displayed on the
2350 bacterial cell surface. Mol Microbiol. **40:**1273-1287.
2351 273. **Attali C, Frolet C, Durmort C, Offant J, Vernet T, Di Guilmi AM.** 2008.
2352 *Streptococcus pneumoniae* choline-binding protein E interaction with
2353 plasminogen/plasmin stimulates migration across the extracellular matrix. Infect
2354 Immun. **76:**466-476.
2355 274. **Jobin MC, Fortin J, Willson PJ, Gottschalk M, Grenier D.** 2005. Acquisition of
2356 plasmin activity and induction of arachidonic acid release by *Streptococcus suis* in
2357 contact with human brain microvascular endothelial cells. FEMS Microbiol Lett.
2358 **252:**105-111.
2359 275. **Magalhaes V, Andrade EB, Alves J, Ribeiro A, Kim KS, Lima M, Trieu-Cuot P,**
2360 **Ferreira P.** 2013. Group B streptococcus hijacks the host plasminogen system to
2361 promote brain endothelial cell invasion. PLoS One. **8:**e63244.
2362 276. **Attali C, Durmort C, Vernet T, Di Guilmi AM.** 2008. The interaction of
2363 *Streptococcus pneumoniae* with plasmin mediates transmigration across endothelial
2364 and epithelial monolayers by intercellular junction cleavage. Infect Immun. **76:**5350-
2365 5356.
2366 277. **Ullberg M, Kuusela P, Kristiansen BE, Kronvall G.** 1992. Binding of plasminogen
2367 to *Neisseria meningitidis* and *Neisseria gonorrhoeae* and formation of surface-
2368 associated plasmin. J Infect Dis. **166:**1329-1334.

2369 278. **Knaust A, Weber MV, Hammerschmidt S, Bergmann S, Frosch M, Kurzai O.**
2370 2007. Cytosolic proteins contribute to surface plasminogen recruitment of *Neisseria*
2371 *meningitidis*. J Bacteriol. **189:**3246-3255.
2372 279. **Drevets DA.** 1999. Dissemination of *Listeria monocytogenes* by infected phagocytes.
2373 Infect Immun. **67:**3512-3517.
2374 280. **Liu PJ, Chen YS, Lin HH, Ni WF, Hsieh TH, Chen HT, Chen YL.** 2013.
2375 Induction of mouse melioidosis with meningitis by CD11b+ phagocytic cells
2376 harboring intracellular *B. pseudomallei* as a Trojan horse. PLoS Negl Trop Dis.
2377 **7:**e2363.
2378 281. **Thwaites G.** 2013. The pathophysiology of tuberculosis meningitis, p 153-167. *In*
2379 Christodoulides M (ed.), Meningitis: cellular and molecular basis, vol. 26. CAB
2380 International, Oxfordshire.
2381 282. **Drevets DA, Jelinek TA, Freitag NE.** 2001. *Listeria monocytogenes*-infected
2382 phagocytes can initiate central nervous system infection in mice. Infect Immun.
2383 **69:**1344-1350.
2384 283. **Drevets DA, Dillon MJ, Schawang JS, Van Rooijen N, Ehrchen J, Sunderkotter**
2385 **C, Leenen PJ.** 2004. The Ly-6Chigh monocyte subpopulation transports *Listeria*
2386 *monocytogenes* into the brain during systemic infection of mice. J Immunol.
2387 **172:**4418-4424.
2388 284. **Join-Lambert OF, Ezine S, Le Monnier A, Jaubert F, Okabe M, Berche P, Kayal**
2389 **S.** 2005. *Listeria monocytogenes*-infected bone marrow myeloid cells promote
2390 bacterial invasion of the central nervous system. Cell Microbiol. **7:**167-180.
2391 285. **Buck L, Axel R.** 1991. A novel multigene family may encode odorant receptors: a
2392 molecular basis for odor recognition. Cell. **65:**175-187.

2393 286. **Nara K, Saraiva LR, Ye X, Buck LB.** 2011. A large-scale analysis of odor coding in
2394 the olfactory epithelium. J Neurosci. **31:**9179-9191.
2395 287. **Hengl T, Kaneko H, Dauner K, Vocke K, Frings S, Mohrlen F.** 2010. Molecular
2396 components of signal amplification in olfactory sensory cilia. Proc Natl Acad Sci U S
2397 A. **107:**6052-6057.
2398 288. **Steinke A, Meier-Stiegen S, Drenckhahn D, Asan E.** 2008. Molecular composition
2399 of tight and adherens junctions in the rat olfactory epithelium and fila. Histochem Cell
2400 Biol. **130:**339-361.
2401 289. **Mackay-Sim A.** 2003. Neurogenesis in the adult olfactory neuroepithelium, p 93-113.
2402 *In* Doty RL (ed.), Handbook of olfaction and gustation. Dekker, NY.
2403 290. **Mackay-Sim A, Kittel P.** 1991. Cell dynamics in the adult mouse olfactory
2404 epithelium: a quantitative autoradiographic study. J Neurosci. **11:**979-984.
2405 291. **Su Z, He C.** 2010. Olfactory ensheathing cells: biology in neural development and
2406 regeneration. Prog Neurobiol. **92:**517-532.
2407 292. **Mackay-Sim A, St John JA.** 2011. Olfactory ensheathing cells from the nose:
2408 clinical application in human spinal cord injuries. Exp Neurol. **229:**174-180.
2409 293. **Firestein S.** 2001. How the olfactory system makes sense of scents. Nature. **413:**211-
2410 218.
2411 294. **Mackay-Sim AR, Royet JP.** 2006. The olfactory system, p 3-27. *In* Brewer W,
2412 Castle D, Pantelis C (ed.), Olfaction and the brain: window to the mind. Cambridge
2413 University Press, Cambridge.
2414 295. **Phillips AT, Stauft CB, Aboellail TA, Toth AM, Jarvis DL, Powers AM, Olson
2415 KE.** 2013. Bioluminescent imaging and histopathologic characterization of WEEV
2416 neuroinvasion in outbred CD-1 mice. PLoS One. **8:**e53462.

2417 296. **Schrauwen EJ, Herfst S, Leijten LM, van Run P, Bestebroer TM, Linster M,**
2418 **Bodewes R, Kreijtz JH, Rimmelzwaan GF, Osterhaus AD, Fouchier RA, Kuiken**
2419 **T, van Riel D.** 2012. The multibasic cleavage site in H5N1 virus is critical for
2420 systemic spread along the olfactory and hematogenous routes in ferrets. J Virol.
2421 **86:**3975-3984.
2422 297. **Munster VJ, Prescott JB, Bushmaker T, Long D, Rosenke R, Thomas T, Scott D,**
2423 **Fischer ER, Feldmann H, de Wit E.** 2012. Rapid Nipah virus entry into the central
2424 nervous system of hamsters via the olfactory route. Sci Rep. **2:**736.
2425 298. **Bathla G, Hegde AN.** 2013. The trigeminal nerve: an illustrated review of its
2426 imaging anatomy and pathology. Clin Radiol. **68:**203-213.
2427 299. **Silver WL, Finger TE.** 2009. The anatomical and electrophysiological basis of
2428 peripheral nasal trigeminal chemoreception. Ann N Y Acad Sci. **1170:**202-205.
2429 300. **Finger TE, St Jeor VL, Kinnamon JC, Silver WL.** 1990. Ultrastructure of
2430 substance P- and CGRP-immunoreactive nerve fibers in the nasal epithelium of
2431 rodents. J Comp Neurol. **294:**293-305.
2432 301. **Schaefer ML, Bottger B, Silver WL, Finger TE.** 2002. Trigeminal collaterals in the
2433 nasal epithelium and olfactory bulb: a potential route for direct modulation of
2434 olfactory information by trigeminal stimuli. J Comp Neurol. **444:**221-226.
2435 302. **Bryant BP, Silver WL.** 2000. Chemesthesis: the common chemical sense, p 73-100.
2436 *In* Finger TE, Silver WL, Restrepo D (ed.), The neurobiology of taste and smell, 2nd
2437 ed. Wiley, New York.
2438 303. **Finger TE, Bottger B, Hansen A, Anderson KT, Alimohammadi H, Silver WL.**
2439 2003. Solitary chemoreceptor cells in the nasal cavity serve as sentinels of respiration.
2440 Proc Natl Acad Sci U S A. **100:**8981-8986.

2441 304. **Tizzano M, Gulbransen BD, Vandenbeuch A, Clapp TR, Herman JP, Sibhatu**
2442 **HM, Churchill ME, Silver WL, Kinnamon SC, Finger TE.** 2010. Nasal
2443 chemosensory cells use bitter taste signaling to detect irritants and bacterial signals.
2444 Proc Natl Acad Sci U S A. **107:**3210-3215.
2445 305. **Kennedy DP, Clement C, Arceneaux RL, Bhattacharjee PS, Huq TS, Hill JM.**
2446 2011. Ocular herpes simplex virus type 1: is the cornea a reservoir for viral latency or
2447 a fast pit stop? Cornea. **30:**251-259.
2448 306. **Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR.** 2010. The
2449 human nasal microbiota and *Staphylococcus aureus* carriage. PLoS One. **5:**e10598.
2450 307. **Lemon KP, Klepac-Ceraj V, Schiffer HK, Brodie EL, Lynch SV, Kolter R.** 2010.
2451 Comparative analyses of the bacterial microbiota of the human nostril and
2452 oropharynx. MBio. **1**.
2453 308. **Rasmussen TT, Kirkeby LP, Poulsen K, Reinholdt J, Kilian M.** 2000. Resident
2454 aerobic microbiota of the adult human nasal cavity. APMIS. **108:**663-675.
2455 309. **Oikawa J, Ishiwada N, Takahashi Y, Hishiki H, Nagasawa K, Takahashi S,**
2456 **Watanabe M, Chang B, Kohno Y.** 2014. Changes in nasopharyngeal carriage of
2457 *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*
2458 among healthy children attending a day-care centre before and after official financial
2459 support for the 7-valent pneumococcal conjugate vaccine and *H. influenzae* type b
2460 vaccine in Japan. J Infect Chemother. **20:**146-149.
2461 310. **Bogaert D, Keijser B, Huse S, Rossen J, Veenhoven R, van Gils E, Bruin J,**
2462 **Montijn R, Bonten M, Sanders E.** 2011. Variability and diversity of nasopharyngeal
2463 microbiota in children: a metagenomic analysis. PLoS One. **6:**e17035.

2464 311. **Button B, Cai LH, Ehre C, Kesimer M, Hill DB, Sheehan JK, Boucher RC,**
2465 **Rubinstein M.** 2012. A periciliary brush promotes the lung health by separating the
2466 mucus layer from airway epithelia. Science. **337:**937-941.

2467 312. **Roy MG, Livraghi-Butrico A, Fletcher AA, McElwee MM, Evans SE, Boerner**
2468 **RM, Alexander SN, Bellinghausen LK, Song AS, Petrova YM, Tuvim MJ,**
2469 **Adachi R, Romo I, Bordt AS, Bowden MG, Sisson JH, Woodruff PG, Thornton**
2470 **DJ, Rousseau K, De la Garza MM, Moghaddam SJ, Karmouty-Quintana H,**
2471 **Blackburn MR, Drouin SM, Davis CW, Terrell KA, Grubb BR, O'Neal WK,**
2472 **Flores SC, Cota-Gomez A, Lozupone CA, Donnelly JM, Watson AM, Hennessy**
2473 **CE, Keith RC, Yang IV, Barthel L, Henson PM, Janssen WJ, Schwartz DA,**
2474 **Boucher RC, Dickey BF, Evans CM.** 2014. Muc5b is required for airway defence.
2475 Nature. **505:**412-416.

2476 313. **Alevy YG, Patel AC, Romero AG, Patel DA, Tucker J, Roswit WT, Miller CA,**
2477 **Heier RF, Byers DE, Brett TJ, Holtzman MJ.** 2012. IL-13-induced airway mucus
2478 production is attenuated by MAPK13 inhibition. J Clin Invest. **122:**4555-4568.

2479 314. **Cervantes-Sandoval I, Serrano-Luna Jde J, Meza-Cervantez P, Arroyo R,**
2480 **Tsutsumi V, Shibayama M.** 2009. *Naegleria fowleri* induces MUC5AC and pro-
2481 inflammatory cytokines in human epithelial cells via ROS production and EGFR
2482 activation. Microbiology. **155:**3739-3747.

2483 315. **Li W, Yan F, Zhou H, Lin X, Wu Y, Chen C, Zhou N, Chen Z, Li JD, Shen H.**
2484 2013. *P. aeruginosa* lipopolysaccharide-induced MUC5AC and CLCA3 expression is
2485 partly through Duox1 *in vitro* and *in vivo*. PLoS One. **8:**e63945.

2486 316. **Lima C, Vital JP.** 1994. Olfactory mucosa response in guinea pigs following
2487 intranasal instillation with *Cryptococcus neoformans*. A histological and
2488 immunocytochemical study. Mycopathologia. **126:**65-73.

317. **Di YP, Zhao J, Harper R.** 2012. Cigarette smoke induces MUC5AC protein expression through the activation of Sp1. J Biol Chem. **287:**27948-27958.

318. **Ganesan S, Comstock AT, Sajjan US.** 2013. Barrier function of airway tract epithelium. Tissue Barriers. **1:**e24997.

319. **Blank F, Rothen-Rutishauser B, Gehr P.** 2007. Dendritic cells and macrophages form a transepithelial network against foreign particulate antigens. Am J Respir Cell Mol Biol. **36:**669-677.

320. **Young BC, Golubchik T, Batty EM, Fung R, Larner-Svensson H, Votintseva AA, Miller RR, Godwin H, Knox K, Everitt RG, Iqbal Z, Rimmer AJ, Cule M, Ip CL, Didelot X, Harding RM, Donnelly P, Peto TE, Crook DW, Bowden R, Wilson DJ.** 2012. Evolutionary dynamics of *Staphylococcus aureus* during progression from carriage to disease. Proc Natl Acad Sci U S A. **109:**4550-4555.

321. **Margolis E, Levin BR.** 2007. Within-host evolution for the invasiveness of commensal bacteria: an experimental study of bacteremias resulting from *Haemophilus influenzae* nasal carriage. J Infect Dis. **196:**1068-1075.

322. **Kim JY, Sajjan US, Krasan GP, LiPuma JJ.** 2005. Disruption of tight junctions during traversal of the respiratory epithelium by *Burkholderia cenocepacia*. Infect Immun. **73:**7107-7112.

323. **Nomura K, Obata K, Keira T, Miyata R, Hirakawa S, Takano K, Kohno T, Sawada N, Himi T, Kojima T.** 2014. *Pseudomonas aeruginosa* elastase causes transient disruption of tight junctions and downregulation of PAR-2 in human nasal epithelial cells. Respir Res. **15:**21.

324. **Sjolinder H, Jonsson AB.** 2010. Olfactory nerve - a novel invasion route of *Neisseria meningitidis* to reach the meninges. PLoS One. **5:**e14034.

2513 325. **Harris JA, West AK, Chuah MI.** 2009. Olfactory ensheathing cells: nitric oxide
2514 production and innate immunity. Glia. **57:**1848-1857.
2515 326. **St John JA, Ekberg JA, Dando SJ, Meedeniya AC, Horton RE, Batzloff M,**
2516 **Owen SJ, Holt S, Peak IR, Ulett GC, Mackay-Sim A, Beacham IR.** 2014.
2517 *Burkholderia pseudomallei* penetrates the brain via festruction of the olfactory and
2518 trigeminal nerves: implications for the pathogenesis of neurological melioidosis.
2519 MBio. **5**.
2520 327. **Herbert RP, Harris J, Chong KP, Chapman J, West AK, Chuah MI.** 2012.
2521 Cytokines and olfactory bulb microglia in response to bacterial challenge in the
2522 compromised primary olfactory pathway. J Neuroinflammation. **9:**109.
2523 328. **Saunders CJ, Christensen M, Finger TE, Tizzano M.** 2014. Cholinergic
2524 neurotransmission links solitary chemosensory cells to nasal inflammation. Proc Natl
2525 Acad Sci U S A. **111:**6075-6080.
2526 329. **Rake G.** 1937. The rapid invasion of the body through the olfactory mucosa. J Exp
2527 Med. **65:**303-315.
2528 330. **van Ginkel FW, McGhee JR, Watt JM, Campos-Torres A, Parish LA, Briles DE.**
2529 2003. Pneumococcal carriage results in ganglioside-mediated olfactory tissue
2530 infection. Proc Natl Acad Sci U S A. **100:**14363-14367.
2531 331. **Briles DE, Novak L, Hotomi M, van Ginkel FW, King J.** 2005. Nasal colonization
2532 with *Streptococcus pneumoniae* includes subpopulations of surface and invasive
2533 pneumococci. Infect Immun. **73:**6945-6951.
2534 332. **Berning AK, Eicher EM, Paul WE, Scher I.** 1980. Mapping of the X-linked
2535 immune deficiency mutation (xid) of CBA/N mice. J Immunol. **124:**1875-1877.

2536 333. **Sundberg-Kovamees M, Holme T, Sjogren A.** 1996. Interaction of the C-
2537 polysaccharide of *Streptococcus pneumoniae* with the receptor asialo-GM1. Microb
2538 Pathog. **21:**223-234.
2539 334. **Macedo-Ramos H, Campos FS, Carvalho LA, Ramos IB, Teixeira LM, De Souza**
2540 **W, Cavalcante LA, Baetas-da-Cruz W.** 2011. Olfactory ensheathing cells as
2541 putative host cells for *Streptococcus pneumoniae*: evidence of bacterial invasion via
2542 mannose receptor-mediated endocytosis. Neurosci Res. **69:**308-313.
2543 335. **Stephens DS, Greenwood B, Brandtzaeg P.** 2007. Epidemic meningitis,
2544 meningococcaemia, and *Neisseria meningitidis*. Lancet. **369:**2196-2210.
2545 336. **Weyand NJ, Wertheimer AM, Hobbs TR, Sisko JL, Taku NA, Gregston LD,**
2546 **Clary S, Higashi DL, Biais N, Brown LM, Planer SL, Legasse AW, Axthelm MK,**
2547 **Wong SW, So M.** 2013. *Neisseria* infection of rhesus macaques as a model to study
2548 colonization, transmission, persistence, and horizontal gene transfer. Proc Natl Acad
2549 Sci U S A. **110:**3059-3064.
2550 337. **Roberts L.** 2008. Infectious disease. An ill wind, bringing meningitis. Science.
2551 **320:**1710-1715.
2552 338. **Dare AO, Balos LL, Grand W.** 2003. Neural-dural transition at the medial anterior
2553 cranial base: an anatomical and histological study with clinical applications. J
2554 Neurosurg. **99:**362-365.
2555 339. **Erlich SS, McComb JG, Hyman S, Weiss MH.** 1986. Ultrastructural morphology of
2556 the olfactory pathway for cerebrospinal fluid drainage in the rabbit. J Neurosurg.
2557 **64:**466-473.
2558 340. **Gal D, Mayo M, Spencer E, Cheng AC, Currie BJ.** 2005. Short report: application
2559 of a polymerase chain reaction to detect *Burkholderia pseudomallei* in clinical

2560 specimens from patients with suspected melioidosis. Am J Trop Med Hyg. **73:**1162-
2561 1164.

2562 341. **Oevermann A, Di Palma S, Doherr MG, Abril C, Zurbriggen A, Vandevelde M.**
2563 2010. Neuropathogenesis of naturally occurring encephalitis caused by *Listeria*
2564 *monocytogenes* in ruminants. Brain Pathol. **20:**378-390.

2565 342. **Antal EA, Loberg EM, Dietrichs E, Maehlen J.** 2005. Neuropathological findings
2566 in 9 cases of *Listeria monocytogenes* brain stem encephalitis. Brain Pathol. **15:**187-
2567 191.

2568 343. **Charlton KM, Garcia MM.** 1977. Spontaneous listeric encephalitis and neuritis in
2569 sheep. Light microscopic studies. Vet Pathol. **14:**297-313.

2570 344. **Jin Y, Dons L, Kristensson K, Rottenberg ME.** 2001. Neural route of cerebral
2571 *Listeria monocytogenes* murine infection: role of immune response mechanisms in
2572 controlling bacterial neuroinvasion. Infect Immun. **69:**1093-1100.

2573 345. **Madarame H, Seuberlich T, Abril C, Zurbriggen A, Vandevelde M, Oevermann**
2574 **A.** 2011. The distribution of E-cadherin expression in listeric rhombencephalitis of
2575 ruminants indicates its involvement in *Listeria monocytogenes* neuroinvasion.
2576 Neuropathol Appl Neurobiol. **37:**753-767.

2577 346. **Otter A, Blakemore WF.** 1989. Observation on the presence of *Listeria*
2578 *monocytogenes* in axons. Acta Microbiol Hung. **36:**125-131.

2579 347. **Jin Y, Dons L, Kristensson K, Rottenberg ME.** 2002. Colony-stimulating factor 1-
2580 dependent cells protect against systemic infection with *Listeria monocytogenes* but
2581 facilitate neuroinvasion. Infect Immun. **70:**4682-4686.

2582 348. **Dramsi S, Levi S, Triller A, Cossart P.** 1998. Entry of *Listeria monocytogenes* into
2583 neurons occurs by cell-to-cell spread: an in vitro study. Infect Immun. **66:**4461-4468.

2584 349. **Jin Y, Lundkvist G, Dons L, Kristensson K, Rottenberg ME.** 2004. Interferon-
2585 gamma mediates neuronal killing of intracellular bacteria. Scand J Immunol. **60:**437-
2586 448.
2587 350. **Shivkumar M, Milho R, May JS, Nicoll MP, Efstathiou S, Stevenson PG.** 2013.
2588 Herpes simplex virus 1 targets the murine olfactory neuroepithelium for host entry. J
2589 Virol. **87:**10477-10488.
2590 351. **Milho R, Frederico B, Efstathiou S, Stevenson PG.** 2012. A heparan-dependent
2591 herpesvirus targets the olfactory neuroepithelium for host entry. PLoS Pathog.
2592 **8:**e1002986.
2593 352. **El-Habashi N, El-Nahass el S, Fukushi H, Hibi D, Sakai H, Sasseville V, Yanai T.**
2594 2010. Experimental intranasal infection of equine herpesvirus 9 (EHV-9) in suckling
2595 hamsters: kinetics of viral transmission and inflammation in the nasal cavity and
2596 brain. J Neurovirol. **16:**242-248.
2597 353. **Chowdhury SI, Onderci M, Bhattacharjee PS, Al-Mubarak A, Weiss ML, Zhou**
2598 **Y.** 2002. Bovine herpesvirus 5 (BHV-5) Us9 is essential for BHV-5
2599 neuropathogenesis. J Virol. **76:**3839-3851.
2600 354. **Boggian I, Buzzacaro E, Calistri A, Calvi P, Cavaggioni A, Mucignat-Caretta C,**
2601 **Palu G.** 2000. Asymptomatic herpes simplex type 1 virus infection of the mouse
2602 brain. J Neurovirol. **6:**303-313.
2603 355. **Lee BJ, Weiss ML, Mosier D, Chowdhury SI.** 1999. Spread of bovine herpesvirus
2604 type 5 (BHV-5) in the rabbit brain after intranasal inoculation. J Neurovirol. **5:**474-
2605 484.
2606 356. **Narita M, Uchimura A, Kawanabe M, Fukushi H, Hirai K.** 2001. Invasion and
2607 spread of equine herpesvirus 9 in the olfactory pathway of pigs after intranasal
2608 inoculation. J Comp Pathol. **124:**265-272.

2609 357. **Al-Mubarak A, Zhou Y, Chowdhury SI.** 2004. A glycine-rich bovine herpesvirus 5
2610 (BHV-5) gE-specific epitope within the ectodomain is important for BHV-5
2611 neurovirulence. J Virol. **78:**4806-4816.
2612 358. **Harberts E, Yao K, Wohler JE, Maric D, Ohayon J, Henkin R, Jacobson S.** 2011.
2613 Human herpesvirus-6 entry into the central nervous system through the olfactory
2614 pathway. Proc Natl Acad Sci U S A. **108:**13734-13739.
2615 359. **Esiri MM.** 1982. Herpes simplex encephalitis. An immunohistological study of the
2616 distribution of viral antigen within the brain. J Neurol Sci. **54:**209-226.
2617 360. **Flowerdew SE, Wick D, Himmelein S, Horn AK, Sinicina I, Strupp M, Brandt T,**
2618 **Theil D, Hufner K.** 2013. Characterization of neuronal populations in the human
2619 trigeminal ganglion and their association with latent herpes simplex virus-1 infection.
2620 PLoS One. **8:**e83603.
2621 361. **Wilson AC, Mohr I.** 2012. A cultured affair: HSV latency and reactivation in
2622 neurons. Trends Microbiol. **20:**604-611.
2623 362. **Iwasaki T, Itamura S, Nishimura H, Sato Y, Tashiro M, Hashikawa T, Kurata T.**
2624 2004. Productive infection in the murine central nervous system with avian influenza
2625 virus A (H5N1) after intranasal inoculation. Acta Neuropathol. **108:**485-492.
2626 363. **Aronsson F, Robertson B, Ljunggren HG, Kristensson K.** 2003. Invasion and
2627 persistence of the neuroadapted influenza virus A/WSN/33 in the mouse olfactory
2628 system. Viral Immunol. **16:**415-423.
2629 364. **Majde JA, Bohnet SG, Ellis GA, Churchill L, Leyva-Grado V, Wu M,**
2630 **Szentirmai E, Rehman A, Krueger JM.** 2007. Detection of mouse-adapted human
2631 influenza virus in the olfactory bulbs of mice within hours after intranasal infection. J
2632 Neurovirol. **13:**399-409.

2633 365. **Shinya K, Makino A, Hatta M, Watanabe S, Kim JH, Hatta Y, Gao P, Ozawa M,**
2634 **Le QM, Kawaoka Y.** 2011. Subclinical brain injury caused by H5N1 influenza virus
2635 infection. J Virol. **85:**5202-5207.
2636 366. **Yamada M, Bingham J, Payne J, Rookes J, Lowther S, Haining J, Robinson R,**
2637 **Johnson D, Middleton D.** 2012. Multiple routes of invasion of wild-type Clade 1
2638 highly pathogenic avian influenza H5N1 virus into the central nervous system (CNS)
2639 after intranasal exposure in ferrets. Acta Neuropathol. **124:**505-516.
2640 367. **Plourde JR, Pyles JA, Layton RC, Vaughan SE, Tipper JL, Harrod KS.** 2012.
2641 Neurovirulence of H5N1 infection in ferrets is mediated by multifocal replication in
2642 distinct permissive neuronal cell regions. PLoS One. **7:**e46605.
2643 368. **Mori I, Goshima F, Imai Y, Kohsaka S, Sugiyama T, Yoshida T, Yokochi T,**
2644 **Nishiyama Y, Kimura Y.** 2002. Olfactory receptor neurons prevent dissemination of
2645 neurovirulent influenza A virus into the brain by undergoing virus-induced apoptosis.
2646 J Gen Virol. **83:**2109-2116.
2647 369. **Leyva-Grado VH, Churchill L, Wu M, Williams TJ, Taishi P, Majde JA,**
2648 **Krueger JM.** 2009. Influenza virus- and cytokine-immunoreactive cells in the murine
2649 olfactory and central autonomic nervous systems before and after illness onset. J
2650 Neuroimmunol. **211:**73-83.
2651 370. **van den Pol AN, Ding S, Robek MD.** 2014. Long-distance interferon signaling
2652 within the brain blocks virus spread. J Virol. **88:**3695-3704.
2653 371. **van Riel D, Leijten LM, Verdijk RM, Geurtsvankessel C, van der Vries E, van**
2654 **Rossum AM, Osterhaus AD, Kuiken T.** 2014. Evidence for influenza virus CNS
2655 invasion along the olfactory route in an immunocompromised infant. J Infect Dis.
2656 372. **Kristensson K, Leestma J, Lundh B, Norrby E.** 1984. Sendai virus infection in the
2657 mouse brain: virus spread and long-term effects. Acta Neuropathol. **63:**89-95.

2658 373. **Mori I, Komatsu T, Takeuchi K, Nakakuki K, Sudo M, Kimura Y.** 1995.
2659 Parainfluenza virus type 1 infects olfactory neurons and establishes long-term
2660 persistence in the nerve tissue. J Gen Virol. **76 (Pt 5):**1251-1254.
2661 374. **Lundh B, Kristensson K, Norrby E.** 1987. Selective infections of olfactory and
2662 respiratory epithelium by vesicular stomatitis and Sendai viruses. Neuropathol Appl
2663 Neurobiol. **13:**111-122.
2664 375. **Weingartl H, Czub S, Copps J, Berhane Y, Middleton D, Marszal P, Gren J,**
2665 **Smith G, Ganske S, Manning L, Czub M.** 2005. Invasion of the central nervous
2666 system in a porcine host by nipah virus. J Virol. **79:**7528-7534.
2667 376. **Dups J, Middleton D, Yamada M, Monaghan P, Long F, Robinson R, Marsh**
2668 **GA, Wang LF.** 2012. A new model for Hendra virus encephalitis in the mouse. PLoS
2669 One. **7:**e40308.
2670 377. **Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith**
2671 **CS, Chua KB, Lam SK, Tan CT, Goh KJ, Chong HT, Jusoh R, Rollin PE,**
2672 **Ksiazek TG, Zaki SR.** 2002. Nipah virus infection: pathology and pathogenesis of an
2673 emerging paramyxoviral zoonosis. Am J Pathol. **161:**2153-2167.
2674 378. **Charles PC, Walters E, Margolis F, Johnston RE.** 1995. Mechanism of
2675 neuroinvasion of Venezuelan equine encephalitis virus in the mouse. Virology.
2676 **208:**662-671.
2677 379. **Roy CJ, Reed DS, Wilhelmsen CL, Hartings J, Norris S, Steele KE.** 2009.
2678 Pathogenesis of aerosolized Eastern Equine Encephalitis virus infection in guinea
2679 pigs. Virol J. **6:**170.
2680 380. **Schafer A, Brooke CB, Whitmore AC, Johnston RE.** 2011. The role of the blood-
2681 brain barrier during Venezuelan equine encephalitis virus infection. J Virol.
2682 **85:**10682-10690.

2683 381. **Conomy JP, Leibovitz A, McCombs W, Stinson J.** 1977. Airborne rabies
2684 encephalitis: demonstration of rabies virus in the human central nervous system.
2685 Neurology. **27:**67-69.
2686 382. **Lafay F, Coulon P, Astic L, Saucier D, Riche D, Holley A, Flamand A.** 1991.
2687 Spread of the CVS strain of rabies virus and of the avirulent mutant AvO1 along the
2688 olfactory pathways of the mouse after intranasal inoculation. Virology. **183:**320-330.
2689 383. **Miragall F, Kadmon G, Husmann M, Schachner M.** 1988. Expression of cell
2690 adhesion molecules in the olfactory system of the adult mouse: presence of the
2691 embryonic form of N-CAM. Dev Biol. **129:**516-531.
2692 384. **Thoulouze MI, Lafage M, Schachner M, Hartmann U, Cremer H, Lafon M.**
2693 1998. The neural cell adhesion molecule is a receptor for rabies virus. J Virol.
2694 **72:**7181-7190.
2695 385. **Plakhov IV, Arlund EE, Aoki C, Reiss CS.** 1995. The earliest events in vesicular
2696 stomatitis virus infection of the murine olfactory neuroepithelium and entry of the
2697 central nervous system. Virology. **209:**257-262.
2698 386. **Yoder JS, Straif-Bourgeois S, Roy SL, Moore TA, Visvesvara GS, Ratard RC,**
2699 **Hill VR, Wilson JD, Linscott AJ, Crager R, Kozak NA, Sriram R, Narayanan J,**
2700 **Mull B, Kahler AM, Schneeberger C, da Silva AJ, Poudel M, Baumgarten KL,**
2701 **Xiao L, Beach MJ.** 2012. Primary amebic meningoencephalitis deaths associated
2702 with sinus irrigation using contaminated tap water. Clin Infect Dis. **55:**e79-85.
2703 387. **Shakoor S, Beg MA, Mahmood SF, Bandea R, Sriram R, Noman F, Ali F,**
2704 **Visvesvara GS, Zafar A.** 2011. Primary amebic meningoencephalitis caused by
2705 *Naegleria fowleri*, Karachi, Pakistan. Emerg Infect Dis. **17:**258-261.
2706 388. **Carter RF.** 1968. Primary amoebic meningo-encephalitis: clinical, pathological and
2707 epidemiological features of six fatal cases. J Pathol Bacteriol. **96:**1-25.

2708 389. **Duma RJ, Ferrell HW, Nelson EC, Jones MM.** 1969. Primary amebic
2709 meningoencephalitis. N Engl J Med. **281:**1315-1323.
2710 390. **Rojas-Hernandez S, Jarillo-Luna A, Rodriguez-Monroy M, Moreno-Fierros L,**
2711 **Campos-Rodriguez R.** 2004. Immunohistochemical characterization of the initial
2712 stages of *Naegleria fowleri* meningoencephalitis in mice. Parasitol Res. **94:**31-36.
2713 391. **Jarolim KL, McCosh JK, Howard MJ, John DT.** 2000. A light microscopy study
2714 of the migration of *Naegleria fowleri* from the nasal submucosa to the central nervous
2715 system during the early stage of primary amebic meningoencephalitis in mice. J
2716 Parasitol. **86:**50-55.
2717 392. **Cervantes-Sandoval I, Serrano-Luna Jde J, Garcia-Latorre E, Tsutsumi V,**
2718 **Shibayama M.** 2008. Mucins in the host defence against *Naegleria fowleri* and
2719 mucinolytic activity as a possible means of evasion. Microbiology. **154:**3895-3904.
2720 393. **Martinez AJ, Nelson EC, Duma RJ.** 1973. Animal model of human disease.
2721 Primary amebic meningoencephalitis, *Naegleria* meningoencephalitis, CNS protozoal
2722 infection. Am J Pathol. **73:**545-548.
2723 394. **Shibayama M, Martinez-Castillo M, Silva-Olivares A, Galindo-Gomez S,**
2724 **Navarro-Garcia F, Escobar-Herrera J, Sabanero M, Tsutsumi V, Serrano-Luna**
2725 **J.** 2013. Disruption of MDCK cell tight junctions by the free-living amoeba *Naegleria*
2726 *fowleri*. Microbiology. **159:**392-401.
2727 395. **Kiderlen AF, Laube U.** 2004. *Balamuthia mandrillaris*, an opportunistic agent of
2728 granulomatous amebic encephalitis, infects the brain via the olfactory nerve pathway.
2729 Parasitol Res. **94:**49-52.
2730 396. **Bravo FG, Seas C.** 2012. *Balamuthia mandrillaris* amoebic encephalitis: an
2731 emerging parasitic infection. Curr Infect Dis Rep. **14:**391-396.

2732 397. **Sabiiti W, May RC.** 2012. Mechanisms of infection by the human fungal pathogen
2733 *Cryptococcus neoformans*. Future Microbiol. **7:**1297-1313.
2734 398. **Fromtling RA, Abruzzo GK, Ruiz A.** 1988. *Cryptococcus neoformans*: a central
2735 nervous system isolate from an AIDS patient that is rhinotropic in a normal mouse
2736 model. Mycopathologia. **102:**79-86.
2737 399. **Dixon DM, Polak A.** 1986. *In vivo* and *in vitro* studies with an atypical, rhinotropic
2738 isolate of *Cryptococcus neoformans*. Mycopathologia. **96:**33-40.
2739 400. **Anderson DA, Sagha HM.** 1988. Persistence of infection in mice inoculated
2740 intranasally with *Cryptococcus neoformans*. Mycopathologia. **104:**163-169.
2741 401. **Gomes NG, Boni M, Primo CC.** 1997. The invasive behaviour of *Cryptococcus*
2742 *neoformans*: a possibility of direct access to the central nervous system?
2743 Mycopathologia. **140:**1-11.
2744 402. **Lima C, Vital JP.** 1994. Olfactory pathways in three patients with cryptococcal
2745 meningitis and acquired immune deficiency syndrome. J Neurol Sci. **123:**195-199.
2746 403. **Gamba JL, Woodruff WW, Djang WT, Yeates AE.** 1986. Craniofacial
2747 mucormycosis: assessment with CT. Radiology. **160:**207-212.
2748 404. **Galetta SL, Wulc AE, Goldberg HI, Nichols CW, Glaser JS.** 1990. Rhinocerebral
2749 mucormycosis: management and survival after carotid occlusion. Ann Neurol.
2750 **28:**103-107.
2751 405. **Yousem DM, Galetta SL, Gusnard DA, Goldberg HI.** 1989. MR findings in
2752 rhinocerebral mucormycosis. J Comput Assist Tomogr. **13:**878-882.
2753 406. **Press GA, Weindling SM, Hesselink JR, Ochi JW, Harris JP.** 1988. Rhinocerebral
2754 mucormycosis: MR manifestations. J Comput Assist Tomogr. **12:**744-749.

2755 407. **Orguc S, Yuceturk AV, Demir MA, Goktan C.** 2005. Rhinocerebral
2756 mucormycosis: perineural spread via the trigeminal nerve. J Clin Neurosci. **12:**484-
2757 486.
2758 408. **McLean FM, Ginsberg LE, Stanton CA.** 1996. Perineural spread of rhinocerebral
2759 mucormycosis. AJNR Am J Neuroradiol. **17:**114-116.
2760 409. **Frater JL, Hall GS, Procop GW.** 2001. Histologic features of zygomycosis:
2761 emphasis on perineural invasion and fungal morphology. Arch Pathol Lab Med.
2762 **125:**375-378.
2763 410. **Teng CH, Cai M, Shin S, Xie Y, Kim KJ, Khan NA, Di Cello F, Kim KS.** 2005.
2764 *Escherichia coli* K1 RS218 interacts with human brain microvascular endothelial cells
2765 via type 1 fimbria bacteria in the fimbriated state. Infect Immun. **73:**2923-2931.
2766 411. **Zou Y, He L, Huang SH.** 2006. Identification of a surface protein on human brain
2767 microvascular endothelial cells as vimentin interacting with *Escherichia coli* invasion
2768 protein IbeA. Biochem Biophys Res Commun. **351:**625-630.
2769 412. **Zou Y, He L, Wu CH, Cao H, Xie ZH, Ouyang Y, Wang Y, Jong A, Huang SH.**
2770 2007. PSF is an IbeA-binding protein contributing to meningitic *Escherichia coli* K1
2771 invasion of human brain microvascular endothelial cells. Med Microbiol Immunol.
2772 **196:**135-143.
2773 413. **Chi F, Bo T, Wu CH, Jong A, Huang SH.** 2012. Vimentin and PSF act in concert to
2774 regulate IbeA+ *E. coli* K1 induced activation and nuclear translocation of NF-kappaB
2775 in human brain endothelial cells. PLoS One. **7:**e35862.
2776 414. **Prasadarao NV, Wass CA, Huang SH, Kim KS.** 1999. Identification and
2777 characterization of a novel Ibe10 binding protein that contributes to *Escherichia coli*
2778 invasion of brain microvascular endothelial cells. Infect Immun. **67:**1131-1138.

2779 415. **Huang SH, Wass C, Fu Q, Prasadarao NV, Stins M, Kim KS.** 1995. *Escherichia*
2780 *coli* invasion of brain microvascular endothelial cells *in vitro* and *in vivo*: molecular
2781 cloning and characterization of invasion gene ibe10. Infect Immun. **63:**4470-4475.
2782 416. **Hoffman JA, Badger JL, Zhang Y, Huang SH, Kim KS.** 2000. *Escherichia coli* K1
2783 aslA contributes to invasion of brain microvascular endothelial cells *in vitro* and *in*
2784 *vivo*. Infect Immun. **68:**5062-5067.
2785 417. **Badger JL, Wass CA, Weissman SJ, Kim KS.** 2000. Application of signature-
2786 tagged mutagenesis for identification of *Escherichia coli* K1 genes that contribute to
2787 invasion of human brain microvascular endothelial cells. Infect Immun. **68:**5056-
2788 5061.
2789 418. **Wang Y, Huang SH, Wass CA, Stins MF, Kim KS.** 1999. The gene locus yijP
2790 contributes to *Escherichia coli* K1 invasion of brain microvascular endothelial cells.
2791 Infect Immun. **67:**4751-4756.
2792 419. **Parthasarathy G, Yao Y, Kim KS.** 2007. Flagella promote *Escherichia coli* K1
2793 association with and invasion of human brain microvascular endothelial cells. Infect
2794 Immun. **75:**2937-2945.
2795 420. **Swords WE, Buscher BA, Ver Steeg Ii K, Preston A, Nichols WA, Weiser JN,**
2796 **Gibson BW, Apicella MA.** 2000. Non-typeable *Haemophilus influenzae* adhere to
2797 and invade human bronchial epithelial cells via an interaction of lipooligosaccharide
2798 with the PAF receptor. Mol Microbiol. **37:**13-27.
2799 421. **Braun L, Ghebrehiwet B, Cossart P.** 2000. gC1q-R/p32, a C1q-binding protein, is a
2800 receptor for the InlB invasion protein of *Listeria monocytogenes*. EMBO J. **19:**1458-
2801 1466.
2802 422. **Shen Y, Naujokas M, Park M, Ireton K.** 2000. InlB-dependent internalization of
2803 *Listeria* is mediated by the Met receptor tyrosine kinase. Cell. **103:**501-510.

2804 423. **Sheen TR, Ebrahimi CM, Hiemstra IH, Barlow SB, Peschel A, Doran KS.** 2010.
2805 Penetration of the blood-brain barrier by *Staphylococcus aureus*: contribution of
2806 membrane-anchored lipoteichoic acid. J Mol Med (Berl). **88:**633-639.
2807 424. **Doran KS, Engelson EJ, Khosravi A, Maisey HC, Fedtke I, Equils O, Michelsen**
2808 **KS, Arditi M, Peschel A, Nizet V.** 2005. Blood-brain barrier invasion by group B
2809 streptococcus depends upon proper cell-surface anchoring of lipoteichoic acid. J Clin
2810 Invest. **115:**2499-2507.
2811 425. **Maisey HC, Hensler M, Nizet V, Doran KS.** 2007. Group B streptococcal pilus
2812 proteins contribute to adherence to and invasion of brain microvascular endothelial
2813 cells. J Bacteriol. **189:**1464-1467.
2814 426. **van Sorge NM, Quach D, Gurney MA, Sullam PM, Nizet V, Doran KS.** 2009. The
2815 group B streptococcal serine-rich repeat 1 glycoprotein mediates penetration of the
2816 blood-brain barrier. Journal of Infectious Diseases. **199:**1479-1487.
2817 427. **Baron MJ, Bolduc GR, Goldberg MB, Auperin TC, Madoff LC.** 2004. Alpha C
2818 protein of group B streptococcus binds host cell surface glycosaminoglycan and
2819 enters cells by an actin-dependent mechanism. J Biol Chem. **279:**24714-24723.
2820 428. **Mori I, Nishiyama Y, Yokochi T, Kimura Y.** 2005. Olfactory transmission of
2821 neurotropic viruses. J Neurovirol. **11:**129-137.
2822 429. **Ekberg JA, Amaya D, Chehrehasa F, Lineburg K, Claxton C, Windus LC, Key**
2823 **B, Mackay-Sim A, St John JA.** 2011. OMP-ZsGreen fluorescent protein transgenic
2824 mice for visualisation of olfactory sensory neurons *in vivo* and *in vitro*. J Neurosci
2825 Methods. **196:**88-98.
2826 430. **Windus LC, Claxton C, Allen CL, Key B, St John JA.** 2007. Motile membrane
2827 protrusions regulate cell-cell adhesion and migration of olfactory ensheathing glia.
2828 Glia. **55:**1708-1719.

2829 **AUTHOR BIOS**

2830 **Samantha J. Dando**

2831 Dr Samantha Dando received her PhD in microbiology in 2012 from Queensland University
2832 of Technology, Australia, where she studied the pathogenic mechanisms of the human
2833 *Ureaplasma* species during intrauterine infection. She was subsequently appointed as a
2834 postdoctoral research fellow at the Institute for Glycomics, Griffith University, and undertook
2835 research to investigate the virulence of *Burkholderia pseudomallei* in a murine model of
2836 acute melioidosis. She has recently accepted a postdoctoral research position at Monash
2837 University, to research dendritic cell biology in the central nervous system during
2838 autoimmune disease.

2839 **Alan Mackay-Sim**

2840 Professor Alan Mackay-Sim obtained his PhD in 1980 at Macquarie University, Australia. He
2841 undertook a postdoctoral position at the University of Pennsylvania and had faculty positions
2842 at the University of Sydney, the University of Wyoming and the University of Adelaide
2843 before moving to Griffith University in 1987 where he has held positions as Deputy Director
2844 of the Eskitis Institute for Cell and Molecular Therapies and Director of the National Centre
2845 for Adult Stem Cell Research. He is currently Professor of Neuroscience, Griffith University.
2846 With over 30 years of research in the field, Professor Mackay-Sim is a leading expert on the
2847 human sense of smell and the biology and development of the olfactory mucosa. In the last
2848 16 years he has concentrated on the clinical applications of olfactory cells and their use for
2849 neural regeneration therapies, as well as the involvement of the olfactory nerve pathway in
2850 the development of disease.

2851 **Robert Norton**

2852 Dr Robert Norton graduated in Medicine in 1980 and has worked in a variety of clinical
2853 positions including five years in Australian indigenous communities. He trained in
2854 Microbiology at the Institute of Medical and Veterinary Science in Adelaide between 1991
2855 and 1995. He gained an MD from the University of Adelaide in 1998. In his current capacity
2856 as Director of Microbiology at Townsville Hospital, Queensland, he has collaborated with
2857 researchers locally and nationally on projects relating to melioidosis, rheumatic fever,
2858 invasive group A streptococcal disease and Q-fever. Dr Norton is part of the Infectious
2859 Diseases and Immunopathogenesis Research Group which includes clinicians and academic
2860 staff of James Cook University. He is an Associate Professor at James Cook University and is
2861 the Chief Examiner in Microbiology for the Royal College of Pathologists in Australasia. He
2862 has over 100 peer-reviewed publications and has been successful in obtaining local and
2863 national collaborative grants.

2864 **Bart J. Currie**

2865 Bart Currie is Head of Infectious Diseases at Royal Darwin Hospital and Professor in
2866 Medicine at the Northern Territory Medical Program, Flinders and Charles Darwin
2867 Universities. He is also Program Leader for Tropical and Emerging Infectious Diseases in the
2868 Global and Tropical Health Division of the Menzies School of Health Research and Director
2869 of RHD Australia. Areas of interest include clinical and epidemiological aspects of tropical
2870 and emerging infections, development of treatment guidelines and clinical toxinology. He
2871 initiated the Darwin Prospective Melioidosis Study in 1989 and this remains the basis for
2872 ongoing multi-disciplinary collaborations on melioidosis.

2873 **James A. St John**

2874 Dr James St John obtained his PhD in Agricultural Science at the University of Melbourne,
2875 Australia in 1996. He has previously held full-time research fellowships at the University of

2876 Melbourne and The University of Queensland. He is currently a Senior Research Fellow at
2877 the Eskitis Institute for Drug Discovery, Griffith University. Since his PhD he has worked in
2878 the field of mammalian olfactory nervous system development and regeneration and has
2879 published over 40 papers on olfactory axon guidance and the role of olfactory glia. He
2880 performs detailed microscopic anatomical studies of the olfactory system and has identified
2881 subpopulations of olfactory glia using live cell imaging of in vitro cultures. He is particularly
2882 interested in the role of olfactory glia in protecting the brain from bacterial infections.
2883 Together with Dr Jenny Ekberg, he recently performed the majority of the work which
2884 identified the intranasal route of infection via the olfactory nerve by *Burkholderia*
2885 *pseudomallei.*

2886 **Jenny A. K. Ekberg**

2887 Dr Jenny Ekberg obtained her PhD in neuroscience/biomedical science from The University
2888 of Queensland in 2005. During her PhD and first postdoctoral appointment, also at UQ, she
2889 studied novel modulatory mechanisms of voltage-gated ion channels in neurons using
2890 electrophysiological techniques. In 2008, she moved to Griffith University as a Research
2891 Fellow, now focussing on neuron-glia interactions and neural regeneration in the olfactory
2892 nervous system. In 2012, she took up a position as Senior Lecturer at Queensland University
2893 of Technology. She continues her work on neural repair, and has expanded into the field of
2894 bacterial infections of the central nervous system. Together with Dr St John and Prof.
2895 Beacham, she recently investigated how *B. pseudomallei* can invade the brain via the
2896 olfactory nerve. The focus of Dr. Ekberg's research is to investigate how cells in the olfactory
2897 nerve prevent microorganisms from entering the brain, whilst simultaneously promoting
2898 nerve regeneration.

2899 **Michael Batzloff**

Dr Michael Batzloff completed his PhD from Griffith University in 2001 after which he accepted a postdoctoral position at the Queensland Institute of Medical Research, Australia. He has been a recipient of two fellowships from the National Heart Foundation of Australia for his research into vaccine development for *Streptococcus pyogenes*. He was subsequently appointed the inaugural head of the Bacterial Vaccines Laboratory at the Queensland Institute of Medical Research and recently accepted a position at the Institute for Glycomics at Griffith University. His research interests include neglected tropical diseases focusing on pathogenesis and vaccine development for the bacterial pathogens *Streptococcus pyogenes* and *Burkholderia pseudomallei*.

Glen C. Ulett

Associate Professor Glen C. Ulett received his PhD from James Cook University (Australia) in 2001, and completed post-doctoral research at St Jude Children's Research Hospital, the University of Queensland, and the University of Alabama at Birmingham. He is currently an Australian Research Council (ARC) Future Fellow in Microbiology at Griffith University where he leads a research team studying bacterial pathogenesis and mechanisms of host defence against infection. His laboratory focuses on infections related to the urogenital tract, programmed cell death, and mechanisms of virulence and disease associated with *Escherichia coli*, *Streptococcus agalactiae* and *Burkholderia pseudomallei*. He has been a microbiology researcher in the field of bacterial pathogenesis for seventeen years and is a two-time-recipient of the George McCracken Infectious Disease Fellow Award from the American Society for Microbiology. His research program in microbiology and infectious diseases is supported by funding from the National Health and Medical Research Council of Australia.

Ifor R. Beacham

2924 Emeritus Professor Ifor Beacham undertook undergraduate studies in biochemistry at the
2925 University of Otago, New Zealand, and obtained his PhD in microbial genetics from the
2926 University of Leicester, UK. He has undertaken postdoctoral research at the University of
2927 Leicester, Washington University, St Louis, and the University of California, Santa Barbara,
2928 and has held teaching positions at the University of Wales, Aberystwyth, UK, and Griffith
2929 University, Australia. He is currently a Research Leader at the Institute for Glycomics,
2930 Griffith University. He has worked in the general area of molecular microbiology on a variety
2931 of bacteria before undertaking work with *Burkholderia pseudomallei* 16 years ago. These
2932 latter studies were motivated by the endemicity of melioidosis in northern Australia,
2933 difficulties with genetic manipulation and the enigmatic status of *B. pseudomallei* as a
2934 pathogen. He hopes his continuing work will contribute to a greater understanding of the
2935 molecular nature of virulence of *B. pseudomallei*.

Bacterial pathogen	Ligand	HBMEC receptor	Function	References
Escherichia coli (and other Gram negative enterics)	FimH	CD48	Adhesion to HBMECs; FimH also promotes invasion by activation of RhoA, which induces host cytoskeletal rearrangements	(154, 410)
	Nlp1	Unknown	Adhesion to and invasion of HBMECs; Nlp1 activates host cytosolic phospholipase A_2	(174)
	OmpA	Gp96 homologue	Loops 1, 2 and 3 of OmpA function in adhesion to, and invasion of, HBMECs; ompA-mediated invasion is due to Rac1 activation and activation of host cytosolic phospholipase A_2	(161, 162, 173, 178)
	CNF-1	37/67 kDa laminin receptor	Invasion of HBMECs; CNF-1 activates RhoA and recruits focal adhesin kinase to the site of bacterial entry	(149, 150, 171, 172)
	IbeA	Vimentin, PSF	Invasion of HBMECs; activates Rac1	(178, 411-413)
	Ibe10	45 kDa Ibe10 receptor	Invasion of HBMECs	(414, 415)
	AslA	Unknown	Invasion of HBMECs	(416)
	TraJ	Unknown	Invasion of HBMECs	(417)
	yijP	Unknown	Invasion of HBMECs	(418)
	Flagella	Unknown	Adhesion to and invasion of HBMECs	(419)
Haemophilus influenzae	Phosphorylcholine	PAF receptor	Potentially involved in invasion of HBMECs	(420)
	OmpP2	37/67 kDa laminin receptor	Adhesion to HBMECs	(151, 152)
Listeria monocytogenes	InlA	E-cadherin	Invasion of polarized human choroid plexus epithelial cells	(232)
	InlB	gC1q-R, met receptor tyrosine kinase	Invasion of HBMECs and	(213, 232,

			polarized human choroid plexus epithelial cells	421, 422)
	Vip	Gp96	Invasion of the brain *in vivo*	(160)
	IspC	Unknown	Adhesion to and invasion of sheep choroid plexus epithelial cells, but not HBMECs	(233)
Mycobacterium tuberculosis	*Rv0980c*, *Rv0987*, *Rv0989c*, *Rv1801*	Unknown	Invasion of HBMECs	(118)
	HBHA	Heparan sulfate glycosaminoglycans	Adhesion to and invasion of BBMECs	(234)
	Rv0311, *Rv0805*, *Rv0931c*, *Rv0986*, *MT3280*	Unknown	Invasion of HBMECs	(235)
	PknD	Laminin	Attachment to, invasion of and survival within HBMECs	(158)
Neisseria meningitidis	Type IV pili	Unknown	Adhesion to HBMECs; type IV pilus-mediated adhesion leads to the formation of cortical plaques and host membrane protrusions	(122, 124)
	PilQ and PorA	37/67 kDa laminin receptor	Adhesion to HBMECs	(151, 152)
	Opc	$\alpha_5\beta_1$ integrin (mediated by binding to the bridging molecules vitronectin and fibronectin)	Adhesion to and invasion of HBMECs; activates JNK1, JNK2 and p38, and protein tyrosine kinase signalling	(155, 191, 192)
Staphylococcus aureus	*ypfP* and LTA	Unknown	Invasion of HBMECs	(423)
Streptococcus agalactiae	Lmb	Laminin	Invasion of HBMECs	(159)
	iagA and LTA	Unknown	Invasion of HBMECs; *iagA* anchors LTA to the streptococcal cell wall and is required for invasion	(424)
	FbsA	Fibrinogen	Adhesion to HBMECs	(156)
	SfbA	Integrins (*via S. agalactiae* binding to	Invasion of HBMECs	(199)

		immobilised fibronectin)		
	PilA	$\alpha_2\beta_1$ integrin (mediated by *S. agalactiae* binding to immobilised collagen)	Adhesion to and invasion of HBMECs; pilA binding stimulates focal adhesion kinase phosphorylation and downstream phosphatidylinositol-3 kinase activation	(109, 425)
	PilB	Unknown	Invasion of HMBECs	(425)
	Srr-1	Fibrinogen (srr-1 binds amino acids 283 – 410 of the fibrinogen α chain *via* a dock, lock and latch mechanism)	Adhesion to and invasion of HBMECs	(200, 201, 426)
	HvgA	Unknown	Adhesion to HBMECs	(198)
	ACP	Glycosaminoglycans	Adhesion to and invasion of HBMECs; invasion potentially occurs following Rho GTPase-dependent actin rearrangements	(157, 427)
Streptococcus pneumoniae	CbpA	37/67 kDa laminin receptor	Adhesion to HBMECs	(151)
	Phosphorylcholine	PAF receptor	Invasion of HBMECs; binding to platelet-activating factor receptor induces the co-localisation of β-arrestin	(193, 197)
	NanA	Unknown	Adhesion to and invasion of HBMECs	(194, 195)

Table 1: Known bacterial ligands and their host receptors for adhesion to and invasion of the blood-brain barrier

ACP = alpha C protein; AslA = aryl-sulfatesulfohydrolase; BBMEC = bovine brain microvascular endothelial cells; CbpA = choline binding protein A; CNF-1 = cytotoxic necrotizing factor-1; FbsA = fibrinogen-binding protein; FimH = type 1 fimbrial adhesin; HBHA = heparin-

binding haemagglutinin adhesin; HBMECs = human brain microvascular endothelial cells; HvgA = hypervirulent GBS adhesion; IbeA = invasion of brain endothelial cell protein A; iagA = invasion associated gene; InlA = internalin A; InlB = internalin B; LTA = lipoteichoic acid; Lmb = laminin-binding protein; NanA = neuraminidase A; Nlp1 = new lipoprotein 1; OmpP2 = outer membrane protein P2; Opc = outer membrane protein C; PAF = platelet activating factor; PilQ = pilus secretin protein; PorA = major outer membrane protein; PSF = polypyrimidine-tract-binding protein (PTB)-associated splicing factor; SfbA = streptococcal fibrinogen binding protein A; Srr-1 = serine-rich repeat 1; Vip = virulence protein.

Figure legends

Figure 1: Anatomical location and organization of the blood-brain and blood-cerebrospinal fluid barriers, and the olfactory portal.

The skull and the meninges, which consist of the dura mater, arachnoid mater and pia mater, protect the brain. The arachnoid mater and pia mater are connected by strands of connective tissue called arachnoid traberculae, which course through the CSF-containing subarachnoid space. At the cellular level, the BBB and BCSFB maintain homeostasis of the brain. The BBB is formed by tight junctions between endothelial cells lining cerebral microvessels, in addition to pericytes and astrocytes. The BCSFB is formed by tight junctions between: (i) epithelial cells at the choroid plexus, (ii) endothelial cells of the veins and venules within the subarachnoid space, and (iii) the epithelial cells of the arachnoid mater. The olfactory system bypasses the cellular barriers of the CNS and provides a direct portal from the nasal cavity to the olfactory bulb within the brain.

Figure 2: Mechanisms of blood-cerebrospinal fluid penetration by bacterial pathogens.

Bacteria (purple) may invade the CNS *via* the BCSFB by (A) transcellular penetration involving either pinocytosis or receptor-mediated mechanisms; (B) by paracellular entry following the disruption of junctions (comprising tight junctions and adherens junctions) between choroidal epithelial cells, endothelial cells of veins/venules within the subarachnoid space, or the cells of the arachnoid membrane; and (C) by the "Trojan horse" mechanism, where microbes may transmigrate with infected leukocytes (such as macrophages, as shown).

Figure 3: The olfactory system is a direct portal for bacterial pathogens to the brain.

(A) The cilia of olfactory sensory neurons penetrate the nasal mucosa and provide a direct pathway from the external environment to the CNS. Olfactory sensory neurons in the

olfactory epithelium are supported by sustentacular cells and replaced by proliferation and differentiation of basal stem cells, and their axons pass through the lamina propria and cribriform plate of the skull to synapse with mitral cells in the glomeruli of the olfactory bulb. Microbial pathogens can potentially access the brain through the olfactory epithelium *via* axonal transport, by travel within olfactory ensheathing cells that surround the axons, or external to these cells within the perineural space and passage through holes in the cribriform plate to access the subarachnoid space. Adapted from (101, 428). (B-C) A coronal section through the olfactory system of transgenic reporter mice OMP-ZsGreen x S100ß-DsRed (429, 430). (B) Primary olfactory neurons (green) reside in the olfactory epithelium, which lines the nasal cavity (NC); septum is indicated as S. The neurons project axons to the olfactory bulb (OB) within the CNS. Boxed region is shown in C. (C) Bundles of olfactory axons project from the olfactory epithelium (OE) through the cribriform plate (CP; chondrocytes are bright red) and enter the nerve fiber layer (NFL), which forms the outer layer of the olfactory bulb. Olfactory ensheathing cells (dull red, arrows) surround the axon bundles. (D) A sagittal section through the olfactory bulb and nasal cavity of a mouse that was intranasally inoculated with *B. pseudomallei* (green) with invasion of the nerve fiber layer of the olfactory bulb in the region indicated by the arrow. (E) A higher power view of the ventral nerve fiber layer shows bacterial infestation within the nasal cavity (NC), the olfactory epithelium (OE) and invasion of the NFL by the bacteria. Inset box shows higher power view of *B. pseudomallei* (green) within the nerve fiber layer. Scale bar is 500 µm in B; 65 µm in C; 750 µm in D; 300 µm in E; 300 µm in E; 70 µm in inset in E.

Figure 4: The trigeminal nerve route of entry.

(A) Schematic showing the three branches of the trigeminal nerve, V1, V2 and V3. The branches V1 and V2 innervate the nasal cavity and project to the brain stem (BS). (B-D) In coronal sections of the mouse nasal cavity, *B. pseudomallei* (green) is localized within

branches of the trigeminal nerve that innervates the septum (S) within the nasal cavity (NC). Boxed area in B is shown in C. The bacteria (arrows) are contained within the trigeminal nerve while adjacent olfactory nerve (arrows with tail) bundles do not contain bacteria. (D) A higher magnification view of the trigeminal nerve in a nearby section. Scale bar is 650 µm in B; 40 µm in C; 15 µm in D. OE = olfactory epithelium.