Astroglipathology in the infectious insults of the brain

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Astroglipathology in the infectious insults of the brain

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Highlights

- Astrocytes, homeostatic cells of the central nervous system contribute to all neurological diseases.
- Astroglipathology is represented by reactive astrogliosis, astrodegeneration with astroglial atrophy and loss of function and pathological remodelling.
- Astrocytes provide the defensive barrier against infectious agents but can be also the main target of neuroinfection.
- Astrocytes can provide a reservoir for proliferation of infectious agents.

Abstract

Astroglia, a heterogeneous type of neuroglia, play key homeostatic functions in the central nervous system (CNS) and represent an important defence system. Impaired homeostatic capacity of astrocytes manifests in diseases and this is mirrored in various astrocyte-based pathological features including reactive astrogliosis, astrodegeneration with astroglial atrophy and pathological remodelling of astrocytes. All of these manifestations are most prominently associated with infectious insults, mediated by bacteria, protozoa and viruses. Here we focus into neurotropic viruses such as tick-borne encephalitis (TBEV) and Zika virus (ZIKV), both belonging to

Flaviviridae and both causing severe neurological impairments. We argue that astrocytes provide a route through which neurotropic infectious agents attack the CNS, since they are anatomically associated with the blood-brain barrier and exhibit aerobic glycolysis, a metabolic specialization of highly morphologically dynamic cells, which may provide a suitable metabolic milieu for proliferation of infectious agents, including viral bodies.

Abbreviations

BBB: blood-brain barrier; CNS: Central nervous system; CXCL-1, CXCL-10: chemokine ligand 1, 10; CVO: circumventricular organs; GFAP: glial fibrillary acidic protein; IL-6, IL-8: interleukin-6,8; TNF- α : tumour necrosis factor α ; STAT1: signal transducer and activator of transcription 1; TBEV: tick-borne encephalitis virus; ZIKV: Zika virus.

Keywords: Astroglia; Neuroinfection; CNS homeostasis; Zika virus; Tick-borne encephalitis virus

1. Astrocytes: Wardens and guarantors of the brain

The function of the brain as an organ is determined by a complex system of cellular networks responsible for neural tissue homeostasis, which includes defence against exogenous pathogens and infectious agents. These defensive systems are represented by blood-brain barrier (BBB) formed by endothelial cells and pericytes and by neuroglia, consisting of microglia and macroglia. The latter group includes oligodendrocytes which myelinate and support axons and astroglia, highly morphologically heterogeneous cells, that act as key homeostatic elements of the CNS; in particular astrocytes control ionic balance (ionostasis), neurotransmitter turnover, energy balance and synaptic connectivity [112]. Evolutionary specialisation of neural cells resulted in division of functions, with majority of homeostatic molecular cascades confined to astroglia, while neurones express proteins needed for electrical excitation (i.e. the triggering action potentials and their propagation along axons), fast neurotransmitter release and synaptic plasticity [111]. Perisynaptic processes of protoplasmic astrocytes cover substantial proportion of (mostly excitatory) synapses throughout the CNS and form the synaptic cradle, which is fundamentally important for homeostatic control of neurotransmission [110]. These astroglial perisynaptic membranes contain Na^+/K^+ pumps and inward rectifier $\text{K}_{\text{ir}} 4.1$ channels, which control local K^+ buffering and K^+ turnover, as well as high densities of solute carrier transporters that provide for uptake of glutamate, release of glutamine, secretion of reactive oxygen species scavengers and regulation of pH [87, 110]. The glutamate(GABA)-glutamine shuttle is imperative for maintenance of neurotransmission, because neurones cannot synthesise glutamate *de novo* and fully rely on astroglia for supply of glutamate precursor glutamine [41]. In addition astroglial cradle controls synaptogenesis through release of numerous trophic factors such as trombospondins, glypicans or hevin, and is involved in synaptic extinction [4, 27]. Moreover, astrocytes are central storage elements for glycogen [43, 57] and are the key site of cholesterol synthesis in the CNS, this cholesterol being ultimately required for synapse formation and remodelling [61].

A single protoplasmic astrocyte in human cortex establishes contacts with up to 2 million of synapses and hence astrocytes are capable of integrating information from multiple neuronal inputs thus tuning astroglial homeostatic cascades to the demands of neuronal networks [72, 113]. Astrocytes establish contacts with brain vasculature through endfeet formed by astroglial perivascular processes; these endfeet contribute to local control of capillaries constriction thereby linking neuronal activity to the blood flow (the phenomenon of functional hyperaemia). Astrocytes are secretory cells of the CNS being able to release ~ 200 molecules from neuromodulators to hormones and trophic factors [109] into the extracellular space, where convective movement distributes them throughout the neural tissue; a mode of action similar to that present in the endocrine system, therefore these cells were termed gliocrine [107]. Finally, astroglial cells are an important part of brain defence system, through both homeostatic response and disease and context-specific astroglial remodelling which is of paramount importance for neuropathology [77].

2. Types of astroglia pathology: an overview

Although the pathological potential of glial cells has been widely recognised in 19th century [2, 5, 6, 45, 71], the principles of astroglia pathology and the fundamental role of neuroglia in neurological diseases have been defined only in a recent decade [12, 29, 30, 32, 77, 85, 96, 97,

106, 115, 116, 118, 119]. Astrogliopathic changes are classified into (i) reactive (astro)gliosis; (ii) astrodegeneration with astroglial atrophy and loss of function and (iii) pathological remodelling of astrocytes [77, 108, 119].

Reactive gliosis is the most studied and most appreciated type of astrogliopathy, which is manifested in many neurological conditions. Reactive astrogliosis is an evolutionary conserved complex remodelling of astroglial biochemistry, morphology and function essentially aimed at neuroprotection and defence of neural tissue [12, 77, 96, 97]. Instigation of astroglial response results in multiple reactive phenotypes that differ depending on the specific disease context and display distinct changes in gene expression, morphology and physiology [56, 76, 77, 122]. Reactive astrocytes provide for neuroprotection and trophic support of stressed nervous tissue, isolate damaged area through formation of astroglial scar, they are instrumental for recovery of the compromised BBB and finally reactive astrocytes support post-lesion remodelling and regeneration of the nervous tissue.

In many neurological conditions the primary astrogliopathy emerges in a form of **astrodegeneration with astroglial atrophy** and loss of function. Particularly important are failures of astroglial homeostatic capabilities, represented for example by deficient uptake of glutamate (which leads to excitotoxicity) or defects in extracellular K^+ buffering. Astroglial atrophy and astrodegeneration are prominent in major neuropsychiatric diseases, in toxic encephalopathies such as Wernicke encephalopathy or hepatic encephalopathy, in addictive disorders and in neurodegenerative disorders such as for example amyotrophic lateral sclerosis or Alzheimer's disease [22, 23, 46, 66, 73, 83, 85, 90, 91, 94, 114, 117, 120].

The third type of astrogliopathy is represented by **pathological remodelling of astroglial cells**, when they acquire specific properties that facilitate and even drive pathological process. The most notable example of astroglial pathological remodelling is observed in Alexander disease caused by expression of sporadically mutated glial fibrillary acidic protein (GFAP), a cytoskeletal intermediate filament. This causes yet uncharacterised changes in astroglial function, likely involving a perturbed vesicle network system which result in severe damage to the white matter and leucomalacia [53, 65, 123].

3. Astrocytes in infectious diseases of the brain

Astrocytes contribute to the infectious lesions of the brain in several ways. First and foremost they are the part of brain defence system and inner part of the BBB. Of note, brain areas of the circumventricular organs (CVOs) are highly vascularised, lacking a tight (endothelial) BBB. These structures include the subfornical organ, the organum vasculosum of the lamina terminalis and the area postrema. The circumventricular organs (CVOs) are atypical brain structures lining the third and fourth ventricles, which exist in the brains of all vertebrates. They play a role in regulating body homeostasis based on blood-brain communication [37]. In the CVOs, the blood-brain interface is formed by a rich capillary plexus harbouring a fenestrated endothelium [20] and lacking tight junction complexes in the endothelial cells [92]. Infectious agents cross the BBB in several ways (including paracellular route, transcytosis, receptor hijacking or infected leukocytes, the Trojan horse route [21, 49]) after entering brain parenchyma are met by astroglial cells. Astrocytes express several receptors for pathogen-associated molecular patterns, and can possibly recognise various bacterial agents. At the same time astrocytes are targets of infectious

agents; and after being infected astroglial cells may either mount astroglial defensive response, which is associated with neuroinflammation [40], or undergo pathological remodelling which may mediate pathological progression or even become the reservoir for infection.

3.1. Bacterial infection of astroglia

Many bacteria which cause brain infection interact with astroglia and trigger astroglial reactivity. Activation of astrocytes was observed, for example, in pneumococcal meningitis caused by *Streptococcus pneumoniae* [44] or *Neisseria meningitidis* [17]. Astroglial reactivity was also induced by infection with *Borrelia burgdorferi*, the Lyme disease spirochete which does not produce lipopolysaccharide [26, 84]; this activation of astroglia resulted in significant increase in secretion of pro-inflammatory factors such as IL-6, TNF- α , IL-8, CXCL-1, and CXCL-10 [11]. Astroglial reactivity in the context of brain infection has clear defensive value, because suppression of astroglial response, for example in mice lacking GFAP, exacerbates the spread of infection and neurological damage [99]. Similarly, genetic deletion of NK-1R receptor activated by Substance P reduced astrogliosis and limited inflammatory response to *N. meningitidis* [17]. Brain infection with *Staphylococcus aureus* results in a down-regulation of astroglial synthesis of connexins and hence in a decrease of astroglial syncytial connectivity between cells surrounding the infection foci [28]. This decrease in gap junctional connectivity may reflect a defensive reaction that limits the spread of the infection.

3.2. Protozoan infection of astrocytes

The *Toxoplasma gondii*, an obligate intracellular, parasitic alveolate that causes the disease toxoplasmosis, seems to have an affinity to astroglia (although see [13], and tachyzoites, the asexual stage microorganism growth, were identified in the astroglial cells [101]; activation of astrocytes and up-regulation of cytokine production however inhibits tachyzoites. Failure of this inhibition, for example in astrocytes lacking gp130 (a protein of IL-6 signalling pathway) results in rapid development of lethal necrotising toxoplasma encephalitis [121]. Parasite replication seems also to be under control of signal transducer and activator of transcription 1 (STAT1) and genetic deletion of this factor facilitates uncontrolled spread of *T. gondii* infection [42]. Infection of astrocytes with *T. gondii* may result in pathological remodelling of the former; infected astrocytes start to produce excessive amount of kynurenic acid, which interacts with glutamate and acetylcholine receptors, thus causing abnormal neurotransmission which may lead to schizophrenia [93]. Astrocytes also contribute to cerebral malaria caused by infection with *Plasmodium falciparum*. In the early stages of infection astrocytes become reactive and increase secretion of TNF- α [63], whereas at late and terminal stages of *cerebra malaris* the loss of astrocytes and possible loss of their protective function occurs, which may contribute to the BBB failure and spread of infection [62, 63].

3.3. Viral infections of the brain: Special case of flavivirus TBEV and ZIKA viruses

A virus is considered to be neuroinvasive when it can enter the nervous system and neurovirulent when it is capable of causing disease within the nervous system. For example, poliovirus is highly neurovirulent but weakly neuroinvasive; rabies virus, also highly neurovirulent, requires a trauma (usually a bite by an animal) for neuroinvasiveness. Many viruses use arthropods, such as ticks and mosquitoes, to enter the human body, and then invade the CNS via the blood system.

The brain infection by arthropod-borne viruses is best presented by the case of Zika virus (ZIKV) epidemic in the Central and South Americas, as this virus causes a prominent neurodevelopmental defect, microcephaly (under-development of the cerebral cortex in newborn babies from infected mothers [67]). Moreover, in adults ZIKV may also induce neurologic impairments known as the Guillain-Barré syndrome, characterized by a rapid-onset of muscle weakness [51, 100]. How ZIKV causes these effects, especially in the adults, where clinical manifestations can occur within hours, remains unclear, although this may reflect a highly efficient neuroinvasiveness of this flavivirus.

3.3.1. Virus entry and cytoplasmic transport

An imperative step in the cascade of complex events in virus neuroinvasion is that the virus enters the CNS, hence it must cross the BBB, which represents a barrier of CNS tissue preventing the direct ingress of viruses circulating in the blood [9, 58]. The parenchymal portion of BBB is represented by astroglial endfeet, which provide an almost complete coverage of the intracranial blood vessels. At the same time astrocytes are not in contact with blood vessels only, but also enwrap synapses, hence they are intermediates and integrators of a neurovascular unit [7, 14].

Both ZIKV and the tick-borne encephalitis virus (TBEV), belong to the family of *Flaviviridae* [67, 88], a human pathogen that causes neuroinfections in Euroasia [19, 36, 58], enters the astrocytes by endocytosis [78] to cause the neuroinfection. Upon the entry of TBEV into astrocytes there is a preceding binding to a receptor, involving many mechanisms, including the interaction with heparan sulfate (HS), a glycosaminoglycan [50, 52, 58, 59]. Endocytosis appears to be common mechanism of neurotropic virus brain infection, since human immunodeficiency virus 1 (HIV-1) enters astroglia by endocytosis as well, albeit the fate of HIV-1 virus seems to be linked with its destruction in the endolysosomes [16]. Some of the HIV-1 virus parts, such as the protein Nef can be propagated to neighbouring cells via vesicle-like structures, and this process is inhibited by elevated cytosolic calcium levels, $[Ca^{2+}]_i$ [98], which contrasts to the case where elevated $[Ca^{2+}]_i$ stimulates vesicle-based secretion [47, 60, 95]. The internalisation of TBEV particles into astrocytes [78] is consistent with the clathrin-dependent endocytotic entry known for several members of *Flaviviridae* family including West Nile virus (WNV) [19], Dengue virus [3, 69], Hepatitis C virus [10] and Bovine Viral Diarrhoea virus (BVDV) [38]. Localization of TBEV in late endosomes/lysosomes is similarly consistent with other flaviviruses: BVDV, [38]; WNV, [18]; DIL-labelled dengue virus, [69]. Therefore, ZIKV may use a similar strategy to enter the human brain via astrocytes, as it is depicted in Fig. 1, which reveals ZIKV particles, characterized by structures of less than 50 nm in diameter, with an electron-dense core, present in an electron-lucent most likely endosomal compartment.

At least in rodent brain, the infection by TBEV has no detrimental effect on astroglial viability and hence rat astrocytes likely represent a reservoir for TBEV from where further infection and re-infection can occur [78]. Why astroglia presents a suitable environment for TBEV and other proliferating microorganisms is discussed below.

3.3.2. Viral infection and cytoplasmic vesicular traffic

Once within a cell virus particles can traffic to different parts in the cytoplasm. In astroglia the TBEV uses the endosomal system for the spread within the cytoplasm and this was monitored in real-time [78]. On Fig. 2, TBEV, labelled by a fluorescent marker DiD, associates with early endosomes (co-localization with early endosome antigen 1, EEA1). If time after post-infection is prolonged, the DiD-TBEV particles also associate with the lysosomal associated membrane protein 1 (LAMP1) marker. These structures are not stationary, but exhibit intracellular mobility, which is similar to that described for secretory vesicles [80]. These compartments may also deliver receptors and transporters to the plasma membrane [81]. Once TBEV is endocytosed, the TBEV-positive organelles may either enter the directional and/or the non-directional mode of mobility in living cells. Directional mobility is considered to be driven by protein motors carrying vesicles along the cytoskeletal elements, including microtubules, actin and intermediate filaments, such as the astroglia-specific GFAP [79]. On the other hand, vesicles exhibiting non-directional mobility are exposed to randomness determined by free diffusion [80]. As a function of the post-infection time, which resulted in increased number of TBEV particles per astrocytes, there was also a pronounced increase in TBEV particle mobility [78].

Changes in virus-particle cytoplasmic traffic may reflect the virus-mediated cell function alterations. Morphological developmental defects, such as microcephally in the case of ZIKV infection [67], may be due to virus-impaired cell shape mechanisms. This is possible since changes in cytoskeleton were reported for several viral infections including herpes virus, [35], TBEV, [89]); the TBEV dependent alteration of astroglial cytoskeleton was also reported; this alterations however, did not affect the viability of rat astrocytes [78]. Whether this is also the case in the TBEV- and ZIKV-infected human astrocytes remains to be investigated.

Similarly to the infiltration of TBEV, endocytosis was recently confirmed to be the mechanism of ZIKV infection of astrocytes and microglia [64]. Here a protein (Axl), a member of the Tyro3 Axl Mer (TAM) family, was identified. The membrane protein Axl, is involved in the removal of apoptotic cells, regulating innate immunity [55]. Abundant expression of Axl in glial cells, and its absence in neural progenitor cells, indicates a cellular astroglial tropism for ZIKV infection [64]. Additional experiments will have to be conducted, ideally on human cells, to establish the mechanism of neurovirulence of flaviviruses, including ZIKV and their putative preferential association with astroglia and the specialized form astroglia, radial glia.

These latter cells are essential in shaping the development of the human neocortex, through the activity of the brainstem nucleus *Locus coeruleus* (LC) [31]. The LC neurones release noradrenaline, which in astroglia elevates cytosolic Ca^{2+} [8, 25, 48, 74, 75], elevated Ca^{2+} in turn stimulates astroglial aerobic glycolysis, resulting in an increase in cytosolic glucose concentration [82, 105], leading to the production of L-lactate [24, 43, 105]. Astroglial excitability is linked to many effector mechanisms including that mediated by L-lactate, that also serves as an extracellular signalling molecule acting through the L-lactate receptor GPR81 or HCAR1 [54, 68]. At least in astrocytes L-lactate also acts through a yet unidentified L-lactate receptor [105]. The increased production of astroglial L-lactate may result in elevated levels of extracellular L-lactate concentration; thus latter may reach (in humans) 5 mM being much higher than in the blood plasma at the rest [1]. Elevated extracellular levels of L-lactate feed-back to the LC neurones in a

manner, which then releases even more noradrenaline [102], an interesting glio-neuronal communication where L-lactate serves as extracellular signalling molecule.

As already highlighted, extracellular L-lactate may have many signalling functions, including playing a role in angiogenesis linked to metabolism [68], to be neuroprotective against various types of brain damage [15] and is required for cancer cell survival [86]. Moreover, the L-lactate mediated “metabolic excitability”, where extracellular L-lactate enhances astroglial aerobic glycolysis, via a yet unidentified receptor [105], may contribute to the maintenance of the L-lactate gradient between the brain interstitium and the plasma [1]. This gradient may also be linked to the neuroinvasiveness and neurovirulence.

3.3.3. Astrocytes as a reservoir for proliferation of infectious agents

Why do astrocytes present a favourable milieu for viral and other microorganism’s infections? To address this question, let us consider that in addition to transmission of ZIKV by mosquitoes, there is evidence for human-to-human sexual ZIKV transmission, as studied in a special case of an infected male [70]. This means that ZIKV has to be present in the cells associated with spermatogenesis. Indeed, it was reported that ZIKV is present in the testes of mice in cells that are highly active in cell division including spermatocytes, Sertoli cells and spermatogonia [33]. For survival, spermatogonial stem cells, exhibiting dynamic proliferation, require an environment that favours glycolysis [39]. Why is glycolysis important for proliferation? Eukaryotic and prokaryotic cell division requires building blocks and material for making new cells, protozoa, bacteria or viruses, therefore it is essential that such intermediates are available, and glycolysis may be a favourable environment for dynamic replication.

As in the case of ZIKV-infected testes [33], where glycolytic environment favours spermatogonial cell division [39], it is likely, that astrocytes provide a favourable environment to support replication of viruses, since they exhibit a special adaptation of glycolysis. Aerobic glycolysis exists in rapidly dividing cells and in cells undergoing plastic morphological changes, despite the presence of adequate levels of oxygen, a phenomenon known as “the Warburg effect” [104], typically present in cancer cells. This metabolic specialisation is also present in astroglia. Although it is not very efficient in producing ATP, it generates biosynthetic intermediates, an essential advantage for developing and growing tissues [103] and supporting the proliferation of infective agents. Aerobic glycolysis, primarily taking place in astrocytes in the CNS, is operative during neurodevelopment and even in adulthood in some areas of the CNS [34]. Therefore, this property of astrocytes may be a reason of why bacteria, protozoa and viruses exhibit a preference for astroglia. In addition, ZIKV-mediated microcephaly, may be a result of impaired radial glia, critical for neurogenesis and the development of the neocortical cell stratification. In addition these cells are also a cell type that generates a favourable environment for virus infection. This in turn affects the cytoskeleton and astroglial cell polarity and shape, which is detrimental for the radial glia playing a guiding role in the neocortex development, when the foetal brain is infected by ZIKV.

4. Conclusions

This short review aimed to highlight that astrogliopathology, a recently acknowledged and growing field, links the evolution of neurological diseases with morphology and function of astroglia. Pathological potential of astrocytes needs to be investigated in much more detail to

understand the processes that are impaired during homeostatic CNS failure. This is essential for the development of new drugs that interfere with these pathobiological processes. Since infections of the nervous system with two main entry points into the CNS (blood and cerebrospinal fluid, Fig. 3), represent an important traditional aspect of neurological diseases, we here focused on bacterial, protozoan and viral infections and brief how astrocytes deal with these pathogens. Specifically, we present the special cases of tick-borne encephalitis and Zika viruses and their interaction with astrocytes, and argue that astrocytic glycolytic milieu is a favourable environment for infectious agents.

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Figure 1. Electron micrograph of Zika virus (strain Uganda 976) in an infected Vero cell; ultrathin section was stained with 0,5 % uranyl acetate. Imaging of the ultrathin section was performed with use of 120-kV JEM-1400 Plus electron microscope (JEOL); magnification 40.000x (horizontal bar represents 200 nm). Photomicrograph was kindly provided by Dr. M. Poljšak Prijatelj and Mr. M. Kolenc (Intitute of Microbiology and Immunology, Medical Faculty, University of Ljubljana).

Figure 2. Time-dependence of the number of endosomes and lysosomes associated with fluorescently labelled TBEV particles in astrocytes.

(Ai,ii). An astrocyte with DiD-labelled TBEV vesicles (TBEV) incubated at 37 °C for 4 h and 18 h and with labelled early endosomes (EEA1) Overlays represents overlapped TBEV and EEA1 fluorescent signals, indicating the association between TBEV and endosomes. Bars: 5 µm. (Aiii) Prolonged incubation increased the average number of TBEV labelled vesicles per cell and also the average number of vesicles co-labelled with TBEV and EEA1. Black bars - TBEV labelled vesicles, white bars - TBEV and EEA1 co-labelled vesicles, $*P<0.05$. (Bi,ii) An astrocyte with DiD-labelled TBEV vesicles (TBEV) incubated at 37 °C for 4 h and 18 h and with LAMP1-labelled lysosomes (LAMP1-lysosomal associated membrane protein 1). Overlays represent overlapped TBEV and LAMP1 fluorescent signals, indicating the association between TBEV and lysosomes. Bars: 5 µm. (Biii) Prolonged incubation increased the average number of TBEV labelled vesicles per cell and the average number of vesicles co-labelled with TBEV and LAMP1. Black bars - TBEV labelled vesicles, white bars - TBEV and LAMP1 co-labelled vesicles, $***P<0.001$. (C) Astrocyte co-labelled with anti-LAMP1 and anti-EEA1. In a single, 1 µm thick optical slice, lysosomes and early endosomes appear to be largely different in size due to different position in z-axis and variable antibody attachments. Arrowheads point to large late lysosomes (LAMP1) and early endosomes (EEA1). Bar (bar inset): 5 µm (2.5 µm). n (n) = number of cells (number of vesicles). Reprinted with permission [78].

Figure 3. Infectious agents may enter the central nervous system through two main routes: the blood (left panel) and cerebrospinal fluid (right panel). Once in the blood infectious particles may cross the endothelial cell (engaging transcytosis) or escape into the CNS parenchyma through fenestrae between endothelial cells. Infectious particles may enter more easily into the cerebrospinal fluid via the circumventricular organs. Right panel is a drawing of the median sagittal aspect of the human brain showing the seven circumventricular organs and choroid plexus (CP). AP, area postrema; ME, median eminence; NL, pituitary neural lobe; OVL T, organum vasculosum of the lamina terminalis; PI, pineal gland; SCO, subcommissural organ; and SFO, subfornical organ. Redrawn from [37].

Figr-1

Figr-2

Figr-3