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Glymphatic and Immun Systems of the Brain

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Abstract
Even though brain lymphatic system has several peculiarities including the construction of the endothelial cells of the lymph vessels, in common with the lymphatic system of the body in general, studies have documented eminent discrepancy. Unique lymphatic system of the brain is called ''the glymphatic system'' due to its lymphatic system-like function and glial-like fluid flow. Collaboratory functions of this system, and CSF and interstitial fluid has been documented. Recent discovery of this system has highlighted the requirement to review the diagnose and treatment procedures of several neuronal diseases. With this discovery, anatomic, physiopathologic, immunologic, and genetic natures of this system have attracted attention of scientists. This review has summarized the accumulating information on the lymphatic system of the brain, particularly focusing on the function of the glymphatic system in fetus and newborn, cranial tumors, neurodegenerative diseases, brain metabolism and its relationship with sleep, and anatomical posture of the body. Yet, the cells responsible with the brain immune system and their relationship with the glymphatic system have been discussed. By summarizing the latest information on the relationship of this system with immunologic, molecular, physiologic, and anatomical aspects of several neuronaldiseases, this paper will surely contribute to the diagnose and treatment of them through shedding light on the researchers.
Keywords: *Brain, Glymphatic System, Immunity, Neuronal Disease.*

Introduction

Lymphatic system plays a critical role in homeostasis and function in peripheral tissue. Although the brain does not have a lymphatic vasculature in traditional thought, it has been understood that the brain contains a unique lymphatic formation among the somatic organs. No anatomical structures of the brain with very high metabolic functions and complexity have

been determined before, facilitates the passage of fluid through the parenchyma and lymphatic clearance of extracellular substances[1]. Central nerve system (CNS), previously thought to have no lymph system, has remained a mystery as a subject of research in the field of neuroscience for many years[2].

The first definition of the lymphatic system on the brain goes back to Italian anatomist and illustrator Paolo Mascagni[3]. He clearly explained the meningeal lymph vessels (MLV) and their functional roles more than 250 years ago, expressing them as ‘’the privilege of the brain immunity’’. Another study done nearly 100 year later, indicated controversially detailed explanation of the absence of a lymphatic circulatory system in the brain and which has misled many scientists for years[4]. Some reasons of this misconception have been cited as the presence of tight connections in blood-brain barrier (BBB), lack of the conventional lymphatic drainage system, and data showing limited rejection of the foreign matter in CNS[5].

With the new data accumulated on the lymphatic system of the CNS surprising findings have been obtained in recent years[5,6,7]. In a study investigating T-cell gateways in the meninges, functional lymphatic vessels lining the subdural sinuses were discovered[8].

This study revealed that MLVs are located specifically around the subdural sinuses at the base of the skull, and that the endothelial cells in these vessels show all the molecular features of their counterparts found in other lymphatic vessels and eventually attach to the deep cervical lymph nodes[9]. These lymphatic vessels take glial fluid and immune cells from the cerebrospinal fluid (CSF). Another study also has confirmed theselinks[10].

The lymphatic vessels of the CNS transport CSF throughout the perivascular and interstitial spaces. The CSF flow then is directed through venous perivascular spaces, and at the same time metabolic waste in the parenchyma through the CSF. This system is called ‘’The Glymphatic System’’ because it functions like glial fluid flow and lymphatic system. It is indeed not a lymphoid tissue but occasionally named as “lymphoid-like” tissue. These lymphatic vessels lie along the dura mater and advance throughout the superior sagittal sinus and peri-sinus space of the lateral subdural sinuses[6].

Recent studies have proved that lymphatic vessels in the skull base of the rodents are responsible for draining the CSF and other waste materials out of the brain[11]. A study done on mice has shown that MLVs have turned into a complex network along the blood vessels in the base of the skull[12]. Yet, certain macromolecules infused into the lateral ventricle have been displayed to reach out the cervical lymph nodes using the foramen of the skull and perineural tracks. Researchers have demonstrated, using magnetic resonance imaging, contrast agents injected into the interstitial fluid, absorbed by the basal meningeal vessels, and later drained out of the brain through the CSF[13,14]. A study has used Prox1 – GFP to explain the function of the lymphatic vessels of the brain in mouse by marking the promotor region of the Prox1 gene, which has a key role in lymphatic development, with green fluorescentprotein[12]. This study has displayed the unification pattern of the endothelial cells of the dorsal MLVs to be like closed zipper while that of the basal MLVs to be basically as a button, as is the case in the lymphatic capillary vessels of the peripheral organs.In general, based on these anatomical and functional experiments, the delivery of macromolecules that cannot pass through the BBB in the brain to the peripheral lymphatic system and the drainage of macromolecules in the CSF are due to basal MLVs[14].

Lymphatic Drainage System of the Brain in Fetus and Newborn

Drainage of the interstitial fluid and CSF of the brain by the lymphatic system is essential in homeostasis. This drainage system may deteriorate with neurologic lacerations in both premature and full-term newborns. Brain damage due to acute hypoxia-ischemia in full-term

newborns is an important death or injury cause[15]. Various cellular and molecular pathologies such as neurovascular and BBB deformations, inflammation, damage in immune cells and lymphatic circulation, and deterioration in the brain restoration capacity, have been suggested in hypoxic-ischemic encephalopathy[16]. Astrocytes, important components of both BBB and glymphatic system, are very essential mediator sources in the inflammation seen right after the cerebral ischemia[17]. Anmagnetic resonance imaging (MRI) study has reported the meningeal lymphatic flow streaming in the sagittal sinus, in reverse direction with the venous flow[14]. Cerebral edema is the most apparent histological finding during the hypoxic ischemia. Animal studies have shown increased aquaporin-4 (AQP-4) expression in this finding after brain damage with different causes[18]. This increase has been associated with the development of the cerebral edema due to the increase in BBB permeability[19]. Therefore, acute brain damage ruins glymphatic function, in turn deteriorating itself due to accumulation of both normal and pathological metabolic wastes. Lymphatic drainage system of the brain has also been associated with several other pathologies such as white matter damage in newborns. Reactive astrogliosis is one of the regenerative mechanisms after the neuronal damage in the white matter of premature. It can change the nature and function of the glymphatic system. Waste metabolites produced during the oxidative stress and inflammation, representing main mechanism of the white matter development, are removed by MLVs, which is very vital in limiting neurological pathology in fetal development[15].

Exclusive Immune System of the Brain

Features that contribute to the opinion that CSN is immunologically privileged: mostly due to the presence of tight junctions in the BBB, the idea of the absence of a classical lymph drainage system, and limited rejection of foreign tissue in the CNS. However, today the concept of immune privilege of the brain has been redefined. As mentioned above, functional lymph vessels have been descripted and antigen offering cells (APC) including microglia, macrophages, astrocytes, and dendritic cells (DCs) have been shown in CNS. It is known that these components are not isolated from T cells, being responsible in emptying CNS antigens locally into the cervical lymph nodes. Exclusive immune system of CNS is not insufficient[5,20]. Either dysfunction or hyperfunction of the MLVs causes to the autoimmune attack, resulting in destroyed meningeal immune activity with irregular ablations[7]. Hence, autochthon production of the T cells and antibodies is present in the brain. Consequently, it is now accepted that presence of the T cells in the brain is autoimmune based[9].

Cytokinin production and migration of DCs to the lymph nodes have also been searched to reveal APC function of the CNS. Invitro studies have shown immunogenetic activity of DC[21]. Addition of certain agents into the medium such as type I interferon, synthetic Toll-like receptor 3 (TLR3) ligand, and polycytidylic acid has enhanced this activity. On the researches dealing with the inflammation that occurs in the vaccine area, granulocyte-macrophage colony stimulant factor (GM-CSF) 127, poly (I: C) 200 and other TLR agonists have been applied to the area to help increase the immunological response[22]. It is obvious, in the light of the information given above, that an isolated immunologic nature is present in the brain. Genetic risk factors are also important regulators of the lymphatic drainage system of the brain. In several of the cognitive Alzheimer Disease (AD) model, the diseases are caused by mutations in the macrophage gene expression[23-26].APOE polymorphic alleles have been APOE major alleles considered as the primary genetic risk factor for AD[27,28]. Among the (e2, e3, and e4), e4 has been suggested as the strongest independent risk factor for the sporadic AD development[20].

New lymphatic concept of the brain has led to reconsider the autoimmune etiology of several neuroinflammatory and neurodegenerative diseases including Multiple Sclerosis (MS) and

AD(9). Aging is considered as an important factor on the lymphatic drainage system of the brain. Fluid and metabolites produced by parenchyma of the brain may fast and effectively be eliminated by multi-lymphatic drainage system in young. With increasing age, changes are observed in the cerebral vessel system. Atherosclerosis and decrease in the amplitude of pulsations lead to the fluid accumulation in the enlarged perivascular space, occlusion of the perivascular pathways along the basal membrane, and functional deterioration in the glymphatic drainage and CSF paths[29].

Literature has reported cognitive inefficiency when MLVs are cut in young mice. MLVs have been documented to have a close relation functionally with these kinds of pathologies including AD in neurodegenerative mouse models[7]. With degeneration of these vessels in aged mice, CSF drainage circulation has been deteriorated[6]. A similar study has showed eminent deteriorated dorsal MLVs and dilated and hyperplastic basal MLVs in the aged animals as compared to the young ones[12]. Another research has revealed breakdown in the connections of the lymphatic endothelial cells because of the impairment in the lymphatic drainage, which is surely in relation with the lymphatic vessel dysfunction and degeneration due to aging[30]. Studies have striven to reveal age related changes. They have reported less zipper type connections and more button type ties between the lymphatic endothelial cells of the aged basal MLVs. These changes are possibly related to decrease in the CSF drainage in aged mice. Another study using MS animal model has associated MS with MLV ablation, emphasizing the fact that MLVs need to be ameliorated to eliminate inflammation in the brain[12].

Accumulation of toxic protein clusters is the common pathologic feature of the neurodegenerative diseases including AD, Parkinson and Huntington diseases, and motor neuron disease. Amyloid-β (Aβ) accumulation is considered as the primary cause of the AD. Recent findings have suggested that extracellular Aβ congregate is removed from the brain by several ways such as enzymatic degradation, phagocytosis by microglia, glycophytic clearance, and BBB and lymphatic ways. The last two ways have been shown to be particularly responsible for the clearing metabolic waste away from the brain[31]. Indeed, one report has revealed ~40 % of the Aβ peptides and ~19 % of the pathologic tau protein removed from the brain through this way[32]. MLVs have also been reported to ease pathologies in relation with Aβ and tau protein in the brain through enhancing Aβ clearance[20]. Therefore, suggesting a new therapeutic approach in neurodegenerative diseases. After all, detail exploration of the brain lymphatic system may lead to new approaches to reveal connections between the brain parenchyma and peripheral tissues and related pathologies[6,33].

There are several pathways between the brain and peripheral tissues for substance exchange such as BBB, choroid plexus, subarachnoid plexuses, and lymphoid system. Effectiveness of these ways decrease with the aging[34].The data accumulated in this review has suggested the presence of certain physiological mechanisms that carry intra- and extra-cellular pathological proteins away from the brain, playing a critical role in the clearance of metabolic wastes and homeostasis. Displaying the vessels of the CNS with the advanced technology may open a road to new approaches for the treatment of neuroinflammatory diseases[6,7]. Revealing the possible relation of the lymphatic system with the other clearance mechanisms is also of essential. Likewise, determining the volume of the waste material cleared by the lymphatic system through the CSF may answer the question whether the brain lymphatic system plays important role in either clearance of the waste materials or immune regulation. Basically, this question will bring about the possible role of the brain lymphatic system on the pathogenesis of neurodegenerative diseases, contributing to the development of the therapeutic agents[20].

Glymphatic system is a perivascular space network supporting CSF flow and clearance of the waste materials[35]. Literature has indicated reciprocal transition of the CSF and brain interstitial fluid through the perivascular structures surrounding cerebral blood vessels. This system is supported by AQP4 localized to the last vascular extensions of the astrocytes. Reports have documented three elements facilitating the CSF passage; the CSF flow path in periarterial space, the perivenous interstitial fluid clearance, and the astrocytic AQP4transparenchymal channel[1,2]. The glymphatic system under the control of circadian rhythm is affected by the cardiovascular system and sleep, both being regulated by that. The space between the brain tissue and CSF compartments is broad enough to provide clearance by the basic diffusion. This is particularly the case for larger molecules such as proteins and peptides with lower diffusion coefficient. A study has shown the relation of the glymphatic flow of the CSF from the cerebellomedullary cistern through the lymph nodes atday and night[35]. This data emphasizes the role of glymphatic system in the interstitial fluid elimination from the brain parenchyma during the sleep[2]. This system is especially effective during the sleep but whether sleep timing promotes it or not is unclear. It has clearly been reported that this system removes waste materials from the brain through increasing CSF activity[36,37]. Supporting this data, another study has found more effectiveness of the rodent brain in removing neuronal metabolism waste particularly during the slow wave sleep, as compared to that of awake status[38].

Brain energy metabolism may also play a role in regulating the function of the glymphatic system on sleep-wakefulness cycle. Originally thought as a waste material, lactate may function, based on the hypothesis of the lactate cycle between astrocyte and neuron, as supplementary fuel, and signal molecule in this metabolism. This idea is supported by the evidence revealing the presence of a lactate concentration gradient between the astrocyte and neuron[39,40]. Dynamic changes in the brain lactate level are regulated by the lymphatic clearance systems[(36)]. Sleep is known to have homeostatic function removing the neural metabolites[41]. It clears harmful metabolites from the brain such as Aβ that occurs during the wakefulness, and strengthen memory[42]. Consequently, enhancing the sleep quality may be helpful through lowering the development risk of the amyloid beta (Aβ) pathology in AD protection. Yet, acute insomnia ruins cognitive functions, and usually is the early result of most neurodegenerative diseases[42].

Effect of Body Posture on Lymphatic System Function

A research has examined the changes in CSF and interstitial fluid in rats at supine, facedown and lateral postures[43]. Of these positions, the lateral one representing sleep-resting in rats, have been shown to be most effective posture in removing Aβ and other metabolites through glymphatic paths. Reversely, it is significantly low at the supine posture which is ‘’upright’’ position at conscious state in rats. The relationship between intracranial pressure (ICP) and body posture has been reported[20]. Yet, another study has indicated ICP variations at different body postures in the idiopathic intracranial hypertension and mild hydrocephaly cases[44].

Intracranial Tumors

Mechanisms in MLVs are used to promote anti-tumor reaction. Several loaded antigen types are applied on DCs vaccine studies for brain tumors. $EGFR^{vIII}$ molecule functions as an ideal tumor specific antigen for glioblastoma patients. It is expressed in nearly 30% of the patients. While vaccine is prepared, $EGFR^{vIII}$ molecule on the T cell, a chimeric antigen receptor (CAR), is marked. Detection of this molecule may be an indicator on therapy options. CAR T cell is one of the immunologic indicators in lymphatic system. Decrease in the expression of that molecule in this cell provokes tumor recurrence. Accordingly, this complex system has been proved to clear off the brain tumors, and at long term can provide protection[45]. Another study has reported increases

in cytokine production and chance of survival after the administration of the DC vaccine during the phase I and II trials of glioma patients[5].

In the light of this information, it can surely be pointed out that future immunotherapeutic studies will help us understand in detail the relationship between the metastatic tumors and lymphatic immune system of the brain.

Function of the brain lymphatic system is broadly affected by several pathological conditions. Changes of the compounds of constructive proteins in vascular diseases like atherosclerosis cause to the decrease in vascular plasticity, deteriorating perivascular flow. Intracranial arterial narrowness not only restrain cerebral blood flow but also obstruct perivascular or paravascularpathways[46]. Traumatic brain damage, hemorrhagic or embolic paralyses and diabetes destroy the glymphatic clearance of neurotoxic metabolites such as Aβ(20). A study has determined irregularity in the expression polarity of the astrocytic AQP4 the glymphatic system, right after the diffuse ischemia in the multiple microinfarcts, leading to neurovascular-neurocognitive disorders[47]. Disorders seen in the lymphatic system drainage of the cerebrum due to either aging or different pathologies, cause to extracellular dyshomeostasis and antigen production. However dysfunction of this drainage system may lead to severe pathophysiological changes including neurodegenerative diseases, neuroinflammation and brain tumors, as reported by the literature[20].

Neurovascular disease and stroke are main reasons of the geriatric disability. Secondary complications including cerebral aneurismal rupture, bleeding, hydrocephaly, and delayed cerebral ischemia related to microthrombus, may developalso[48]. Lymphatic system drainage of the cerebrum can be disrupted by acute brain injuries, neurovascular diseases, and various stroke cases. This disruption may trigger secondary complications, ending up with more severe neuropathology. Functions of the cytokines produced by both macrophages and microglia in the neuroinflammation seen after ischemic stroke, have been documented[49,50]. There are two polarization phenotypes of the macrophages documented; one, classically activated macrophages (M1 type), the other, alternatively activated macrophages (M2 type)[51]. It has been reported that as ischemic infarcts develops, M1 phenotype produces proinflammatory cytokines, and M2 phenotypeanti-inflammatory cytokines[49]. Besides, a broad variation is observed in the timing and contribution rates of the microglia/macrophage to the different phases of ischemic stroke. A study performed on mice has reported intense localized microglia very active at the first day of the acute ischemic stroke induced by the obstruction of the middle cerebral artery[52].Blood originated macrophages have congregated thereby 3-7 days after the stroke. Microglia/macrophage activations and polarizations that occur at mechanical and time wise fashion, have been shown to coincide with the dynamic changes in their phenotypes during the progression and development of the ischemic stroke. Perivascular spaces are essential regions for the initiation of neuroinflammation. Microglia may instantly become active as a reaction to hypoxia stimulation right after ischemic damage begins. Blood originated macrophages migrate to the paravascular spaces. Cytokines produced by the perivascular macrophages after the cerebral ischemia, have been thought to orientate infiltration of the inflammatory cells. Intravascular inflammation also activates intracerebral microglia after the subarachnoid hemorrhage. Activation and survival of microglia induced by ischemic stimulus may be through the signals of CSF-1 (colony stimulant factor-1) receptor[53]. Several researches have examined effects of the blockage of the lymphatic drainage in the cerebrum during the ischemic and hemorrhagic strokes[54,55]. Models of the cervical lymphatic blockage (CLB) have been demonstrated to deteriorate the cerebral lymphatic drainage directly, leading to excessive fluid and waste material accumulation in the peri- and

paravascular spaces, ending up with the intracranial hypertension and brain edema[20]. Likewise, effects of the blockage in the model of the ischemic stroke originating from the middle cerebral artery have been reported[54]. Brain edema and oxidative stress in the ischemic region has been documented in this literature. Additionally, severe neuronal damages have been observed. The blocking of drainage of the cervical lymphatic flow and middle cerebral artery has made the mentioned changes more noticeable. Another study has introduced a visualization procedure to measure interstitial fluid drainage of the brain dynamically at multiphoton microscope. In the stroke model induced by Rose Bengal which acts as a sensitizer against light through photo-thrombose, it has been shown to obstruct a single vessel segment, lowering the drainage of the interstitial fluid profoundly through deteriorating focal vascular perfusion, and not affecting the flow of other vessels[56]. Another report has documented the function of the glymphatic system in different stroke models through administrating contrast substance into the cerebellomedullary cistern, using MRI. The system has significantly been deteriorated in the acute phase of embolic ischemic stroke and after the subarachnoid hemorrhage[57]. These results have indicated the cerebral arterial pulsation to be an important force in affecting the flow of glymphatic drainage in the brain. In the meantime, AQP4 allows cytotoxic edema at the early phase of the cerebral ischemic stroke while removing vasogenic edema in the BBB at the late phase. Fine-tuning of AQP4 expression level in paravascular spaces is suggested to use in the treatment of the brain edema caused by various stroke types[58].

Conclusion

In conclusion, this review article has attempted to gather latest data on the brain glymphatic and immune systems. We wanted to emphasize that the lymphatic system of the brain performs the task of clearing metabolites from the brain. We have described the latest developments reported in the relevant literature that guide the diagnosis and treatment of many diseases. This review has documented immunologic, molecular, physiologic, and anatomical relations of several neurotic pathologies including neurodegenerative diseases, tumors and related metastases, which will surely shed light on researchers dealing with this area.

Conflict of Interest

The authors declare that they have no conflicts interest.

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