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Gap Junction Coupled Cells, Barriers and Systemic Inflammation

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Abstract

The blood-brain barrier (BBB), the blood-retinal barrier (BRB), and the blood-nerve barrier (BNB) may have similarities in systemic chronic inflammation, as seen in a variety of diseases. A disturbance of the barriers lead to a disruption of the cells coupled with gap junctions in syncytium. The network coupling may be principal to understand the homeostatic imbalance in systemic inflammation. This review high-lightens the role of gap junction network coupled cells in inducing and maintaining barrier properties under physiological conditions as well as their involvement in inflammatory pathologies.

Keywords: Gap junction, Blood-brain barrier, Blood-retinal barrier, Blood-nerve barrier, Inflammation

Introduction

Many human diseases, stroke, infectious and inflammatory processes, Alzheimer's disease, Parkinson's disease, HIV, obesity, type 2 diabetes, atherosclerosis, allergy, age-related macular degeneration (AMD), glaucoma, osteoarthritis (OA), systemic vasculitis, and cardiovascular diseases seem to have features in common, where there is a disruption of homeostasis, and they are nearly universally associated with systemic chronic inflammation [1-8]. Inducers of inflammation trigger the production of inflammatory mediators, which in turn alter the functionality of tissues and organs. Very mild inflammation might be handled by tissue-resident cells, mainly macrophages and mast cells, whereas more extensive malfunctions or damage might require additional leukocytes to be recruited and plasma proteins to be delivered locally. When inflammation turns to be extreme, help provided by local macrophages might be insufficient, and the tissues might call for the recruitment of additional cells and cell interactions between different cell types will arise.

Cell-cell interactions lead to induction of specialized features of several barriers such as the blood-brain barrier (BBB) [1] and the blood-retinal barrier (BRB) [8]. The BBB controls transport processes between the central nervous system (CNS) and peripheral blood. The BRB controls the exchange of metabolites and waste products between the vascular lumen and the neural retina and is formed by the interaction of retinal glia and pericytes with the endothelium. The blood-nerve barrier (BNB) consists of blood vessels and sensory nerves but gap junction coupled cells in networks, such as the articular cartilage in joints, might be involved in these cell interactions [3].

Future studies elucidating barrier induction and maintenance will provide a framework for rational therapies in several diseases, to restore the barriers with influence of gap junction syncytium coupled cells on the barrier functions.

Similarities between the BBB, BRB and BNB

The BBB is a selective barrier formed by endothelial cells that line microvessels in the CNS. It acts as a physical barrier as tight junctions between adjacent endothelial cells force most molecular traffic to take a transcellular route across the BBB [9]. Small molecules such as gaseous, small lipophilic agents and some selective drugs can diffuse freely through the lipid membranes. Specific transport systems on both the luminal and abluminal membranes regulate the transcellular traffic of small hydrophilic molecules [1] and proteins like occludin, claudins and zonula occludens may have an important role. The micro vessels are closely surrounded by other cell types: the perivascular end feet of astrocytes, pericytes, microglia and neuronal processes. In larger blood vessels the pericytes are replaced by smooth muscle cells. The cells are surrounded by basement membranes. The astrocytes are, however, necessary for correct association of the endothelial cells and the interaction between endothelial cells, astrocytes and pericytes are required for proper capillary differentiation [10]. The BBB regulates the ion balance in the nervous system to maintain ion gradients [11].

The BRB is composed of vascular cells (pericytes and endothelial cells), macroglia (Müller cells and astrocytes), neurons (photoreceptors, bipolar cells, amacrine cells, horizontal cells, and ganglion cells), and pigment epithelium as well as microglia or resident macrophages [12-16].

It is divided into two parts; an inner component with tight junctions between retinal capillary endothelial cells and an outer

component located in the retinal pigment epithelium with tight junctions between retinal pigment epithelial cells.

The inner BRB, a well-developed blood-neural barrier, controls permeability from the retinal blood vessels and consists of a well-developed junctional complex in the vascular endothelial cells with limited or no fenestrations. It acts as a selective barrier. The regulation of flux of blood-borne metabolites into the retina is controlled by the retinal pigment epithelium (RPE), which controls the flow of fluid and nutrients from the highly vascularized choroid into the outer retina, and utilize well-developed junctional complexes to regulate permeability and maintain the neural environment of the retina. The junctional complexes that create these barriers are composed of distinct proteins; the claudins in forming the barrier, occludin in regulating permeability, and zonula occludens proteins in assembling the barrier by interacting with the transmembrane proteins. The necessity of glia and pericytes to induce the barrier has been demonstrated but it is just in its beginning [8]. The capillaries of the BRB seem to be similar to the capillaries of the BBB [14, 17].

An intact BRB plays a crucial role in maintaining a normal visual acuity and function of the eye. Disturbance of the BRB plays a central role in retinal diseases, like neovascular age-related macular degeneration (AMD) and diabetic retinopathy. Both diseases lead to macular edema with a loss in visual acuity [18].

There are factors that indicate that some retinal diseases are due to an inflammatory process causing leakage of the BRB. Aqueous humor, in patients with neovascular AMD, contained elevated concentrations of vascular endothelial growth factor (VEGF) and angiogenin, as well as specific cytokines (IP-10, Mig MIP-1β and MCP-1) [19]. VEGF and interleukin-6 (IL-6) is also present in the aqueous humor in patients with diabetic retinopathy [20, 21].

The BNB consists of microvessels in the endoneurium in nerves and are permeable capillaries. This internal microenvironment in peripheral nerves is regulated to maintain transmission to and from the CNS, and is regulated by tight junctions. The perineurium is less permeable than the endoneurial capillaries. Cellular components are secreted and soluble factors are responsible for maintaining the integrity of the tight junctional complexes. These tight junctions are also associated with the barrier proteins claudins, occludin and zonula occludens proteins [22]. In contrast to the BBB and BRB, the BNB is not directly supported by gliallimitans [23].

Syncytium Coupled Cells

The astrocytes with their perivascular end feet line the microvessels and make the BBB together with capillary endothelial cells. The close astrocyte cell-cell relationship mediates the induction of specific features of the barrier phenotype in the capillary endothelium of the brain. Substances released to the blood centrally or in the periphery, influence the BBB, and give rise to changed permeability of the tight junctions of the microvessel endothelial cells [1]. Immune competent cells from the blood pass the BBB and are converted into microglia, which produce cytokines upon stimulation [24]. The capillary endothelial cells release substances that influence the astrocyte endfeet, and are thereby influenced by the cytokines interleukins, transforming growth factor β (TGFβ), tumor necrosis factor-α (TNF-α) and inflammatory substances; proteases, tryptases, chymases, and matrix metalloproteinases (MMPs or matrixins) within the CNS [25]. Ca^{2+}-mediated signaling in astrocytes and propagation of Ca^{2+} fluctuates between astrocytes via gap junctions, where connexin 43 (Cx43), and ATP and its purinergic receptors play important roles [26-28]. Via intercellular communication astrocytes act as a syncytium for modulating neuronal and vascular function. The degree of coupling between astrocytes may influence neuronal activity [28, 29].

Both astrocytes and Müller cells in the retina cover the blood vessels with their endfeet forming the BRB [30]. The Müller cells are specialized radial glial cells and span through the entire thickness of the retina. They regulate the tightness of the BRB and are the link between the neurons and the compartments and represent around 90% of the glia in the retina [8]. Retinal astrocytes are found around blood vessels and the distribution depends on the density of nerve fibers. They are absent in the fovea and the avascular zone to maintain high acuity vision [31]. A main difference between astrocytes and Müller cells is that only the astrocytes are formed with gap junctions, where the astrocytes are coupled in syncytium and have a Ca^{2+} signaling system. Astrocytes and Müller cells do not seem to be coupled to each other via gap junctions. The astrocytes in the retina seem otherwise to share similar properties as astrocytes in other regions in the CNS. The BNB does not seem to be surrounded by end feet belonging to syncytium coupled cells but are most likely involved in the impact by substances released into the blood. The importance of the effect is seen in the chondrocytes in joints, that are connected to each other via cell-to-cell interactions and form functional gap junctions that express Cx43 [32]. They can sustain the propagation of intercellular Ca^{2+} waves in rabbits, humans, and equines [33, 34] and form hemichannels that exchange signals within the extracellular space [35, 36]. In the adult cartilage, chondrocytes exist as individual cells embedded in the extracellular matrix, and gap junctions are mainly expressed by the flattened chondrocytes facing the outer cartilage layer where intercellular communication occurs [37].

Tight Junction Disturbances during Inflammation

During inflammation the electrical resistance is decreased and tight junctions in the endothelial cells are opened [11]. The perivascular cells, pericytes and glial cells, have a major impact on BBB functions. The astrocyte endfeet ensheath the microvessels and protect the BBB, the gliovascular units [38].

A wide variety of secreted factors such as TNF-α, IL-1β, IL-6, IL-8, and prostaglandins induce barrier properties and can promote barrier breakdown and facilitate immune cell transmigration. In inflammation that causes disruption of tight junctions a down regulation of Cx43 occurs in the gap junction regions and an up

regulation of Cx43 in the hemichannels [39, 40].

The tight junction-coupled endothelial cells are equipped with transporters that supply the brain, retina and nerves with nutrients: glucose carriers (GLUT), amino acid carriers (LAT, L-system for large neutral amino acids), and transporters for nucleosides, nucleobases [1, 14].

Glucose is the main energy source for the brain and an upregulation of the glucose transporter 1 (GLUT1) expression has been observed during starvation and hypoxia [41]. It is also the main energy source for the retina [14]. Glucose is taken up by the astrocytes and converted to lactate, the end-product of glycolysis. Astrocytes are one of the major cells that produce lactate in the brain. Lactate can be released from the cells via Cx43 hemichannels and can affect the functioning of the BBB [42].

Microglia/Macrophages

Microglia are tissue-resident macrophages in the CNS and are found in close proximity to CNS vasculature, which suggest that they are involved in the BBB regulation. Both systemic inflammation and chronic neurodegeneration activates microglia. However, their role in maintaining BBB properties is less known [10]. Microglia express different Toll-like receptors (TLRs) and the TLR4 seem to be of importance when microglia exert its effects on astrocytes [43].

Microglia are also of importance in the retina where the TLRs allow them to monitor the microenvironment. When they get activated they convert from a resting state to an active form [5].

Macrophages in the periphery as well as microglia in the CNS act as lines of defense upon detection of signs of injury. At signs of injury, as extracellular Ca^{2+} waves and ATP release from neighboring cells, microglial processes rapidly move towards the lesion site and are activated [44]. Perivascular macrophages in the retina are also resident-immune cells. They are regularly replaced by circulating monocytes [5].

Invasion of White Blood Cells

Under normal physiological conditions the BBB acts as a barrier to the immune system where leukocytes cannot penetrate. Although, during inflammatory processes leukocytes migrate into the nervous system where they are transformed into microglia.

An initial contact has been observed between blood circulating leukocytes and the vascular endothelium and disorganization in the tight junctions occurs [10]. Absence of astroglial Cx43 promotes the recruitment of leukocytes via activation of the BBB and endothelial cells.

Leukocytes are the main source of cytokines in the inflamed CNS, but activated astrocytes and microglia are known to secrete an array of pro-inflammatory molecules, including reactive oxygen species (ROS), IL-1β, IL-6, and IL-17A.

At start of an inflammatory-mediated disease in the eye an infiltration of leukocytes occur through the BRB. Distortion of the endothelial tight junctions, breakdown of the barrier, and release of pro-inflammatory cytokines such as TNF-α and IL-1β is a fact [45].

Leukocyte-endothelial cell interactions are observed in the BNB during different inflammatory diseases [7].

Inflammatory Inducers and Mediators

Inducers of inflammation can be exogenous or endogenous and have been extensively reviewed recently [2]. The exogenous inducers can be microbial or non-microbial, and the endogenous, which seem to be of importance of inflammation can be signals produced by stressed, damaged or malfunctioning tissues.

Inflammatory inducers trigger the production of inflammatory mediators, which can change the functionality of several tissues or organs. They have an influence on specialized leukocytes, macrophages and mast cells. Endogenous inducers can be vasoactive amines, for example histamine and 5-hydroxytryptamine (5-HT), vasoactive peptides, for example substance P, complement fragments, such as C3a, C4a and C5a, lipid mediators, for example eicosanoids and platelet-activating factors, inflammatory cytokines such as TNF-α, IL-1, IL-6, chemokines, proteolytic enzymes such as elastin, cathepsins, and MMP demonstrated in the extracellular matrix.

Diseases involved in Barrier Disruption

Several diseases characterized by degeneration of neurons in the nervous system are associated with inflammatory processes. The substances that produce these processes induce glial activation and damage of different barriers leading to infiltration of peripheral immune cells. Several inflammatory mediators are proposed and in the Parkinson's disease ROS seem to be of importance [46].

Disruption or breakdown of the BRB is seen in diabetic retinopathy and neovascular AMD leading to macular edema and threatening visual acuity [47, 48]. Calcium-dependent zinc endopeptidases known as MMPs degrade and remodulate the extracellular matrix in different tissues. During neurodegeneration MMPs seem to have important roles, so far only studied in some diseases for example glaucoma, where genes associated with cytokines and TNF-α in glial cells are up-regulated and a low-grade inflammation may play a crucial role [49]. In glaucoma, a degeneration of retinal ganglion cells and retinal nerve fibers are seen and an up-regulation of MMP-2, -9, and -13 have been observed [50].

In several diseases it is leukocyte trafficking into the peripheral nervous system, with an influence on leukocyte-endothelial cells leading to disruption in the BNB that lead to inflammatory neuropathies, seen in Guillain-Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy, and vasculitic neuropathies [4, 7]. In OA it is a loss of osteochondral integrity that removes the barrier between intra-articular and subchondral compartments, and an invasion by blood vessels and sensory nerves occur [3]. This pathological cross-talk leads to inflammation and in the prolongation to chronic pain. In cardiovascular diseases such as atherosclerosis

and hypertension, the barrier between the vascular lumen and the vascular wall formed by endothelial cells is disturbed and leukocytes migrate across the barrier [51].

Down Regulation of Signaling Systems in Syncytium Coupled Cells

Astrocytes are one of the most-studied syncytium coupled cells with gap junctions. In inflammatory processes the Ca^{2+} signaling in the astrocyte networks is overactivated and triggers astrocytes and microglia to become inflammatory reactive. Down-regulations of Na^{+} transporters occur, and disruption of the cytoskeleton, which abolishes the Ca^{2+} oscillations by changing the balance between the Ca^{2+} regulating processes. Thus there is a down-regulation of Cx43. There is also an increased release of pro-inflammatory cytokines. The resulting increased neuronal excitability leads to increased glutamate release. Astrocyte uptake of excessive extracellular glutamate plays a critical role in preventing glutamate excitotoxicity. The inflammatory induced dysfunction of glial cells can lead to pathogenic chronic neuroinflammation [52, 53].

A huge amount of receptors are found on the astrocytes and cause increased intracellular Ca^{2+} release when activated [29]. The spread of Ca^{2+} waves through the astrocyte syncytium has a propagating rate of about 100 μm/s [27, 54]. Astrocytes in most part of the nervous system use two types of Ca^{2+} communication. During normal physiological conditions intercellular communication through gap junctions occurs [27], and at disturbed properties extracellular communication through enhanced release of ATP. Astrocytic Ca^{2+} waves are mediated by autocrine activation of purinoceptors [26].

In the retina, intracellular Ca^{2+} communication occurs through the astrocytes, and extracellular Ca^{2+} communication occurs between astrocytes and Müller cells [55]. An increased production of VEGF, nitric oxide (NO) and ROS lead to reduced occludin in the tight junction of the BRB during inflammatory processes and leakage of plasma proteins from the blood vessels occur [14].

Astrocytes also exhibit Na^{+} transients, but they have a longer duration compared to the Ca^{2+} transients. The Na^{+} transients persist for tens of seconds while the Ca^{2+} transients persist for hundreds of milliseconds [56].

Examples of other syncytium coupled cells are chondrocytes, keratinocytes, synovial fibroblasts, osteoblasts, connective tissue cells, cardiac and corneal fibroblasts, myofibroblasts, hepatocytes, and different types of glandular cells. These cells are targets for inflammation, which can be initiated after injury or in disease. If the inflammation reaches the CNS, it develops into neuroinflammation and can be of importance in the development of systemic chronic inflammation, which can manifest as pain and result in changes in the expression and structure of cellular components [52].

Disorganization of Actin Filaments

The actin filaments in syncytium coupled cells are dominated by F-actin stress fibers [52, 57]. The actin family belongs to the cytoskeleton family, actin filaments, myosin and microtubules, which represent one component of the tight junction complex. Actin is present in two forms, globular monomeric actin, G-actin, and filamentous polymeric actin, F-actin [58]. Dynamic rearrangements in the actin cytoskeleton are huge and are regulated by a large group of more than hundred binding proteins. Both endothelial and epithelial cell membrane localization of F-actin filaments is necessary for tight junction formation. Changes in the F-actin organization results in changes in transelectrical endothelial resistance (TEER) [23].

Cell cultures of astrocytes stimulated with the endotoxin lipopolysaccharide show a more diffuse organization of the actin filaments and ring structures are more pronounced. The morphologic changes include retraction of the cell bodies and also changes in stress fiber distribution. The ratio between the polymer form of the F-actin and the monomer form of the G-actin was increased [25]. Similar changes of the actin filament structure are observed in chondrocytes [36]. Different stimuli that cause inflammation result in endocytosis of junction proteins, which causes barrier dysfunction. Recently two processes were highlighted: cleavage of transmembrane junction proteins via MMPs, and proteolysis of junctional proteins via lysosomes or proteasomes [58].

Barrier Regulation

Glial cell line-derived neurotrophic factor (GDNF), a growth factor in the TGF-β family is produced and secreted from astrocytes [59] and regulates the permeability of the BBB [60] and the BRB [17]. GDNF acts by a two-component receptor complex; 1) glycosylphospatidylinositol-linked cell surface molecule, the GDNF receptor GFRα-1, which acts as a ligand-binding domain, and 2) the receptor protein tyrosine kinase Ret, which acts as the signal transducing domain [61]. GFRα-1 is preferentially localized on capillary endothelial cell membranes in the brain and it may be a differential factor of endothelial cells of the BBB by enhancing the tight junction function [60]. GDNF is a basic protein of 134 amino acids and does not penetrate the BBB. GDNF may play an important role in neuropathic pain and it is endogenously enhanced at electroacupuncture [61]. GDNF protects and repairs dopamine-containing neurons, which degenerate in Parkinson's disease, and motorneurons, which die in amyotrophic lateral sclerosis [62]. GDNF, IL-6 and basic fibroblast growth factor (bFGF) are involved in the BRB regulation [14]. Furthermore, different N-methyl-D-aspartate (NMDA) antagonists have been shown to enhance the production of GDNF in astrocytes [63-65]. Also, TNF-α released from microglia stimulates astrocytes in a paracrine regulation of GDNF production in activated astrocytes [66].

Endothelial cells of the BNB are capable to respond to GDNF and enhance the TEER recovery after injury and thereby restore the BNB [23]. Nicotine, stimulating the α7-nicotinic acetylcholine receptor (α7-nAChR) on astrocytes [67], protects GDNF downregulation, which may be of importance in inflammatory diseases [68].

The combination of three compounds; a μ-opioid receptor antagonist at ultralow concentrations, naloxone, a μ-opioid receptor agonist, endomorphin-1/morphine or (-)-linalool, and an agent attenuating IL-1β release, the anti-epileptic agent levetiracetam, can restore the cellular parameters induced by lipopolysaccharide back to physiological homeostatic levels. The combination of the pharmaceutical compounds has been tested in network coupled astrocytes in vitro [25, 69]. We have tested two of the pharmaceutical compounds in post-surgical neuropathic pain patients with promising results [70]. The combination that is identified can resolve and restore disordered cellular inflammatory pathways in network coupled cells, which might be the target cells.

Another signaling system, the platelet-derived growth factor (PDGF)-CC system has been suggested as target to restore the BBB by inhibition with a tyrosine-kinase inhibitor [71]. Furthermore, brain endothelial microRNAs have been suggested to play critical roles in the regulation under neuroinflammation conditions and in restoration of the BBB [72]. Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) have both been shown to protect and prevent damage of the BRB [73]. Interestingly, we have earlier shown that PACAP could attenuate the 5-HT-, histamine-, and ATP-evoked Ca^{2+} responses in syncytium coupled astrocytes. The results show that PACAP can regulate Ca^{2+} dynamics in astrocytes and be a modulator for chemical substances released during physiological processes or in pathophysiology [74]. Moreover, VIP might modulate second messenger response in astrocytes through intracellular mechanisms [75].

Conclusion

The BBB, the BRB and the BNB seem to play a crucial role in several diseases where inflammatory processes are a key feature. During inflammation the electrical resistance is decreased causing more permeability of the tight junctions in the endothelial cells. The damaged barriers will have leukocytes migrating into the nervous system where they transform into microglia, and cytokines will be released. This inflammatory process with the distortion of the barriers have been seen in diseases like Alzheimer's disease, Parkinson's disease, type 2 diabetes, atherosclerosis, AMD, OA, systemic vasculitis and cardiovascular diseases. The understanding of the inflammatory processes is a step towards the key features of many diseases although more studies are needed to elucidate the more specific mechanisms in the different diseases.

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