Introduction for the Special Issue on the Blood-Brain Barrier

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The blood-brain barrier (BBB) serves to maintain brain homeostasis, which is crucial for the functionality of neuronal networks, and thus the regulation of body functions. It consists of a polarized monolayer of brain microvascular endothelial cells that separates the blood from the brain. The BBB tightly regulates the transport of substances between blood and brain, serving brain homeostasis but –at the same time– hindering the treatment of brain diseases by precluding the entry of therapeutics. An improved understanding of the natural transport pathways that exist at the BBB in both health and disease may help to design methods for the transfer of therapeutic and toxic compounds into and out of the brain, respectively, for the treatment of CNS diseases. For this purpose, *in vitro* BBB models that accurately represent the physiological situation in human are desired. Several lines of research need to converge in order to study and understand brain diseases, to develop proper *in vitro* BBB model systems, and to develop treatment strategies.

This Special Issue of *Tissue Barriers* is dedicated to the Blood-Brain Barrier (BBB). It contains 11 reviews that altogether summarize and discuss recent advances in understanding normal structure, development and functions of the BBB and the mechanisms underlying disruption of this barrier in CNS diseases. In addition, potential therapeutic interventions and gateways for drug delivery into the brain, including strategies to bypass the BBB and strategies to cross the BBB, are discussed. Challenges in the development of physiologically relevant *in vitro* BBB model systems, including BBB-on-a-chip, and challenges in the imaging of nanoparticle transport at the BBB are revealed.

The first article of this special issue, by Stamatovic et al., provides an in depth review of the molecular composition of the junctional protein complexes, i.e., tight junctions (TJs), adherens junctions (AJs) and gap junctions (GJs), that are present at the BBB, and how the redistribution of junctional proteins leads to BBB dysfunction.¹ Typically, redistribution of junctional proteins directly affects barrier integrity by enhancing paracellular leakage. Interestingly, the redistribution of junctional proteins may also lead to new functions such as in the case of JAM-A that, following its translocation to the luminal membrane of the BBB, functions as a receptor for leucocyte infiltration, thereby indirectly enhancing barrier permeability. An emerging field is the role of redistributed junctional proteins in cell signaling processes that may aggravate barrier dysfunction, although it should be noted that at present most evidence for this new hypothesis is obtained from studying epithelial and not endothelial cells.

The next two reviews by Hou et al. and McRae^{2,3} discuss how viruses make use of TJ modulation to enhance BBB permeability in order to enter the brain. TJ modulation occurs by viral factors and host immune factors, including cytokines, and often coincides with enhanced extravasation of (infected) immune cells into the brain. McRae specifically focuses on the interaction between HIV and the BBB. HIV and the HIV proteins Tat and gp120 alter the expression and function of TJ proteins and efflux transporters, affecting both paracellular and transcellular transport at the BBB. Because antiretroviral therapy directed against HIV makes use of drugs that are inducers, inhibitors, or substrates for efflux transporters, it is important to examine how HIV infection affects the penetration of antiretroviral medicines into the brain. Hou et al. recommend the use of high resolution imaging approaches, such as scanning ion conductance microscopy, to reveal the host responses against viral infections at the molecular level, which will be instrumental in finding novel compounds that can correct for BBB permeation.

In CNS diseases BBB integrity is often compromised, suggesting that compounds that prevent or restrict BBB breakdown may represent a promising therapy against not only infections, but also other CNS disorders, including neurodegenerative diseases. In their review, Wevers and De Vries show that the supporting cells of the BBB, i.e., the astrocytes and pericytes, secrete soluble factors, so-called morphogens, that play a role in barrier formation and maintenance, and therefore may have a therapeutic value in the treatment of CNS diseases.⁴ The authors consider the semaphorins as the best option for treating BBB breakdown in CNS disorders, and clinical trials with semaphorin-modulating therapy are described. However, because the effects of morphogens are not CNS-specific but may also affect other parts in the body, the use of morphogens as therapeutics may require specific targeting of the morphogens to the BBB,

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e.g. by fusing the therapeutic drug to a ligand that binds to a specific receptor on the BBB, or by encapsulating the drug in nanocarriers that can be targeted to the BBB using similar ligands.

Yet, except for regional differences in brain function, there likely also is heterogeneity of the BBB throughout the brain, as exemplified in the paper by Wilhelm and colleagues.⁵ For example, within the CNS there is great diversity of astrocytes and (consequently) GFAP expression, which was shown to have an impact on BBB function. This heterogeneity of the BBB may explain for the regional restriction of certain brain pathologies, and may similarly cause therapeutic interventions to differently affect the BBB of different brain regions. This should be taken into account when designing therapeutic interventions.

The less broadly studied delivery methods that are described by Pandey et al.,⁶ including heterotypic mucosal grafting and intranasal delivery, may circumvent the problem of BBB heterogeneity. In addition, the authors discuss quantitative *in vivo* imaging techniques for tracking of drug delivery devices.

In contrast to the aim to repair BBB integrity in CNS diseases, BBB permeation can be exploited for paracellular transport of therapeutic molecules into the brain. The discovery of structural and regulatory proteins in TJs and AJs has led to the development of TJ modulators that can alter BBB function to allow for paracellular transport of molecules, as reviewed by Greene and Campbell.⁷ They describe how enhanced paracellular transport through the opening of TJs may offer a promising way for the delivery of drugs into the CNS and may also facilitate the clearance of pathogenic molecules, such as β -amyloid, from the brain. They discuss the therapeutic potential of 'biological' TJ modulation by a.o. siRNAs and peptides, and 'mechanical' TJ modulation by focused ultrasound (FUS).

Many CNS compounds need to be protected from degradation during circulation in the blood, need to be taken up into the CNS via the BBB, and need to be targeted to specific sites of action while preventing (lysosomal) degradation. The encapsulation of therapeutics into nanocarrier systems provides major advantages over the use of free drugs, including protection of the therapeutics against degradation, and reduced toxicity at non-target tissues. The review by Grabrucker et al. summarizes the applications of 'artificial' nanoparticles over the last 20 years in *in vivo* brain experiments,⁸ while the review by András and Toborek summarizes the use of 'biological' delivery vehicles, specifically BBB-derived exosomes.⁹ Exosomes are extracellular vesicles derived from endosomes that contain mRNAs, miRNAs, lipids, and proteins. They play a role in cell-cell communication and have shown potential for the delivery of therapeutic molecules. Exosomes from different cellular origins were shown to cross the BBB via the process of transcytosis. András and Toborek provide an overview of the published literature on BBB-derived extracellular vesicles as biological delivery vehicles, and their possible role in A β pathology. In addition, they discuss commercial tools that are available for the loading of exosomes with therapeutic content.

The following review by Aberg summarizes the current shortcomings of different imaging techniques in connection with the shortcomings of the current *in vitro* BBB models, for the quantitative analysis of nanoparticle transport across the BBB.¹⁰ To correct for leakage of nanoparticles through imperfections in the BBB monolayer or adherence of nanoparticles to the Transwell filters that are typically used to support the BBB monolayer, investigation by electron microscopy or live cell imaging is proposed, although the areas of the barrier that can be covered using these techniques are limited. In addition, the author stresses the effect the protein corona may have on nanoparticle transport. Since the protein corona is very much influenced by the administration route via which the nanoparticle is administered, nanoparticles should be pre-exposed to the relevant biological fluids when measuring their transport across the *in vitro* BBB.

In the final review van der Helm et al. provide recommendations for further standardization in BBB model characterization.¹¹ Because of the poor reproduction of human pathophysiology by animals together with species-to-species variation, they opt for the use of human cells and tissues in *in vitro* BBB models. The review discusses the papers that are published on BBB-on-a-chip models, i.e., microfluidic devices in which BBB cell types are cultured under physiologically relevant conditions, such as shear stress imposed by blood flow. Most microfluidic devices make use of a filter with a thickness of several micrometers (similar to the Transwell filters) to support the cells. However, in order to limit nanoparticle adherence to filters, the use of thinner membranes or no membranes in microfluidic devices would be preferred. This would also allow for the intimate contact between different cell types in case brain endothelial cells are co-cultured with pericytes and astrocytes, to better mimic the *in vivo* situation. Furthermore, the use of patient-derived induced pluripotent stem cells (iPSCs) for the generation of (disease-specific) BBB cell types can contribute to the building of more relevant *in vitro* model systems.

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