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Review

Exit pathways of therapeutic antibodies from the brain and retention strategies

Linda Schellhammer,[1,4] Michal Beffinger,[1,2] Ulisse Salazar,[1] Jon D. Laman,[3] Thorsten Buch,[1] and Johannes vom Berg[1,2,*]

SUMMARY

Treating brain diseases requires therapeutics to pass the blood-brain barrier (BBB) which is nearly impermeable for large biologics such as antibodies. Several methods now facilitate crossing or circumventing the BBB for antibody therapeutics. Some of these exploit receptor-mediated transcytosis, others use direct delivery bypassing the BBB. However, successful delivery into the brain does not preclude exit back to the systemic circulation. Various mechanisms are implicated in the active and passive export of antibodies from the central nervous system. Here we review findings on active export via transcytosis of therapeutic antibodies - in particular, the role of the neonatal Fc receptor (FcRn) - and discuss a possible contribution of passive efflux pathways such as lymphatic and perivascular drainage. We point out open questions and how to address these experimentally. In addition, we suggest how emerging findings could aid the design of the next generation of therapeutic antibodies for neurologic diseases.

AIMS AND SCOPE

Brain delivery strategies for therapeutic antibodies and other protein-based therapeutics are intensely investigated in both academia and the pharmaceutical industry and are well-covered in scientific literature. In comparison, much less is known about the fate of these drugs once they have entered the brain parenchyma. This review article summarizes the current understanding of the fate of antibody-based therapeutics for neurological diseases of the central nervous system (CNS) once they have passed the blood-brain barrier (BBB). After a brief review of relevant anatomical structures and the most common delivery strategies to pass or bypass the BBB, the main focus of this article is, therefore, on the CNS to blood antibody efflux. We point out open questions and potential experimental strategies to address these, as well as implications for drug development to target brain diseases.

THE CENTRAL NERVOUS SYSTEM AND ITS BARRIERS

The mammalian CNS is isolated from the periphery by unique anatomical structures: 1) The BBB, which limits direct access of substances in the blood to the brain parenchyma[1]; 2) a distinctive barrier formed between spinal cord parenchyma and the surrounding vasculature[2]; 3) the cerebrospinal fluid (CSF)-brain barrier and 4) the blood-CSF barrier, which separate the brain parenchyma from the CSF space and the CSF from the blood, respectively.[3,4] Furthermore, in the vicinity of the cribriform plate and along the cranial nerves and venous sinuses of the dura mater run the meningeal lymphatic vessels, which drain CSF to the regional lymph nodes.[5,6] This system was prominently described by Michael Bradbury and by Helen Cserr in the 1980s and in the early 1990s,[7–9] and has been steadily gaining attention over the last decades. The history and complexities of the field have been discussed recently.[10]

The BBB is a highly specialized vascular system of brain endothelial cells supported by pericytes and bolstered up by astrocytes forming the glia limitans.[11] It serves as an interface between the CNS and the periphery while at the same time isolating the CNS from potentially toxic substances in the blood, including metabolites, but also inflammatory molecules and infectious agents. The barrier function of the BBB relies on firmly interconnected endothelial cells, which form many adherens junctions and tight junctions, preventing the passage of most molecules other than small lipophilic compounds. The BBB endothelium contains multiple ion pumps and transporters, including ATP-binding cassette (ABC) and solute carrier (SLC) families of influx and efflux transporters, which shuttle nutrients into and clear metabolites out of the brain.[3,12]

Importantly, endothelial cells forming the BBB express the neonatal Fc receptor (FcRn). FcRn is a heterodimeric protein formed of a heavy/alpha chain (encoded by the gene FCGRT) and a light chain (beta2 microglobulin).[13,14] FcRn was initially discovered for intestinal IgG uptake in neonatal rodents[15] and continues to function during adult life beyond the neonatal period. In the endolysosome, FcRn binds the Fc moiety of IgG antibodies (Abs) at lysosomal pH (pH ~5.5), which allows salvage from the sorting endosome and release back into the circulation at

[1]Institute of Laboratory Animal Science, University of Zurich, 8952 Schlieren, Switzerland
[2]InCephalo AG, 4123 Allschwil, Switzerland
[3]Department of Pathology & Medical Biology, University of Groningen, University Medical Center Groningen, Groningen 9713, the Netherlands
[4]Present address: Novo Nordisk Pharma AG, 8058 Zurich, Switzerland
*Correspondence: johannes.vomberg@uzh.ch
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neutral pH, thus prolonging their half-life. A similar mechanism has been described for albumin, although the binding sites of IgG Fc and albumin on FcRn are not overlapping.[16] While recycling and transcytosis of IgG in epithelial cells and endothelial cells through FcRn is well established, for albumin mostly the recycling function has been so far experimentally confirmed.[17]

The blood-spinal cord barrier is morphologically and functionally analogous to the BBB, including the presence of non-fenestrated endothelium and glia limitans. However, its barrier function appears to be less stringent, presumably due to reduced expression of adherens and tight junction proteins.[2]

Two additional barriers confine the CSF compartment toward the blood and toward the brain parenchyma

The blood-CSF barrier is present throughout the arachnoid mater, formed by a layer of cells called the arachnoid barrier layer. A different type of blood-CSF barrier exists within the choroid plexus: Blood vessel endothelial cells in this organ express carrier receptors, but, in contrast to the endothelium of the BBB, are fenestrated and do not form tight junctions, thus allowing macromolecule permeability.[1] The barrier function is in this case ensured by the choroid plexus epithelium, which is also responsible for the secretion of CSF into the ventricles. Those epithelial cells, in addition to various transporters, also express FcRn.[3,14]

Toward the brain parenchyma, forming a CSF-brain barrier, the subarachnoid space is isolated by the pia mater and glial cells. A similar barrier composed solely of astroglia endfeet and their associated basement membrane is present along the perivascular spaces limiting free exchange of molecules with the brain parenchyma. Within the ventricles, the brain parenchyma is loosely separated from CSF by a lining of ependymal cells, which do not form tight junctions and permit exchange of macromolecules between brain interstitial fluid and CSF.[1,3,6]

In summary, tight control over the traffic across CNS barriers is maintained through specialized anatomical and cellular structures including tight junctions, ion pumps, channels, and transporter proteins. Under these circumstances, many therapeutics, including intravenously injected antibodies, are kept from entering the CNS.

THE CENTRAL NERVOUS SYSTEM BARRIERS IN DISEASE

CNS diseases can trigger changes in the function of the CNS barrier systems. These alterations are often related to increased permeability and have been observed in autoimmune neuropsychiatric disorders, epilepsy, neurodegenerative disorders, and brain cancer, among others.[18,19] Infection-induced BBB permeability triggers the deposition of immunoglobulin G (IgG) within the brain in a model of autoimmune psychiatric disorder.[20] In epilepsy, dysfunctions of the BBB are etiopathologically central to the disease and cause as well as aggravate seizures.[21] In multiple sclerosis and Parkinson's disease, increased barrier permeability has been observed and may play a role in the development of these diseases.[18,22] In a preclinical marmoset model of multiple sclerosis, systemically administered therapeutic antibodies were detectable in the white matter lesions.[23] Furthermore, in Alzheimer's disease, the blood-CSF barrier also appears to be affected.[24]

Perhaps the most severe cases of CNS barrier breakdown occur in malignant brain cancers. Indeed, BBB permeability can be used for tumor staging and diagnosis of glioblastoma, using magnetic resonance imaging with gadolinium as a BBB-non-permeable contrast agent.[19,25]

Notably, the disease-triggered barrier dysfunction can be spatially or temporally limited, observable as focal increase in BBB permeability in preclinical Alzheimer's disease models.[26] Also in multiple sclerosis, barrier permeability is increased temporally, depending on the phase of the disease.[21] Even in high-grade gliomas, some tumor regions do not show BBB dysfunction.[27] In general, the exposure of brain tissue to systemically present substances can be both a triggering factor as well as a consequence of a disease.

STRATEGIES TO INCREASE ANTIBODY DELIVERY TO THE BRAIN

The favored way for administering therapeutic biologics, in particular antibodies and Fc-fusion constructs, is often parenteral, which is advantageous over oral administration, as it bypasses the proteolytic environment of the digestive tract and provides better control of dosing. Yet, an active ingredient still must pass the BBB to enter the brain. As only small and lipophilic molecules passively cross the BBB, it is inherently difficult to bring biologics into the CNS parenchyma.[1,28,29] Nonetheless, there are various approaches to overcome this limitation. We summarize four fundamental methods for biologics: (1) induction of BBB permeability, (2) charge-optimization to allow adsorption-mediated transcytosis (AMT), (3) receptor-mediated transcytosis (RMT), and (4) bypassing the BBB via direct CNS delivery. For detailed reviews refer to.[30–32]

(1) Inducing BBB permeability (Figure 1A). One of the best-studied approaches is focused ultrasound (FUS). Mechanistically, FUS affects the tight junctions in the BBB endothelium by the application of ultrasound with or without the administration of microbubbles into the vasculature.[33] While most FUS research has been focused on delivering non-antibody therapeutics and nanoparticles, preclinical data and recent trials suggest that FUS is also well-suitable for the safe delivery of antibodies.[34,35] An alternative method to enhance BBB permeability is the use of pharmacotherapeutics, such as the potassium channel inhibitor minoxidil sulfate or the osmotic pressure regulator mannitol.[36,37] Using osmotic BBB disruption, therapeutic antibodies were successfully delivered into the parenchyma, which improved the outcome of bevacizumab (anti-VEGF) treatment in patients with glioma.[37]

(2) Adsorption-mediated transcytosis (AMT, Figure 1B). Instead of permeabilizing the BBB, the therapeutic itself is modified for efficient transport across the BBB in this approach. Positively charged molecules are endocytosed and transported through the BBB via AMT. In mice, charge optimization increased brain uptake of chemically modified antibodies with an isoelectric point above 9.5.[47] However,

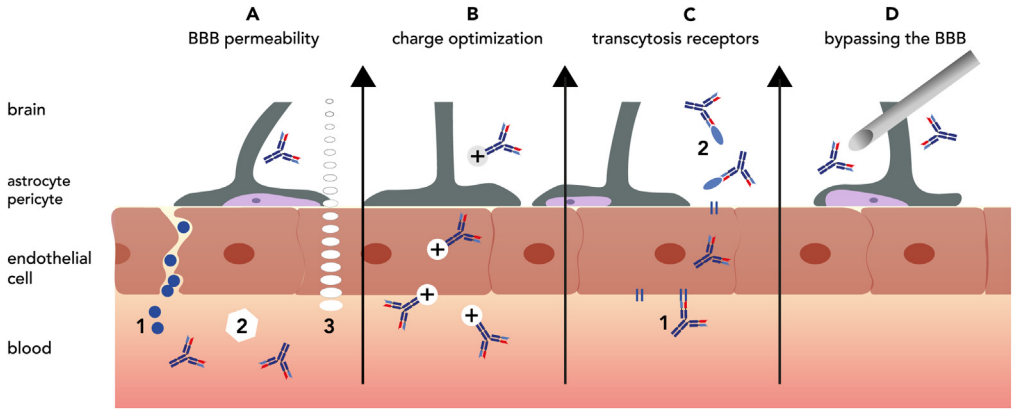

Figure 1. Strategies to increase antibody delivery to the brain across the BBB

(A) Permeabilization: (1) Focused ultrasound (FUS) induces a steady oscillation of intravenously administered microbubbles, which transiently permeabilize the BBB (reviewed in[30]; 2) Pharmacological reduction of BBB function, e.g., using potassium channel agonists to increase brain uptake of an anti-Her-2 Ab.[36] (3) Osmotic permeabilization of the BBB showed positive effects on the delivery of therapeutic antibodies.[37]

(B) Charge-optimization: During adsorption-mediated transcytosis (AMT), positively charged molecules are endocytosed and transported through the BBB.[32]

(C) Engaging transcytosis receptors: Receptor-mediated transcytosis (RMT) is often used with the IR, LRP1, or TfR. The affinity to the receptor determines release in the parenchyma or accumulation in the endothelium.[38] (1) High-affinity antibodies to TfR accumulate in the BBB endothelium instead of penetrating the parenchyma.[38,39] (2) This is avoided by lowering the TfR affinity and adding a second specificity to direct homing to the CNS. This requires a bior multispecific antibody format, such as the modular brain shuttle system.[38,40] A similar engineering approach is necessary for IR,[41] LRP1,[42] CD98hc[43] or other targets.

(D) Bypassing: Direct delivery via the olfactory epithelium[44] or by direct injection into the CSF[45] or the brain parenchyma.[46]

AMT also increased uptake in the liver, kidney, lung, and heart, thus decreasing the delivery specificity. Furthermore, the cationization of an antibody can interfere with its target specificity, toxicity, and immunogenicity and thus needs to be carefully evaluated.[32]

(3) Receptor-mediated transcytosis (RMT, Figure 1C). RMT leverages influx receptors for nutrient carriers present at the BBB. Antibodies targeting receptors such as the insulin receptor (IR),[41] low-density lipoprotein receptor-related protein type 1 (LRP1),[42] or the transferrin receptor (TfR)[48] are transported from the luminal to the abluminal side of the endothelium. With only 1–10% of antibodies detected in the CNS parenchyma after systemic dosing, the fraction of biologics that pass the BBB with RMT still remains substantially lower compared to what the body periphery is exposed to.[38–43]

(4) Bypassing the BBB via direct CNS delivery (Figure 1D). Another option is to circumvent the BBB altogether and to directly administer biologics into the CNS. This also obliterates the necessity for continuous and high systemic exposure. One administration route is intranasal application. In mice, antibodies cross the olfactory epithelia via paracellular or transcellular transport, the latter being potentially FcRn-dependent.[44,49,50] However, distribution in the parenchyma forms a steep concentration gradient starting from the brain entry points around the olfactory and trigeminal nerves.[44] In addition, murine and human nasal anatomy differ substantially. Currently, the mechanism governing the antibody penetration of the CNS through the nasal cavity in humans remains only partially understood.[51]

Another approach is direct injection into the CNS parenchyma or the CSF. Apart from intrathecal injections in the lumbar spinal cord, these are invasive neurosurgical interventions. Intracranial delivery and surgical resection can be combined in the context of brain tumors. Alternatively, catheters can be implanted, allowing intracerebral injection, microperfusion, or administration by convection-enhanced delivery.[46,52,53] Convection-enhanced delivery also has been employed to treat other CNS diseases, such as Parkinson's disease.[54] Recent improvements in catheter design and placement techniques enable acute brain injections, but also long-term repeated therapy in an outpatient setting (over 18 months).[55]

Direct injection into the CSF requires the antibody to cross the CSF-brain barrier and penetrate the target site, a slow and inefficient process that appears to depend on the diffusion and convective flow around leptomeningeal blood vessels.[45] This route is potentially useful for high-affinity antibodies which target the CNS, as antibodies applied to CSF are usually rapidly cleared into the blood circulation.[56] In rats, antibodies injected into the CSF of the lumbar spinal cord reached the brain tissue at concentrations 3.8-fold higher than upon intravenous injection.[57] In humans, intrathecal delivery of trastuzumab (anti-HER2) has been tested against leptomeningeal disease, and rituximab (anti-CD20) for recurrent CNS lymphoma.[58,59] Unfortunately, the apparent CSF half-life of trastuzumab is only 4.1 $\pm$ 3.0 h, and also for rituximab, CSF concentration in these studies dropped to approximately 5% of the initial dose within three days post-application.[58,60]

Beyond neuro-oncology, the intraventricular administration of an anti-Nogo-A antibody in a phase I clinical trial for the treatment of acute spinal cord injury demonstrated good tolerability, but also this trial revealed an overall short half-life and a high variability in drug levels in the CSF among patients.[61]

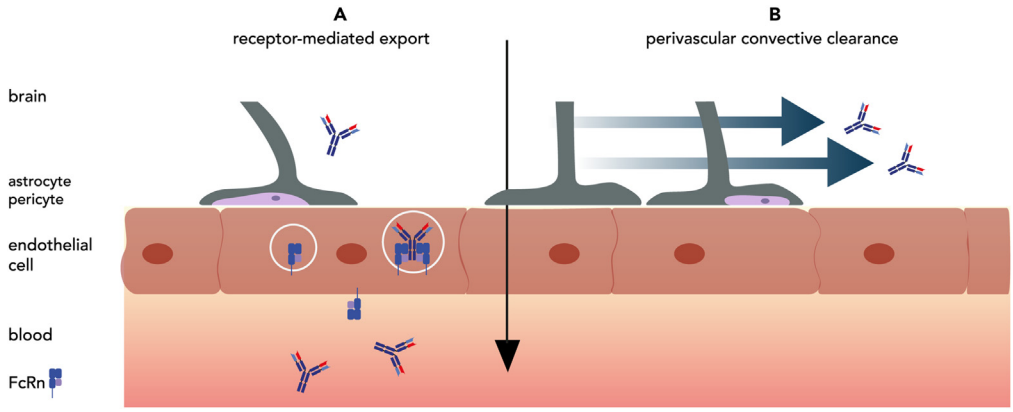

Figure 2. Mechanisms of antibody clearance from the CNS

(A) Receptor-mediated export: IgG antibodies administered directly into the parenchyma end up in the blood due to receptor-mediated export, potentially by FcRn. In addition, the presence of the cognate antigen critically influences the distribution and clearance.[62]

(B) Perivascular convective clearance: antibodies can be transported by passive diffusion through the brain parenchyma or by convection via the interstitial fluid (ISF).[63,64]

Taken together, multiple options exist for introducing therapeutic antibodies into the CNS. The desired CNS concentration, acceptability of systemic exposure, and feasibility of invasive interventions define the preferred delivery method. Yet, the fate of the antibody within the CNS is governed by additional factors.

MECHANISMS OF ANTIBODY CLEARANCE FROM THE CENTRAL NERVOUS SYSTEM

While research has focused on the delivery of antibodies to the CNS to enhance therapeutic efficacy, the fate of such therapeutic antibodies after reaching the brain remains largely understudied. Two main concepts describe the passage of antibodies from the CNS into the systemic circulation: receptor-mediated passage (Figure 2A) and passive convective clearance (Figure 2B). Importantly, there is a striking difference between experimental findings, depending on the delivery setting. This is exemplified by work focusing on the role of FcRn: While preclinical studies using local delivery provide experimental evidence for a receptor-mediated export of antibodies from the brain to the blood, results from studies using the systemic application of antibodies suggest no relevant role of receptor-mediated brain-to-blood export.

Receptor-mediated export

Zhang and Pardridge were among the first to study IgG efflux from the brain to the blood in 2001.[65] They applied a mouse monoclonal IgG2a antibody that did not recognize an epitope in the brain via intraparenchymal administration into rat brains and analyzed its efflux into the circulation. They observed an Fc-dependent efflux mechanism, which neither interfered with albumin, Fab, nor TfR. Kinetic analysis distinguished two phases, a high- and a low-affinity efflux phase. The measured intracranial half-life of IgG was below 1 h, whereas medium molecular weight dextran (~70 kDa) and albumin (66 kDa) showed half-lives of 10–12 h. These efflux rates were too high to be explained by passive leakage, pointing toward an Fc-specific receptor-based transport mechanism. Only one year later, the same group confirmed the expression of the neonatal Fc receptor (FcRn) in the BBB.[13] In retrospect, the Fc-receptor-based mechanism described by Zhang and Pardridge strongly resembles the later described FcRn-mediated efflux.

In 2005, Deane et al. investigated the brain clearance of radiolabeled β-amyloid complexed with an anti-β-amyloid antibody (mIgG2b) in mice.[62] They found the clearance to depend on two mechanisms: a) interaction of β-amyloid with the LRP1 receptor and b) interaction of IgG with FcRn. Accordingly, blockade of either LRP1 by deleting the RAP encoding gene, or blockade of the IgG-FcRn interaction by deleting *fcgrt* or *b2m* genes in mice reduced clearance by up to 68%. They also assessed brain clearance of an intracranially applied mIgG2b antibody in the absence of antigen and found that it is transcytosed across the BBB. This export was observed to be saturable by adding excess antibody. In contrast to the β-amyloid-antibody complexes, the clearance of the unbound antibody was not affected by blocking the LRP1 receptor. It was, however, reduced in mice lacking either the α or the β_2m subunit of FcRn. These data point toward the involvement of FcRn in exporting IgG antibodies from the brain parenchyma to the blood, similar to its function in another CNS immune-privileged site, the eye.[66] The active involvement of FcRn has been confirmed with other β-amyloid binding IgG constructs.[67,68] However, as binding to FcRn takes place in the intracellular compartment, Deane et al. also hypothesized that an additional receptor may exist that facilitates IgG endocytosis.

Finally, in 2013, Cooper et al. conducted a study similar to the one reported by Zhang and Pardridge, but used antibodies with modified FcRn affinity.[69] Using amino acid substitutions in the IgG-FcRn binding interface, they generated FcRn-high- and FcRn-low-binding variants that they injected intracranially into rat brains. In line with previous findings, they observed approximately 2-fold increased brain tissue retention of the FcRn-low-binding antibody variant compared to the FcRn-high-binding variant. They also demonstrated that this mechanism was saturable by excess IgG.

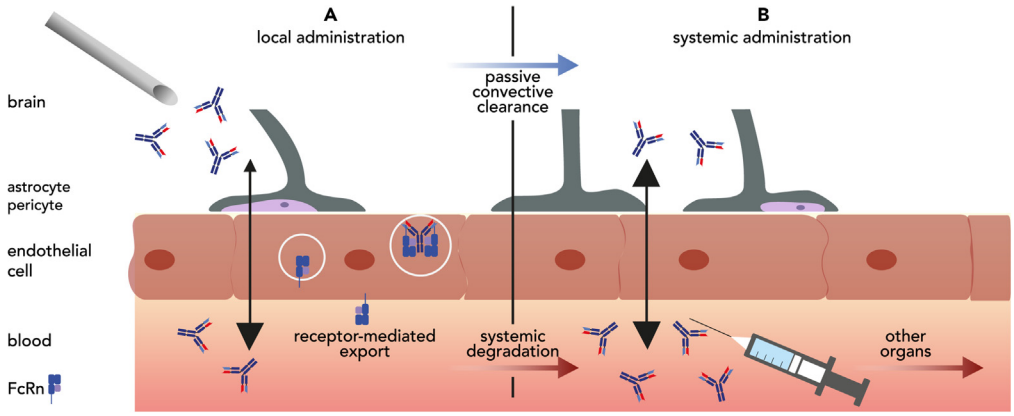

Figure 3. Different findings from experimental approaches relying on local or systemic dosing

(A) Local administration: Studies suggesting a role of FcRn in exporting antibodies from brain/CSF to the systemic circulation used experimental methods of the local administration of antibodies into the CNS.[62,65,69]

(B) Systemic administration: Studies using the systemic administration of antibodies concluded that FcRn does not influence the distribution of IgG in the brain and did not identify an alternative export receptor.[70,71,74,75]

Taken together, these studies provided experimental evidence for an FcRn-mediated export of antibodies from the brain to the blood. In contrast, studies using the systemic application of antibodies suggest that FcRn is irrelevant for brain-to-blood export.

Garg and Balthasar investigated the transcytosis of mIgG1 across the BBB in wild-type (WT) and $\beta_2m^{-/-}$ mice by assessing the distribution of an intravenously administered radiolabeled IgG in blood and brain.[70] They compared the brain-to-plasma ratio as a primary outcome. Their results confirmed that FcRn plays a role in prolonging the serum half-life of IgG, showing significantly reduced IgG levels over time in $\beta_2m^{-/-}$ mice. However, the brain-to-plasma exposure ratio did not differ between WT to $\beta_2m^{-/-}$ mice, and they concluded that FcRn does not affect the deposition of IgG in the brain. The same group extended their analysis by studying also *FcRn*$^{-/-}$ animals, deficient in the FcRn alpha chain, and *FcγRIIb*$^{-/-}$, and *FcγRI/RIII*$^{-/-}$ animals, deficient in the respective Fcγ receptors, in a similar setting.[71] Fcγ receptors are another group of receptors able to bind IgG and are also expressed in the CNS: FcγRI, FcγRIIa, FcγRIIb, and FcγRIIIa have been described on microglia, whereas FcγRI and FcγRIIb are found on astrocytes and neurons.[72] In contrast to IgG-FcRn interaction, binding of IgG to FcγR occurs at the cell surface at neutral pH. The interaction of IgG with FcγR can induce endocytosis, trigger cell activation (high-affinity receptor FcγRI, low affinity receptors FcγRIIa, and FcγRIIIa), or inhibition (FcγRIIb and low affinity) in various myeloid cell types, lymphocytes, and non-immune cells.[73] In a second study by the Balthasar group[71] the low brain antibody levels could be explained by rapid local catabolism or swift convective elimination but also supported the hypothesis of an active IgG export from the brain. Yet, the authors excluded the role of both FcRn and FcγR as no differences compared to WT control animals were observed.[71]

Furthermore, a study by Chen et al. compared antibody levels in multiple organs of WT and *FcRn*$^{-/-}$ animals upon the systemic application of human IgG1.[74] They showed a significant role of FcRn in the distribution of IgG in various tissues, including the liver and spleen. However, the participation of FcRn in IgG deposition in brain tissue appeared negligible in this study. Eigenmann and colleagues measured tissue uptake of systemically applied untargeted human IgG1 antibodies, modified to be either FcRn binding or non-binding.[75] They confirmed the importance of FcRn for the clearance of antibodies from organs such as the liver but did not find a significant contribution of FcRn to brain antibody levels.

The authors of this second group of studies in which the consequence of systemic IgG delivery (Figure 3B) was investigated, concluded that FcRn did not influence the distribution of IgG in the brain and did not identify an alternative export receptor.[70,71,74,75] This conclusion contrasts with studies performed using direct delivery of IgG into the brain parenchyma (Figure 3A). One potential explanation for this discrepancy is that upon systemic delivery, the impact of FcRn on blood pharmacokinetics of IgG is much more substantial and therefore masks any effect at the BBB/brain parenchyma. As only minute amounts of IgG pass the BBB, influx and efflux are in equilibrium without the apparent involvement of FcRn-mediated export.

A recent study demonstrated substantial antibody brain deposition upon the systemic administration of a human IgG1 FcRn-high-binding variant with higher serum prevalence.[76] This effect was abolished in *FcRn*$^{-/-}$ mice and the same antibody with an unmodified Fc or with reduced FcRn affinity showed in comparison lower brain deposition. While the authors in this case suggest an involvement of FcRn in brain-to-blood transcytosis, it is conceivable that high systemic recycling and thus higher serum levels result in a shorter time to reach equilibrium between the blood and CNS compartments.

Developing novel *in vitro* models enabled the investigation of influx and efflux effects independently. Using such an approach, Finke and colleagues showed that the efflux can be saturated by excess IgG.[77] This saturation depends on the sialylation of the Fc and can be potentially explained by the glycosylation-related changes in the intracellular trafficking of endocytosed IgG. However, a confounding factor might be a relatively low level of sialylation in the tested antibody and the sialylation-dependent changes in the FcRn affinities between fully sialylated and desialylated proteins.[78] Moreover, human and rodent FcRn exhibit fundamental differences in glycosylation, which influences the cellular

distribution and direction of transcytosis of IgGs,[79] a factor to be considered in particular when comparing human and rodent models of FcRn-mediated transcytosis.

Finally, FcRn is likely not the only factor involved in IgG efflux. Transcytosis receptors do not necessarily need to bind the Fc part of the IgG.[62,71,80] Such receptors will likely play a role in endocytosis or endosomal trafficking in the BBB and may even interact with FcRn.[62] For a summary of the studies discussing IgG transport mechanisms in the CNS context, see Table 1.

Passive convective clearance and leakage

Whereas the studies discussed above identified receptor-mediated export, studies of *in vitro* BBB models also support the possibility of passive IgG efflux ([83]; Figure 2B). These observations are essential for understanding the fate of therapeutic IgG antibodies in the brain parenchyma, as the egress can be driven not solely by directional shuttling but also by the endocytosis and trafficking of intracellular vesicles, independently of FcRn.

In addition, next to passive diffusion through the interstitial fluid (ISF) of the brain parenchyma, antibodies and other macromolecules can be transported by advection, including bulk flow along the white matter tracts and along the perivascular spaces toward the CSF. This efflux route to the CSF is impaired for immune complexes, which may have direct functional implications for an antibody binding to targets such as β-amyloid.[64] In rats, swift clearance of both an IgG antibody (150 kDa) and the low molecular weight tracer inulin (~5 kDa) from the CSF with a half-life around 50 min has been described. While a direct passage from the ventricle into the circulation has not been demonstrated, the apparent independence of molecular weight appears unexpected.[84] Yet, for substantially larger molecules there may be a size dependency for CSF to blood drainage: Upon intrathecal administration, the efflux rate for an IgM format antibody (950 kDa) was approximately two times lower than for an IgG antibody.[85]

One of the most extensive studies on the fate of intrathecally administrated antibodies has been conducted by Pizzo and colleagues in rats, using a variety of methods including *ex vivo* fluorescence and magnetic resonance imaging of Alexa Fluor 488 and gadolinium labeled antibodies, respectively.[45] Next to important findings on direct entry of antibodies from the CSF into the perivascular space, the authors confirmed drainage of IgG to the deep cervical lymph nodes as had been demonstrated decades earlier with isotope-labelled albumin in rabbits.[8] In addition, IgG and single domain antibodies also appeared to efflux via the cribriform plate to the nasal mucosa and often also to the superficial cervical lymph nodes.[45]

While only few recent studies focused on passive convective clearance of antibodies in detail, a larger body of literature studied isotope labeled albumin or fluorescent dyes as tracers. When 125I isotope-labelled albumin was injected into the lateral ventricle of sheep, only approximately 8–25% of the total amount appear to efflux from the brain and was recovered either in the cervical and thoracic duct or in the blood.[86] Similar results were obtained when 125I-HSA was injected in the lumbar CSF. When taking into account the amounts of macromolecular tracer recovered in blood and lymphatic vessels, the authors concluded that around 50% of the CSF drainage in sheep is through the lymphatic system.[86] Employing high-resolution stereomicroscopy in lymphatic-reporter mice, a more recent study concluded that the major CSF outflow route of fluorescent dyes, such as 3kDa-AF680 or near-infrared labeled PEGylated tracers is through lymphatic vessels.[87] Furthermore, the flow of the interstitial fluid also carries macromolecules toward the ventricles as has been shown for albumin labeled with gadolinium or fluorescent dyes, which may serve as a sink for solutes, including antibodies, in the brain.[88,89]

Overall, unspecific, non-receptor-mediated processes appear to contribute to the observed clearance of macromolecules of a wide size range up to 150 kDa, a size corresponding to human IgG.[90,91] As passive egress of macromolecules from the CNS has been mainly studied using fluorescent dyes the transferability of the findings to therapeutic antibodies is limited. Further studies are needed to specify the exact contribution of each drainage path to the swift clearance of therapeutic antibodies from the brain. For example, in the context of local application, the differential drainage from distinct brain regions to deep and superficial cervical lymph nodes may benefit from further investigation when different doses of antibody are applied. Similarly, drainage from the spinal cord to lumbar lymph nodes may be playing an important role in antibody clearance. In addition, direct injection into the CNS parenchyma may provoke tissue damage depending on the infusion volume, rate, and the target region.

Overall, despite considerable efforts to increase CNS exposure of therapeutic antibodies, our current understanding of the different mechanisms that drive antibody egress back into circulation is surprisingly limited and several open questions remain to be answered with existing and novel experimental approaches (Box 1).

IMPLICATIONS FOR DRUG DEVELOPMENT TO TARGET BRAIN DISEASES

Reaching sufficient CNS exposure with therapeutic antibodies remains challenging due to the poor BBB permeability of biologics, followed by their rapid removal from the CNS through passive efflux or active receptor-mediated transcytosis. Increasing the intraparenchymal half-life of therapeutic antibodies could thus play a major role in developing novel therapies for CNS diseases. Indeed, several approaches are conceivable to prevent the efflux of antibodies from brain tissue.

One strategy is the saturation of FcRn by adding excess binders, e.g., free Fc fragments or specific blocking peptides, thus preventing the FcRn:Fc interaction. A local co-injection of antibodies and FcRn-blocking or FcRn-saturating agents, such as antibody (fragments) or intravenous immunoglobulin (IVIg), would potentially reduce the net export. However, if the therapeutic is to be applied systemically, the added blocking compounds must cross the BBB to reach their site of action. Furthermore, systemic FcRn blockade will also affect half-lives of all other IgGs in the system, as well as gut transcytosis and cellular uptake in organs such as the liver and spleen.[75,92] This effect creates a dilemma for systemic dosing: Only a small fraction of systemically applied antibody is being shuttled into the brain, even if increased via RMT approaches.

Table 1. Summarized findings on BBB transcytosis of IgG antibodies

Reference	Findings and hypothesized mechanism	Saturation	Route	Type of drug/Ab	Model	Readout
Zhang, 2001[65]	Fc receptor-mediated transcytosis of IgG from brain to blood	yes	i.c.	mIgG2a TfR-specific and isotype ctrl	rat	efflux
Banks, 2002[81]	anti-β-amyloid Ab influx through extracellular pathways; efflux detected but mechanism not elucidated	N/A	i.v.	anti-β-amyloid IgG goat polyclonal	mouse β-amyloid overexpression (SAMP8 and ICR strains)	influx
Deane, 2005[62]	IgG efflux only by FcRn, not LRP; β-amyloid-complexes by LRP and FcRn; age-dependency	yes	i.c. (ISF microinjections)	anti-β-amyloid mIgG2b 4G8	Mouse APPswe$^{+/-}$ (Tg2576) *β2m−/− FcRn$^{-/-}$* *RAP$^{-/-}$* *FcRγ$^{-/-}$*	efflux
Boado, 2007[68]	FcRn plays a significant role in IgG efflux from the brain	yes	i.c.	anti-β-amyloid IgG	rat	efflux
Garg, 2009[70]	FcRn is not involved in the uptake of IgG into the brain	N/A	i.v.	mIgG1 7E3	mouse β2m−/−	tissue-to-blood ratio
Abuqayyas, 2013[71]	FcRn, FcγRI, FcγRIIb, or FcγRIII are not involved in the limited brain uptake of IgG	N/A	i.v.	mIgG1 8C2	mouse C57BL/6 FcgRI/RIII−/− FcgRIIb−/− FcRn−/−	tissue-to-blood ratio
Proulx, 2012[80]	LRP1 receptor-mediated internalization, recycling, and transcytosis	yes	N/A	IVIg polyclonal	MEF-1 cells	internalization and recycling *in vitro*
St-Amour, 2013[29]	Mechanism unknown, maybe FcRn or LRP1 or antigen-specificity due to polyclonal IVIg	yes	i.p.	IVIg polyclonal	mouse 3xTg-AD *APP_{swe}* x *$PS1_{M146V}$* x *tau_{P301L}*	influx
Cooper, 2013[69]	FcRn plays a significant role in IgG efflux from the brain	yes	i.c. and i.n.	hIgG1 N434A and H435A (high and low FcRn binder) without a target in the CNS	rat	efflux
Strazielle, 2013[82]	Unidirectional transport mechanism, correlated with FcRn expression	N/A	N/A	IVIg polyclonal	*in vitro* model of choroidal endothelial cells	influx and efflux
Chen, 2014[74]	FcRn is not involved in IgG distribution to the brain	N/A	i.v.	hIgG1	mouse *FcRn$^{-/-}$*	tissue-to-blood ratio
Eigenmann, 2017[75]	FcRn is involved in IgG clearance from the liver but not from the brain	N/A	i.v.	Humanized IgG1 with normal and reduced FcRn binding	mouse	tissue clearance modeling
Finke, 2017[77]	Unclear mechanism; FcRn inhibition by sialylation?	N/A	N/A	anti-Aβ mIgG2b 4G8 (sialylated) and mIgG1 6E10 (asialylated)	*in vitro* model of brain vascular endothelial cells	influx and efflux
Tien, 2023[76]	Unidirectional transport mechanism, brain deposition correlated with FcRn expression and affinity	yes	i.v.	Humanized IgG1, high (YTE) and abolished FcRn affinity (AA) mutants	*mouse, FcRn$^{-/-}$*	influx

efflux, brain-to-blood transport; i.c., intracranial; ICR, mouse strain; i.n., intranasal; ISF, interstitial fluid; i.v., intravenous; i.p., intraperitoneal; influx, blood-to-brain transport; IVIg, intravenous immunoglobulin; LRP1, Low Density Lipoprotein Receptor-related Protein 1; MEF-1 cells, mouse embryonic fibroblasts; n/a, not applicable; RAP, Lrpap1^{tm1Her} targeted mutation affecting LRP1; SAMP8, Senescence Accelerated Mouse-Prone 8; 3xTg-AD *APP_{swe}* x *$PS1_{M146V}$* x *tau_{P301L}*, transgenic model with three mutations associated with early onset familial Alzheimer's Disease, APPswe, Amyloid Beta Precursor Protein with Swedish mutation, $PS1_{M146V}$, *Psen1* gene for Presenilin-1 with M146V mutation which is linked to human AD, tau_{P301L} protein equivalent to human mutant tau.

Box 1. Open questions and potential experimental strategies to address mechanisms that drive antibody egress from CNS back into the circulation

- What is the quantitative influence of individual leakage pathways at a given dose?
- How does the dose affect the contribution of individual pathways?
- Do other mechanisms contribute to leakage and export of therapeutic antibodies from the CNS?
- Test a defined set of antibodies from the same species as the experimental model used, modified to answer particular questions, e.g.,
  specificity: binding or not binding a brain antigen
  role of Fc receptors: mutants with a range of affinities to the particular Fc receptor
  charge
  RMT targets
  competitor reagents (e.g., for co-administration)
- Experimental *in vivo* models, including transgenic animals (allowing e.g., conditional receptor deletion in endothelium) and large animal models and microsurgical manipulation of potential drainage pathways
- Use of different observation modalities resolving temporal dynamics, such as
  short term processes in high resolution vs.
  long term processes with whole animal imaging and
  continuous administration vs. pulse-chase setting
  … employing detection modalities such as isotope labels and PET-CT imaging vs. live imaging of fluorescently labeled test reagents or tissue clearing in combination with light sheet microscopy

A reduced blood concentration due to global FcRn blockade will directly translate into lower quantities of antibody that are available for RMT to reach the CNS parenchyma. In this case, a constant saturation of the systemic compartment with antibodies is necessary, requiring a high dosing frequency. On the other hand, if a high systemic antibody titer is tolerable, an increase in FcRn affinity to reach higher serum prevalence and eventually also higher brain exposure[76] may represent a viable strategy for systemic dosing and could be even combined with RMT.

An alternative approach is to directly prevent FcRn-mediated export by reducing the affinity of the IgG to FcRn. As demonstrated by Cooper and colleagues, reducing IgG:FcRn interaction can improve the brain retention of locally applied IgG antibodies.[69] However, this approach increases the retention in the CNS at the cost of fast systemic degradation. Yet, such FcRn-non-binding therapeutics would be well-suited for direct administration behind the BBB. Direct intracranial delivery is feasible, not only in the case of brain cancer but also in neurodegenerative diseases such as Parkinson's disease.[52,54] Further studies would need to validate if the increase in retention due to FcRn affinity reduction translates to improvements in the efficacy and screening of individual mutations maybe be necessary in the context of a particular antibody, as FcRn affinity is not exclusively governed by the IgG constant domain.[78,93] The situation is similar for IgG effector functions such as antibody-dependent cell-mediated cytotoxicity, any new Fc mutation may require independent optimization in the context of the whole therapeutic molecule.[94]

Brain tissue retention of antibodies also improves by adding a moiety binding to a brain-expressed epitope. This approach is auspicious, exemplified by the long intracranial half-life of systemically applied anti-β-amyloid antibodies, approximately 30 days in the CNS versus 6 days systemically.[95] As IgGs are naturally bivalent, designing bifunctional molecules and precise tuning of the affinity of a specific binding moiety conferring sufficient immobilization while ensuring sustained on-target bioactivity is conceivable. For antibodies that act via the multimerization of the target, for example in the case of agonistic antibodies against growth factor receptors,[96,97] further design adjustments will be necessary.

An archetypical example of biologics with two (or more) specificities are antibodies designed for RMT. Here the interplay between the transport entity and the therapeutically relevant binding part(s) needs to be well-balanced to avoid sequestration within the BBB.[38] Also, it appears that the choice of the individual RMT receptors to target may have a profound effect on intraparenchymal half-life. Compared to TfR, CD98hc may in this regard confer longer CNS exposure of transcytosed antibodies.[43]

On a sidenote, it is tempting to speculate about the fate of albumin-based biologics or biologics that entail an albumin binding domain to increase serum half-life by piggy backing onto FcRn based albumin recycling. Assuming that albumin, unlike IgG, is not transcytosed but rather recycled,[17] such therapeutics may only be subject to passive efflux upon local intraparenchymal administration.

While in most cases the development of therapeutics may be aimed at bringing them to and retaining them in the brain, in some cases, active export from the CNS is required for their mechanism of action. A well-studied example is Alzheimer's disease, where antibody therapies were designed to facilitate the removal of beta-amyloid deposits from the brain.[62,67,68]

Whether an efflux blockade is necessary and which approach to use must be carefully evaluated for each disease and therapeutic independently. For example, a successful therapy against brain cancer with immune checkpoint-blocking antibodies might require high local concentrations but not necessarily high systemic exposure. The latter might trigger generalized adverse events. Even more so, delivery of an agonistic, proinflammatory antibody requires establishing an optimal dose in a narrow range, in which the activation of the immune system does not lead to overt adverse events. In such cases, bypassing the BBB via local delivery to spare the periphery may be advantageous and reducing FcRn-mediated export at the expense of low systemic prevalence is desirable. Different requirements may apply to other CNS diseases and necessitate the careful balancing of the therapeutic dose, route of administration, and optimization of the local brain retention properties. Finally, increased permeability of the BBB and blood-CSF barrier in some diseases will have a major impact on the efflux route.

In conclusion, the efflux of IgG antibodies from within the CNS underlines that getting in is only part of the journey. Equally important is how to ensure sustained exposure to a therapeutic. We need to carefully evaluate the way biologics are designed for brain targeting. Novel approaches and rigorous, systematic testing to answer open questions are required to guide the design of the next generation of therapeutics and to better help patients suffering from brain disorders.

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AUTHOR CONTRIBUTIONS

Conceptualization: L.S., M.B., J.D.L, and J.v.B.; data curation: L.S., M.B., U.S., and J.v.B.; writing-original draft: L.S., M.B., U.S., and J.v.B.; writing-review and editing: L.S., M.B., U.S., T.B., J.D.L, and J.v.B.; visualization: L.S.; supervision: J.v.B.

DECLARATION OF INTERESTS

L.S., M.B., T.B. and J.v.B. are named inventors on patents in the field of immuno-oncology (WO2013053775, WO2020201167, WO2020201168). M.B. and J.v.B. are part time employees and M.B., T.B. and J.v.B. have equity interest in InCephalo AG. J.v.B. has received honoraria from Bristol Meyer Squibb.

REFERENCES

1. Bourasset, F., Auvity, S., Thorne, R.G., and Scherrmann, J.M. (2022). Brain Distribution of Drugs: Brain Morphology, Delivery Routes, and Species Differences, Part I chapter1. Handb. Exp. Pharmacol. *273*, 97–120. https://doi.org/10.1007/164_2020_402.
2. Bartanusz, V., Jezova, D., Alajajian, B., and Digicaylioglu, M. (2011). The blood-spinal cord barrier: morphology and clinical implications. Ann. Neurol. *70*, 194–206. https://doi.org/10.1002/ana.22421.
3. Hladky, S.B., and Barrand, M.A. (2014). Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. Fluids Barriers CNS *11*, 26. https://doi.org/10.1186/2045-8118-11-26.
4. Tietz, S., and Engelhardt, B. (2015). Brain barriers: Crosstalk between complex tight junctions and adherens junctions. J. Cell Biol. *209*, 493–506. https://doi.org/10.1083/jcb.201412147.
5. Engelhardt, B., Carare, R.O., Bechmann, I., Flügel, A., Laman, J.D., and Weller, R.O. (2016). Vascular, glial, and lymphatic immune gateways of the central nervous system. Acta Neuropathol. *132*, 317–338. https://doi.org/10.1007/s00401-016-1606-5.
6. Engelhardt, B., Vajkoczy, P., and Weller, R.O. (2017). The movers and shapers in immune privilege of the CNS. Nat. Immunol. *18*, 123–131. https://doi.org/10.1038/ni.3666.
7. Bradbury, M. (1981). Lymphatics and the Central Nervous-System. Trends Neurosci. *4*, 100–101. https://doi.org/10.1016/0166-2236(81)90032-1.
8. Bradbury, M.W., Cserr, H.F., and Westrop, R.J. (1981). Drainage of cerebral interstitial fluid into deep cervical lymph of the rabbit. Am. J. Physiol. *240*, F329–F336. https://doi.org/10.1152/ajprenal.1981.240.4.F329.
9. Cserr, H.F., and Knopf, P.M. (1992). Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. Immunol. Today *13*, 507–512. https://doi.org/10.1016/0167-5699(92)90027-5.
10. Proulx, S.T. (2021). Cerebrospinal fluid outflow: a review of the historical and contemporary evidence for arachnoid villi, perineural routes, and dural lymphatics. Cell. Mol. Life Sci. *78*, 2429–2457. https://doi.org/10.1007/s00018-020-03706-5.
11. Mastorakos, P., and McGavern, D. (2019). The anatomy and immunology of vasculature in the central nervous system. Sci. Immunol. *4*, eaav0492. https://doi.org/10.1126/sciimmunol.aav0492.
12. Geier, E.G., Chen, E.C., Webb, A., Papp, A.C., Yee, S.W., Sadee, W., and Giacomini, K.M. (2013). Profiling solute carrier transporters in the human blood-brain barrier. Clin. Pharmacol. Ther. *94*, 636–639. https://doi.org/10.1038/clpt.2013.175.
13. Schlachetzki, F., Zhu, C., and Pardridge, W.M. (2002). Expression of the neonatal Fc receptor (FcRn) at the blood-brain barrier. J. Neurochem. *81*, 203–206. https://doi.org/10.1046/j.1471-4159.2002.00840.x.
14. Latvala, S., Jacobsen, B., Otteneder, M.B., Herrmann, A., and Kronenberg, S. (2017). Distribution of FcRn Across Species and Tissues. J. Histochem. Cytochem. *65*, 321–333. https://doi.org/10.1369/0022155417705095.
15. Jones, E.A., and Waldmann, T.A. (1972). The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. J. Clin. Invest. *51*, 2916–2927. https://doi.org/10.1172/JCI107116.
16. Pyzik, M., Kozicky, L.K., Gandhi, A.K., and Blumberg, R.S. (2023). The therapeutic age of the neonatal Fc receptor. Nat. Rev. Immunol. *23*, 415–432. https://doi.org/10.1038/s41577-022-00821-1.
17. Sand, K.M.K., Bern, M., Nilsen, J., Noordzij, H.T., Sandlie, I., and Andersen, J.T. (2015). Unraveling the interaction between FcRn and albumin: opportunities for design of albumin-based therapeutics. Front. Immunol. *5*, 682. https://doi.org/10.3389/fimmu.2014.00682.
18. Lochhead, J.J., Yang, J., Ronaldson, P.T., and Davis, T.P. (2020). Structure, Function, and Regulation of the Blood-Brain Barrier Tight Junction in Central Nervous System Disorders. Front. Physiol. *11*, 914. https://doi.org/10.3389/fphys.2020.00914.
19. Arvanitis, C.D., Ferraro, G.B., and Jain, R.K. (2020). The blood-brain barrier and blood-tumour barrier in brain tumours and metastases. Nat. Rev. Cancer *20*, 26–41. https://doi.org/10.1038/s41568-019-0205-x.
20. Dileepan, T., Smith, E.D., Knowland, D., Hsu, M., Platt, M., Bittner-Eddy, P., Cohen, B., Southern, P., Latimer, E., Harley, E., et al. (2016). Group A Streptococcus intranasal infection promotes CNS infiltration by streptococcal-specific Th17 cells. J. Clin. Invest. *126*, 303–317. https://doi.org/10.1172/JCI80792.
21. Profaci, C.P., Munji, R.N., Pulido, R.S., and Daneman, R. (2020). The blood-brain barrier in health and disease: Important unanswered questions. J. Exp. Med. *217*, e20190062. https://doi.org/10.1084/jem.20190062.
22. Ma, Y., Sannino, D., Linden, J.R., Haigh, S., Zhao, B., Grigg, J.B., Zumbo, P., Dündar, F., Butler, D., Profaci, C.P., et al. (2023). Epsilon toxin-producing Clostridium perfringens colonize the multiple sclerosis gut microbiome overcoming CNS immune privilege. J. Clin. Invest. *133*, e163239. https://doi.org/10.1172/JCI163239.
23. Laman, J.D., t Hart, B.A., Brok, H., Meurs, M.v., Schellekens, M.M., Kasran, A., Boon, L., Bauer, J., Boer, M.d., and Ceuppens, J. (2002). Protection of marmoset monkeys against EAE by treatment with a murine antibody blocking CD40 (mu5D12). Eur. J. Immunol. *32*, 2218–2228. https://doi.org/10.1002/1521-4141(200208)32:8<2218::AID-IMMU2218>3.0.CO;2-0.
24. Chalbot, S., Zetterberg, H., Blennow, K., Fladby, T., Andreasen, N., Grundke-Iqbal, I., and Iqbal, K. (2011). Blood-cerebrospinal fluid barrier permeability in Alzheimer's disease. J. Alzheimers Dis. *25*, 505–515. https://doi.org/10.3233/JAD-2011-101959.

25. Essig, M., Dinkel, J., and Gutierrez, J.E. (2012). Use of contrast media in neuroimaging. Magn. Reson. Imag. Clin. N. Am. *20*, 633–648. https://doi.org/10.1016/j.mric.2012.08.001.
26. Bien-Ly, N., Boswell, C.A., Jeet, S., Beach, T.G., Hoyte, K., Luk, W., Shihadeh, V., Ulufatu, S., Foreman, O., Lu, Y., et al. (2015). Lack of Widespread BBB Disruption in Alzheimer's Disease Models: Focus on Therapeutic Antibodies. Neuron *88*, 289–297. https://doi.org/10.1016/j.neuron.2015.09.036.
27. Sarkaria, J.N., Hu, L.S., Parney, I.F., Pafundi, D.H., Brinkmann, D.H., Laack, N.N., Giannini, C., Burns, T.C., Kizilbash, S.H., Laramy, J.K., et al. (2018). Is the blood-brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data. Neuro Oncol. *20*, 184–191. https://doi.org/10.1093/neuonc/nox175.
28. Pardridge, W.M. (2007). Drug targeting to the brain. Pharm. Res. (N. Y.) *24*, 1733–1744. https://doi.org/10.1007/s11095-007-9324-2.
29. St-Amour, I., Paré, I., Alata, W., Coulombe, K., Ringuette-Goulet, C., Drouin-Ouellet, J., Vandal, M., Soulet, D., Bazin, R., and Calon, F. (2013). Brain bioavailability of human intravenous immunoglobulin and its transport through the murine blood-brain barrier. J. Cerebr. Blood Flow Metabol. *33*, 1983–1992. https://doi.org/10.1038/jcbfm.2013.160.
30. Curley, C.T., Sheybani, N.D., Bullock, T.N., and Price, R.J. (2017). Focused Ultrasound Immunotherapy for Central Nervous System Pathologies: Challenges and Opportunities. Theranostics *7*, 3608–3623. https://doi.org/10.7150/thno.21225.
31. Karmur, B.S., Philteos, J., Abbasian, A., Zacharia, B.E., Lipsman, N., Levin, V., Grossman, S., and Mansouri, A. (2020). Blood-Brain Barrier Disruption in Neuro-Oncology: Strategies, Failures, and Challenges to Overcome. Front. Oncol. *10*, 563840. https://doi.org/10.3389/fonc.2020.563840.
32. Hervé, F., Ghinea, N., and Scherrmann, J.M. (2008). CNS delivery via adsorptive transcytosis. AAPS J. *10*, 455–472. https://doi.org/10.1208/s12248-008-9055-2.
33. Sheikov, N., McDannold, N., Sharma, S., and Hynynen, K. (2008). Effect of focused ultrasound applied with an ultrasound contrast agent on the tight junctional integrity of the brain microvascular endothelium. Ultrasound Med. Biol. *34*, 1093–1104. https://doi.org/10.1016/j.ultrasmedbio.2007.12.015.
34. Jordão, J.F., Thévenot, E., Markham-Coultes, K., Scarcelli, T., Weng, Y.Q., Xhima, K., O'Reilly, M., Huang, Y., McLaurin, J., Hynynen, K., and Aubert, I. (2013). Amyloid-beta plaque reduction, endogenous antibody delivery and glial activation by brain-targeted, transcranial focused ultrasound. Exp. Neurol. *248*, 16–29. https://doi.org/10.1016/j.expneurol.2013.05.008.
35. Schoen, S., Jr., Kilinc, M.S., Lee, H., Guo, Y., Degertekin, F.L., Woodworth, G.F., and Arvanitis, C. (2022). Towards controlled drug delivery in brain tumors with microbubble-enhanced focused ultrasound. Adv. Drug Deliv. Rev. *180*, 114043. https://doi.org/10.1016/j.addr.2021.114043.
36. Ningaraj, N.S., Rao, M.K., and Black, K.L. (2003). Adenosine 5'-triphosphate-sensitive potassium channel-mediated blood-brain tumor barrier permeability increase in a rat brain tumor model. Cancer Res. *63*, 8899–8911.
37. Boockvar, J.A., Tsiouris, A.J., Hofstetter, C.P., Kovanlikaya, I., Fralin, S., Kesavabhotla, K., Seedial, S.M., Pannullo, S.C., Schwartz, T.H., Stieg, P., et al. (2011). Safety and maximum tolerated dose of superselective intraarterial cerebral infusion of bevacizumab after osmotic blood-brain barrier disruption for recurrent malignant glioma. J. Neurosurg. *114*, 624–632. https://doi.org/10.3171/2010.9.JNS101223.
38. Yu, Y.J., Zhang, Y., Kenrick, M., Hoyte, K., Luk, W., Lu, Y., Atwal, J., Elliott, J.M., Prabhu, S., Watts, R.J., and Dennis, M.S. (2011). Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. Sci. Transl. Med. *3*, 84ra44. https://doi.org/10.1126/scitranslmed.3002230.
39. Bien-Ly, N., Yu, Y.J., Bumbaca, D., Elstrott, J., Boswell, C.A., Zhang, Y., Luk, W., Lu, Y., Dennis, M.S., Weimer, R.M., et al. (2014). Transferrin receptor (TfR) trafficking determines brain uptake of TfR antibody affinity variants. J. Exp. Med. *211*, 233–244. https://doi.org/10.1084/jem.20131660.
40. Niewoehner, J., Bohrmann, B., Collin, L., Urich, E., Sade, H., Maier, P., Rueger, P., Stracke, J.O., Lau, W., Tissot, A.C., et al. (2014). Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. Neuron *81*, 49–60. https://doi.org/10.1016/j.neuron.2013.10.061.
41. Pardridge, W.M., Kang, Y.S., Buciak, J.L., and Yang, J. (1995). Human insulin receptor monoclonal antibody undergoes high affinity binding to human brain capillaries in vitro and rapid transcytosis through the blood-brain barrier in vivo in the primate. Pharm. Res. (N. Y.) *12*, 807–816. https://doi.org/10.1023/a:1016244500596.
42. Dehouck, B., Fenart, L., Dehouck, M.P., Pierce, A., Torpier, G., and Cecchelli, R. (1997). A new function for the LDL receptor: Transcytosis of LDL across the blood-brain barrier. J. Cell Biol. *138*, 877–889. https://doi.org/10.1083/jcb.138.4.877.
43. Pornnoppadol, G., Bond, L.G., Lucas, M.J., Zupancic, J.M., Kuo, Y.-H., Zhang, B., Greineder, C.F., and Tessier, P.M. (2023). Bispecific antibody shuttles targeting CD98hc mediate efficient and long-lived brain delivery of IgGs. Preprint at bioRxiv. https://doi.org/10.1101/2023.04.29.538811.
44. Kumar, N.N., Lochhead, J.J., Pizzo, M.E., Nehra, G., Boroumand, S., Greene, G., and Thorne, R.G. (2018). Delivery of immunoglobulin G antibodies to the rat nervous system following intranasal administration: Distribution, dose-response, and mechanisms of delivery. J. Contr. Release *286*, 467–484. https://doi.org/10.1016/j.jconrel.2018.08.006.
45. Pizzo, M.E., Wolak, D.J., Kumar, N.N., Brunette, E., Brunnquell, C.L., Hannocks, M.J., Abbott, N.J., Meyerand, M.E., Sorokin, L., Stanimirovic, D.B., and Thorne, R.G. (2018). Intrathecal antibody distribution in the rat brain: surface diffusion, perivascular transport and osmotic enhancement of delivery. J. Physiol. *596*, 445–475. https://doi.org/10.1113/JP275105.
46. Bastiancich, C., Malfanti, A., Préat, V., and Rahman, R. (2021). Rationally designed drug delivery systems for the local treatment of resected glioblastoma. Adv. Drug Deliv. Rev. *177*, 113951. https://doi.org/10.1016/j.addr.2021.113951.
47. Pardridge, W.M., Kang, Y.S., Yang, J., and Buciak, J.L. (1995). Enhanced cellular uptake and in vivo biodistribution of a monoclonal antibody following cationization. J. Pharmacol. Sci. (Tokyo, Jpn.) *84*, 943–948. https://doi.org/10.1002/jps.2600840808.
48. Broadwell, R.D., Baker-Cairns, B.J., Friden, P.M., Oliver, C., and Villegas, J.C. (1996). Transcytosis of protein through the mammalian cerebral epithelium and endothelium. III. Receptor-mediated transcytosis through the blood-brain barrier of blood-borne transferrin and antibody against the transferrin receptor. Exp. Neurol. *142*, 47–65. https://doi.org/10.1006/exnr.1996.0178.
49. Maigler, F., Ladel, S., Flamm, J., Gänger, S., Kurpiers, B., Kiderlen, S., Völk, R., Hamp, C., Hartung, S., Spiegel, S., et al. (2021). Selective CNS Targeting and Distribution with a Refined Region-Specific Intranasal Delivery Technique via the Olfactory Mucosa. Pharmaceutics *13*, 1904. https://doi.org/10.3390/pharmaceutics13111904.
50. Ye, L., Zeng, R., Bai, Y., Roopenian, D.C., and Zhu, X. (2011). Efficient mucosal vaccination mediated by the neonatal Fc receptor. Nat. Biotechnol. *29*, 158–163. https://doi.org/10.1038/nbt.1742.
51. Lochhead, J.J., and Thorne, R.G. (2012). Intranasal delivery of biologics to the central nervous system. Adv. Drug Deliv. Rev. *64*, 614–628. https://doi.org/10.1016/j.addr.2011.11.002.
52. Souweidane, M.M., Kramer, K., Pandit-Taskar, N., Zhou, Z., Haque, S., Zanzonico, P., Carrasquillo, J.A., Lyashchenko, S.K., Thakur, S.B., Donzelli, M., et al. (2018). Convection-enhanced delivery for diffuse intrinsic pontine glioma: a single-centre, dose-escalation, phase 1 trial. Lancet Oncol. *19*, 1040–1050. https://doi.org/10.1016/S1470-2045(18)30322-X.
53. Sendelbeck, S.L., and Urquhart, J. (1985). Spatial distribution of dopamine, methotrexate and antipyrine during continuous intracerebral microperfusion. Brain Res. *328*, 251–258. https://doi.org/10.1016/0006-8993(85)91036-4.
54. Whone, A., Luz, M., Boca, M., Woolley, M., Mooney, L., Dharia, S., Broadfoot, J., Cronin, D., Schroers, C., Barua, N.U., et al. (2019). Randomized trial of intermittent intraputamenal glial cell line-derived neurotrophic factor in Parkinson's disease. Brain *142*, 512–525. https://doi.org/10.1093/brain/awz023.
55. Whone, A.L., Boca, M., Luz, M., Woolley, M., Mooney, L., Dharia, S., Broadfoot, J., Cronin, D., Schroers, C., Barua, N.U., et al. (2019). Extended Treatment with Glial Cell Line-Derived Neurotrophic Factor in Parkinson's Disease. J. Parkinsons Dis. *9*, 301–313. https://doi.org/10.3233/JPD-191576.
56. Weinmann, O., Schnell, L., Ghosh, A., Montani, L., Wiessner, C., Wannier, T., Rouiller, E., Mir, A., and Schwab, M.E. (2006). Intrathecally infused antibodies against Nogo-A penetrate the CNS and downregulate the endogenous neurite growth inhibitor Nogo-A. Mol. Cell. Neurosci. *32*, 161–173. https://doi.org/10.1016/j.mcn.2006.03.007.
57. Wahl, A.S., Correa, D., Imobersteg, S., Maurer, M.A., Kaiser, J., Augath, M.A., and Schwab, M.E. (2020). Targeting Therapeutic Antibodies to the CNS: a Comparative Study of Intrathecal, Intravenous, and Subcutaneous Anti-Nogo A Antibody Treatment after Stroke in Rats.

Neurotherapeutics *17*, 1153–1159. https://doi.org/10.1007/s13311-020-00864-z.

58. Rubenstein, J.L., Li, J., Chen, L., Advani, R., Drappatz, J., Gerstner, E., Batchelor, T., Krouwer, H., Hwang, J., Auerback, G., et al. (2013). Multicenter phase 1 trial of intraventricular immunochemotherapy in recurrent CNS lymphoma. Blood *121*, 745–751. https://doi.org/10.1182/blood-2012-07-440974.
59. Lazaratos, A.M., Maritan, S.M., Quaiattini, A., Darlix, A., Ratosa, I., Ferraro, E., Griguolo, G., Guarneri, V., Pellerino, A., Hofer, S., et al. (2023). Intrathecal trastuzumab versus alternate routes of delivery for HER2-targeted therapies in patients with HER2+ breast cancer leptomeningeal metastases. Breast *69*, 451–468. https://doi.org/10.1016/j.breast.2023.04.008.
60. Kumthekar, P.U., Avram, M.J., Lassman, A.B., Lin, N.U., Lee, E., Grimm, S.A., Schwartz, M., Bell Burdett, K.L., Lukas, R.V., Dixit, K., et al. (2023). A phase I/II study of intrathecal trastuzumab in human epidermal growth factor receptor 2-positive (HER2-positive) cancer with leptomeningeal metastases: Safety, efficacy, and cerebrospinal fluid pharmacokinetics. Neuro Oncol. *25*, 557–565. https://doi.org/10.1093/neuonc/noac195.
61. Kucher, K., Johns, D., Maier, D., Abel, R., Badke, A., Baron, H., Thietje, R., Casha, S., Meindl, R., Gomez-Mancilla, B., et al. (2018). First-in-Man Intrathecal Application of Neurite Growth-Promoting Anti-Nogo-A Antibodies in Acute Spinal Cord Injury. Neurorehabilitation Neural Repair *32*, 578–589. https://doi.org/10.1177/1545968318776371.
62. Deane, R., Sagare, A., Hamm, K., Parisi, M., LaRue, B., Guo, H., Wu, Z., Holtzman, D.M., and Zlokovic, B.V. (2005). IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor. J. Neurosci. *25*, 11495–11503. https://doi.org/10.1523/JNEUROSCI.3697-05.2005.
63. Hladky, S.B., and Barrand, M.A. (2018). Elimination of substances from the brain parenchyma: efflux via perivascular pathways and via the blood-brain barrier. Fluids Barriers CNS *15*, 30. https://doi.org/10.1186/s12987-018-0113-6.
64. Carare, R.O., Teeling, J.L., Hawkes, C.A., Püntener, U., Weller, R.O., Nicoll, J.A.R., and Perry, V.H. (2013). Immune complex formation impairs the elimination of solutes from the brain: implications for immunotherapy in Alzheimer's disease. Acta Neuropathol. Commun. *1*, 48. https://doi.org/10.1186/2051-5960-1-48.
65. Zhang, Y., and Pardridge, W.M. (2001). Mediated efflux of IgG molecules from brain to blood across the blood-brain barrier. J. Neuroimmunol. *114*, 168–172. https://doi.org/10.1016/s0165-5728(01)00242-9.
66. Kim, H., Robinson, S.B., and Csaky, K.G. (2009). FcRn receptor-mediated pharmacokinetics of therapeutic IgG in the eye. Mol. Vis. *15*, 2803–2812.
67. Sumbria, R.K., Hui, E.K.W., Lu, J.Z., Boado, R.J., and Pardridge, W.M. (2013). Disaggregation of amyloid plaque in brain of Alzheimer's disease transgenic mice with daily subcutaneous administration of a tetravalent bispecific antibody that targets the transferrin receptor and the Abeta amyloid peptide. Mol. Pharm. *10*, 3507–3513. https://doi.org/10.1021/mp400348n.
68. Boado, R.J., Zhang, Y., Zhang, Y., Xia, C.F., and Pardridge, W.M. (2007). Fusion antibody for Alzheimer's disease with bidirectional transport across the blood-brain barrier and abeta fibril disaggregation. Bioconjugate Chem. *18*, 447–455. https://doi.org/10.1021/bc060349x.
69. Cooper, P.R., Ciambrone, G.J., Kliwinski, C.M., Maze, E., Johnson, L., Li, Q., Feng, Y., and Hornby, P.J. (2013). Efflux of monoclonal antibodies from rat brain by neonatal Fc receptor. Brain Res. *1534*, 13–21. https://doi.org/10.1016/j.brainres.2013.08.035.
70. Garg, A., and Balthasar, J.P. (2009). Investigation of the influence of FcRn on the distribution of IgG to the brain. AAPS J. *11*, 553–557. https://doi.org/10.1208/s12248-009-9129-9.
71. Abuqayyas, L., and Balthasar, J.P. (2013). Investigation of the role of FcgammaR and FcRn in mAb distribution to the brain. Mol. Pharm. *10*, 1505–1513. https://doi.org/10.1021/mp300214k.
72. Fuller, J.P., Stavenhagen, J.B., and Teeling, J.L. (2014). New roles for Fc receptors in neurodegeneration-the impact on Immunotherapy for Alzheimer's Disease. Front. Neurosci. *8*, 235. https://doi.org/10.3389/fnins.2014.00235.
73. Bruhns, P., and Jönsson, F. (2015). Mouse and human FcR effector functions. Immunol. Rev. *268*, 25–51. https://doi.org/10.1111/imr.12350.
74. Chen, N., Wang, W., Fauty, S., Fang, Y., Hamuro, L., Hussain, A., and Prueksaritanont, T. (2014). The effect of the neonatal Fc receptor on human IgG biodistribution in mice. mAbs *6*, 502–508. https://doi.org/10.4161/mabs.27765.
75. Eigenmann, M.J., Fronton, L., Grimm, H.P., Otteneder, M.B., and Krippendorff, B.F. (2017). Quantification of IgG monoclonal antibody clearance in tissues. mAbs *9*, 1007–1015. https://doi.org/10.1080/19420862.2017.1337619.
76. Tien, J., Leonoudakis, D., Petrova, R., Trinh, V., Taura, T., Sengupta, D., Jo, L., Sho, A., Yun, Y., Doan, E., et al. (2023). Modifying antibody-FcRn interactions to increase the transport of antibodies through the blood-brain barrier. mAbs *15*, 2229098. Artn 2229098. https://doi.org/10.1080/19420862.2023.2229098.
77. Finke, J.M., Ayres, K.R., Brisbin, R.P., Hill, H.A., Wing, E.E., and Banks, W.A. (2017). Antibody blood-brain barrier efflux is modulated by glycan modification. Biochim. Biophys. Acta Gen. Subj. *1861*, 2228–2239. https://doi.org/10.1016/j.bbagen.2017.06.008.
78. Wada, R., Matsui, M., and Kawasaki, N. (2019). Influence of N-glycosylation on effector functions and thermal stability of glycoengineered IgG1 monoclonal antibody with homogeneous glycoforms. mAbs *11*, 350–372. https://doi.org/10.1080/19420862.2018.1551044.
79. Kuo, T.T., de Muinck, E.J., Claypool, S.M., Yoshida, M., Nagaishi, T., Aveson, V.G., Lencer, W.I., and Blumberg, R.S. (2009). N-Glycan Moieties in Neonatal Fc Receptor Determine Steady-state Membrane Distribution and Directional Transport of IgG. J. Biol. Chem. *284*, 8292–8300. https://doi.org/10.1074/jbc.M805877200.
80. Proulx, D.P., Rouleau, P., Paré, I., Vallières-Noël, M.M., and Bazin, R. (2012). Interaction between intravenous immunoglobulin (IVIg) and the low-density lipoprotein receptor-related protein 1: a role for transcytosis across the blood brain barrier? J. Neuroimmunol. *251*, 39–44. https://doi.org/10.1016/j.jneuroim.2012.06.009.
81. Banks, W.A., Terrell, B., Farr, S.A., Robinson, S.M., Nonaka, N., and Morley, J.E. (2002). Passage of amyloid beta protein antibody across the blood-brain barrier in a mouse model of Alzheimer's disease. Peptides *23*, 2223–2226. https://doi.org/10.1016/s0196-9781(02)00261-9.
82. Strazielle, N., and Ghersi-Egea, J.F. (2013). Physiology of blood-brain interfaces in relation to brain disposition of small compounds and macromolecules. Mol. Pharm. *10*, 1473–1491. https://doi.org/10.1021/mp300518e.
83. Ruano-Salguero, J.S., and Lee, K.H. (2020). Antibody transcytosis across brain endothelial-like cells occurs nonspecifically and independent of FcRn. Sci. Rep. *10*, 3685. https://doi.org/10.1038/s41598-020-60438-z.
84. Noguchi, Y., Kato, M., Ozeki, K., and Ishigai, M. (2017). Pharmacokinetics of an intracerebroventricularly administered antibody in rats. mAbs *9*, 1210–1215. https://doi.org/10.1080/19420862.2017.1345834.
85. Bergman, I., Burckart, G.J., Pohl, C.R., Venkataramanan, R., Barmada, M.A., Griffin, J.A., and Cheung, N.K. (1998). Pharmacokinetics of IgG and IgM anti-ganglioside antibodies in rats and monkeys after intrathecal administration. J. Pharmacol. Exp. Therapeut. *284*, 111–115.
86. Boulton, M., Young, A., Hay, J., Armstrong, D., Flessner, M., Schwartz, M., and Johnston, M. (1996). Drainage of CSF through lymphatic pathways and arachnoid villi in sheep: measurement of 125I-albumin clearance. Neuropathol. Appl. Neurobiol. *22*, 325–333. https://doi.org/10.1111/j.1365-2990.1996.tb01111.x.
87. Ma, Q., Ineichen, B.V., Detmar, M., and Proulx, S.T. (2017). Outflow of cerebrospinal fluid is predominantly through lymphatic vessels and is reduced in aged mice. Nat. Commun. *8*, 1434. https://doi.org/10.1038/s41467-017-01484-6.
88. Magdoom, K.N., Brown, A., Rey, J., Mareci, T.H., King, M.A., and Sarntinoranont, M. (2019). MRI of Whole Rat Brain Perivascular Network Reveals Role for Ventricles in Brain Waste Clearance. Sci. Rep. *9*, 11480. https://doi.org/10.1038/s41598-019-44938-1.
89. Bedussi, B., van Lier, M.G.J.T.B., Bartstra, J.W., de Vos, J., Siebes, M., VanBavel, E., and Bakker, E.N.T.P. (2015). Clearance from the mouse brain by convection of interstitial fluid towards the ventricular system. Fluids Barriers CNS *12*, 23. https://doi.org/10.1186/s12987-015-0019-5.
90. Barua, N.U., Bienemann, A.S., Hesketh, S., Wyatt, M.J., Castrique, E., Love, S., and Gill, S.S. (2012). Intrastriatal convection-enhanced delivery results in widespread perivascular distribution in a pre-clinical model. Fluids Barriers CNS *9*, 2. https://doi.org/10.1186/2045-8118-9-2.
91. Cserr, H.F., Cooper, D.N., Suri, P.K., and Patlak, C.S. (1981). Efflux of radiolabeled polyethylene glycols and albumin from rat brain. Am. J. Physiol. *240*, F319–F328. https://doi.org/10.1152/ajprenal.1981.240.4.F319.
92. Hornby, P.J., Cooper, P.R., Kliwinski, C., Ragwan, E., Mabus, J.R., Harman, B., Thompson, S., Kauffman, A.L., Yan, Z., Tam, S.H., et al. (2014). Human and non-human

primate intestinal FcRn expression and immunoglobulin G transcytosis. Pharm. Res. (N. Y.) *31*, 908–922. https://doi.org/10.1007/s11095-013-1212-3.

93. Schoch, A., Kettenberger, H., Mundigl, O., Winter, G., Engert, J., Heinrich, J., and Emrich, T. (2015). Charge-mediated influence of the antibody variable domain on FcRn-dependent pharmacokinetics. Proc. Natl. Acad. Sci. USA *112*, 5997–6002. https://doi.org/10.1073/pnas.1408766112.
94. Hayes, J.M., Cosgrave, E.F.J., Struwe, W.B., Wormald, M., Davey, G.P., Jefferis, R., and Rudd, P.M. (2014). Glycosylation and Fc receptors. Curr. Top. Microbiol. Immunol. *382*, 165–199. https://doi.org/10.1007/978-3-319-07911-0_8.
95. Bard, F., Fox, M., Friedrich, S., Seubert, P., Schenk, D., Kinney, G.G., and Yednock, T. (2012). Sustained levels of antibodies against Abeta in amyloid-rich regions of the CNS following intravenous dosing in human APP transgenic mice. Exp. Neurol. *238*, 38–43. https://doi.org/10.1016/j.expneurol.2012.07.022.
96. Spaargaren, M., Defize, L.H., Boonstra, J., and de Laat, S.W. (1991). Antibody-induced dimerization activates the epidermal growth factor receptor tyrosine kinase. J. Biol. Chem. *266*, 1733–1739.
97. Opaliński, Ł., Sokołowska-Wędzina, A., Szczepara, M., Zakrzewska, M., and Otlewski, J. (2017). Antibody-induced dimerization of FGFR1 promotes receptor endocytosis independently of its kinase activity. Sci. Rep. *7*, 7121. https://doi.org/10.1038/s41598-017-07479-z.