BRAIN
REVIEW ARTICLE

Editing a gateway for cell therapy across the blood–brain barrier

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Stem cell therapy has been shown to improve stroke outcomes in animal models and is currently advancing towards clinical practice. However, uncertainty remains regarding the optimal route for cell delivery to the injured brain. Local intracerebral injections are effective in precisely delivering cells into the stroke cavity but carry the risk of damaging adjacent healthy tissue. Systemic endovascular injections, meanwhile, are minimally invasive, but most injected cells do not cross CNS barriers and become mechanically trapped in peripheral organs. Although the blood–brain barrier and the blood–CSF barrier tightly limit the entrance of cells and molecules into the brain parenchyma, immune cells can cross these barriers especially under pathological conditions, such as stroke. Deciphering the cell surface signature and the molecular mechanisms underlying this pathophysiological process holds promise for improving the targeted delivery of systemic injected cells to the injured brain. In this review, we describe experimental approaches that have already been developed in which (i) cells are either engineered to express cell surface proteins mimicking infiltrating immune cells; or (ii) cell grafts are preconditioned with hypoxia or incubated with pharmacological agents or cytokines. Modified cell grafts can be complemented with strategies to temporarily increase the permeability of the blood–brain barrier. Although these approaches could significantly enhance homing of stem cells into the injured brain, cell entrapment in off-target organs remains a non-negligible risk. Recent developments in safety-switch systems, which enable the precise elimination of transplanted cells on the administration of a drug, represent a promising strategy for selectively removing stem cells stuck in untargeted organs. In sum, the techniques described in this review hold great potential to substantially improve efficacy and safety of future cell therapies in stroke and may be relevant to other brain diseases.

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Introduction

The average human brain is composed of 86 billion neurons and 85 billion glia—indispensable cells that require a special protective environment to ensure proper brain function.[1] This protection is guaranteed by a 650-km (400-mile) network of specialized blood vasculature designed to selectively restrict the entry of potentially toxic substances into the brain.[2] This blood–brain barrier (BBB) is fundamental in maintaining homeostasis and physiological brain function, with BBB disruption being a hallmark of most major neurological disorders.[3] Unfortunately, the BBB is, at the same time, a barrier for most systemically applied drugs. An intact BBB is impermeable for 98% of all small molecule drugs and for nearly all large substances, such as peptides, antibodies or gene- and cell-based therapeutics.[4]

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Therefore, many research laboratories are developing diverse strategies to improve therapeutic drug delivery to the brain, ranging from targeted ultrasound and alteration of administration routes to biochemical or genetic modifications. For instance, various drug delivery systems have been tested to transport small molecules across the BBB based on nanoparticles, such as liposomes or cyclodextrins. For larger molecules, such as antibodies, the brain shuttle technology has been recently developed and is currently being clinically tested for Alzheimer's disease to enable efficient crossing of the BBB by binding the transferrin receptor.[5] By contrast, the delivery of cells to the brain has been considerably less explored, even though cell therapy holds great promise to restore damaged neural networks and become a promising regenerative strategy for acute neurological injuries, such as stroke. In total, more than 80 clinical trials in the field of cell therapy for stroke have already been conducted or are currently ongoing. Although the initial clinical results have demonstrated the safety of cell therapies, further validating their translational potential, promising results from preclinical animal research have not been confirmed beyond doubt in a clinical setting.

The reasons for this limited translational success are multifaceted. For example, it is poorly understood which is the most promising cell source for transplantation. Some clinical trials have used primary, non-neuronal cells, such as mesenchymal stem cells (MSCs), which can be obtained from various tissues, such as bone marrow (BM, BD-MSCs), adipose tissue (AD-MSCs) or umbilical cord blood (UCB-MSCs), while other experimental settings have favoured the use of neural cells, including neural stem/progenitor cells (NSCs/NPCs). With advances in reprogramming technology, the use of induced pluripotent stem cell (iPSC)–derived NPCs is gaining more popularity, but this approach is not yet in the clinical phase for stroke therapy. An overview of the cell sources used in cell therapies for stroke is provided in Table 1. In addition to the cell source itself, the delivery of transplants to injured brain regions has been recognized as a major hurdle by experts in the field.[6] Despite the great promise of cell-based therapies for stroke, there is currently no clinical 'gold standard' for how to deliver cells most efficiently to injured brain regions.

In this review, we discuss promising approaches by focusing on the newest strategies in cell delivery and cellular engineering that may shape a new generation of cell therapies capable of reaching injured brain regions more efficiently, thus constituting improved future strategies for stroke therapy.

Delivery routes for the cell graft to the injured brain

There are several feasible routes for delivering a cellular graft to the recipient of a cell therapy. The most commonly used routes for cell delivery in animal models and in humans are the intracerebral (IC), intracerebroventricular (ICV), intrathecal (IT), intra-arterial (IA), intravenous (IV) and intranasal (IN) deliveries (Fig. 1). Although intraperitoneal and subcutaneous injections are the preferred routes for drug administration in rodent models, their use for cell delivery is not effective enough and is even less so in humans[13]; therefore, we will not consider these routes in this review. Each route of cell delivery has its advantages and disadvantages in terms of efficacy and safety—a careful weighing of these factors is essential in developing successful cell therapy (Table 2).

Intracerebral transplantation

IC delivery enables the precise injection of cells into the damaged brain parenchyma, which is the main advantage of this route. This route entails the smallest injection volume, which may prompt the need for multiple injections to reach the therapeutic threshold (~10^6 cells). A comparative preclinical study showed that an IC NPC injection resulted in a higher number of transplanted cells in the ischaemic brain compared to ICV and IV administration.[15] However, it remains unclear to what extent the graft migrates and spreads throughout the whole lesion. Some preclinical studies have shown that ESC, BM-MSC and NSC have the potential to migrate to the damaged brain regions.[22–26] At the same time, other authors have suggested that ESC-derived NPCs and oligodendrocyte precursor cells injected IC migrated only short distances[27,28] or only towards the peri-infarct region rather than to the stroke core when ESCs, NPCs or BM-MSCs were injected.[25,29,30] A large body of research confirms that local IC transplantation of BM-MSCs or NSCs improved functional recovery and decreased infarct volume of stroked rodents.[15,23,31,32] However, the stereotaxic IC injection is an invasive procedure that can be challenging or even impossible to perform depending on the stroke location. This technique also carries the risk of damaging adjacent healthy tissue and causing unexpected adverse events. Although some studies have demonstrated a good safety profile,[33,34] other clinical trials have revealed adverse effects related to surgical procedures, including headache, partial seizures and subdural haematoma.[35–38] Regarding efficacy of cell therapies, some studies demonstrated improved recovery in at least one of the functional scores compared to the baseline when BM-MSCs, NSCs and $CD34^+$ cells were locally transplanted in subacute or chronic stroke patients (Table 3 and Supplementary Table 1).[34,36–38] However, a major limitation in reliably assessing efficacy is the absence of a placebo-controlled group in IC cell therapies due to the invasiveness of the procedure. Additionally, IC injections cannot be carried out in acute stroke patients due to the associated risks with the procedure, which limits the therapeutic application of IC cell transplantations to the subacute and chronic stroke phase.

Intracerebroventricularand intrathecal transplantation

In rodents, cells can enter the CSF through ICV transplantation via the ventricles (fourth and lateral), the subdural space, the cisterna magna (subarachnoid space) or by IT administration.[64–68] The most common way of accessing the CSF in humans is through the IT route by lumbar puncture, but ICV administration has also been performed. Compared to direct parenchymal delivery, ICV cell injection is less invasive and allows for a higher cell injection volume. Furthermore, this route entails higher levels of grafted cells being delivered into the ischaemic brain compared to systemic IV engraftment.[15,68] Stem cells are widely distributed throughout the brain depending on the fluidity of the CSF.[69] NPCs grafted by ICV injections migrated post-stroke to the damaged tissue in a middle cerebral artery occlusion (MCAO) mouse model.[15] The injection of UCB-MSCs and iPSCs into the CSF can improve motor functions and reduce lesion size after cerebral ischaemia.[66,68] Administered cells also exhibit an indirect effect by activating endogenous NSCs and oligodendrocyte progenitors localized at the subventricular zone.[65,70] Conversely, the effect of injected cells is considered insufficient in the damaged areas further away from the ventricles.[14] Furthermore, NSC ICV injection showed no beneficial

Table 1 Stem cells used for treating stroke

Cell type	ESC	Primary NSC	iPSC	iPSC- NPC	DPSC	MSC	MNC (=MSC + HSC)
Cell source[7]	Embryonic tissue	Embryo or foetal tissue	Diverse, often fibroblasts	Diverse, often fibroblasts	Dental Pulp	BM-MSC AD-MSC UCB-MSC	BM, cord blood, peripheric blood
Differentiation potential[8]	++	+	++	+	+	+	+
Low risk of oncogenesis[7,9,10]	–	+	–	+	+	+	+
In vitro expansion[9,11]	++	+	++	+	++	+	++
Immunotolerance[7]	–	–	–	–	+	++	–
Reduction of infarct volume[7,11,12]	+	++	+	++	+	++	++
Improved functional recovery[7,11,12]	+	++	+	++	++	++	++
Anti-inflammatory factor secretion[7,11,12]	+	+	+	+	++	++	++
Trophic factor secretion[7,11,12]	+	+	+	++	++	++	++
Cell replacement[7,11,12]	+	++	+	++	++	–	–

ESC = embryonic stem cell; DPSC = dental pulp stem cells; HSC = haematopoietic stem cells. (++) Advantage described in >60% of the studies; (+) Advantage described in <60% of the studies; (–) Absent advantage or disadvantage.

outcomes in improving the sensorimotor function of stroked rats, whereas NSC IC transplantation resulted in a significant improvement.[71] A pilot clinical study on treating stroke with IT injection of autologous $CD34^+$ cells improved National Institutes of Health Stroke Scale (NIHSS) and Barthel index from baseline, without reporting adverse events.[41] Another clinical trial on ischaemic and haemorrhagic stroke determined that IT injection of bone marrow-mononuclear cells (BM-MNCs) is safe and leads to a better functional recovery in ischaemic stroke patients.[39] ICV administration of autologous BM-MSCs improved NIHSS from baseline in some haemorrhagic stroke patients (Table 3 and Supplementary Table 1).[40] However, all these studies lack a control cohort which is crucial for determining efficacy.

Intra-arterial injection

Cells can enter arterial circulation through the common carotid artery in mice or the external carotid artery in rats.[72] In humans, cells are delivered to the middle cerebral artery through cannulation entering via the femoral artery.[47] Endovascular administration allows at least a seven-times higher injection volume compared to IC graft.[73] In a rat model, the percentage of NPCs found in the ischaemic brain following IA injection was relatively low (1–10% of injected cells).[16] NPCs and UCB-MSCs IA injected into experimentally stroked rats presented a broader distribution throughout the lesion compared to cells grafted into the cisterna magna, IC or IV.[16,67] The IA route circumvents the venous circulation that entails mechanical entrapment in peripheric organs[74]; this advantage is of most interest when stem cells present a substantial adhesion capacity.[75] Higher levels of transplanted cells in the ischaemic brain via the IA route compared to IV administration were correlated with better neurological recovery in animal models.[76,77] Furthermore, IA Injection of BM-MSCs and BM-MNCs after rodent MCAO led to better outcomes in various behavioural tests, such as the rotarod, a standard test for assessing neuromuscular coordination after stroke in a rodent.[16,77–79] In some preclinical studies, IA cell injection has been associated with complications, such as reduction of cerebral blood flow, microembolisms or even death,[67,80,81] while other studies have not reported any adverse effects.[77,78,82] More recently, procedural refinements of IA cell injections, such as replacing microcatheters by microneedles, have been found to improve the safety of the procedure.[80] Cell size, cell dose and cell infusion velocity should all be considered for reducing the number of micro-embolisms and Lacunar strokes that may occur when cells are injected via the IA route.[19,81,83] Clinical trials using BM-MNCs for treating acute stroke have reported two cases of partial seizures and some moderate adverse events, e.g. urinary infection and pneumonia following IA injection.[45,46] Nevertheless, the IA injection of BM-MNCs for treating subacute stroke has been reported to be safe in other clinical trials, which highlights the importance of determining the optimal timing for cell engraftment.[43,44] Regarding efficiency, the IA administration of $CD34^+$ HSCs or BM-MNCs significantly improved NIHSS, modified ranking scale and/or Barthel index from baseline.[46,47] Nevertheless, the inclusion of a control arm, determined that although there is a trend to better improvement in patients treated with BM-MNCs, there are no significant differences compared to control cohort (Table 3 and Supplementary Table 1).[43–45] A biodistribution clinical study determined that IA administration of BM-MNCs resulted in high cell counts in liver and spleen, but relatively low cell number in the brain, which might explain the mixed results in clinical trials.[84]

Intravenous injection

IV injection in rodents is usually performed via the tail vein, but the femoral vein can also be used.[85] In patients, IV administration is usually performed via the three main veins of the antecubital fossa on the anterior surface of the elbow joint. The main drawback in IV delivery is the increased entrapment of injected cells in peripheral organs, mainly in the lungs, liver and spleen.[17,86] This downside can also be considered an advantage due to the anti-inflammatory effect observed in the spleen following IV NSC injection.[17] Due to cell entrapment and brain-barrier impediment, only 1% of injected cells arrive at the ischaemic brain.[18] Therefore, systemic IA and IV administration requires a higher cell dose (~10^8 cells)than the IC route to reach the therapeutic threshold, which entails higher costs and potentially more side effects.[87] In rodents, the cells grafted using this route are distributed throughout the brain parenchyma and can migrate to the damaged area.[88–92] Despite the low number of grafted cells in the lesion, IV injections of BM-MSCs or NSCs have been shown to promote functional recovery post-stroke in rodents,[88,92–95]

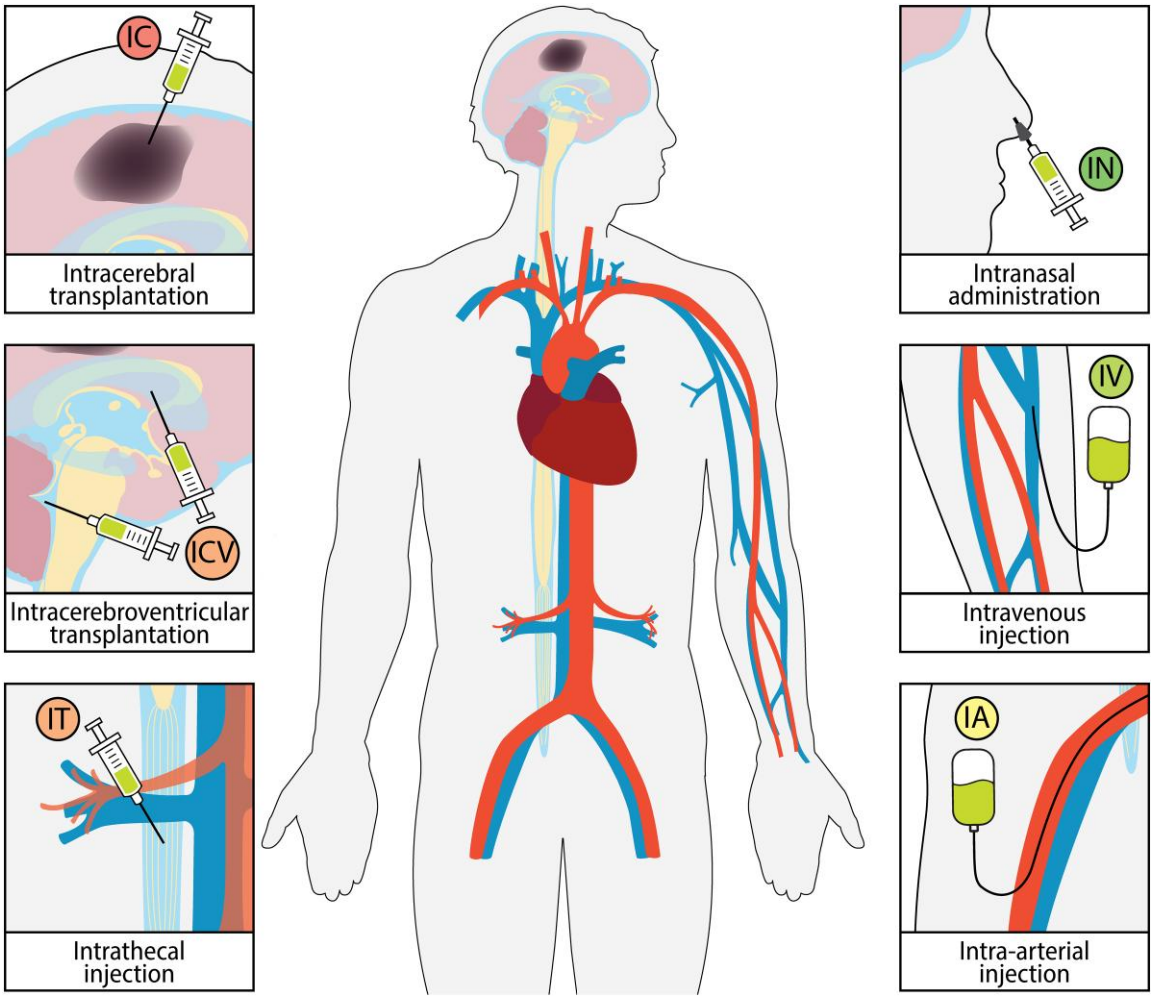

Figure 1 Stem cell administration routes for treating stroke in clinics. Cell grafts can be transplanted into the ischaemic area (IC) or CSF via the ventricles (ICV) or IT injection. Stem cells can also be injected systematically through the IA or IV routes, or cells can be administered IN.

Table 2 Pros and cons of stem cell delivery routes for treating stroke

Route of administrtion	Advantages	Disadvantages
IC	Precise graft placement[14] High levels of grafted cells at the lesion[15]	Poor cell distribution throughout the lesion[14] Small volume of injected cells[16] Adverse events in clinical trials (Supplementary Table 1)
Intra-cerebroventricular, ICV, IT	Cell distribution throughout different parts of the CNS[14]	Mainly for lesions close to ventricles[14]
Intravascular	Good cell distribution throughout the lesion[16,17]	1–10% of grafted cells in the lesion[16,18]
IA	Immunomodulation via spleen (IV)[17]	Adverse events in preclinical studies (IA)[19]
IV	Big volume of injected cells[16] Safe in clinical trials (Supplementary Table 1)	Cell entrapment in lungs, liver and spleen[17]
IN	Bypass BBB[20] No entrapment in other organs[20]	Only preclinical trials[21]

which may be at least partially explained by the secretion of immunomodulatory or trophic factors.[18,94] In clinical settings, the IV route is the most preferred one and has been described as a feasible and safe technique in several clinical trials.[49–51,55–57,59–63] IV injections of BM-MSCs or BM-MNCs for treating subacute or chronic stroke have shown mixed results.[50,54,56,57,59,60] However, in other studies, the IV injection of adipose-derived-MSCs, BM-MSCs or BM-MNCs for treating acute, subacute or chronic stroke have not achieved significant improvement in any of the studied functional outcome scores compared to the control cohort.[48,49,51,52,55] A biodistribution clinical study determined that IV administration of BM-MNCs resulted in lung entrapment and low cell counts in brain which might explain the failure observed in clinical trials (Table 3 and Supplementary Table 1).[84] However, the determination of the optimal cell type, cell

Table 3 Resume of clinical trials using stem cells for treating stroke in the last 10 years

Route	Cell type	Controlled study	Stroke phase
IC	BM-MSC[33,36,37]	No	Subacute
	$CD34^+$ cells[34]	Yes	Chronic
	NSC[35,38]	No	Subacute/chronic
ICV, IT	BM-MNC[39]	No	Subacute/chronic
	BM-MSC[40]	No	Chronic (haemorrhagic)
	$CD34^+$ cells[41]	No	Chronic
IA	BM-ALD401[42]	Yes	Subacute
	BM-MNC[43–45]	Yes	Acute/subacute
	BM-MNC[46]	No	Acute
	$CD34^+$ HSC[47]	No	Acute
IV	AD-MSC[48]	Yes	Acute/subacute
	BM-MNC[49–51]	Yes	Subacute/chronic
	BM-MNC[52,53]	No	Acute/subacute
	BM-MSC[54–57]	Yes	Acute/subacute
	BM-MSC[58–60]	No/Yes	Chronic
	BM-MSC or EPC[61]	Yes	Subacute
	BM multipotent cell[62]	Yes	Acute
	UCB cells[63]	No	Acute/subacute

BM-ALD401 = BM-derived aldehyde dehydrogenase bright stem cells; EPC = endothelial progenitor cells; HSC = haematopoietic stem cells. Stroke phase: acute <7 days; subacute 7 days to 6 months; chronic > 6 months.

dose and injection timing are also crucial for improving the recovery of stroke patients following endovascular cell administration.

Intranasal administration

The pathways and mechanisms underlying cell migration to the brain parenchyma after IN administration are not fully understood. IN-administered cells migrate from nasal mucosa through the cribriform plate into the olfactory bulb and other brain regions or go into the CSF to eventually enter the brain parenchyma.[20] Cells can also enter the peripheral trigeminal system spread throughout the nasal epithelium, then migrate again to the CSF or to the brainstem and the spinal cord.[96] IN delivery is the least invasive route, but it entails low injection volumes (<20 µl in mice).[73] It has also been demonstrated that IN administration enables a faster homing of BM-MSC (1.5 h) compared to intravascular delivery (from 24 h to 10 days).[97] Moreover, IN administration enables the homing of MSCs towards the ischaemic area[98] and improves functional recovery after stroke.[97,99] However, the IN route has not yet been studied as thoroughly as the other routes and requires further investigation to determine key parameters, such as the optimal dose, timing and adverse events. IN cell grafts have only been performed in rodents, which possess a more developed olfactory system than humans.[21] Thus, a possible translation into clinical practice is still far away.

Overall, all current routes of cell delivery to the brain have advantages and disadvantages. Contemporary strategies must balance the potential effectiveness of cell therapy against the procedure's invasiveness and safety risks for stroke patients. BBB is the major obstacle to non-invasive and endovascular routes of applications, such as IV cell delivery. Recent studies on BBB function and advances in cellular engineering hold significant potential for enabling the modification of graft properties, which would facilitate the development of targeted delivery to injured brain regions, thus improving the effectiveness of minimally invasive cell therapies.

Cell trafficking across the barriers of the CNS

To develop an effective cell therapy capable of overcoming the barriers of the CNS, a promising strategy is to outline the molecular mechanism underlying the physiological cell trafficking of, for example, peripheral immune cells. The cell surface signature and molecular pathways can be used as a template for improving the migratory abilities of therapeutic cells. Depending on the selected route for delivering cells, there are different obstacles to reaching the ischaemic area. IC-injected cells can be delivered directly to the damaged area, whereas ICV and IN require a further step in migration from the CSF towards the injury site. Cells injected into the systemic blood circulation (IA and IV) must overcome the additional hurdle of brain barriers, which include the BBB and the brain–CSF barrier (BCSFB) located at the choroid plexus.[100] Although the brain parenchyma was considered to be immune privileged,[101] studies have demonstrated that immune cells such as neutrophils, monocytes and T cells can migrate across the vascular endothelial monolayer and, under pathological conditions such as stroke, can enter the CNS parenchyma.[102–105]

Immune cell trafficking across an intact blood–brain barrier

The extravasation of immune cells from the bloodstream into target tissues (including the brain) depends on the orchestrated interaction of circulating leucocytes (e.g. neutrophils and monocytes) with vascular endothelial cells (ECs). This process, first described 30 years ago,[106] starts with (i) the capture and rolling of circulating leucocytes along the EC surface and is followed by (ii) the integrin activation in leucocytes necessary for (iii) the subsequent leucocyte arrest and crawling, which leads to (iv) leucocyte diapedesis across the EC monolayer, before the eventual leucocyte migration through the extracellular matrix located between the blood vessels and the parenchyma (Fig. 2).

The capture and rolling of leucocytes

The first step is regulated by the transient interaction between selectins and glycoproteins. P-selectin (expressed on an activated platelet and EC) and E-selectin (expressed on an activated EC) interact with P-selectin glycoprotein ligand-1 (PSGL-1) containing the tetrasaccharide sialyl Lewis × (sLe^x), which is the minimal glycan determinant for selectin interaction.[107] The molecules involved also depend on cell type and inflammatory status—for example, in a mouse model of multiple sclerosis (a CNS inflammatory disease), leucocyte rolling was also mediated by endothelial α_4 integrins.[108]

Integrin activation in leucocytes

The main actors in the second phase are chemokines, which can be secreted by ECs, especially during the inflammatory response. Damaged parenchymal cells can also secrete chemokines, which can directly diffuse or be transcytosed by ECs into the bloodstream.[109] The chemokine family is classified into four subfamilies (CXC, CC, C and CX_3C)[110] and their receptors are G-coupled protein receptors located at leucocyte surfaces.[111] The binding of chemokines to their receptors starts a intracellular signalling cascade in leukocytes that results in the conformational change of integrins.[112] The activation of leukocytic integrins via chemokine signalling is necessary for the subsequent step of the extravasation process.

Leucocyte arrest and crawling

Integrin activation in leucocytes is necessary for their interaction with immunoglobulin gene superfamily (IgSF) molecules present in ECs. There are four main integrins involved in leucocyte arrest: lymphocyte function-associated antigen 1 (LFA-1, CD11a/CD18, $\alpha_L\beta_2$-integrin), macrophage-1 antigen (Mac1, CD11b/CD18, $\alpha_M\beta_2$-integrin), very late antigen-4 (VLA-4, CD49d/CD29, $\alpha_4\beta_1$-integrin) and lymphocyte Peyer's patch adhesion molecule-1 (LPAM-1, $\alpha_4\beta_7$-integrin). LFA-1 interacts with intercellular adhesion molecule-1 (ICAM-1), ICAM-2 and ICAM-3; Mac-1 binds to ICAM-1; VLA-4 interacts with vascular cell adhesion molecule-1 (VCAM-1); and LPAM-1 links VCAM-1 and mucosal addressin cell adhesion molecule-1 (MadCAM-1).[113] The link between leucocytic activated integrins and endothelial IgSF molecules results in a firm arrest of immune cells. Following this step, through a process known as crawling, leucocytes can laterally move towards the nearest junction or towards a permissive site of transmigration.[114] In the initial characterizations, researchers thought that Mac-1, LFA-1 and their respective counter-receptors (ICAM-1 and ICAM-2) were involved in this process.[114] However, a subsequent study suggested that LFA-1 is mainly involved in firm adhesion, whereas crawling mainly depends on the interaction of Mac-1 with ICAM-1.[115]

Leucocyte diapedesis

Before leucocyte diapedesis, an ICAM-1 cluster forms on the EC membrane surrounding the leukocytic filopodia-like structures.[116] Diapedesis can occur in two ways: across ECs (transcellular) or between ECs (paracellular). Transcellular diapedesis is thought to be preferable when cell junctions are very tight—for example, at the BBB.[117] This pathway requires the homophilic interaction of platelet EC adhesion molecule-1 (PECAM-1, CD31) and CD99 between leucocytes and ECs.[116] Junctional adhesion molecule A (JAM-A, JAM-1) is also necessary for transcellular migration, but its role remains controversial.[118] As for the paracellular diapedesis, the IgSF–integrin interaction induces the loosening of the adherent junctions.[116] In addition to the already mentioned PECAM-1, CD99 and JAM-A, other molecules, such as ICAM-2, VCAM-1 and JAM-C, are involved in paracellular transmigration.[116]

Leucocyte migration through the extracellular matrix towards the brain parenchyma

After crossing the EC monolayer, leucocytes must cross the basement membrane, a highly cross-linked sheet of extracellular matrix composed of collagen IV, laminin, nidogen and perlecan, to reach the target tissue. Except for the capillaries, all CNS blood vessels have the following two different basement membranes: (i) the endothelial basement membrane in contact with ECs; and (ii) the parenchymal basement membrane in contact with the endfeet of astrocytes, which delimitates a CSF-drained perivascular space in between. As immune cell trafficking through the BBB occurs at post-capillary venules, following vascular monolayer extravasation, immune cells enter the perivascular space[119] where they seek permissive areas. For example, T cells migrate across endothelial BM through areas containing laminin-α4 and low laminin-α5 levels via α6β1-integrin.[120] Once in the perivascular space, immune cells interact with resident antigen-presenting cells before traversing the parenchymal basement membrane.[121] In physiological conditions, there is no antigen recognition and, thus, immune cells undergo apoptosis or are drained out of the CNS due to the connection of the perivascular and leptomeningeal/subarachnoidal space with the cervical lymph node or via the glymphatic system.[122] However, after antigen recognition, leukocytes can secrete matrix metalloproteinases (MMPs) such as MMP2 and MMP-9 to break down the parenchymal basement membrane and enter the CNS parenchyma.[123]

Much less is known about leukocytic extravasation through the BCSFB, whose structure differs from that of the BBB. BCSFB presents fenestrated capillaries, followed by a monolayer of tightly joined epithelial cells. Choroid plexus vessels constitutively display E- and P-selectin,[124] and epithelial cells constitutively express ICAM-1 and VCAM-1, but only on the apical surface[125]; therefore, the extravasation molecular pathway remains uncertain. For instance, interleukin 17–producing T helper (T_H-17) cells can go through the BCSFB via the interaction of CCR6 with CCL20, which is constitutively expressed in choroid plexus epithelial cells.[126] This pathway allows the entrance of T_H-17 cells into the uninflamed brain, which eventually leads to a pro-inflammatory state and the entrance of more immune cells via the activation of different pathways.[126] Severe dysregulation of leukocyte migration and brain barriers occurs after major brain injuries, such as stroke. In the acute phase, immune cell extravasation from blood vessels exacerbates the pro-inflammatory environment and increases BBB permeability. This pathological condition could be used to improve the migratory abilities of therapeutic cells.

Immune cell trafficking across barriers in the ischaemic brain

Important hallmarks of ischaemic stroke include a local and systemic inflammatory response and a temporal disruption of the BBB that eventually leads to increased permeability for molecules and immune cells.[127] However, it remains unclear whether the infiltration of immune cells is due to the pro-inflammatory state or the absence of brain barriers, or both.

Post-ischaemic neurons and glial cells secrete damage-associated molecular patterns that activate astrocytes and microglia, which, in turn, secrete cytokines, chemokines and metalloproteinases,[128] similar to the molecule secretion following traumatic brain injury.[129] The main pro-inflammatory cytokines secreted in the acute phase are interleukin-1beta (IL-1β) and tumour necrosis factor-alpha (TNF-α).[130] Another two released pro-inflammatory cytokines are interferon-gamma (IFN-γ) and IL-6, but IL-6 can also present a neuroprotective effect.[128] In addition, high levels of IL-23 activate γδ T cells (an 'unconventional' subtype of T cells) that produce IL-17, which results in increased neutrophil infiltration.[131] At the same time, the secretion of anti-inflammatory cytokines such as IL-4, IL-10, TGF-β and IFN-β by immune cells and microglia may exert a protective effect.[128,130] The classification of cytokines in pro- or anti-inflammatory mediators is a quite simplistic vision for determining their aversive/protective function, as for example, TNF-α present a detrimental role in the acute phase but it is necessary for long-term recovery.[132] The inflammatory environment generated by the microglia, astrocytes and leukocytes can, in turn, activate endothelial and immune cells, increasing the levels of the molecules involved in transmigration.[133]

The capture and rolling of leucocytes after an experimental stroke

After MCAO, E-selectin and P-selectin expression levels are increased in the brain vasculature adjacent to the ischaemic

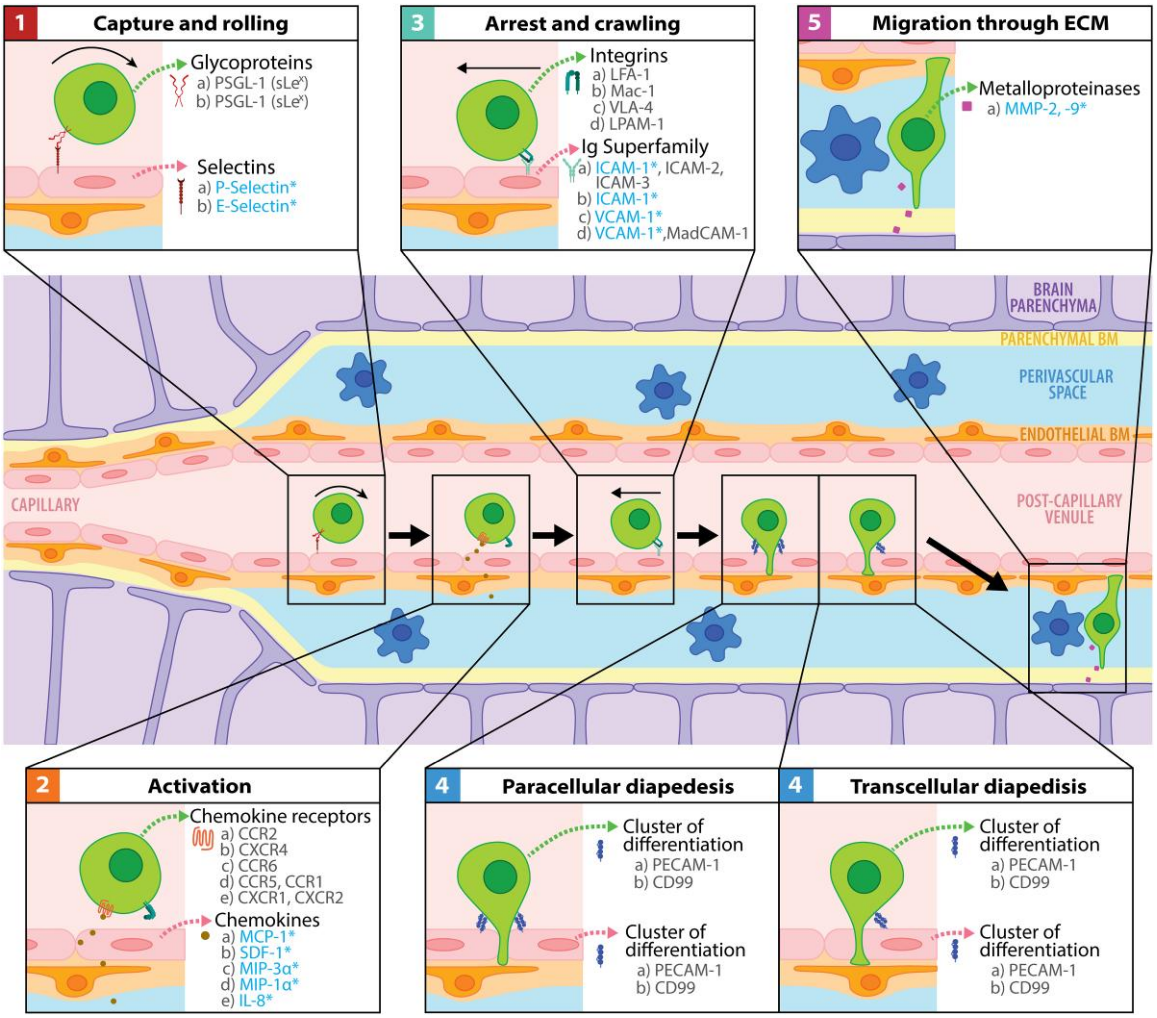

Figure 2 Immune cell transmigration across the BBB. The first step during the transmigration process is known as capture and rolling and is mediated by selectins located in ECs. Second, the interaction between the chemokines and their receptors in immune cells activates the integrins necessary for firm adhesion. Next, diapedesis can occur between ECs (paracellular) or through ECs (transcellular). After interacting with antigen-presenting cells (blue flower-shaped cells), immune cells can secrete metalloproteinases to break the components of the endothelial and parenchymal basement membrane (BM) and eventually enter the brain parenchyma. Upregulated molecules in stroke are indicated with an asterisk in blue. The same letter indicates the interaction partners.

area.[134–139] Following inflammation, P-selectin is rapidly localized at the surface of ECs containing Weibel–Palade bodies. In addition to P-selectin, these granules contain von Willebrand factor, which interacts with PSGL-1 and β_2-integrins, triggering further extravasation steps.[140] Similar pathways are involved in neuroinflammatory diseases such as multiple sclerosis, as in an experimental autoimmune encephalomyelitis mouse model it was demonstrated that interaction between E-/P-selectin and PSGL-1 is crucial for T-cell rolling in inflamed spinal cord, but not for the onset of the disease.[141]

Integrin activation in leucocytes in the ischaemic brain

Chemokines play a key role in the second step (activation) and in the directed migration of cells to the lesion. Monocyte chemoattractant protein-1 (MCP-1, CCL2) is one of the most studied chemokines whose levels increase after ischaemic brain injury.[142–147] The interaction between MCP-1 and its receptor CCR2 leads to a higher permeability of the BBB and a higher leukocyte infiltration,[148–151] which also have an essential role in multiple sclerosis.[152] Other post-ischaemic upregulated molecules are stromal cell-derived factor 1 (SDF-1, CXCL12),[153,154] macrophage inflammatory protein 3α (MIP-3α, CCL20) and MIP-1α (CCL3),[142,143,155,156] whose receptors are CXCR4, CCR6 and CCR5 and CCR1, respectively. After reperfusion, levels of cytokine-induced neutrophil chemoattractant (CINC) were increased, which was associated with neutrophil infiltration.[147,157] CINC is thought to be part of the IL-8 (CXCL8) family, which binds leukocytic CXCR1 and CXCR2[158] and whose mRNA and serum protein levels are higher in patients suffering from ischaemic stroke.[157,159]

Leucocyte arrest and crawling after an experimental stroke

The integrin activation on the leucocyte surface enables their interaction with IgSF proteins, whose expression by ECs is also modified after ischaemic injury. Cytokines, including TNF-α and IL-1,

increase the ICAM-1 and VCAM-1 levels.[160] Following a stroke, the ICAM-1 mRNA and protein levels are increased not only in rodent models but also in primates.[139,161,162] Regarding VCAM-1, after MCAO in rodents, mRNA levels remain unmodified, but protein levels increase, mainly in the ischaemic microvasculature.[135,163] The interaction of integrins with IgSF proteins is an indispensable step, as the blockade of CD49d with a monoclonal antibody (MAb) (Natalizumab) is an approved treatment that prevents the extravasation of immune cells into the CNS in multiple sclerosis.[164]

Leucocyte diapedesis in the ischaemic brain

Little is known about the upregulation of molecules involved in diapedesis following ischaemic brain damage. CD99, PECAM-1 and ICAM-1 were significantly upregulated in an immortalized cell line of endothelial brain cells after oxygen–glucose deprivation.[165] However, depending on the animal strain or the inflammatory model, leukocyte transmigration can occur in a PECAM-1-dependent or a PECAM-1-independent manner (e.g. in the presence of IL-1β or TNF-α, respectively).[166]

Leucocyte migration through the extracellular matrix towards the parenchyma post-stroke

After ischaemic damage, MMP-2 and MMP-9 activation results in increased migration through the basement membranes and in BBB disruption.[167] MMP-9 can be upregulated not only by cytokines (TNF-α and IL-6)[168] but also by a tissue plasminogen activator (the main treatment for removing the blood clot in acute stroke), which has been associated with several complications, such as cerebral oedema and haemorrhagic transformation.[169] Regarding MMP-2, it may play a role in the early opening of the BBB or in a later stage concerning the glial scar formation.[168,170]

Leucocyte migration through the brain parenchyma under pathological conditions

After entering the brain parenchyma, stem cells still have to migrate to the injured area. This process is regulated by cytokines. For instance, the MCP-1/CCR-2 axis may play a crucial role in the macrophage and, to a lesser extent, in neutrophil migration following transient cerebral ischaemia.[171] Astrocytic and endothelial SDF-1α expression following ischaemic brain injury is involved in NSC migration towards the lesion.[154] Another receptor expressed in NSC/NPCs is c-kit, which can direct their migration towards the stem cell factor whose expression is increased in neurons within the injured brain area.[172]

The complex transmigration mechanism is carried out not only by immune cells but also by cancer cells that can escape from the primary tumour and metastasize to the CNS. In addition to the upregulation of certain molecules involved in immune cell extravasation (e.g. VLA-4, MMP-2 and MMP-9),[173,174] three specific proteins, namely cyclooxygenase-2, epidermal growth factor receptor (EGFR) ligand and α2,6-sialyltransferase (ST6GALNAC5), were identified as mediators of BBB transmigration.[175] Deciphering the molecular pathways of cell extravasation into the CNS can be used as a template for improving the migration of stem cells.

Approaches to improving stem cell migration

Stem cells can also pass through brain barriers, but only 1–10% of systemically injected stem cells migrate to the ischaemic brain.[16] Differences in MSC and leukocyte migration have been extensively reviewed.[176] In short, rolling is not required by MSC, crawling patterns are different compared to leucocytes (absence of lateral migration) and the duration of the process is longer in MSC (1–2 h versus 20 min for leucocyte).[176] Depending on the source, type and handling, the stem cells exhibit different sizes and surface-protein patterns, which can influence their migration capacities. For example, BM-MSCs cultured for 24 h *in vitro* and IV injected into sub-lethally irradiated mice presented lower migration capacity to BM and the spleen compared to primary MSCs.[177] Several approaches have been developed to enhance stem cell homing by focusing on increasing the levels of molecules involved in post-ischaemic extravasation through genetic modifications, surface cell engineering, preconditioning or cell selection (Fig. 3). A different approach that does not involve the modification of the transplanted cells is to increase the permeability of the BBB.

Genetic engineering

One of the most widespread techniques for overexpressing proteins is genetic engineering, which enables the introduction of nucleic acids into the cell. Starting with the proteins involved in capture and rolling, primary MSCs have been transfected with a mRNA containing PSGL-1, sLe^x and IL-10.[178] This construct resulted in not only higher cell homing to the inflamed ear but also to immunomodulation via the anti-inflammatory cytokine IL-10.[178] Second, several chemokine receptors have been overexpressed in UCB- and BM-MSC, including CXCR1, CCR2 and CXCR4, using a non-viral, lentiviral or adenoviral vector, respectively.[179–182] Regarding the firm adhesion step, CD49d was cloned into a recombinant adeno-associated virus for achieving a transient expression in BM-MSCs.[183] CD49d dimerized with endogenous CD29 to form VLA-4, resulting in enhanced BM-MSC homing to BM.[183] Furthermore, transfection of VLA-4 into human glial precursor cells induced higher homing to an inflamed brain following IA injection.[184]

Cell membrane engineering

Different techniques have enabled the attachment of extravasation-involved molecules to the surface of stem cells. One approach for enhancing MSC rolling involved the development of biotin-streptavidin-biotin bridges for anchoring selectin ligands to the cell membrane. Free amine groups on the MSC surface were biotinylated with sulfonated biotinyl-*N*-hydroxy-succinimide; afterwards, the cells were incubated with streptavidin and finally with biotinylated sLe^x.[185] This technique was further developed by fusing biotinylated lipid vesicles with MSCs instead of using BNHS[186] or by designing a nanoscale polymer to link biotin to sLe^x, which promoted robust rolling *in vitro* and *in vivo*.[187] Another approach consisted of transfecting the first 19 amino acids of PSGL-1 and the tail of IgG1 into HEK cells overexpressing α1,3-fucosyltransferase (FUT7) to obtain a fusion protein correctly

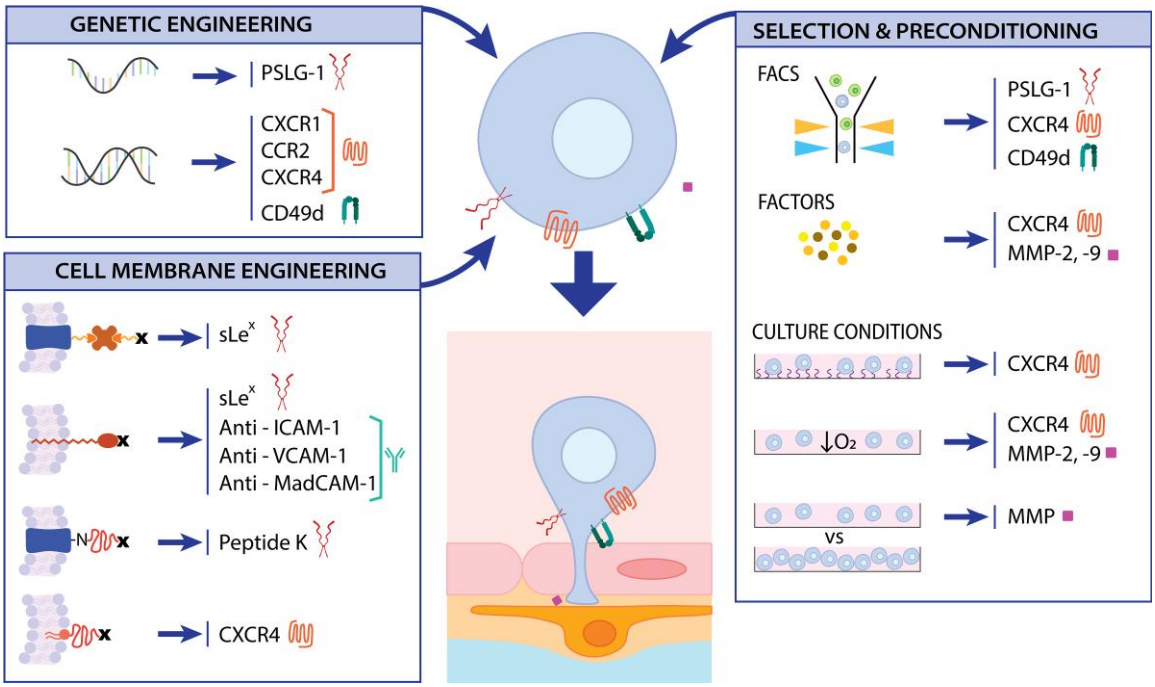

Figure 3 Approaches to enhancing stem cell transmigration to the brain. Stem cells can be modified via genetic or cell membrane engineering to increase the levels of selectin ligands, chemokine receptors, integrins and metalloproteins. These molecules can also be upregulated by preconditioning cells with specific factors or by adjusting culture conditions. These modifications may enable a more efficient migration of cell grafts from the blood circulation to the brain.

glycosylated with sLe^x. Then, palmitated protein G (PPG) was incorporated into the MSC membrane, which enabled the non-covalent coupling of the glycosylated fusion protein, which, in turn, enhanced rolling via selectin interaction.[188] Another technique required reacting NHS-PEG_2-maleimide with amine residues at the MSC surface for anchoring peptides containing N-terminal thiols; this approach was used to anchor peptide K, an E-selectin ligand involved in rolling and capture.[189] Cell membrane engineering was also used to increase these levels of chemokine receptors, which play a key role in integrin activation. Dimyristoyl-sn-glycero-3-phosphoethanolamine was chemically conjugated to CXCR4 to subsequently coat the MSC membrane.[190] PPG was also used for coating an immortalized MSC line (BMC-9) with antibodies against ICAM-1, VCAM-1 and MadCAM-1, which promoted the adhesion and delivery of these modified cells to inflamed tissues.[191,192] Similar to PPG conjugation use to improve the rolling and adhesion steps, all the described techniques can be used to increase the levels of other molecules involved in the extravasation process.

Stem cell selection and cell culture preconditioning

Instead of directly modifying stem cells via genetic or cell surface engineering, stem cells can also be selected according to the presence of molecules involved in the transmigration process. The selection of NSCs expressing sLe^x and CXCR4 via fluorescence activated cell-sorting increased their migration towards the brain previously injected with SDF-1 after IV administration.[193] Furthermore, fluorescence activated cell-sorting selection of $CD94^+$ NSCs allowed higher numbers of NSCs into the ischaemic hemisphere compared to $CD94^-$ NSCs following IA injection, which improved stroke outcomes.[75]

Another alternative is adjusting cell culture conditions or preconditioning stem cells with molecules that activate the intracellular pathways involved in promoting migration. These molecules, such as Toll-like receptor ligands[194] or IL-1β that upregulate CXCR4 in BM-MSCs or UCB-MSCs, respectively, can be involved in the inflammatory response, resulting in an enhanced migration to the inflamed tissue.[195] BM-MSC exposure to a cytokine cocktail composed of stem cell factor, IL-3, IL-6, Flt-3 ligand and hepatocyte growth factor increased CXCR4 expression and thus enhanced their migration to BM after IV injection in a sub-lethally irradiated mouse.[196] Preconditioning or co-injection of Insulin-like growth factor 1 (IGF-1) promoted BM-MSCs homing to injured sites[197,198] and towards SDF-1 via CXCR4 upregulation.[199] BM-MSC exposure to Glycogen synthase kinase 3 beta inhibitors resulted in the upregulation of CXCR4, MMP2 and MT1-MMP.[200] Complement component 1 subcomponent q also promoted SDF-1-directed migration in UCB-MSCs by increasing the expression of CXCR4 and MMP-2.[201] Pre-incubation of MSCs with valproate or lithium upregulated CXCR4 or MMP9, respectively, and resulted in enhanced graft migration to the ischaemic brain following IV injection in an MCAO rat model.[202] A screening method was developed to identify molecules that upregulate the expression of CD11a in MSC to increase the interaction with IgSF molecules in ECs. Ro-31-8425 was chosen as the lead candidate, and when it was used to precondition MSCs, it enhanced their homing to inflamed tissues.[203] NPCs cultured using a hyaluronic acid and laminin hydrogel presented upregulated CXCR4 and an increased *in vivo* migration rate towards exogenous SDF-1α.[204,205] Acidic preconditioning of BM c-kit+ also increased CXCR4 levels and chemotaxis towards SDF-1.[206] Hypoxic preconditioning of BM-MSCs not only increased their survival, proliferative and differentiation rates but also upregulated CXCR4, leading to higher homing to the ischaemic brain.[207] By

contrast, the high culture confluency of BM-MSCs increased tissue inhibitor of metalloproteinase-3 (TIMP-3), which reduces MMP activity and thus transendothelial migration.[208] Most of the studies are focused on increasing CXCR4 levels, which can be explained by the pleiotropic involvement of SDF-1/CXCR4 axis in development, metastasis of cancer cells and NSC migration towards damaged areas.[209]

Increasing blood–brain barrier permeability

Increasing BBB permeability may be an alternative means of promoting cell homing to the brain without having to modify the transplanted cells. Administration of cyclophilin A triggers the transient opening of the BBB by reducing the levels of the vascular tight junction protein Claudin-5.[210] Accordingly, IV injection of cyclophilin A enabled a higher incorporation of the hydrophilic molecule doxorubicin into the brain parenchyma.[210] Mannitol has been proven to open the BBB in a safe way, and when it was co-injected with adipose-derived-MSCs, behavioural functions improved following experimental stroke. However, mannitol administration did not increase the number of cells entering the brain, which suggests a higher penetration of trophic factors instead of cells.[211] Focused ultrasound has also been shown to transiently open the BBB, and it has been demonstrated that this process enabled NSC entrance to the brain parenchyma.[212] Nevertheless, the transient opening of the BBB may entail several side effects, especially in pathological conditions, as in the case of stroke, when the BBB is already impaired. In fact, BBB disruption leads to higher levels of inflammatory factors, vasogenic oedema and a higher risk of haemorrhagic transformation, which worsen stroke outcomes and increase the mortality rate.[213]

Reduction of stem cell off-target entrapment following systemic injection

Systemic cell injection poses the risk of cells clogging vessels or becoming trapped in peripheral organs. The pulmonary first-pass effect refers to the mechanical entrapment in lungs of objects displaying a larger diameter than lung capillaries (ø = 8 µm in humans and ø = 4 µm in mice).[214–216] As the average diameter of stem cells ranges from 6 to 20 µm (e.g. NPC ø = 16 µm),[7] they are likely to become trapped in the lungs following vascular infusion. *Ex vivo* expansion can influence cell morphology and physiology, giving rise to even larger diameters (MSC diameter can reach up to 53 µm).[217] Furthermore, these techniques for improving stem cell migration can also increase the cellular adherence properties, leading to a higher entrapment rate.[218] In addition to the first uptake by the lungs, stem cells can undergo secondary redistribution to other organs, including the liver, kidneys and the spleen,[219] which may entail further adverse effects. Endovascular administration of MSCs can lead to pulmonary embolism in small animal models,[217] and can facilitate the tumour growth and promote metastasis.[220,221] Therefore, although systemically injected cells can also exhibit a beneficial effect without entering the CNS,[222] the potential adverse events related to peripheral entrapment is still considered as a non-negligeable risk. It can be addressed by reducing relative cell diameters and thus the probability of cell entrapment or by selectively removing cells that have become trapped in organs other than the brain.

Decreasing the probability of cell entrapment

The diameter of cells and blood vessels is a key factor in the occurrence of mechanical entrapment. On the one hand, increasing blood vessel diameter with the vasodilator sodium nitroprusside decreased cell entrapment in pulmonary microvasculature.[215,219] On the other hand, smaller cells are likelier to avoid the pulmonary first-pass effect. For instance, a comparison between BM-MSCs and BM-MNCs revealed that BM-MNCs (ø = 7 µm) exhibited a 30-fold higher pulmonary passage than BM-MSCs (ø = 18 µm).[216] Culture conditions can also be optimized to decrease cell volume—for example, by adapting cell confluency or growing cells in suspension.[223–226] However, cell entrapment is determined not only by cell and vasculature size but also by cell deformability.[227] Preconditioning haematopoietic stem cells with SDF-1a or H_2O_2 can increase their deformability, which may result in better properties for passing even thinner blood vessels.[228]

Safety-switch systems for selective specific ablation of cells

Reducing cell entrapment by modifying cell size or deformability properties does not guarantee the absence of cells in off-target organs. Therefore, the selective removal of cells trapped in organs other than the brain is required. Suicide gene therapy, first developed for selectively removing cancer cells,[229–231] has great potential for accomplishing this goal. The application of genes encoding proteins that induce cell death following the administration of a specific molecule has enabled the development of 'safety switches'. This technology has already been applied to reduce graft-versus-host disease after allogenic transplantation of BM cells.[232,233] The impermeability of brain barriers could be considered an advantage because it can prevent the passage of the safety switches to the brain parenchyma, leading to a selective removal of transplanted cells in the periphery and not in the brain.

There are three main techniques for the selective ablation of cells via transgene insertion: enzyme/prodrug system, MAb-mediated system and inducible dimerization system[234] (Fig. 4).

Enzyme–prodrug system

The enzyme–prodrug system enabled the development of gene-directed enzyme–prodrug therapy. The system consists of delivering suicide genes to cancer cells for their subsequent ablation by prodrug administration, the prodrug is then converted into a toxic metabolite. The most studied suicide genes are herpes simplex virus thymidine kinase (HSV-TK), cytosine deaminase (CD), nitroreductase (NTR), carboxypeptidase G2, purine nucleoside phosphorylase, Cytochrome P450 (P450) and a mutated version of thymidylate monophosphate kinase (TMPK). These enzymes can metabolize Ganciclovir (GCV), 5-fluorocytosine, CB1954, a nitrogen mustard-based prodrug (e.g. CMDA), 6-methylpurine deoxyriboside, Oxazaphosphorine drugs (e.g. cyclophosphamide) and azidothymidine, respectively, leading to cell death.[235,236]

One of the major concerns of the enzyme/prodrug system is the source of the suicide gene. Exogenous sequences coming from a virus (HSV-TK), bacteria (NTR, carboxypeptidase G2, purine nucleoside phosphorylase, CD) or yeast (CD) are more immunogenic than endogenous genes, which could lead to a premature elimination of the administered cells by the recipient's immune system.[237] GCV is already clinically approved for the treatment of cytomegalovirus

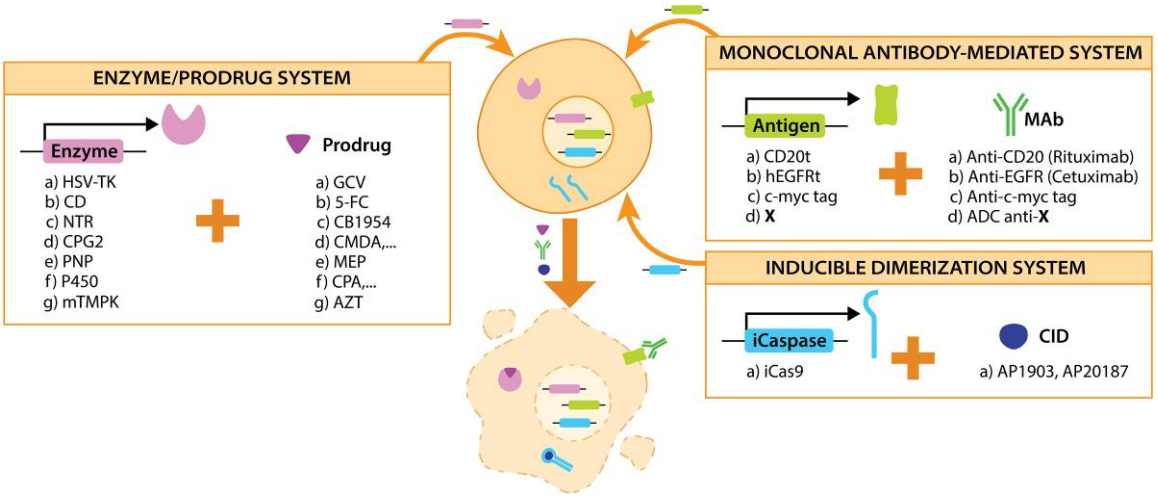

Figure 4 Genetic safety systems for eliminating cell grafts in untargeted organs. After cloning a gene encoding for a suicide enzyme, a specific antigen or inducible caspases, cells can be eliminated by adding the corresponding prodrug, MAb or a CID, respectively.

infections in immunocompromised individuals, which can also result in an undesired ablation of transgenic cells.[238] At the same time, although the human enzyme P450 is less immunogenic, it is mainly expressed in the liver, which may result in liver toxicity. Catalytically improved variants of the enzymes, as is the case with human TMPK, may help to overcome this issue.[236] Another point to consider is cell cycle dependency. HSV-TK and P450 are only active in dividing cells, while the other enzymes are also functional in non-dividing cells.[235,236] A noteworthy feature of the enzyme–prodrug system is the bystander effect, which refers to the ability to induce apoptosis in cells lacking the suicide gene by the diffusion of the converted toxic metabolite. This effect is favourable for oncotherapy but is less applicable for the selective removal of entrapped stem cells, as it can result in the death of endogenous healthy cells. In general terms, bacterial systems exhibit a stronger bystander effect compared to P450, but the result depends on the chosen prodrug.[239] Regarding BBB passage, GCV and 5-fluorocytosine have been widely used to treat brain tumours,[240,241] which shows their ability to enter the brain parenchyma and potentially ablate therapeutic cells in the CNS. Therefore, the obtention of BBB-impermeable prodrugs would be favourable for the removal of transgenic cells exclusively in peripheric organs.

Monoclonal antibody-mediated system

Rituximab is a MAb against CD20 that has been widely used to treat B-cell lymphoma. Rituximab can lead to cell death via complement activation and antibody-dependent cell cytotoxicity.[242] The latter is caused by the interaction between the Fc region of the MAb attached to the target cell with the Fc receptor of an effector cell, such as NK cells.[243] Rituximab's mechanism of action has been used to develop safety switches by adding the CD20 truncated gene to T cells to avoid the graft-versus-host disease after transplantation.[244–246] This approach was further developed by combining the epitopes of CD34 and CD20 antigens (RQR8) to obtain T cells containing a selection marker and a suicide gene.[247] However, as CD20 is present in endogenous B cells, a similar system was developed by introducing a truncated human EGFR gene into chimeric antigen receptor T cells, followed by Cetuximab (anti-EGFR) administration.[248,249] Another strategy consists of adding a 10 amino acid tag of c-myc to the chimeric antigen receptor followed by the administration of a MAb against c-myc.[250]

An antibody–drug conjugate refers to a MAb that is chemically linked to a toxic payload, which was initially developed to reduce off-target toxicities in cancer treatment.[251] In 2017, the Food and Drug Administration had approved only three antibody–drug conjugates for the treatment of different cancer types, but the number of approved treatments has now increased to 11.[252] Therefore, another strategy involves developing antibody–drug conjugates against specific molecules present in the surface of therapeutic stem cells. Antibody–drug conjugates can be engineered to specifically recognize the previously described proteins for enhancing migration, which might improve the efficacy and safety of cell therapies at the same time. Furthermore, antibody–drug conjugates have a limited ability to cross the BBB, which can be considered a downside for treating glioblastomas[253] but an advantage for the selective removal of entrapped cells outside the brain.

Inducible dimerization system

Human caspase-9 was fused to an FK506 binding protein to enable conditional dimerization in the presence of a small molecule known as the chemical inducer of dimerization (CID).[254] The administration of CIDs (AP1903 or AP20187)[255] leads to the activation of the inducible caspase-9 (iCas9), resulting in the rapid induction of apoptosis. The main advantages of this approach are its low immunogenicity (human gene) and the use of an inert small molecule. Although this system is gaining popularity, the available clinical data are still limited. However, CIDs are lipid-permeable synthetic ligands that are likely to cross the BBB. Accordingly, AP20187 was used to selectively ablate macrophages in an intracranial glioma mouse model.[256]

To conclude, features such as the bystander effect, efficacy, immunogenicity and suicide molecule hydrophobicity should be considered when choosing the ideal ablation system for a specific disease. A comparative study analysed some of these characteristics in HSV-TK, mTMPK–azidothymidine, CD20–MAb and iCas9–CID systems. The results showed that mTMPK was the least effective system, while for the other three systems,

the main difference was that HSV-TK required 3 days of constant GCV administration instead of having an immediate effect, as was the case with CD20 and iCas9.[257]

Conclusion

Both preclinical data and initial clinical trials using cell therapy for brain regeneration show highly promising results. Importantly, the transplantation of stem cells has proven to be safe, without major adverse events. Nevertheless, there are still some hurdles to overcome before cell therapy can be established as a standard clinical strategy for treating brain diseases. In addition to the choice of the ideal cell source, which may differ depending on the disease in question, the administration route is of utmost importance. Systemic injection of cells represents the method with the highest translational potential because it is the least invasive and highly practicable in everyday clinical practice. However, the low permeability of the BBB and BCSFB prevents the entry of systemically applied cells into the brain. Therefore, genetic engineering and cell surface modification of transplanted cells will be necessary to increase the BBB/BCSFB penetration and homing to the injury site using similar mechanisms as observed in different types of immune cells. In parallel, it is essential to prevent unwanted entrapment of transplanted cells in peripheral organs. Safety switches, such as the incorporation of suicide genes into cell grafts, can be applied to specifically remove cells that did not reach their target region.

The techniques described in this review have great potential to increase the efficacy and safety of a cell therapy and to establish next-generation cell therapies as standard treatments for treating other brain diseases in the future.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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