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REVIEW

Clinical Trials of Immunomodulation in Ischemic Stroke

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Abstract Inflammatory mechanisms are currently considered as a prime target for stroke therapy. There is evidence from animal studies that immune signals and mediators can have both detrimental and beneficial effects in particular stages of the disease process. Moreover, several of these mechanisms are turned on with sufficient delay after ischemia onset to make them amenable to therapeutic intervention. Several clinical proof-of concept trials have investigated the efficacy of different immunomodulatory approaches in patients with stroke. Trials targeting the innate immune system have focused on reduction of microglial activation, inhibition of neutrophil migration, and interleukin-1 receptor blockade, suggesting that interleukin-1 receptor blockade may be a promising strategy. Studies aiming at halting T-cell migration have also been undertaken with controversial findings regarding prevention of infarct growth in neuroimaging studies. Consistently, recent proof-of-concept trials targeting lymphocytes with drugs such as natalizumab and fingolimod have yielded some promising results on clinical endpoints, but confirmation in larger trials is needed. At present, the understanding of the role of immune mechanisms in neurorepair and neurodegeneration is limited. Improving long-term brain function by mitigating prolonged neuroinflammation that was triggered by acute brain injury could be a strategy in addition to neuroprotection.

Keywords Immunomodulation · immune system · ischemic stroke · stroke

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Introduction

Ischaemic stroke typically results from thrombotic or thromboembolic blockage of a cerebral artery. Primarily, viability of the ischemic brain depends on the duration and the severity of blood flow reduction because brain energy metabolism is critically dependent on continuous oxygen supply. Rapid restoration of cerebral blood supply by intravenous thrombolysis and, more recently, by mechanical thrombectomy is currently the mainstay of acute stroke therapy [1, 2]. Beyond failure of aerobic energy metabolism, ischaemia triggers a wide array of secondary molecular, cellular, and systemic processes, many of which substantially increase brain damage in experimental stroke models [3, 4]. Protection of the brain against deleterious processes during or after ischemia has been a main concept in the development of new stroke therapies [5]. So far, however, the results of medical and physical treatments in experimental stroke models have not been translated successfully into the clinical setting [6, 7] (Table 1).

Inflammatory mechanisms are currently considered as a prime target for stroke therapy [3, 4]. The vast number of cellular and molecular processes commonly referred to as inflammatory precludes that inflammation as a whole can be categorized into either good or bad [3, 4, 8]. Nevertheless, there is evidence from animal studies that certain immune signals and mediators have profound detrimental effects, at least in certain stages of the disease process. Moreover, several of these mechanisms are turned on with sufficient delay after ischemia onset to make them amenable to therapeutic intervention. However, there are concerns that immune modulation in patients with stroke may exacerbate poststroke immune depression and result in increased infectious complications [9–12].

Several clinical proof-of concept trials have investigated the efficacy of different immunomodulatory approaches in

Table 1 Summary of clinical trials of immunomodulation in ischemic stroke

Drug	Mechanism	Trial	Design	Route of administration, time window, dose, duration	Outcome	Reference(s)
Minocycline	Anti-inflammatory effects: reduction of microglial activation, inhibition of MMP activity, reduced NO production, and inhibition of apoptotic cell death	Minocycline treatment in acute stroke: an open-label, evaluator-blinded study	Phase II: open-label, evaluator-blinded study of 152 patients	Route: oral Dose: 200 mg Time window: 6–24 h Duration: 5 days	Lower NIHSS and mRS at 7 days and 30 days	[36]
		Study of a neuroprotective drug to limit the extent of damage from an ischemic stroke (MINOS)	Phase II: dose-finding study with tiers of 3, 4.5, 6, or 10 mg/kg in a total of 60 patients, with 41 at the highest dose (10 mg/kg) and 60 % receiving concurrent tissue plasminogen activator	Route: IV Dose: 3, 4.5, 6, or 10 mg/kg Time window: 0–6 h Duration: 3 days	Well-tolerated; compatible with TPA Half-life of approximately 24 h	[37]
		Neuroprotection with minocycline therapy for acute stroke recovery trial (NeuMAST)	Phase IV: multicenter prospective randomized open-label blinded endpoint evaluation (PROBE) pilot study of 152 patients	Route: oral Dose: 200 mg Time window: 3–48 h Duration: 5 days	No benefit at 90 days, as measured by mRS, NIHSS or BI; terminated at interim analysis for futility	[38]
Recombinant neutrophil inhibitory factor (UK-279, 276)	Recombinant inhibitor of the CD11b/CD18 receptor, thus blocking neutrophil adhesion to endothelium and infiltration to the site of infarction	Acute stroke therapy by inhibition of neutrophils (ASTIN)	Phase II: Bayesian sequential design for double-blind, randomized, adaptive allocation of 966 patients (204 co-thrombolysed) to 1 of 16 dose tiers or placebo and early termination for efficacy or futility	Route: IV Dose: 1 of 15 doses of 10–120 mg Time window: 0–6 h Duration: single dose	No benefit at 90 days, as measured by change in SSS adjusted for baseline; terminated at interim analysis for futility	[50, 51]
Monoclonal antibody (humanized) against the neutrophil CD11/CD18 cell adhesion molecule (Hu23F2G, LeukArrest®)	Humanized monocolonal antibody against neutrophil β2 integrin CD18, thus blocking neutrophil adhesion to endothelium infiltration to the site of infarction		Phase II: dose-escalation study	Route: IV Dose: Variable Time window: 0–12 h Duration: single dose	Concentration of 1.5 mg/kg well-tolerated; twice-daily infusion improved mRS	[6, 49]
		Hu23F2G phase III stroke trial (HALT)	Phase III		Terminated in interim analysis for futility; results not disclosed	[6, 49]
E-selectin	Induction of mucosal tolerance to human E-selectin to minimize inflammation and risk of further cerebral insult	E-selectin nasal spray to prevent stroke recurrence	Phase II: dose-finding study with 4 doses of intranasal recombinant human	Route: nasal spray Dose: variable	Terminated; results not disclosed	[54]

Table 1 (continued)

Drug	Mechanism	Trial	Design	Route of administration, time window, dose, duration	Outcome	Reference(s)
			E-selectin spray, estimated enrolment of 60 people	Time window: 1–4 months Duration: 30 days		
		E-Selectin nasal instillation to prevent secondary stroke	Phase I: single-center, open-label, dose-escalation trial assessing safety profile of 4 doses of intranasal recombinant human E-selectin, estimated enrolment 58 people	Route: nasal spray Dose: 0, 5, 15, or 50 μg Time window: 1–4 months Duration: 30 days	Ongoing	[55]
IL-1 receptor antagonist (rhIL-1Ra, Anakinra)	IL-1 receptor blockade to reduce cerebral inflammation	A randomized phase II study of IL-1 receptor antagonist in patients with acute stroke	Phase II: randomized, double-blind, placebo-controlled trial in 34 patients	Route: IV Time window: 0–6 h Dose: 100 mg loading followed by a 2 mg/kg/h infusion over 72 h Duration: 3 days	No adverse events attributed to treatment; greater reduction in NIHSS at 3 months; more patients with mRS 0–1 at 3 months	[46]
		SC-IL STROKE study	Phase II: randomized, controlled trial with planned sample size of 120 (80 stroke patients and 40 healthy volunteers)	Route: SC Time window: 0–6 h Dose: 100 mg twice daily for 72 h Duration: 3 days	Ongoing	[47]
Murine anti-ICAM-1 (Enlimomab)	Murine monoclonal antibody against intercellular adhesion molecule ICAM-1 to block leukocyte attachment and migration through the cerebral endothelium	Safety, pharmacokinetics, and biological activity of enlimomab (anti-ICAM-1 antibody)	Phase II: open-label, dose-escalation study in 32 patients hospitalized for acute stroke (ischemic or hemorrhagic)	Route: IV Time window: 0–24 h Dose: 140–480 mg Duration: 5 days	Between 140 and 480 mg of Enlimomab administered over 5 days was safe at 30 days; a loading dose of 160 mg followed by 4 daily maintenance doses of 40 mg suggested suitable for further study	[57]
		Enlimomab acute stroke trial (EAST)	Phase III: double-blind, randomized,	Route: IV	Mortality higher in enlimomab-treated	[58]

Table 1 (continued)

Drug	Mechanism	Trial	Design	Route of administration, time window, dose, duration	Outcome	Reference(s)
			placebo-controlled, parallel-group, 90-day follow-up trial of 625 patients	Time window: 0–6 h Dose: 160 mg (day 1), and 40 mg daily (days 2–5) Duration: 5 days	patients; significantly more adverse events with Enlimomab; terminated	
The sphingosine-1-phosphate receptor (S1PR) regulator Fingolimod (FTY720)	Reduced egress of T cells, B cells, NK cells, and other S1PR-expressing cells from the lymph nodes	Efficacy and safety of FTY720 for acute stroke	Phase II: randomized, open-label, evaluator-blinded, parallel-group clinical pilot trial of 22 patients	Route: oral Time window: 4.5–72 h Dose: 0.5 mg Duration: 3 days	Well-tolerated; no increase in infections; milder neurological deficit at 7 days; limited lesion enlargement at 7 days; decreased microvascular permeability at 7 days	[67]
		Combination of the Immune Modulator Fingolimod With Alteplase in Acute Ischemic Stroke: A Pilot Trial	Phase II: randomized, open-label, parallel-group, 90-day follow up trial of 47 patients	Route: oral Time window: 0–4.5 h Dose: 0.5 mg Duration: 3 days	Well-tolerated; attenuated reperfusion; improved clinical outcomes	[68]
Natalizumab	Blockade of the α4-β1 integrin	Effect of Natalizumab on Infarct Volume in Acute Ischemic Stroke (ACTION)	Phase II: randomized, double-blinded, parallel-assignment clinical trial of 161 patients	Route: IV Time window: 0–9 h Dose: 300 mg Duration: single dose	No reduction in focal infarct growth from day 1 to day 5; functional outcome improvement sustained over 90 days	[62, 63]
		Safety and Efficacy of Intravenous Natalizumab in Acute Ischemic Stroke (ACTION II)	Phase II: randomized, double-blinded, parallel-assignment clinical trial with estimated enrolment of 240 patients	Route: IV Time window: 0–9 h Dose: 300 mg Duration: single dose	Ongoing	[64]

MMP = matrix metalloproteinase; NO = nitric oxide; IV = intravenous; NIHSS = National Institutes of Health Stroke Scale; mRS = modified Rankin Score; TPA = tissue plasminogen activator; BI = Barthel index; SSS = Scandinavian Stroke Scale; IL = interleukin; SC = subcutaneous; ICAM-1 = intercellular adhesion molecule 1; NK = natural killer

patients with stroke. The present review summarizes the results of previous and ongoing trials targeting different components of the innate and adaptive immune system. Conceptual consequences for future translational research from the findings of these early-phase trials will be drawn.

Immune Mechanisms

Stroke activates multiple inflammatory cascades in the brain and in the systemic immune system [3, 4, 13]. Upon injury, neurons and other damaged brain cells release a number of molecules that function as danger-associated molecular patterns or alarmins [14]. These mediators bind to pattern recognition receptors on various cells, including microglia and endothelial cells, and lead to their activation [14]. Microglia sense changes in the ischemic brain [15]. They upregulate major histocompatibility complex class II molecules, and express and secrete cytokines, including tumour necrosis factor-α and interleukin (IL)-1 [16]. Activated cerebral microvessels become more permeable to molecules that are normally prevented from crossing the blood–brain barrier [17]. In particular, the immunological blood–brain barrier is substantially altered after ischemia [18]. Together with the secretion of chemokines this promotes the successive entry of systemic leukocytes including neutrophils, macrophages, and lymphocytes [3, 13, 19, 20]. Corresponding to upregulation of adhesion molecules on endothelial cells, integrins serve similar functions on activated leukocytes. The sequence of leukocyte recruitment into the brain after experimental stroke has been well characterized [21], whereas the temporal and spatial profile immune cell recruitment after stroke in humans requires better characterization [22].

A substantial role for the adaptive immune response after stroke is increasingly recognized. Transgenic animals deficient in lymphocytes consistently have smaller infarcts in different stroke models [23–26]. Moreover, antibody-mediated depletion of $CD4^+$, $CD8^+$, and γδ T cells reduced infarct volume and improved functional outcome [25, 27–29]. The dynamics of this deleterious role of different proinflammatory T cells have not been fully elucidated. Interestingly, this effect was evident 24 h after ischemia onset in some studies, suggesting an antigen-independent effect of T cells. Other studies consistently describe a delayed mechanism of tissue injury. Further evidence points to a role for B cells, though perhaps in a regulatory capacity [30]. Cytokines are key mediators in the inflammatory response to stroke [31]. While recent research provides solid support for a deleterious role for interferon-γ in poststroke inflammation [32], evidence for an inciting effect of IL-17 is also mounting, with innate γδ T cells likely to be a main source [33]. Currently, the target of immunomodulation translated into clinical trials is focused on the early phase of toxic neuroinflammation. However, the neuroinflammatory reaction after acute brain injury continues for months [4, 34, 35], and the complex effect on repair and degeneration after stroke remains to be unravelled. This further highlights the dualistic nature of the immune response to stroke, acting not only to exacerbate damage with detrimental effects, but also to propagate repair and recovery.

Stroke Trials Addressing Innate Immune Mechanisms

Microglia as a Target

Minocycline is a second-generation derivative of tetracycline that has a protective effect in animal models of stroke through a variety of mechanisms, including anti-inflammatory effects, reduction of microglial activation, matrix metalloproteinase reduction, nitric oxide production, and inhibition of apoptotic cell death [36]. An open-label, evaluator-blinded study of 152 patients showed minocycline, when administered orally for 5 days at a dosage of 200 mg within 6 to 24 h of onset of stroke, to be associated with significantly lower National Institutes of Health Stroke Scale (NIHSS) score and modified Rankin Score (mRS) compared with placebo [36]. This pattern was apparent on day 7 of follow-up, and continued to day 30 [36]. Furthermore, there was no difference in the incidence of observed complications [36]. These findings prompted further study into the safety and dose range of minocycline [37]. In a phase IIb trial, "Minocycline to Improve Neurologic Outcome in Stroke" (MINOS), minocycline was administered intravenously within 6 h of stroke symptom onset in preset dose tiers of 3, 4.5, 6, or 10 mg/kg daily over 72 h [37]. A total of 60 patients were recruited, with 41 at the highest dose (10 mg/kg) and 60 % receiving concurrent thrombolysis with tissue plasminogen activator. Minocycline infusion was well tolerated with only 1 observation of dose limiting toxicity in the 10-mg/kg regimen. Furthermore, there were no incidences of severe hemorrhage in the thrombolysed patients, confirming compatibility with tissue plasminogen activator [37]. Pharmacokinetic analysis revealed a half-life of approximately 24 h, thus allowing once-daily dosing [37].

These encouraging results prompted a multicentre randomized, double-blind, placebo controlled trial, "Neuroprotection With Minocycline Therapy for Acute Stroke Recovery Trial" (NeuMAST), in which patients with ischemic stroke were randomized to treatment with either oral minocycline or placebo within 3 to 48 h of symptom onset. The assigned treatment was administered for 5 consecutive days after enrolment, and the primary efficacy endpoint was an mRS of 0–1 for all randomized patients at 90 days, with secondary endpoints including NIHSS and Barthel Index at 90 days, analysed using ordinal shift analysis. Unfortunately, the study did not show minocycline to have any efficacy in improving long-term

recovery, and the trial was abandoned in May 2013 after interim analysis suggested futility [38].

Interleukin-1

IL-1 is a proinflammatory mediator with 2 main ligands—IL-1α and IL-1β [39]—as well as a third naturally occurring competitive antagonist, IL-1Ra [40]. Stroke causes upregulation of the IL-1 receptor and its ligands in animal models [41], with expression of IL-1α seen in microglia within 4 h postreperfusion [42]. Furthermore, exogenous administration of IL-1β exacerbates ischaemic damage in rodent models, with the absence of IL-1α or IL-1β in knockout mice ameliorating damage [43]. A recent meta-analysis showed that IL-1Ra administration was associated with a 38.2 % reduction in mean infarct volume across 16 published preclinical studies [44]. It has been hypothesized that the inhibition of IL-1β generated centrally following an acute ischemic stroke would reduce further cerebral injury mediated by inflammation. IL-1 receptor antagonist has long been available for clinical use in arthritis [45]. However, clinical development of IL-1 receptor antagonist after initial promising results in various cerebrovascular proof-of-concept trials, including ischemic stroke and subarachnoid haemorrhage, has been slowed by the replacement of the intravenous by a subcutaneously injected formula.

In a phase II study, 34 patients were block randomized to receive either recombinant human IL-1 receptor antagonist (rhIL-1Ra; Anakinra) administered intravenously with a 100-mg loading dose over 60 s, followed by a 2 mg/kg/h infusion over 72 h, or matching placebo, within 6 h of the onset of symptoms of acute stroke. No adverse events were attributed to treatment and the recombinant human IL-1Ra was deemed safe. Ready transfer across the blood–brain barrier was shown [46]. Furthermore, systemic markers of biological activity (including neutrophil and total white cell counts, C-reactive protein, and IL-6 concentrations) were lower in the treatment arm. Clinical outcomes after at least 3 months were better in the treatment group. Median NIHSS score was reduced to 4 *versus* 1 in the placebo arm, and more patients receiving anakinra had mRS 0–1 at 3 months (30 % *vs* 7 %) [46].

After the intravenous formulation of anakinra was discontinued an ongoing phase II randomized, controlled trial was started which investigated the effects of subcutaneous administration at doses of 100 mg twice daily, administered for 3 days [47]. The primary outcome measure is reduction of inflammatory biomarkers (including IL-6) between 6 h and 5–7 days after stroke; secondary outcomes also include 3-month clinical outcomes (mRS, survival, and length of stay) [6, 47].

Blockade of Neutrophils

Recombinant Neutrophil Inhibitory Factor

Recombinant neutrophil inhibitory factor (UK-279, 276) is a recombinant glycoprotein with selective binding to the CD11b integrin of macrophage-1 antigen (CD11b/CD18) [48]. Demonstration of its ability to reduce neutrophil infiltration and infarct volume in rat models of stroke led to its consideration in humans [49].

The phase II clinical trial, "Acute Stroke Therapy by Inhibition of Neutrophils" (ASTIN), took a Bayesian sequential design allowing for double-blind, randomized, adaptive allocation to 1 of 16 dose tiers (range 10–120 mg) or placebo and early termination for efficacy or futility [50, 51]. The primary endpoint was change from baseline to day 90 on the Scandinavian Stroke Scale (DeltaSSS), adjusted for baseline Scandinavian Stroke Scale. The study aimed for a 3-point additional mean recovery above placebo [50]. A total of 966 patients with stroke were included (887 ischemic infarcts, 204 co-treated with intravenous tissue plasminogen activator) and treated within 6 h of symptom onset. Though UK-279,276 was generally well tolerated, there was no dose–rate effect compared with placebo, and the trial was stopped early after inclusion of 966 patients with acute stroke [50].

Neutrophil β2 Integrin CD18

Similar to the mechanism underpinning Recombinant neutrophil inhibitory factor, use of the humanized monoclonal antibody against neutrophil β2 integrin CD18 (Hu23F2G) was hypothesized to improve long-term outcomes after ischemic stroke [6]. The safety and dose efficacy of this approach was investigated in a phase II dose-escalation study, where Hu23F2G was administered within 12 h of ictus [6, 49]. A concentration of 1.5 mg/kg Hu23F2G in single dose was found to be generally safe, though noted to increase fever. Furthermore, twice-daily infusion of this dose appeared to improve mRS. Consequently, a phase III study was performed. However, the study was terminated after the first interim analysis, when no likely benefit of treatment was observed [49]. Unfortunately, there has been no public information release about the results of the study, specifically the outcomes and safety issues [49].

E-selectin

E-selectin is a cell adhesion molecules expressed on endothelial cells that have been activated by inflammatory cytokines [52]. In humans, L-selectin is the main ligand, and with glycolipids constitutes more than half of the E-selectin receptors on neutrophils [53]. Although serum levels of E-selectin are not elevated following symptom onset in stroke patients [52],

their systemic expression after focal cerebral ischaemia in animal models, along with the attenuation of ischaemic damage with transnasal administration has prompted clinical trials to explore their use in secondary prevention [54, 55]. The hypothesis driving this work is that induction of mucosal tolerance may divert any inflammatory response from contributing to further cerebral insult. While the first of 2 studies investigating recombinant human E-selectin delivered intranasally was terminated prematurely [54] the second is currently ongoing in exploring the maximum safe dose in patients older than 45 years of age who have suffered an ischemic stroke or transient ischemic attack within 30–120 days [55]. Participants are randomly assigned to receive E-selectin at a dose level of 5, 15, or 50 micrograms or a placebo on alternate days for 5 doses, with this repeated a total of 3 times; follow-up is at 1 and 3 months [55].

Intercellular Adhesion Molecule 1

Enlimomab is a murine monoclonal antibody against intercellular adhesion molecule 1. Studies in rat models of acute ischemic stroke suggested improved neurological outcomes [56]. A consequent open, uncontrolled, dose titration study was undertaken in 32 patients hospitalized for ischemic or hemorrhagic stroke, with the aim of clarifying the safety, pharmacokinetics, and biological activity of enlimomab in this context [57]. Patients received 1 of 4 regimens of enlimomab; a loading dose infused within 24 h of symptom onset was followed by 4 daily maintenance doses, with the total dose ranging from 140 to 480 mg. The results suggested that doses of 140 to 480 mg administered over 5 days did not increase the risk of adverse events during the observation period of 30 (±10) days [57]. Furthermore, the loading dose of 160 mg followed by 4 daily maintenance doses of 40 mg was deemed to be suitable for further study [57].

In a subsequent phase III study, 625 patients with ischemic stroke were randomized to receive 5 days of either enlimomab (317 patients) or placebo (308 patients) within 6 h of stroke symptom onset [58]. At day 90, mRS was worse in patients treated with enlimomab than with placebo. Fewer patients had symptom-free recovery on enlimomab, and more patients died [58]. These results were apparent on days 5, 30, and 90 of follow-up. Significantly more adverse events occurred in the enlimomab arm, most notably infections and fever [58]. Work in rodent models subsequently demonstrated that sequential infusion of heterologous antibodies following focal ischemia increaseS cerebral injury volume [59], suggesting that the detrimental effects of enlimomab may be attributable to the development of human antimouse antibodies, with consequent activation of neutrophils through complement-dependent mechanisms.

Stroke Trials Targeting Adaptive Immune Cells

The current concept is that lymphocytes exert their deleterious effects after entering the brain. Blockade of α4-β1 integrin (also referred to as very late antigen 4) is effective in relapsing-remitting multiple sclerosis (MS), and is believed to mediate its effect by restricting leukocyte access through the blood–brain barrier, thus limiting neuroinflammation [60]. Such strategies may also be applicable to stroke. An alternative approach is to arrest lymphocyte egress from lymphatic organs. Again, this principle has been successfully applied in MS using the sphingosine receptor blocker fingolimod [61].

Blocking of α4-β1 Integrin

Blockade of the α4-β1 integrin on leukocytes is a potent strategy to attenuate neuroinflammation and to prevent relapses in MS. The recently completed ACTION study was a randomized controlled phase IIa trial comparing a single injection of 300 mg of intravenous natalizumab against placebo within a 9-h time window after symptom onset [62, 63]. Patients underwent serial magnetic resonance imaging (MRI) at baseline and on days 1, 5, and 30. Based on findings in rodent stroke models the trial intended to prove the concept that blockade of α4 integrin on leukocytes would prevent delayed infarct growth. Moreover, functional outcome parameters were determined While the trial found no effect of natalizumab on infarct growth between day 1 and day 5 (the primary study endpoint), patients receiving natalizumab were more likely to have an excellent clinical outcome at 30 and 90 days. This was particularly evident in subgroups of patients with smaller infarcts and with more exposure to the drug in pharmacokinetic area under the curve studies. No safety issues, including infectious complications, were noted.

The phase IIb ACTION trial has started in mid-2016 to test beneficial effects of 2 doses of natalizumab (single dose of 300 mg and 600 mg, respectively) on functional outcome after stroke [64].

Fingolimod

The sphingosine 1-phosphate (S1P) lipid is secreted extracellularly following the metabolism of sphingomyelin from cell membrane structures. Signaling via G protein-coupled S1P receptors, it regulates a multitude of responses, including cell migration, differentiation, and survival [65]. Fingolimod (FTY720, Gilenya; Novartis, Basel, Switzerland) is an oral S1P receptor modulator that sequesters lymphocytes to lymph nodes and has been approved for therapy of relapsing-remitting MS. It has more recently also been shown to be protective in a number of preclinical stroke studies [66].

In an open-label, 3-center pilot study enrolling 22 patients in China, fingolimod (0.5 mg/day for 3 days) on top of routine medical management or routine management alone was administered to patients within a time window of between at least 4.5 h and 72 h after symptom onset [67]. Candidates for intravenous thrombolysis were excluded. Fingolimod restricted enlargement of the infarct volume on sequential MRI between day 1 and day 7 and reduced blood–brain barrier permeability on contrast MRI [67]. The treatment was also associated with short-term neurological improvements, and no significant excess of adverse events was noted [67].

In a subsequent open pilot study by Zhu et al. [68], 47 patients were randomized in 3 centers to receive either fingolimod (0.5 mg/day for 3 days) or nothing on top of intravenous thrombolysis. Again, patients receiving fingolimod had substantially less lesion volume growth at day 1 and day 7, less hemorrhagic transformation, and profoundly better outcomes on the mRS at 90 days. The effect of fingolimod was also tested in a pilot study of patients with intracerebral hemorrhage. In a 2-arm proof-of-concept clinical study, 11 participants were treated with 0.5 mg oral fingolimod daily for 3 days after intracerebral hemorrhage; the first dose was administered within 72 h of the ictus [69]. Again, short- and long-term neurological functions were better in participants who received fingolimod than in participants who did not.

Comparison of Natalizumab and Fingolimod

The discrepancy between the impact of natalizumab and fingolimod on imaging endpoints in early phase II stroke trials is striking. Although both drugs have T lymphocytes as the presumed key target immune cell population, their effects on early infarct growth differed markedly. First, the sample size of trials was small, and findings should be considered preliminary for both drugs. Second, the trials with fingolimod were open investigator-initiated studies, which implies a different regulatory environment. Third, the ACTION trial was performed predominantly in caucasian patients, whereas the fingolimod trials enrolled only South-East Asian patients. Leaving these formal differences among trials aside, the discrepancy of outcomes may point to different biological effects of both drugs. For example, S1P receptors are also present on other brain cells, and there is some evidence suggesting that direct effects of fingolimod on astrocytes and neurons may be protective. Fingolimod reduces lymphocytes in the blood, which may have beneficial effects even before brain invasion. Finally, fingolimod was repetitively given over 3 days with a cease of action within days, whereas natalizumab blocks the α-4 integrin for at least 4 weeks.

Although both deleterious and protective regulatory roles of B lymphocytes are increasingly recognized, translation into clinical trials in stroke has not happened yet.

Future Directions

There is little doubt now that immune mechanisms profoundly affect the pathophysiology in the ischemic brain. Previous trials of immunomodulatory therapies have focused on attenuating the detrimental effects of certain immune cells and inflammatory mediators on the extent of tissue damage in the acute phase. Despite solid evidence, including meta-analyses [44], and preclinical randomized trials for the effectiveness of this neuroprotective approach in experimental studies, the translational validity of neuroprotection by immune modulation is presently uncertain [70]. Findings in proof-of-concept neuroimaging studies in T lymphocytes are controversial and require further investigation. If confirmed, discrepancies between absent effects on the size of the macroscopic lesion and effects on functional outcomes in the ACTION trial may prompt a different conceptual approach towards the role of neuroinflammation in stroke in which inflammation causes more prolonged interference with brain function. A major limitation at present is a very limited understanding of the involvement of neuroinflammation in neurorepair and neurodegeneration.

A better understanding of the immune biology in experimental and human stroke is needed to identify additional targets for early and delayed interventions. However, present data suggest that the risks of immune modulation in the acute phase of stroke, including increased susceptibility to infections are limited. The growing number of immunomodulatory interventions that are already established for other indications in humans provides a unique opportunity to fast-track innovative proof-of-concept trials. Ideally, these trials are accompanied by surrogate marker studies, including molecular neuroimaging, to evaluate the translational validity of concepts derived from studies in animals. Extensive crosstalk between preclinical and clinical work is needed to elucidate the pathophysiology of the multifaceted poststroke immune response.

Summary

The current understanding of the immunological processes involved in brain injury and repair is still limited. Previous trials of immune modulation in stroke using IL-1Ra and lymphocyte-targeted approaches have yielded some promising results, but confirmation in larger trials is needed. Conceptually, improving brain function by mitigating prolonged neuroinflammation triggered by acute brain injury could be an additional strategy to neuroprotection.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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