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STROKE AND NEUROCOGNITIVE IMPAIRMENT COMPENDIUM

Immune Pathways in Etiology, Acute Phase, and Chronic Sequelae of Ischemic Stroke

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ABSTRACT: Inflammation and immune mechanisms are crucially involved in the pathophysiology of the development, acute damage cascades, and chronic course after ischemic stroke. Atherosclerosis is an inflammatory disease, and, in addition to classical risk factors, maladaptive immune mechanisms lead to an increased risk of stroke. Accordingly, individuals with signs of inflammation or corresponding biomarkers have an increased risk of stroke. Anti-inflammatory drugs, such as IL (interleukin)-1β blockers, methotrexate, or colchicine, represent attractive treatment strategies to prevent vascular events and stroke. Lately, the COVID-19 pandemic shows a clear association between SARS-CoV2 infections and increased risk of cerebrovascular events. Furthermore, mechanisms of both innate and adaptive immune systems influence cerebral damage cascades after ischemic stroke. Neutrophils, monocytes, and microglia, as well as T and B lymphocytes each play complex interdependent roles that synergize to remove dead tissue but also can cause bystander injury to intact brain cells and generate maladaptive chronic inflammation. Chronic systemic inflammation and comorbid infections may unfavorably influence both outcome after stroke and recurrence risk for further stroke. In addition, stroke triggers specific immune depression, which in turn can promote infections. Recent research is now increasingly addressing the question of the extent to which immune mechanisms may influence long-term outcome after stroke and, in particular, cause specific complications such as poststroke dementia or even poststroke depression.

Key Words: COVID-19 ■ depression ■ humans ■ microglia ■ monocytes

Stroke is a severe and life-threatening disease. Worldwide, it is the second leading cause of death, and it is also the leading cause of disability in adulthood.[1] In many countries of the Western world, 1 in 4 individuals will experience a stroke by the end of their lives. Although a number of effective therapies are available for both the prevention and treatment of stroke, the number of new cases and the number of patients living with the consequences of stroke is steadily increasing.

Significant therapeutic breakthroughs in prevention, treatment, and also rehabilitation and follow-up of ischemic stroke have been achieved over the past decades.[2] Treatment of vascular risk factors represents a significant approach to stroke prevention. In the field of acute therapy, the introduction of intravenous thrombolysis (1995) and thrombectomy (2015) established 2 effective therapies for reopening the occluded vessel (recanalization) and improved reperfusion. This is complemented by a worldwide effort to establish specialized stroke units. In addition to reducing neurological deficits directly caused by the stroke, complications such as pneumonia and other infections, thrombosis, or epileptic seizures must continue to be prevented or treated. Rehabilitation and aftercare serve not only to reduce the neurological deficit but also to restore everyday functions and participation, as well as to prevent and treat long-term consequences such as spasticity, poststroke depression, or poststroke dementia.[2]

Especially in animal models of ischemic stroke, a variety of pathobiological mechanisms have been identified that represent attractive therapeutic targets.[3] Approaches to so-called neuroprotection have led to impressive reductions in ischemic brain tissue damage and improvements in functional outcome in experimental stroke. However, this is contrasted by the sobering fact that in a large number of clinical trials, none of these substances provide a therapeutic advantage in patients with stroke. The reasons for this translational roadblock are

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Nonstandard Abbreviations and Acronyms

ACC1	acetyl coenzyme A carboxylase 1
ACE-2	angiotensin-converting enzyme 2
AIM2	absent in melanoma 2
BM	bone marrow
CCL2	C-C chemokine ligand
CCRC-C	chemokine receptor
CHIP	clonal hematopoiesis of indeterminate potential
CLRC-type	lectin receptor
CRPC-reactive	protein
CX3CR1	C-X3-C chemokine receptor 1
DAMPdamage-associated	molecular pattern
EGFR	epidermal growth factor receptor
FasL	fas ligand
G-CSFgranulocyte	colony-stimulating factor
GM-CSFgranulocyte	-macrophage colony-stimulating factor
HDLhigh-density	lipoprotein
HLA-DRhuman	leukocyte antigen–DR isotype
HMGB1	high mobility group box 1
Hsp70	heat shock protein 70
IFN-γ	interferon gamma
IL	interleukin
LDLlow-density	lipoprotein
Ly6C	lymphocyte antigen 6 complex
MCP-1	monocyte chemoattractant protein-1
MyD88	myeloid differentiation primary response 88
NET	neutrophil extracellular trap
NF-κB	nuclear factor kappa B
NLR	nucleotide-binding oligomerization domain, leucine-rich repeat-containing receptor
NLRP3	NOD-, LRR-, and pyrin domain-containing protein 3
NOD	nucleotide oligomerization domain
PCT	procalcitonin
PISCES	Pneumonia in Stroke Consensus
PRR	pattern recognition receptor
RAGE	receptor for advanced glycation end product
SAA	serum amyloid A
SAPstroke-associated	pneumonia
STAT6	signal transducer and activator of transcription 6
TLRToll-like	receptor
TNF-α	tumor necrosis factor alpha
tPA	tissue-type plasminogen activator
TRIF	TIR domain-containing adapter-inducing interferon-β
VLA4	α4β1 integrin

complex and multifaceted.[3,4] Thus, at present, recanalizing therapies remain the only available proven therapies for ischemic stroke.

Inflammation and maladaptive immune mechanisms are among the most important pathological mechanisms in the damage cascades of the acute phase, whereas complications as well as in regeneration dominate in the subacute and chronic phases after stroke.[5,6] These comprise inflammation of the brain as well as changes in the immune system, involve the innate and adaptive immune system, and can have both damaging and protective functions, depending on the specific molecules or cells involved, as well as the affected brain regions and phases after the stroke. Thus, the active field of research on inflammation and immunity in stroke not only contributes to better pathophysiological understanding but, in particular, helps to identify individuals at increased risk of stroke and to identify new therapeutic strategies for both prevention and treatment of stroke. In this review article, we will introduce and discuss inflammation and immune mechanisms along the cerebrovascular continuum from stroke pathogenesis through the acute cascades to chronic sequelae (Figure 1). First, we will briefly review the immune mechanisms leading to the development of ischemic stroke and ongoing studies using anti-inflammatory agents to reduce stroke risk. SARS-CoV2 causing coronavirus disease 2019 is a recent and prominent example of how an (specific) infection can lead to ischemic stroke. Along the cerebrovascular continuum, we will then examine how different immune cells contribute both detrimentally and protectively to the acute ischemic cascade, how stroke-induced immunodepression promotes poststroke infections, and how chronic inflammation and adaptive immunity lead to long-term outcomes such as poststroke depression and dementia. We will focus on studies performed in patients and will rely on the animal literature for explanations of the underlying pathobiology or when clinical data are not available.

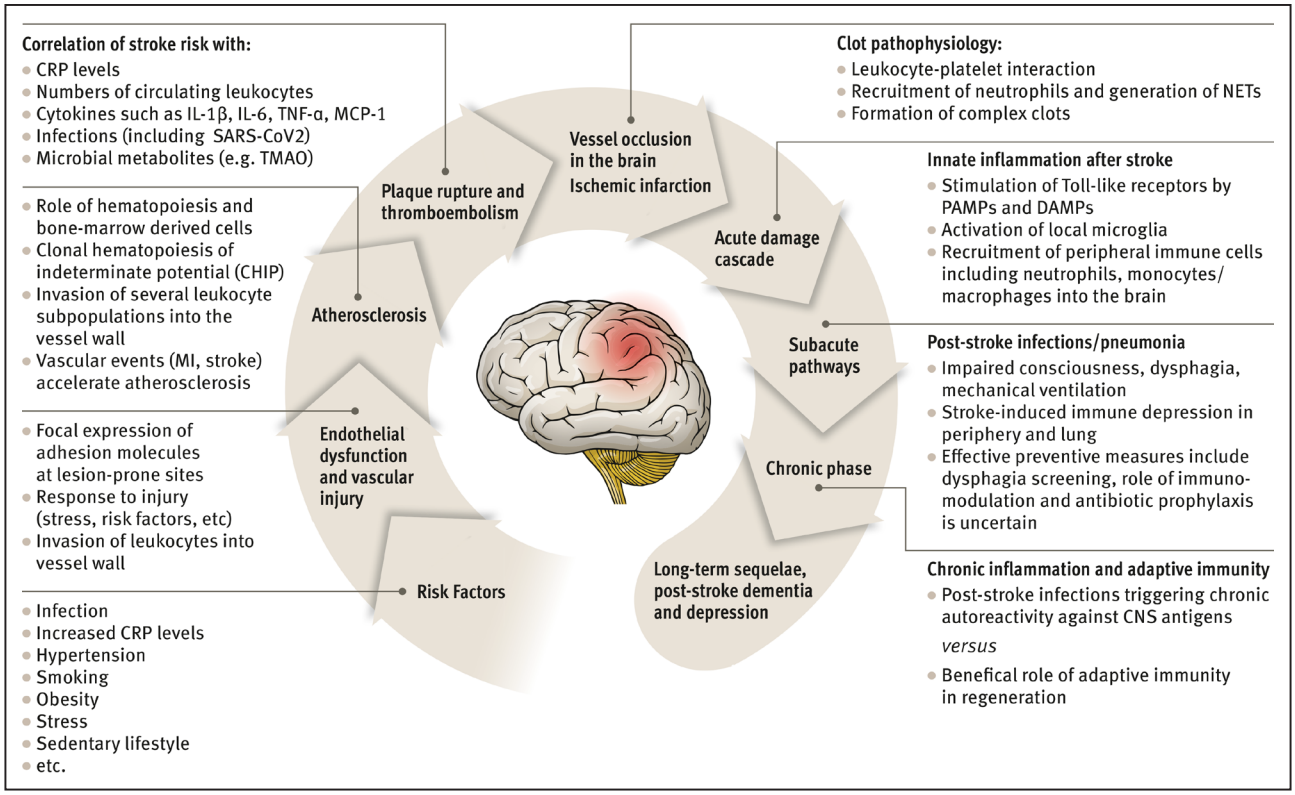

Figure 1. Inflammation and maladaptive immune mechanisms over the cerebrovascular disease continuum.
CHIP indicates clonal hematopoiesis of indeterminate potential; CNS, central nervous system; CRP, C-reactive protein; DAMP, damage-associated molecular pattern; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MI, myocardial infarction; NET, neutrophil extracellular trap; PAMP, pathogen-associated molecular pattern; TMAO, trimethylamine N-oxides; and TNF-α, tumor necrosis factor alpha.

ATHEROSCLEROSIS, INFLAMMATION, AND STROKE DEVELOPMENT

Atherosclerosis is one of the leading causes of death and disability worldwide and contributes to stroke by causing thromboembolism from the aortic arch and the cervical vessels and both thromboembolic and occlusive disease in large and small intracranial vessels.[6] For example, >50% stenosis of the carotid artery is found in 12% to 20% of all patients with stroke.[7] Microatheromas play an important role in small vessel disease of the brain. These often involve the long penetrating vessels and may lead to lacunar infarcts or more diffuse changes in the subcortical white matter due to lipohyalinosis of the vessels.

In 1856, Rudolf Virchow—the founder of cellular pathology—identified inflammatory changes as an important characteristic of atherosclerotic lesions.[8] Ross[9] first referred to atherosclerosis as a primary inflammatory disease. In fact, inflammation and immune mechanisms are involved in all stages of the disease: from initial development of endothelium dysfunction and fatty streaks to destabilization of atherosclerotic plaques and thromboembolism. Overall, these mechanisms are not specific for the pathogenesis of ischemic stroke but apply for all cardiovascular diseases and have been reviewed in detail elsewhere.[10,11]

In the further course of atherosclerosis, the picture is completed by a multitude of inflammatory mechanisms including the migration of additional inflammatory cells into the vessel wall, such as monocytes, as well as T cells and other lymphocyte subtypes.[9,10] It also appears that the early focal expression of adhesion molecules occurs precisely in sites in the vascular tree that are particular predilection sites for the development of such lesions, such as the carotid bifurcation.[12,13] Macrophages are the central regulators of the local inflammatory response penetrating the vessel wall via interaction with VLA4 (α4β1 integrin), CCR (C-C chemokine receptor) 2, as well as CCR5, but to a smaller extent, they can also proliferate in the vessel wall itself.[14] Cholesterol crystals in lipid-laden foamy macrophages in turn activate the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3)-containing inflammasome, which subsequently leads to upregulation of a number of proinflammatory ILs (interleukins).[15] In addition, neutrophils secrete a variety of inflammatory mediators (such as CCL2 [C-C chemokine ligand 2], cathepsin G, and α-defensins)[16] and neutrophil extracellular traps (NETs) induce massive macrophage-dependent activation of the NLRP3 inflammasome and IL-1β production.[17] As the process continues, a lipid-rich core is formed by apoptosis and accumulation of cell debris and lipid pools in the vessel wall. Due to the production of lytic enzymes

such as metalloproteinases and release of cytokines, the fibrous cap above such necrotic cores can then rupture, leading to atherothrombosis and further thromboembolic events through activation of platelets and coagulation factors.[9] Especially in the carotid bifurcation, so-called complicated but nonstenosing carotid artery plaques with surface defects, hemorrhage, or thrombus can develop. These plaques can be detected by high-resolution, contrast-enhanced magnetic resonance imaging and have been identified as a cause of cryptogenic stroke.[18]

Leukocytes and Hematopoiesis

A particular driver of atherosclerosis is leukocytes migrating into the vessel wall.[19] Diabetes, hypercholesterolemia, and hypertension, but also lifestyle factors such as smoking, sedentary lifestyle, unhealthy eating, stress, or lack of sleep, are relevant risk factors for the development of atherosclerosis, and in turn, all lead to maladaptive changes in the immune system and the hematopoietic stem cell niche in the bone marrow (BM; for review, see References[11,19,20]). While atherosclerosis is typically a slowly and steadily progressive disease, crisis deteriorations may also occur.[21] Following an experimental acute stroke or myocardial infarction, sympathetic stimulation of the BM triggers the release of BM-derived cells into the circulation and can accelerate underlying atherosclerosis.[11,19,22] In patients with stroke, the hypothalamic-pituitary-axis mediates B lymphopoiesis defects, and increased cortisol levels correlate with decreased blood lymphocyte numbers.[23] Recently, the so-called clonal hematopoiesis of indeterminate potential (CHIP) has emerged as a novel cardiovascular risk factor (reviewed in detail in References[24,25]). CHIP is due to a series of somatic mutations in various genes in myeloid cells (such as *DNMT3a*, *TET2*, or *ASCL1*) that causes clonal expansion, which in turn displaces regular hematopoietic cells in the BM during aging.[25] Individuals with CHIP have a higher risk of vascular events, likely mediated by inflammation and cytokine pathways.[24] There are, however, few studies linking CHIP with ischemic stroke, and the specific role of CHIP in the pathogenesis of stroke remains to be determined.[26]

Inflammatory Biomarkers and Stroke Risk Prediction

Individuals with elevated leukocytes as well as various inflammatory biomarkers such as CRP (C-reactive protein) in the blood have a higher risk of coronary heart disease, stroke, and mortality.[27,28] Importantly, CRP levels are independent of LDL (low-density lipoprotein) cholesterol: both patients with normal or low LDL cholesterol levels but elevated CRP levels show an increased absolute vascular risk, and hs-CRP (high-sensitive C-reactive protein) shows an additional vascular risk across all ranges of LDL cholesterol elevation.[29]

The inflammatory cascade is characterized by a release of cytokines, such as IL-1β, IL-6, and TNF-α (tumor necrosis factor alpha), and again, correlations with vascular events, including stroke, have been demonstrated.[30] Recently, blood MCP-1 (monocyte chemoattractant protein-1) was identified to be predictive of future stroke risk, especially large-artery and cardioembolic strokes.[31] This was the result of a Mendelian randomization study, which provides causal relationships between exposures and outcomes.[31] This has also been confirmed in large-scale observational studies, and MCP-1 and its receptor CCR2 are thus attractive therapeutic targets for stroke prevention.[31] In addition, the gut microbiome with its metabolites such as trimethylamine N-oxides[32] and several infectious diseases affecting vessels are associated with an increased stroke risk. These include various viral diseases, for example, varicella zoster virus, cytomegalovirus, hepatitis B and C, HIV, as well as various fungal and bacterial infections, for example, *Chlamydia pneumoniae* and *H influenzae* (Haemophilus influenzae).[33]

SARS-CoV2 and Ischemic Stroke Risk

SARS-CoV2 is an important new member of this group. Evidence for a link between SARS-CoV2 and an increased risk for acute ischemic stroke is accumulating. An association between coronaviruses and stroke was suggested as early as 2004, when patients with SARS-CoV type 1 developed stroke.[34] In patients with COVID-19, meta-analyses found a stroke incidence rate of 1.1% to 1.6%.[35] Compared with noninfected contemporary or historical controls, patients with COVID-19 had a 3.6-fold increased risk of ischemic stroke.[36] Although reporting bias must be considered, the adjusted risk of stroke after SARS-CoV2 infection appears to be higher (1.3%) than after influenza infection (0.2%).[37] A nationwide Danish registry found a significantly increased incidence of stroke in patients with SARS-CoV2 infection and one-third experience stroke as the first symptom of the infection.[38] Importantly, patients with COVID-19 who experience stroke have a worse prognosis than patients without stroke.[39]

Of note, stroke prevalence during the COVID-19 pandemic also increased in people without relevant vascular risk factors, and more severe strokes were observed.[36,38] These observations support a mechanism that is independent of atherosclerotic pathways and rather involves the innate immunity and immune-mediated hypercoagulopathy.

Immune-Mediated Thromboembolism and Hypercoagulopathy in COVID-19

Immune-mediated activation of coagulation is part of the physiological host response to pathogens. Indeed, patients with stroke with SARS-CoV2 infection show

higher levels of coagulation factors such as D-dimers, fibrinogen, factor VIII, von Willebrand Factor, antiphospholipid antibodies, and lupus anticoagulant.[35,40] In addition, thrombotic complications such as deep venous thrombosis (DVT) and pulmonary embolism (PE) are hallmarks of COVID-19.[41] This led to the hypothesis of a specific COVID-19–associated, immune-mediated coagulopathy. Although COVID-19–associated coagulopathy shares features with sepsis-induced coagulopathies or thrombotic microangiopathy, several differences have been noted. For example, not only is the incidence of thrombotic events higher than in sepsis but also some of the inflammatory mediators are different.[42] Patients with COVID-19 often have elevated fibrinogen, D-dimers, and von Willebrand Factor levels early on but only minor changes in prothrombin time and platelet count if compared with acute bacterial sepsis with thrombocytopenia, prolonged prothrombin times, and decreased antithrombin levels.[42] Hyperactivation of fibrinolysis was found with increased levels of plasminogen activators and D-dimer in the course of COVID-19 but not in sepsis-induced coagulopathy or disseminated intravascular coagulation.[43] This is of interest because immune-mediated coagulopathy is generally considered to be independent of the type of invading pathogen. With respect to inflammation in general, this interaction will be described in more detail below.

SARS-CoV2 Endothelialitis

Of note, endothelial cells are prone to SARS-CoV2 infection as they express the ACE-2 (angiotensin-converting enzyme 2) receptor, through which SARS-CoV-2 enters cells preferably. Normal function of endothelial cells is impaired by endothelialitis, especially the antithrombotic activity of the endothelial surface.[44] At the surface, endothelial NO release prevents leukocyte and platelet adhesion, inflammatory cell migration into the vessel wall, and smooth muscle cell proliferation and suppresses apoptosis and inflammation.[45] After overcoming the first line of defence, SARS-CoV2 forces its intracellular viral replication and its release into the bloodstream. Activated macrophages trigger proinflammatory (TNF-α) and chemotactic cytokines, which promote an increase of IL-6, which may in turn induce GM-CSF (granulocyte-macrophage colony-stimulating factor), thereby perpetuating the inflammatory process.[46] Such perpetuation with persistent viremia may ultimately lead to a cytokine storm and a systemic inflammatory response syndrome that exacerbates the hypercoagulopathy.[47] During the inflammatory state, NETs are released in patients with COVID-19.[48] NETs, in turn, stimulate the extrinsic coagulation cascade and lead to thrombus formation.[49] A distinct neutrophil-platelet activation pattern reflects COVID-19 disease severity and systemic hypercoagulability[50] (also see below and Figure 2). Furthermore, macrophages were found around the arteries and in thrombi of patients with COVID-19. Complement activation correlates with the severity of COVID-19,[51] and these results support the notion that complement activation is involved in the persistent inflammation and coagulation in these patients.[52] Overall, the interplay between innate immunity, platelets, and endothelial cells in the maladaptive host immune system leads to excessive activation of microvascular immune-mediated thrombosis and hypercoagulopathy.[46,53]

The efficiency with which the coronavirus binds to ACE-2 is a key factor of transmissibility.[54] ACE-2 also plays a critical role in the alternative RAS pathway regulating blood pressure. ACE-2 converts angiotensin II to angiotensin 1–7 and stimulates its vasodilating and anti-inflammatory effects. SARS-CoV2 causes downregulation of ACE-2 expression in affected cells, leading to a reduction in the conversion of angiotensin II by ACE, which in turn decreases inhibition of adhesion and migration of leukocytes.[55] Downregulation of ACE-2 also impairs the reduction of platelet aggregation and NO release.[56] Thus, SARS-CoV-2 induces a hypercoagulate state by inactivating ACE-2 receptors. In addition, RAS activation may impair atherosclerotic plaque stability leading to plaque rupture and thromboembolism.

Cardiac tissue also expresses ACE-2. Magnetic resonance imaging of the heart revealed cardiac involvement in 78% of patients with COVID-19, with 60% having persistent inflammation with lymphocytic infiltration.[57] Therefore, inflammatory mechanisms leading to arrhythmias or cardiac thrombi may contribute to the increased incidence of stroke in patients with COVID-19, too. In conclusion, SARS-CoV2 infection is a prominent and prime example proving the close association between inflammation and coagulation, with respect to COVID-19 in particular because direct vascular (endothelial) infection occurs. This argues in favor of anti-inflammatory therapies.

Anti-Inflammatory Therapies for Prevention of Vascular Events and Stroke

Due to its relationship with peripheral inflammation, as well as to direct vascular infection, vascular inflammation is a highly attractive target to prevent stroke and other vascular events. Secondary prevention trials investigated a number of anti-inflammatory agents including statins, canakinumab (an IL-1β antibody), colchicine (a tubulin disruptor with pleiotropic anti-inflammatory effects), or methotrexate (for details, see Table 1). However, most of these trials predominantly studied patients after myocardial infarction or with stable coronary artery disease, so there is less evidence for secondary prevention of stroke. In addition to reductions in myocardial infarctions and vascular death, however, a reduction in secondary strokes was observed in some of these trials (for

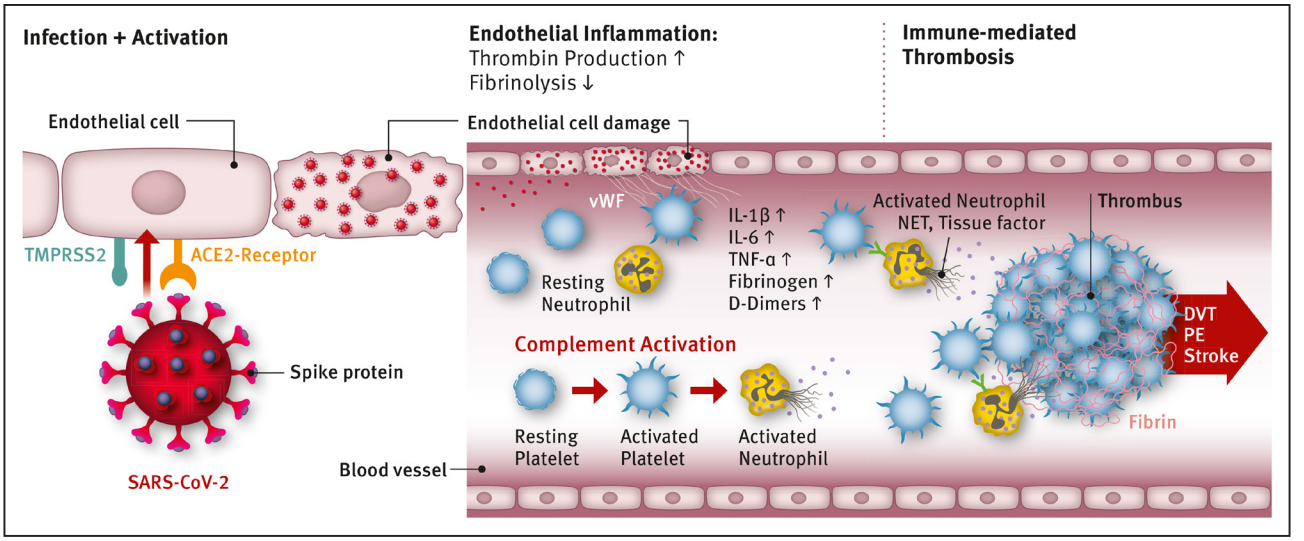

Figure 2. SARS-CoV2 and thromboembolism.
SARS-CoV2 expresses a spike protein that binds to the endothelial cells' ACE-2 (angiotensin-converting enzyme 2). Virus cell entry is facilitated by TMPRSS2 (transmembrane protease serine 2). Invasion of endothelial cells activates exposure of the VWF (von Willebrand Factor). Endothelial cells are forced to replicate and release further viruses that are finally detected by immune cells that upregulate the inflammasome including pro-IL (interleukin)-1β. Inflammasome and neutrophil activation further induce production of IL-6, TNF-α (tumor necrosis factor alpha), and chemokines (not shown) perpetuating the inflammasome. Endothelial inflammation increases thrombin production and reduces fibrinolysis. Platelets are activated and interact with neutrophils via P-selectin. Neutrophils release neutrophil extracellular traps (NETs) and tissue factor inducing thrombus formation. Thrombus may cause vessel blockage causing ischemic stroke, systemic embolism, or pulmonary embolism (PE). DVT indicates deep venous thrombosis.

details, see Table 1). The ongoing CONVINCE trial (Colchicine for Prevention of Vascular Inflammation in Non-CardioEmbolic Stroke) seeks to fill this gap by including patients 3 to 28 days after noncardioembolic strokes or high-risk transient ischemic attack randomized to treatment with 0.5 mg colchicine or placebo and follow-up for 36 months.[58] Other anti-inflammatory agents or targets being tested in vascular high-risk cohorts include P-selectin, p38 mitogen-activated protein (MAP) kinase, phospholipases, salicylates, or n-3 fatty acids.[6,59,60] However, global anti-inflammatory therapies may ultimately remain a blunt instrument because of the important role of both context-dependent and time-dependent changes in innate and adaptive immunity. A better understanding of these complexities is needed to target therapies that can enhance beneficial aspects of the immune response while inhibiting deleterious aspects.

INNATE INFLAMMATION AFTER STROKE

Damage-Associated Molecular Patterns and Innate Inflammation After Stroke

Once ischemic stroke has developed, an intricate innate immune response is triggered. Focal brain ischemia elicits necrotic cell death leading to the uncontrolled release of intracellular molecules. Their abnormal extracellular presence stimulates what is called sterile inflammation.[61] Key intracellular molecules released by damage act as damage-associated molecular patterns (DAMPs) and initiate inflammatory responses via PRRs (pattern recognition receptors). In the context of stroke, different PRRs are involved, such as NLRs (nucleotide-binding oligomerization domain, leucine-rich repeat-containing receptors) or NOD (nucleotide oligomerization domain)-like receptors and TLRs (Toll-like receptors).[62] TLR4 and TLR2 are activated by stroke-associated DAMPs, such as HMGB1 (high mobility group box 1), heat shock proteins, mainly Hsp70 (heat shock protein 70), peroxiredoxins, purines, and several endogenous TLR2/TLR4 ligands, such as fibronectin, defensin or heparin sulfate proteoglycan.[63] After human stroke, DAMPs are elevated and HMGB1 levels have been associated with outcome.[64,65]

The stimulation of TLRs initiates innate immune responses by inducing proinflammatory genes, cytokines, adhesion molecules, and the activation of adaptive immunity.[66] Upon ligand binding, TLR4, the most abundantly described TLR in relation to stroke, dimerizes and forms a complex with MD2, initiating downstream MyD88 (myeloid differentiation primary response 88)- or TRIF (TIR-domain-containing adapter-inducing interferon-β)-dependent signaling, involved in the early and late phases of NF-κB (nuclear factor kappa B) activation. In either case, NF-κB activation is required for inflammatory cytokine production.[67] TLR4 absence reduces brain damage and inflammation after experimental stroke[68] and TLR4 expression on human circulating immune cells correlates with subsequent inflammatory response and outcome of stroke patients,[69] strongly supporting TLR4 activation as a determinant for the initiation of innate inflammation in stroke. Of note, an aptamer developed as TLR4 antagonist[70] is currently under investigation in

Table 1. Selected Trials on Anti-Inflammatory Therapies for the Prevention of Vascular Events and Stroke

Trial	Drug/design	Population	Outcome	Result (primary end point/ stroke)	Trial registry
JUPITER	Rosuvastatin 20 mg vs placebo	17 802 individuals, no cardiovascular disease, normal LDL-C, and CRP ≥2 mg/L	MACE plus revascularization and hospitalization	Positive trial; HR, 0.56 for primary end point; HR, 0.52 for stroke	NCT00239681[196]
CANTOS	Canakinumab 50, 150, and 300 mg (IL-1β antibody) vs placebo	10 061 patients with stable CAD, MI >30 d, and CRP ≥2 mg/L	MACE	Positive trial; HR, 0.85 for primary end point (150-mg dose); HR, 0.93 for stroke (NS); lower CRP, IL-1β, and IL-6	NCT01327846[197]
COLCOT	Colchicine 0.5 mg (tubulin disrupter) vs placebo	4745 patients with recent MI <30 d	MACE plus hospitalization for revascularization	Positive trial; HR, 0.77 for primary end point; HR, 0.26 for stroke	NCT02551094[198]
LoDoCo	Colchicine 0.5 mg vs placebo	532 patients with stable CAD	MACE	Positive trial; HR, 0.33 for primary end point; HR, 0.23 for noncardioembolic stroke	ACTRN12610000293066[199]
LoDoCo2	Colchicine 0.5 mg vs placebo	5522 patients with chronic CAD	MACE plus coronary revascularization	Positive trial; HR, 0.69 for primary end point; HR, 0.66 for ischemic stroke	ACTRN12614000093684[200]
CONVINCE	Colchicine 0.5 mg vs placebo	3154 patients with noncardioembolic ischemic stroke or TIA	MACE plus rehospitalization for unstable angina	Ongoing (results expected in 2023)	NCT02898610[58]
CIRT	Methotrexate 15–20 mg vs Placebo	4786 patients with stable CAD plus type 2 diabetes or metabolic syndrome	MACE plus revascularization	Negative trial; HR, 0.96 for primary end point; HR, 0.91 for nonfatal stroke	NCT01594333[60]
LILACS	Aldesleukin (human recombinant IL-2)	patients with stable CAD (part A with 25 patients) and ACS (part B with 32 patients)	Safety and tolerability, circulating Treg numbers	Ongoing	NCT03113773[201]

ACS indicates acute coronary syndrome; CAD, coronary artery disease; CANTOS, Canakinumab Antiinflammatory Thrombosis Outcomes Study; CIRT, Cardiovascular Inflammation Reduction Trial; COLCOT, Colchicine Cardiovascular Outcomes Trial; CONVINCE, Colchicine for Prevention of Vascular Inflammation in Non-CardioEmbolic Stroke Trial; CRP, C-reactive protein; HR, hazard ratio; IL, interleukin; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; LDL-C, low density lipoprotein (LDL) - cholesterol; LILACS, low-dose interleukin-2 in patients with stable ischaemic heart disease and acute coronary syndromes; LoDoCo2, Low-Dose Colchicine 2 Trial; MACE, major adverse cardiovascular event; MI, myocardial infarction; NS, nonsignificant; TIA, transient ischemic attack; and Treg, regulatorty T-cell.

phase Ib/IIa clinical trial (NCT04734548; Table 2). After stroke, DAMPs not only activate brain resident immune cells (microglia) but also recruit peripheral immune cells to the brain when released into the bloodstream (Figure 3). Circulating immune cells are activated peripherally and recruited to the brain where they contribute to poststroke inflammation and resolution.[63,71]

Microglia

In the injured brain, inflammatory responses involve an early activation of microglia, the resident myeloid immune cells and first responder after injury. Since these resident cells are not replaced by bone marrow–derived cells, they are likely to be endowed with an extensive set of brain-related functions that peripheral myeloid cells lack. Whereas in homeostasis they contribute to maintain brain healthy function, after sensing adjacent cell damage, they release cytokines and chemokines, their processes quickly and autonomously converge on the site of injury, and their morphology evolves from a ramified to an ameboid appearance.[72] In stroke, several receptors mediate this microglial activation. Experimental evidence showing that TLR4 is present in microglia after exposure to pathogen-associated molecular patterns such as LPS,[73] and that TLR4 inhibition reduced LPS-induced microglia activation and reactive oxygen species production,[74] suggested that TLR-induced microglia activation leading to a M1-like proinflammatory phenotype may happen in human brain after stroke and may exacerbate the pathology. Of note, studies in mice suggest that the microglia response is highly dynamic, whereby early M2-like phenotypes are temporally followed by a transition to pathological M1 subsets.[75] However, rather than the simplistic proinflammatory M1 versus anti-inflammatory M2 phenotypic dichotomy, in vivo microglia display a much larger functional and phenotypic diversity, with dynamic temporal features.[76] Thus, a range of different transcriptional programs allow these cells to participate in brain injury but also to promote resolution of local inflammation, for example, through release of proresolving cytokines,[5] phagocytosis of dead cells, and secretion of trophic factors for tissue repair.[77] An additional protective role of microglia, mediated by purinergic receptors, is to contain the lesion by projecting their processes toward the injured blood vessels, forming rosettes, an effect attributed to astrocytic ATP release and subsequent P2RY12 microglia signaling.[78] P2RY12 expression in microglia is decreased in the lesion core of postmortem human stroke brains, where round myeloid cells were found, in contrast to ramified microglia at the

Table 2. Selected Trials on Immunomodulatory Therapies for Acute Stroke

Trial name	Mode of action	Drug/design	Population	Primary end point	Result	Trial registry
STEMTHER	Neuroprotective, stimulates neurogenesis and angiogenesis (1)	Filgrastim (G-CSF) 10 µg/kg subcutaneously once daily×5 d vs no intervention (phase I)	20 stroke patients	Day 180 mRS	Negative trial; no impact on outcome	NCT00901381[202]
AXIS 2		Filgrastim (G-CSF) 135 µg/kg vs placebo (phase II)	324 treated stroke patients (161 G-CSF/163 placebo)	Day 90 mRS, NIHSS	Negative trial; no benefit in clinical outcome	NCT00927836[203]
	Reduces peripheral inflammatory response (2)	IL-1Ra 100 mg+2 mg/kg per h over 72 h vs placebo (phase II)	34 stroke patients (17 IL-1Ra/17 placebo)	Safety, NIHSS	Safe and well tolerated	[204]
SCIL-STROKE		IL-1Ra 6×100 mg vs placebo (phase II)	80 stroke patients (39 IL-1Ra/41 placebo)	Plasma IL-6 level	Interaction of IL-1Ra with tPA; reduced IL-6 level	ISRCTN74236229[205]
ACTION	Blocking leukocyte infiltration (3)	Natalizumab 300 mg vs placebo (phase II)	161 stroke patients (79 natalizumab/82 placebo)	Change in infarct volume	Negative trial; no reduced infarct growth	NCT01955707[206]
ACTION II	Blocking leukocyte infiltration (3)	Natalizumab 300 or 600 mg vs placebo (phase II)	267 stroke patients (88 natalizumab 300 mg/89 natalizumab 600 mg/90 placebo)	Day 90 mRS, Barthel index	Negative trial; no benefit in clinical outcome	NCT02730455[207]
	Reducing lymphocyte infiltration (3)	Fingolimod 3×0.5 mg vs placebo (all r-tPA; phase II)	47 (22 fingolimod+tPA/25 placebo+tPA)	Day 90 mRS	Less infarct growth; better mRS	NCT02002390[208]
		Fingolimod 3×0.5 mg vs placebo (all r-tPA; phase II)	118 stroke patients	Day 90 mRS	Ongoing (recruiting)	NCT04675762
REPAIR FAST		Fingolimod 3×0.5 mg+intravascular therapy vs placebo+intravascular therapy	20 stroke patients	Day 90 mRS	Ongoing (not yet recruiting)	NCT04718064
MASTERS	Multipotent adult progenitor cells modulating immune system (4)	MultiStem 400 million or 1.2 billion allogeneic, adult stem cells vs placebo (phase II)	137 (67 cells/62 placebo)	Safety, mRS	Safe but no improvement in mRS	NCT01436487[209]
MASTERS-2		MultiStem 1.2 billion allogeneic, adult stem cells vs placebo (phase III)	300 participants	Day 90 mRS	Recruiting	NCT03545607
	Endothelin-B receptor agonist improving neural cell survival and proliferation (4)	Sovateltide 0.9 µg/kg per d on 3 d vs placebo (phase II)	36 stroke patients (18 sovateltide/18 placebo)	Safety and efficacy	Improved mRS and BI	NCT04046484[210]
	Aptamer (single-stranded DNA molecule) targeting TLR4 (1)	ApTOLL 0.025–0.2 mg/kg vs placebo (phase Ib); 2 doses ApTOLL vs placebo (phase IIa)	34 (24 ApTOLL/8 placebo; phase Ib); 119 (70 ApTOLL/49 placebo; phase IIa)	Safety, infarct size	Recruiting	NCT04734548
AMASCIS-02	MSCs improve neural cell survival and regeneration (4)	allogeneic adipose tissue–derived stem cells 1×10^6 cells/kg vs placebo (phase IIb)	30 stroke patients (15/15)	Safety and tolerability, mRS, NIHSS	Recruiting	NCT04280003[211]
EAISE	Antibody against RGMa (4)	Elezanumab 13 doses every 4 wk vs placebo (phase II)	120 stroke patients	NIHSS, mRS	Recruiting	NCT04309474
	Pleiotropic immunomodulatory effects reducing inflammatory responses (3)	Dimethyl fumarate 6×240 mg vs placebo (phase II)	50 stroke patients	NHISS, mRS, infarct size	Not yet recruiting	NCT04890353
		Intra-arterial treatment+dimethyl fumarate 6×240 mg vs intra-arterial treatment+placebo (phase II)	50 stroke patients	Infarct size, hemorrhage volume, NIHSS	Not yet recruiting	NCT04891497
		r-tPA+dimethyl fumarate 6×240 mg vs r-tPA+placebo (phase II)	50 stroke patients	Infarct size, hemorrhage volume, NIHSS	Not yet recruiting	NCT04890366
IV IG/AIS	Pleiotropic immunomodulatory effects reducing inflammatory responses (3)	IV IG (Privigen) 1.0 g/kg vs placebo (phase I)		Day 90 mRS, NIHSS	Withdrawn (difficult recruitment and new black box warning for IV IG)	NCT01628055

ACTION indicates Effect of Natalizumab on Infarct Volume in Acute Ischemic Stroke; ApTOLL, aptamer targeting TLR4; AMASCIS-02, Allogeneic Adipose Tissue-derived Mesenchymal Stem Cells in Ischemic Stroke; AXIS 2, AX200 for the Treatment of Ischemic Stroke; BI, Barthel index; EAISE, A Safety and Efficacy Study of Intravenous (IV) Elezanumab Assessing Change in Neurologic Function in Adult Participants With Acute Ischemic Stroke; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; IL-1Ra, IL-1 receptor antagonist; IV IG, intravenous immunoglobulin; MASTERS, MultiStem® Administration for Stroke Treatment and Enhanced Recovery Study; mRS, modified Rankin Scale; MSC, mesenchymal stem cell; NIHSS, National Institutes of Health Stroke Scale; REPAIR FAST, Revascularization Pretreated With Fingolimod in Acute Stroke; r-tPA, recombinant tissue-type plasminogen activator; RGMa, repulsive guidance molecule A; SCIL-STROKE, Subcutaneous Interleukin-1 Receptor Antagonist in Ischemic Stroke Trial; STEMTHER, Granulocyte-Colony Stimulating Factor for Stem Cells Therapy for Acute Ischemic Stroke Trial; TLR, Toll-like receptor; and tPA, tissue-type plasminogen activator.

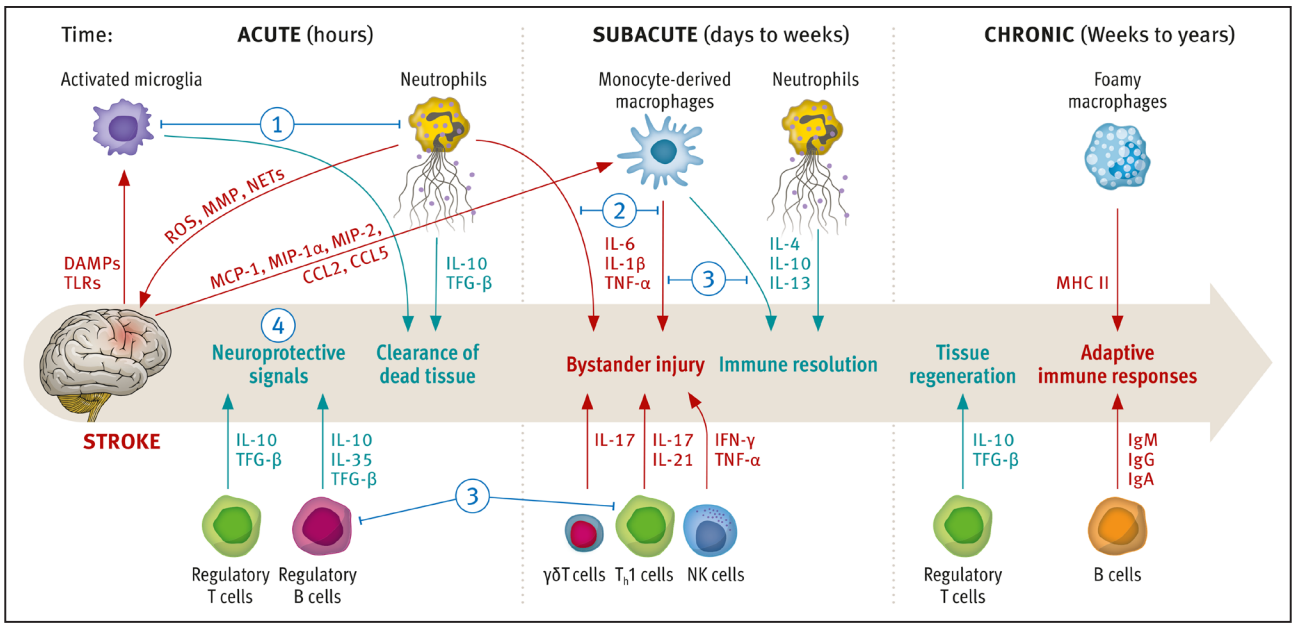

Figure 3. Signaling cascades of acute and chronic inflammation after ischemic stroke.
Cells of resident (microglia) and peripheral innate (neutrophils, monocytes) and adaptive (lymphocytes, NK cells) immunity interact following a spatiotemporal pattern to orchestrate an appropriate response to the ischemic brain. Immediately released damage-associated molecular patterns (DAMPs) activate microglia and recruit neutrophils and monocytes, which interact to remove dead tissue and promote resolution. The proinflammatory cytokines released in this process cannot also directly damage intact nerval cells but also trigger infiltration of NK and T cells, which in turn contribute to cell damage. Infiltrating regulatory T and B cells may attenuate inflammatory responses via secretion of IL (interleukin)-10 and TFG-β in the acute phase and contribute to regeneration in the chronic phase after stroke. However, autoreactive central nervous system antigen-specific T- and B-cell responses act in parallel to negatively affect long-term outcome after stroke. On the basis of these mechanisms, immunomodulatory strategies (1 - 4; see Table 2 for explanation) have been tested in clinical trials aimed at improving functional outcome after ischemic stroke. CCL indicates C-C chemokine ligand; IFN-γ, interferon gamma; MCP-1, monocyte chemoattractant protein-1; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; NET, neutrophil extracellular trap; NK, natural killer; ROS, reactive oxygen species; TLR, Toll-like receptor; TFG-β, transforming growth factor; and TNF-α, tumor necrosis factor alpha.

lesion rim.[79] In addition, STAT6 (signal transducer and activator of transcription 6) activation in microglia/macrophages from patients with stroke was described to induce an anti-inflammatory phenotype, promoting the resolution of inflammation in the ischemic brain.[80] Microglial activation has been detected with selective radiolabeled tracers and positron emission tomography (PET) in the infarct of patients with stroke[81,82] suggesting the presence of anti-inflammatory microglia.[79] Supporting the importance of microglial heterogeneity, different transcriptomic studies indicate that aging, sex-, region-, and disease-dependent phenotypic differences of microglia also exist.[83,84] Since transcriptional heterogeneity has been described in human microglia,[85] further studies are needed to elucidate whether this diverse heterogeneity has an impact on human stroke outcome.

Peripheral Myeloid Subsets

Stroke-induced inflammation also involves mobilization and recruitment of peripheral circulating neutrophils and inflammatory monocytes to the injury site, which participate in both acute injury and later lesion resolution.

Neutrophils arrive early and are key in the innate immune response (Figure 3). They have a short half-life and are highly heterogeneous, so their involvement in pathology is dependent on phenotype rather than numbers. In homeostasis, apart from the BM niches, neutrophils may be found at the intravascular level, either circulating or marginated in some locations, or within certain tissues,[86] but not in the brain.[87] However, in response to stroke, activated neutrophils are among the first peripheral cells recruited into the brain after experimental and human stroke.[71–73,88] In the first stages, early intravascular activation after interaction with platelets is one of the initial drivers of stroke inflammation and subsequent injury.[19] Once in the ischemic area, neutrophils participate in thrombus formation and expansion and also release NETs,[89] which in turn impair poststroke revascularization and vascular remodeling.[90] In the brain parenchyma, neutrophils contribute to tissue and blood-brain barrier damage by DAMP-induced TLR activation in resident cells, release of leukotrienes, cytokines, and chemokines, and production of reactive oxygen species.[73] In contrast with animal studies, evidence from ischemic human brains argues against a massive presence of neutrophils in the injured tissue,[91–93] an apparent controversy that may be due to the difficulties in studying human brains, the nature of the ischemic insult, methodological problems, and to the short-lived nature of these cells.

Neutrophil function is also important to ensure resolution of inflammation, scar formation, and repair in murine and human stroke,[94] a function in which neutrophil heterogeneity may play an important role. Importantly, recent studies using mass cytometry have identified early neutrophil progenitors at the BM, which can contribute to neutrophil heterogeneity not only by their potential to be mobilized in disease states but as targets of the thus termed trained immunity, by which these subsets build immune memory features evoked by exogenous or endogenous insults.[86] Beyond the BM, the presence of neutrophils with noncanonical functions such as homeostatic and stress-induced angiogenesis in lung and intestine likely aimed to mediate vascular repair suggests that these proangiogenic subsets could be mobilized from to other tissues.[95] Therefore, a wide range of neutrophil subpopulations exists that might have different impacts on stroke outcome—a fact that could be leveraged for therapeutic benefit rather than blanket targeting of neutrophil numbers.

Monocyte-derived macrophages, upon activation by DAMPs and other stress signals, infiltrate into brain infarcts early after murine stroke.[71,73] Primary infiltrating monocytes during the acute inflammatory response in mice are subsets of CCR2^{+}Ly6C (lymphocyte antigen 6 complex)^{hi} monocytes,[96–98] followed by CX3CR1^{+} (C-X3-C chemokine receptor 1) cells accumulating at the infarct core border 2 weeks after stroke onset. CCR2^{+} monocytes undergo a phenotypic transformation into CX3CR1^{+}CCR2^{-} cells following infiltration to the ischemic tissue.[98] These findings strengthen the idea that different effector functions of monocytes/macrophages contribute to both brain injury and repair.[73] CCR2^{+} monocytes may play an important role in anti-inflammatory and recovery processes after cerebral ischemia in mouse models, as their absence has been associated with poorer functional outcome.[99] However, reduced monocyte infiltration after stroke due to the absence of CCR2 or its ligand CCL2 correlates with better outcomes and reduced proinflammatory response.[100] CD68^{+} monocytes infiltrate human ischemic lesions at early stages of stroke.[91] Resident microglia and recruited monocytes differentiate into proinflammatory macrophages in earlier stages; in contrast, at later time points, monocyte-derived macrophages disappear and microglia-derived cells tend to restore the homeostatic state. In addition, markers of anti-inflammatory phenotypes were found in lesions at late resorption stages,[93] consistent with a role in resolution. An important caveat to keep in mind when analyzing human stroke samples is that the distinct role of infiltrating monocytes/macrophages versus resident microglia is not clearly established. The identity of pro- versus anti-inflammatory subsets and mechanisms of transition between them are also controversial. Identification of specific myeloid subsets and their functions is important to unravel the diversity of the myeloid compartment in stroke pathogenesis.[71,73]

Source and Mobilization of Immune Myeloid Cells in Stroke

Increased circulating monocyte and neutrophil counts have been consistently described in the bloodstream of acute stroke patients[80,94] and are associated with poor outcome.[69,88] Interestingly, monocyte subtypes can also predict clinical outcomes.[101] Experimental research showed that mechanisms mediating neutrophilia and monocytosis involve mobilization of cells from spleen and BM niches (femur and tibia) at the periphery.[22,102,103] After mobilization, several routes have been proposed for the entry of leukocytes into the central nervous system. Peripheral leukocytes have been traditionally proposed to infiltrate the injured brain parenchyma by transendothelial migration through the blood-brain barrier. In addition, the meninges enveloping the central nervous system operate as a portal where such peripheral immune cells pile up and thereafter infiltrate the brain, mainly in pathological conditions.[104,105] Furthermore, the skull BM niches constitute an increasingly recognized source of immune cells, from which they mobilize using microscopic vascular channels crossing the skull-dura interface.[106] Mouse meninges have been shown to contain a pool of monocytes and neutrophils derived from the adjacent skull and vertebral BMs with a specific transcriptional signature distinct from cells derived from the peripheral blood,[107] as well as a lymphopoietic niche hosting B cells.[108]

Emerging evidence shows that various cardiovascular diseases promote an alteration of the BM niche that causes an overproduction of proinflammatory myeloid subsets and systemic leukocytosis,[109] suggesting that the presence of cardiovascular risk factors may induce vascular BM remodeling with deleterious impact on subsequent strokes. In particular, in the skull, recent data demonstrate that the BM niches are different from those in other bones and house a highly heterogeneous set of immune cells displaying unique transcriptional signatures, which are also manifested in specific disease-associated patterns in the human skull, including in patients with stroke.[110] Cranial hematopoiesis and subsequent cellular transcriptional signatures may be modulated by dural cerebrospinal fluid efflux,[111] which may be important as a therapeutic target in neuroinflammatory conditions.

Meningeal lymphatics, involved in drainage of blood solutes in pathological conditions, may also participate in immune cell circulation after brain damage—a process in which meningeal lymphangiogenesis might be involved.[112] However, observations on the effects of meningeal lymphatics impairment in animal models are so far contradictory.[112]

Leukocyte-Platelet Interactions and Clot Pathophysiology

Platelets are active immune elements in both innate and adaptive arms; their interactions with leukocytes,

specifically with neutrophils, are critical checkpoints during the early stages of inflammation at the intravascular level.[113] As regards innate immunity, platelets are endowed with PRRs, including TLRs, NLRs, and CLRs (C-type lectin receptors), which allow them to respond when bound by DAMPs or pathogen-associated molecular patterns. Specifically, when TLRs are activated, the release or surface expression of granule proteins occur, and aggregates between platelets and neutrophils and monocytes form and promote tissue infiltration. In addition, platelet TLRs participate in the process of NETosis, in the enhancement of blood-brain barrier permeability, and in the amplification of the inflammatory signaling by releasing further DAMPs such as HMGB1 and ATP.[114]

Leukocyte adhesion is accompanied by the recruitment of rolling and adherent platelets. A potential consequence of platelet attachment to leukocytes is an enhanced cell activation and platelet-leukocyte aggregate formation within cerebral venules that could enter the circulation.[115] Consequently, platelet-leukocyte aggregates are significantly increased in peripheral blood of patients with stroke.[116] These interactions may play a role in the no-reflow phenomenon described in some ischemic stroke patients with incomplete tissue reperfusion even after clot removal.[117] The importance of the clot composition, with different molecular (eg, fibrin, von Willebrand Factor, NETs) and cellular components (eg, red blood cells, platelets, leukocytes, bacteria), is increasingly considered as target to be explored, as it may be important for the failure of stroke therapies and outcome in the chronic phase.[118]

Several studies demonstrated that NETs and neutrophils are important elements of cerebral thrombi[119] and NET content accounts for resistance to reperfusion therapies.[89] Consistently, NET's presence and amount in thrombi were also associated with patients' outcome after ischemic stroke and myocardial infarction.[120] Human platelet-rich recombinant tPA (tissue-type plasminogen activator)-resistant thrombi from ischemic stroke patients are effectively lysed by DNase-I.[89] This, together with the demonstration that platelet TLR4 mediates NET formation after experimental stroke,[89] poses novel therapies for the treatment of acute stroke caused by platelet-rich thrombosis.

ADAPTIVE INFLAMMATION AFTER STROKE

Both T and B lymphocytes enter the brain within the first weeks after stroke, T cells within a few days, and B cells after 7 days (Figure 3). Both types then accumulate over weeks and months. Various T-cell subtypes have been identified, with proinflammatory subtypes such as the CD4+ Th1 and Th17 cells, as well as γδ and CD8+ T cells being harmful and worsening stroke size and outcomes, while Tregs (regulatory T-cells) are conversely beneficial.[121,122] This beneficial effect of T cells has been attributed to their ability to confer tolerance to brain antigens and to produce IL-10.[123] There is strong evidence that IL-10 production is important, however, and that boosting Tregs improves stroke outcomes by limiting neuroinflammation via IL-10 production.[124] And although most studies demonstrate that Tregs improve stroke outcome, they may also worsen strokes by causing microvascular dysfunction in the acute phase of stroke.[125] Once in the brain, T cells accumulate and proliferate for at least 1 month after stroke in rodents and humans[126] and are present for at least 12 weeks.[127]

Commensal gut bacteria can influence adaptive immune responses to stroke, including via stroke-induced autonomic stimulation of the gut, which causes changes in gut immune cells.[128,129] Disbalanced gut microbiota (dysbiosis) after stroke induces a proinflammatory T-cell polarization in the intestinal immune compartment resulting in an increased migration of IL-17(+) γδ T cells from the gut, via the leptomeninges, and into ischemic brain tissue, while Tregs are decreased.[128,129] Systemically administered antibiotics change gut microbiota, with both positive and negative effects on outcome after experimental stroke.[128,129] Retrospective clinical data suggest that antibiotic classes may cause both negative (eg, carbapenems) and positive effects (eg, macrolides) on long-term outcome after stroke. Direct effects on the nervous cells and indirect effects via immunity and the gut microbiome may play a role.[130]

B cells reside in the dura[131] and are very sparse in uninjured parenchyma.[127] During the first week after stroke, IL-10 producing regulatory B cells are protective[132]; however, over the next weeks, and increasing in number over at least 7 weeks, B lymphocytes accumulate in the stroke scar in mice, often forming structures similar to tertiary lymphoid follicles with IgG and IgA class-switched B cells.[127,133] These delayed responses are dependent on CD4-expressing T cells.[134] There is no (yet) conclusive evidence that autoimmune responses to brain antigens play a major role in chronic sequelae (see below), but they do adversely affect initial stroke outcomes. Brain antigens are released by dying brain cells into the bloodstream. Antigen-presenting cells occur in association with brain antigens very rapidly not only in the brain but also in the deep cervical lymph nodes.[135] Furthermore, a proinflammatory state at the time of stroke can increase the production of Th1 cells and autoantibodies that are associated with worsened stroke size and motor outcomes.[136] This proinflammatory state in humans may be due to circulating proinflammatory factors induced by cardiovascular risk factors, including obesity, diabetes, and hypertension, but peri-stroke infection is likely the greatest inducer of autoimmunity after stroke.[33]

The beneficial effects of the adaptive immune system are not limited to cytoprotective functions in the acute phase but may also include the orchestration of multiple distinct regenerative processes in chronic stroke (Figure 3). For example, the accumulation of Treg cells in the brain promotes neurological recovery after experimental

stroke by suppressing neurotoxic astrogliosis via stimulation of EGFR (epidermal growth factor receptor) by amphiregulin.[137] Osteopontin produced by Treg cells enhances oligodendrogenesis via microglial repair activity, thus promoting white matter repair and functional recovery after stroke. This effect can be boosted by administration of IL-2:IL-2 antibody complexes.[138] Proregenerative effects after experimental stroke have also been proposed for B lymphocytes. Stroke might induce significant bilateral diapedesis of endogenous B cells from ischemic tissue to distant brain regions regulating motor and cognitive functions via neurogenesis. B-cell depletion not only reduces stroke-induced neurogenesis in remote regions such as the hippocampus but also worsens motor and cognitive recovery.[139]

Differentiation and function of immune cells require dynamic reprogramming of cellular metabolism. Cellular reprogramming may also occur due to the hypoxic-hypoglycemic microenvironment in ischemic brain tissue.[140] For example, metabolic reprogramming of CD4+ T cells is involved in imbalanced differentiation of CD4+ T cells after stroke. Here, the ACC1 (acetyl coenzyme A carboxylase 1) and the RAGE (receptor for advanced glycation end products) play an essential role. Blocking AAC1 as well as the administration of soluble RAGE reverses the ischemia-induced Treg/Th17 imbalance and improves functional recovery after stroke.[141] Metabolic reprogramming can also be induced by the intestinal microbiome via fermentation products, such as short-chain fatty acids. Short-chain fatty acids can stimulate neuronal plasticity after a stroke via circulating lymphocytes and the activation of microglia.[142]

STROKE-INDUCED IMMUNE DEPRESSION AND POSTSTROKE INFECTIONS

Bacterial infections, particularly urinary tract infections and pneumonia, are among the most common complications after stroke, with pneumonia having a major impact on prognosis. Stroke-associated pneumonia (SAP) occurs in ≈10% of patients, increasing mortality and worsening outcome in survivors.[143,144] Stroke-induced changes that directly affect lung tissue integrity are poorly characterized. However, well-studied predisposing factors and systemic changes allow conclusions to be drawn about pulmonary defense dysfunction and occurrence of pneumonia. In addition to the general risk factors, such as impaired consciousness, dysphagia, and mechanical ventilation, stroke-induced suppression of peripheral immunity independently contributes to the occurrence of SAP[145,146] (Figure 4). In the acute phase of stroke, catecholamines, corticosteroids, and cytokines are released, which induce a systemic immunosuppressive state associated with severe outcomes and increasing risk for infections. The impaired immunity following central nervous system injury involves alterations of innate and adaptive immune responses.[147]

Bacterial killing, surface HLA-DR (human leukocyte antigen–DR isotype), or CD11b expression, all important factors of the innate defense by neutrophils and monocytes, are diminished after stroke.[148] Suppression of adaptive immune responses includes apoptotic loss of peripheral and splenic lymphocyte subpopulations, splenic atrophy, and functional impairment of the remaining lymphocytes.[69,149] In addition to direct proapoptotic effects of β-adrenergic signaling from stress axis overactivation, stroke induces a FasL (Fas ligand)-expressing monocyte population that also leads to T-cell apoptosis. This mechanism is driven by AIM2 (absent in melanoma 2) inflammasome-dependent secretion of IL-1β triggered by cell-free DNA.[150] Functional impairment of lymphocytes is characterized by a reduced proliferative capacity that is mediated through soluble CD163 released from activated monocytes.[151]

Moreover, a cytokine shift, particularly diminished IFN-γ (interferon gamma) production by T and natural killer (NK) cells and increased IL-10 secretion by iNKT (invariant natural killer T cells) and Treg cells, causes increased susceptibility to poststroke infection.[152–154] Interestingly, while these mechanisms are detrimental because they lead to infection, they may also represent an endogenous mechanism to reduce the risk of poststroke autoreactive immune mechanisms caused by overactivation of adaptive immunity in the ischemic brain.[151,155]

Over the last decade, significant progress has been made in characterizing immunologic changes in stroke patients with and without infection. Immune markers predicting the severity of immunosuppression and onset of SAP may be utilized to improve the design of clinical trials to prevent poststroke infections. For example, white blood cell count or neutrophil-to-lymphocyte ratio has been discussed as a useful biomarker of complications in acute stroke patients.[156] Other commonly measured blood markers, such as hyperglycemia and low HDL (high-density lipoprotein) cholesterol, as well as monocyte–to-HDL ratio, are also significantly associated with immunosuppression and SAP.[157,158] Clinical studies have also examined the classic acute-phase proteins CRP, SAA (serum amyloid A), and PCT (procalcitonin).[159–161] Additionally, typical pro- and anti-inflammatory cytokines like IL-6, IL-10, and IFN-γ alone or in varying combinations with other markers were found to be predictive for the development of SAP.[162,163]

Many efforts have been undertaken to prevent lower respiratory tract infections and to identify high-risk patients early. To overcome the differences in diagnosis and terminology of SAP used in many studies, the PISCES (Pneumonia in Stroke Consensus) group proposed to use the term SAP for all lower respiratory tract infections within the first week after stroke and diagnosis according to modified Centers for Disease Control and Prevention criteria.[164] A meta-analysis of 4197 stroke patients enrolled in randomized controlled phase II and III trials investigating preventive antibiotic treatment demonstrates that this approach reduced urinary tract infection but not SAP and

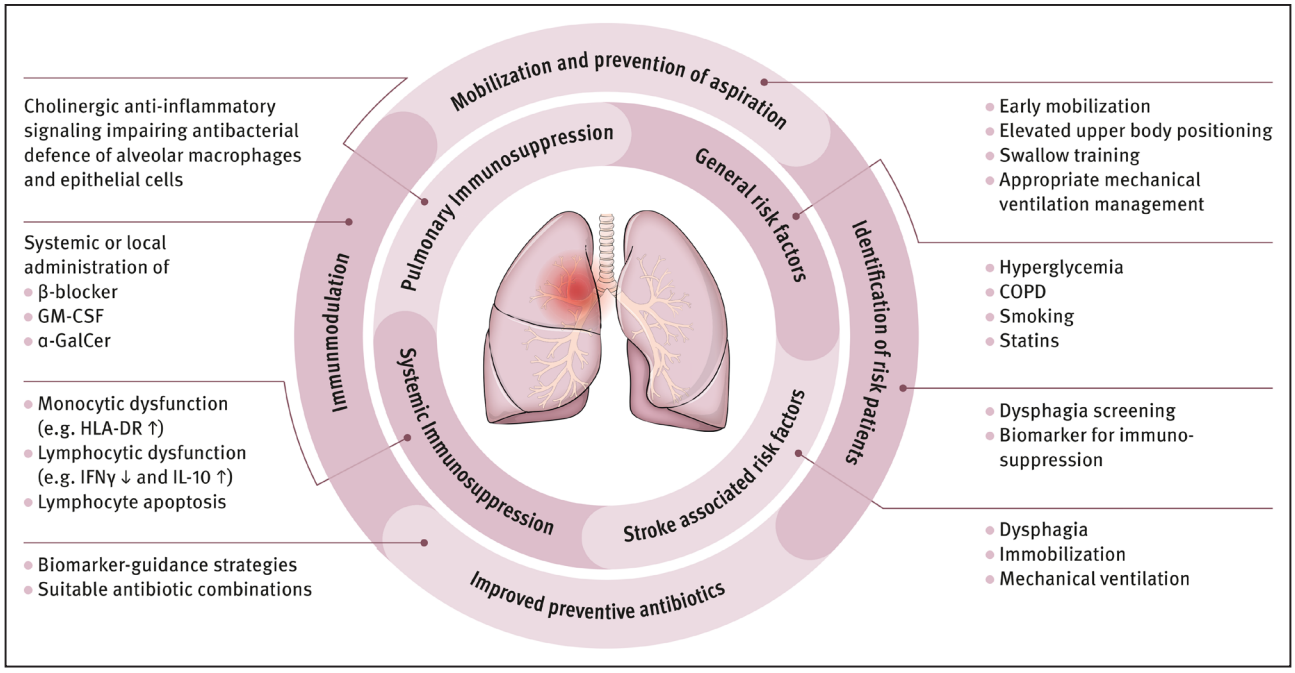

Figure 4. Strategies for prediction, prevention, and treatment of stroke-associated pneumonia.
Stroke-related risk factors and stroke-induced depression of systemic and local pulmonary immune responses are responsible for stroke-associated pneumonia (SAP). From these insights, scores and biomarkers can be derived to predict patients at risk of SAP. Although simple strategies such as dysphagia screening are partially effective, pneumonia remains the most important medical complication after stroke, leading to poor outcome. Optimized preventive antibiotic therapy and immunomodulatory approaches are promising concepts that should be tested in clinical trials. COPD indicates chronic obstructive pulmonary disease; GM-CSF, granulocyte-macrophage colony-stimulating factor; HLA-DR, human leukocyte antigen–DR isotype; IFN-γ, interferon gamma; and IL, interleukin.

did not improve outcome.[165] In this regard, the class of antibiotics used can potentially influence the neurological outcome after poststroke infections.[130]

LONG-TERM SEQUELAE

In addition to motor disability, which is closely tied to damage to corticospinal tract neurons,[166] there are several long-term sequelae after stroke that cause significant morbidity. The risks of dementia, depression, seizures, and fatigue all rise after stroke, and although none is well-understood, all may be linked to neuroinflammation. Poststroke fatigue occurs in up to 70% of stroke survivors in the first year after stroke and appears unrelated to stroke size or disability.[167] Its mechanism or mechanisms remain unknown, but IL-1β, TLR4, low IL-10, and high Th17/Treg ratio have been implicated.[33]

After a stroke, the risk of dementia is approximately doubled even after controlling for cardiovascular risk factors,[168] with a larger effect in older individuals, higher National Institutes of Health Stroke Scale score, and hemorrhagic strokes.[169] Dementia has a biphasic onset, with the initial risk occurring within ≈6 months and tightly associated with stroke severity and location, while the later risk is independent of stroke size and location.[170] The earlier effect on incident dementia is likely due to strategic infarcts that impair results during cognitive testing rather than due to a neurodegenerative disease, as elegantly demonstrated by Weaver et al.[171] In contrast, the later dementia risk appears linear for at least a decade and is thus more likely to be due to a neurodegenerative process. This may account for as much as 20% absolute risk of incident poststroke dementia.[172]

In rodent stroke models that do not cause immediate cognitive impairment, there is later cognitive decline due to aberrant neurogenesis and chronic B lymphocyte activity, perhaps related to their invasion of the stroke scar late after stroke.[127,173] It is not known whether the aberrant neurogenesis is a result of inflammation, but inflammatory mediators can inhibit neurite outgrowth and rewiring, as well as inhibit normal neurogenesis.[174] The immune-mediated B cell–dependent effect is hypothesized to cause cognitive decline due to autoimmunity. People with increased anti-brain antigen antibodies, themselves associated with peri-stroke inflammation and infection, have a higher risk of cognitive decline between 3 months and 1 year after stroke.[175] Also, patients with more peripheral monocytic activation 2 days after stroke, presumed to act as an adjuvant to brain antigens released by the stroke, are more likely to have cognitive decline over the same 90-day to 1-year time period.[176] However, much remains to be learned about how B cells cause cognitive decline in both mice and humans.

Poststroke depression is a major neuropsychiatric consequence of stroke, occurring in at least 30% of survivors, and associated with increased mortality.[177]

Risk factors include a history of depression, female sex, stroke severity, and lack of social support.[178] It is likely multifactorial; however, peripheral inflammation is implicated, with the strongest evidence for increased CRP, TNFα, and IL-6 in blood. All are associated with poststroke depression in meta-analyses.[179]

Poststroke seizures and epilepsy can occur both early and late after stroke.[180] Early seizures occur in up to 17%, with about a fifth of these asymptomatic[181] and are more common with intracerebral hemorrhage or hemorrhagic transformation, alcoholism, and worse stroke severity.[182] Late seizure risk is higher with more severe strokes and strokes with cortical involvement, and occurs in 6% to 16% of stroke survivors.[181,183] Interestingly, patients who received thrombolytic or intra-arterial therapy are at higher risk of poststroke epilepsy, even after adjustment for the National Institutes of Health Stroke Scale score, age, and 3-month modified Rankin scale.[184] This could mean that reperfusion is acting as a proinflammatory stimulus here as it is associated with complement activation and thus more innate immune activation.[185] Indeed, in humans, IL-1β levels within 24 hours of admission confer an increased risk of poststroke epilepsy.[183] In animal models, both blood-brain barrier breakdown and entrance of TGFβ into the brain and neuroinflammation associated with thalamic circuit reorganization and seizure generation after cortical stroke are implicated in epilepsy.[186,187] However, precise inflammatory mechanisms that can successfully be targeted in humans have not been identified.

Immunomodulatory Therapies for Improving Neurological Long-Term Outcome and Prevention of Poststroke Infections

Based on the understanding of neuroinflammation and stroke-induced immunodepression, various immunomodulatory therapeutic approaches have been tested in clinical trials. In particular, stimulation of neuroprotection by immune mediators and growth hormones (eg, TLR4 inhibitor and G-CSF [granulocyte colony-stimulating factor]), inhibition of peripheral inflammatory response (eg, IL-1R blockers), or leukocyte infiltration (eg, natalizumab) and pleiotropic immunomodulators (eg, allogeneic adult stem cells) were used for improving neurological outcome by modulating neuroinflammation (Figure 3). To date, no immunotherapy targeting neuroinflammation has been able to achieve a relevant effect on neurological outcome (Table 2).

Because preventive antibiotic therapy has not only failed to improve long-term outcomes but also has not reduced SAP (see above) and stroke-induced immunodepression plays a causal role in SAP, immunomodulation is also a promising strategy for this indication. The essential role of the sympathetic nervous system in stroke-induced immunodepression and experimental data suggest the use of β-blockers. However, observational studies do not support a clinically relevant immunomodulation by β-blockers, and randomized controlled trials would have to be performed.[188–191] More specific therapeutic approaches, for example, with IFN-γ, caspase inhibitors, and GM-CSF, have been used to prevent experimental SAP.[192]

However, experimental data suggest that immunomodulation after acute stroke needs to consider potentially opposing effects of immunity. Targeting immune cells or mechanisms known to positively affect neurological outcome may simultaneously impair pulmonary defenses and promote infections and vice versa.[193] Two promising experimental approaches have been described recently. CD200-CD200R1 is an important checkpoint in the regulation of innate and adaptive inflammation, which is involved in both stroke and lung disease. The CD200-CD200R1 signaling cascade is an interesting drug target because it both prevents spontaneous bacterial infections and promotes resolution of neuroinflammation, thereby improving recovery after stroke.[194] The second example is based on the finding that stroke suppresses splenic marginal zone B cells, resulting in reduced circulating IgM levels. Their reconstitution with intravenous immunoglobulins (IgM-IVIg) experimentally prevents SAP and has no negative effect on neurological outcome.[195]

CONCLUSIONS AND THE FUTURE OF NEUROINFLAMMATION RESEARCH IN STROKE

Overall, inflammation has a triphasic relationship with stroke (Figure 1). Inflammation and infections are involved in atherosclerosis and blood clotting and may promote stroke. Subacute inflammation after stroke is triggered by the innate immune system (Figure 3), which then engages adaptive immune cells, with both arms of the immune system leading to beneficial effects, such as clearance of dead tissue but also to deleterious collateral injury that damages cells and synapses in the vicinity of the stroke. The changes in the peripheral inflammatory state induced by stroke can cause both immunodepression as well as infections and increased peripheral inflammation, which in turn may cause harmful autoimmune responses (Figure 4). Finally, both peripheral and central inflammation long after stroke is associated with and can cause significant long-term sequelae. Much progress has been made in understanding the interactions between the interdependent and multidimensional immune response and stroke. However, much remains to be learned because, despite many attempts, there are currently no immune-related therapies to help people afflicted by stroke.[5] A bright spot for the future is that there are an increasing number of human studies. Comparing humans and model organisms will shed light on how to best model the complex relationship between stroke and inflammation and thus help researchers developing successful therapies for stroke. One of the most important aspects for the

successful development of immunomodulatory therapies will be to consider both the signaling cascades of neuroinflammation and peripheral immunodeficiency. Many experimental findings demonstrate that these signaling cascades can have opposing consequences for the brain and peripheral organs such as the lungs. Thus, the same approach can have positive effects on neuroinflammation but enhance immunodepression and vice versa. In addition to the need of an accurate understanding of these complex signaling cascades and their precise timings, immune markers may help to identify appropriate patient populations for specific immune therapies.

ARTICLE INFORMATION

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