

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)-1 β 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
DAMP	damage-associated molecular pattern
EGFR	epidermal growth factor receptor
FasL	fas ligand
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
HDL	high-density lipoprotein
HLA-DR	human leukocyte antigen-DR isotype
HMGB1	high mobility group box 1
Hsp70	heat shock protein 70
IFN-γ	interferon gamma
IL	interleukin
LDL	low-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
SAP	stroke-associated pneumonia
STAT6	signal transducer and activator of transcription 6
TLR	Toll-like receptor
TNF-α	tumor necrosis factor alpha
tPA	tissue-type plasminogen activator
TRIF	TIR domain-containing adapter-inducing interferon- β
VLA4	α 4 β 1 integrin

complex and multifaceted.^{3,4} Thus, at present, recanalization 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.

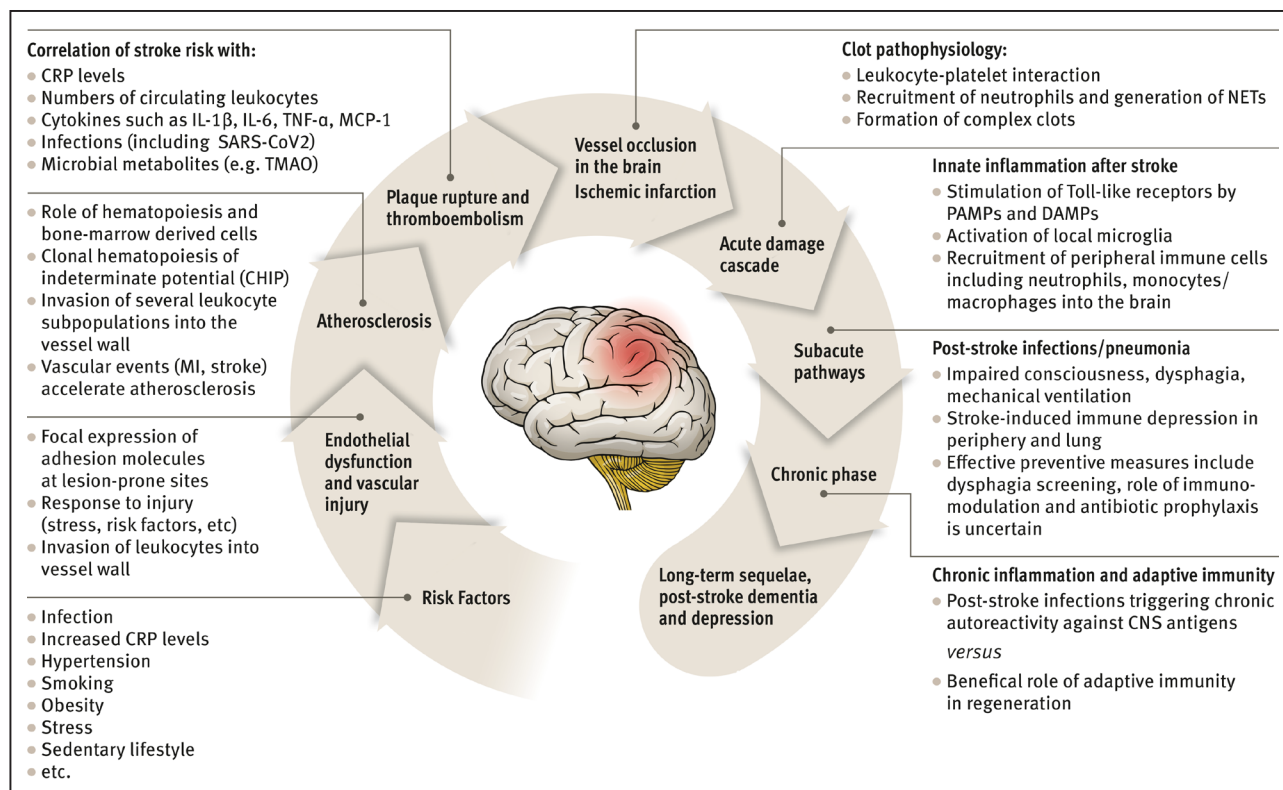


Figure 1. Inflammation and maladaptive immune mechanisms over the cerebrovascular disease continuum.

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 Ross⁹ 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 ($\alpha 4\beta 1$ 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-1 β 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-1 β , 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-oxides³² 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 hypercoagulability⁵⁰ (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-1 β 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

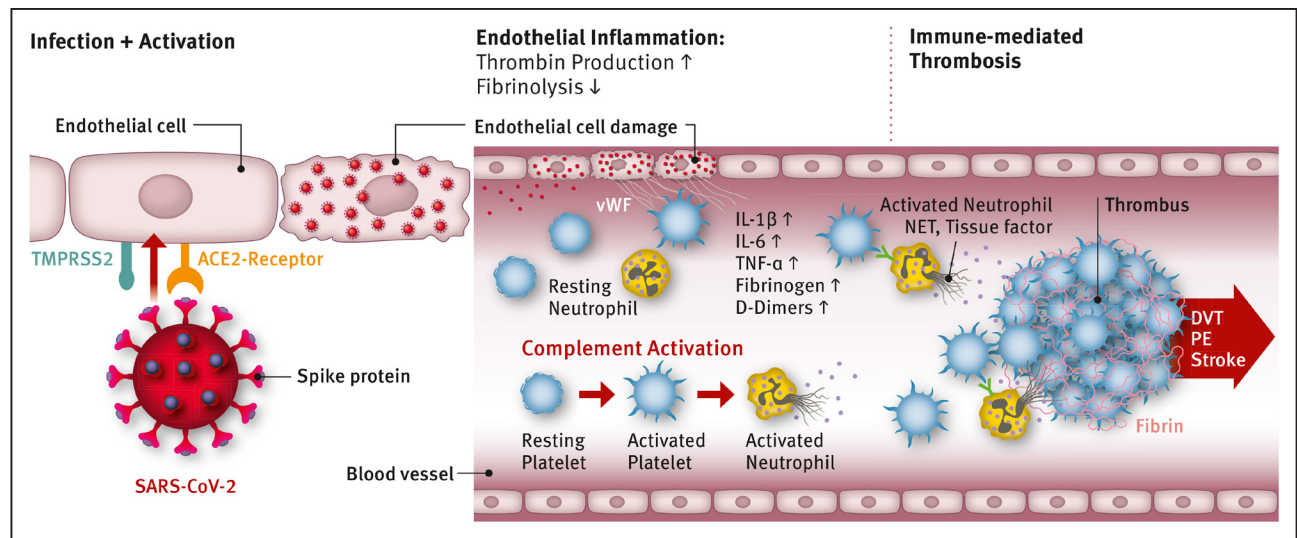


Figure 2. SARS-CoV2 and thromboembolism.

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)-1 β . 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- κ B (nuclear factor kappa B) activation. In either case, NF- κ B activation is required for inflammatory cytokine production.⁶⁷ TLR4 absence reduces brain damage and inflammation after experimental stroke⁶⁸ 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 antagonist⁷⁰ 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, regulatory T-cell.

phase Ib/Ila 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 amoeboid 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

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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
SCIL-STROKE	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
		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
REPAIR FAST	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
		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	NHSS, 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.

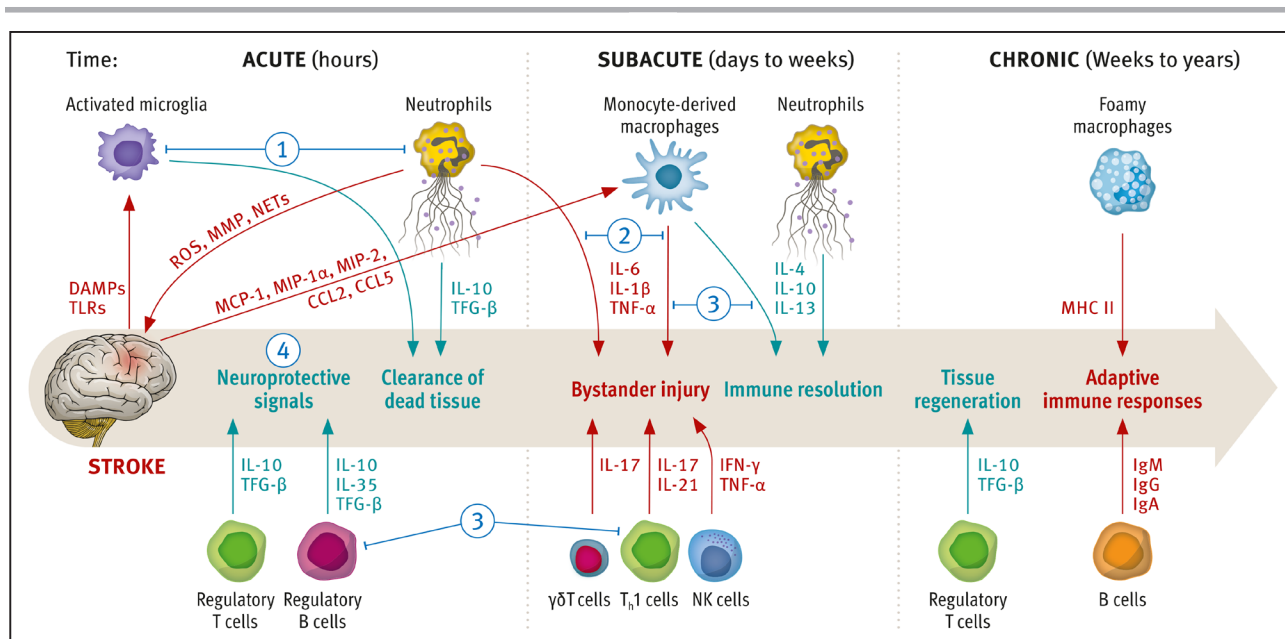


Figure 3. Signaling cascades of acute and chronic inflammation after ischemic stroke.

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 neural 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 thrombi¹¹⁹ 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 $\gamma\delta$ 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 humans¹²⁶ 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(+) $\gamma\delta$ 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 dura¹³¹ and are very sparse in uninjured parenchyma.¹²⁷ During the first week after stroke, IL-10 producing regulatory B cells are protective¹³²; 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 Pro-regenerative 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-1 β 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

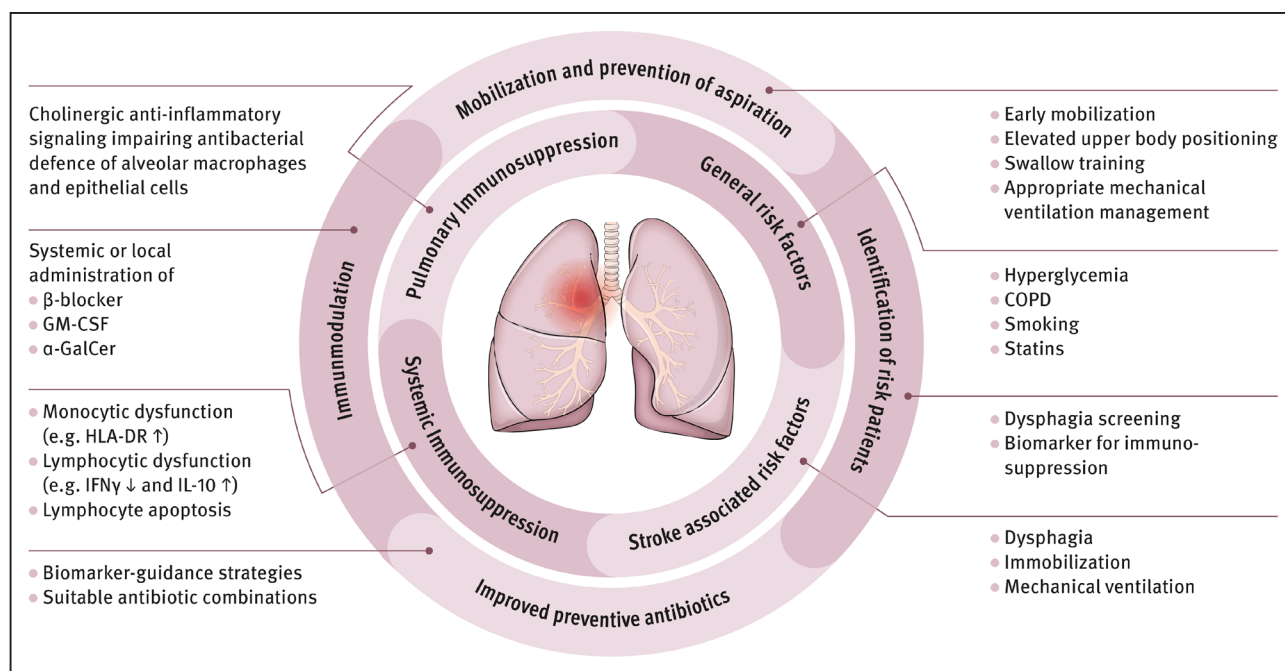


Figure 4. Strategies for prediction, prevention, and treatment of stroke-associated pneumonia.

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-1 β , 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 \approx 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, TNF α , and IL-6 in blood. All are associated with post-stroke 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 asymptomatic¹⁸¹ 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-1 β 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 TGF β 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 immunoglobulin (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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REFERENCES

- Collaborators GBDLROs; Feigin VL, Nguyen G, Cercy K, Johnson CO, Alam T, Parmar PG, Abajobir AA, Abate KH, Abd-Allah F, et al. Global, regional, and country-specific lifetime risks of stroke, 1990 and 2016. *N Engl J Med*. 2018;379:2429–2437. doi: 10.1056/NEJMoa1804492
- Emmrich JV, Knauß S, Endres M. Current advances, challenges, and opportunities in stroke research, management, and care. *Nova Acta Leopoldina*. 2021;2:1–16
- Dirnagl U, Endres M. Found in translation: preclinical stroke research predicts human pathophysiology, clinical phenotypes, and therapeutic outcomes. *Stroke*. 2014;45:1510–1518. doi: 10.1161/STROKEAHA.113.004075
- Howells DW, Sena ES, O'Collins V, Macleod MR. Improving the efficiency of the development of drugs for stroke. *Int J Stroke*. 2012;7:371–377. doi: 10.1111/j.1747-4949.2012.00805.x
- Iadecola C, Buckwalter MS, Anrather J. Immune responses to stroke: mechanisms, modulation, and therapeutic potential. *J Clin Invest*. 2020;130:2777–2788. doi: 10.1172/JCI135530
- Kelly PJ, Murphy S, Coveney S, Purroy F, Lemmens R, Tsigoulis G, Price C. Anti-inflammatory approaches to ischaemic stroke prevention. *J Neurol Neurosurg Psychiatry*. 2018;89:211–218. doi: 10.1136/jnnp-2016-314817
- Kelly PJ, Crispino G, Sheehan O, Kelly L, Marnane M, Merwick A, Hannon N, Ní Chróinín D, Callaly E, Harris D, et al. Incidence, event rates, and early outcome of stroke in Dublin, Ireland: the North Dublin population stroke study. *Stroke*. 2012;43:2042–2047. doi: 10.1161/STROKEAHA.111.645721
- Virchow R. Der atheromatöse prozeß der arterien. *Wien Med Wochenschr*. 1856;6
- Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med*. 1999;340:115–126. doi: 10.1056/NEJM199901143400207
- Soehnlein O, Libby P. Targeting inflammation in atherosclerosis - from experimental insights to the clinic. *Nat Rev Drug Discov*. 2021;20:589–610. doi: 10.1038/s41573-021-00198-1
- Schloss MJ, Swirski FK, Nahrendorf M. Modifiable cardiovascular risk, hematopoiesis, and innate immunity. *Circ Res*. 2020;126:1242–1259. doi: 10.1161/CIRCRESAHA.120.315936
- Endres M, Laufs U, Merz H, Kaps M. Focal expression of intercellular adhesion molecule-1 in the human carotid bifurcation. *Stroke*. 1997;28:77–82. doi: 10.1161/01.str.28.1.77
- Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol*. 1998;18:842–851. doi: 10.1161/01.atv.18.5.842
- Winter C, Silvestre-Roig C, Ortega-Gomez A, Lemnitzer P, Poelman H, Schumski A, Winter J, Drechsler M, de Jong R, Immler R, et al. Chrono-pharmacological targeting of the CCL2-CCR2 axis ameliorates atherosclerosis. *Cell Metab*. 2018;28:175–182.e5. doi: 10.1016/j.cmet.2018.05.002
- Duewelling P, Kono H, Rayner KJ, Sirois CM, Vladimirov G, Bauernfeind FG, Abela GS, Franchi L, Nuñez G, Schnurr M, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464:1357–1361. doi: 10.1038/nature08938
- Christ A, Günther P, Lauterbach MAR, Duewelling P, Biswas D, Pelka K, Scholz CJ, Oosting M, Haendler K, Baßler K, et al. Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell*. 2018;172:162–175.e14. doi: 10.1016/j.cell.2017.12.013
- Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science*. 2015;349:316–320. doi: 10.1126/science.aaa8064
- Kopczak A, Schindler A, Bayer-Karpinska A, Koch ML, Sepp D, Zeller J, Streckler C, Hempel JM, Yuan C, Malik R, et al. Complicated carotid artery plaques as a cause of cryptogenic stroke. *J Am Coll Cardiol*. 2020;76:2212–2222. doi: 10.1016/j.jacc.2020.09.532
- Poller WC, Nahrendorf M, Swirski FK. Hematopoiesis and cardiovascular disease. *Circ Res*. 2020;126:1061–1085. doi: 10.1161/CIRCRESAHA.120.315895
- Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420:868–874. doi: 10.1038/nature01323
- Dutta P, Courties G, Wei Y, Leuschner F, Gorbatov R, Robbins CS, Iwamoto Y, Thompson B, Carlson AL, Heidt T, et al. Myocardial infarction accelerates atherosclerosis. *Nature*. 2012;487:325–329. doi: 10.1038/nature11260
- Courties G, Herisson F, Sager HB, Heidt T, Ye Y, Wei Y, Sun Y, Severe N, Dutta P, Scharff J, et al. Ischemic stroke activates hematopoietic bone marrow stem cells. *Circ Res*. 2015;116:407–417. doi: 10.1161/CIRCRESAHA.116.305207
- Courties G, Frodermann V, Honold L, Zheng Y, Herisson F, Schloss MJ, Sun Y, Presumey J, Severe N, Engblom C, et al. Glucocorticoids regulate bone marrow B lymphopoiesis after stroke. *Circ Res*. 2019;124:1372–1385. doi: 10.1161/CIRCRESAHA.118.314518
- Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, McConkey M, Gupta N, Gabriel S, Ardissino D, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017;377:111–121. doi: 10.1056/NEJMoa1701719

25. Watson CJ, Papula AL, Poon GYP, Wong WH, Young AL, Druley TE, Fisher DS, Blundell JR. The evolutionary dynamics and fitness landscape of clonal hematopoiesis. *Science*. 2020;367:1449–1454. doi: 10.1126/science.aay9333
26. Bhattacharya R, Zekavat SM, Haessler J, Fornage M, Raffield L, Uddin MM, Bick AG, Niroula A, Yu B, Gibson C, et al. Clonal hematopoiesis is associated with higher risk of stroke. *Stroke*. 2022;53:788–797. doi: 10.1161/STROKEAHA.121.037388
27. Friedman GD, Klatsky AL, Siegel AB. The leukocyte count as a predictor of myocardial infarction. *N Engl J Med*. 1974;290:1275–1278. doi: 10.1056/NEJM197406062902302
28. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J; Emerging Risk Factors Collaboration. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet*. 2010;375:132–140. doi: 10.1016/S0140-6736(09)61717-7
29. Bohula EA, Giugliano RP, Leiter LA, Verma S, Park JG, Sever PS, Lira Pineda A, Honarpour N, Wang H, Murphy SA, et al. Inflammatory and cholesterol risk in the FOURIER trial. *Circulation*. 2018;138:131–140. doi: 10.1161/CIRCULATIONAHA.118.034032
30. Ridker PM, Lüscher TF. Anti-inflammatory therapies for cardiovascular disease. *Eur Heart J*. 2014;35:1782–1791. doi: 10.1093/eurheartj/ehu203
31. Georgakakis MK, Malik R, Björkbacka H, Pana TA, Demissie S, Ayers C, Elhadad MA, Fornage M, Beiser AS, Benjamin EJ, et al. Circulating monocyte chemoattractant protein-1 and risk of stroke: meta-analysis of population-based studies involving 17 180 individuals. *Circ Res*. 2019;125:773–782. doi: 10.1161/CIRCRESAHA.119.315380
32. Haghikia A, Li XS, Liman TG, Bledau N, Schmidt D, Zimmermann F, Kränkel N, Widera C, Sonnenschein K, Haghikia A, et al. Gut microbiota-dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. *Arterioscler Thromb Vasc Biol*. 2018;38:2225–2235. doi: 10.1161/ATVBAHA.118.311023
33. Elkind MSV, Boehme AK, Smith CJ, Meisel A, Buckwalter MS. Infection as a stroke risk factor and determinant of outcome after stroke. *Stroke*. 2020;51:3156–3168. doi: 10.1161/STROKEAHA.120.030429
34. Umapathi T, Kor AC, Venketasubramanian N, Lim CC, Pang BC, Yeo TT, Lee CC, Lim PL, Ponnudurai K, Chuah KL, et al. Large artery ischaemic stroke in severe acute respiratory syndrome (SARS). *J Neurol*. 2004;251:1227–1231. doi: 10.1007/s00415-004-0519-8
35. Nannoni S, de Groot R, Bell S, Markus HS. Stroke in COVID-19: a systematic review and meta-analysis. *Int J Stroke*. 2021;16:137–149. doi: 10.1177/1747493020972922
36. Katsanos AH, Palaodimou L, Zand R, Yaghi S, Kamel H, Navi BB, Turc G, Romoli M, Sharma VK, Mavridis D, et al. The impact of SARS-CoV-2 on stroke epidemiology and care: a meta-analysis. *Ann Neurol*. 2021;89:380–388. doi: 10.1002/ana.25967
37. Merkle AE, Parikh NS, Mir S, Gupta A, Kamel H, Lin E, Lantos J, Schenck EJ, Goyal P, Bruce SS, et al. Risk of ischemic stroke in patients with coronavirus disease 2019 (COVID-19) vs patients with influenza. *JAMA Neurol*. 2020;77:1–7. doi: 10.1001/jamaneurol.2020.2730
38. Shahjouei S, Tsigoulis G, Farahmand G, Koza E, Mowla A, Vafaei Sadr A, Kia A, Vaghefi Far A, Mondello S, Cernigliaro A, et al. SARS-CoV-2 and stroke characteristics: a report from the multinational COVID-19 stroke study group. *Stroke*. 2021;52:e117–e130. doi: 10.1161/STROKEAHA.120.032927
39. Perry RJ, Smith CJ, Roffe C, Simister R, Narayanamoorthi S, Marigold R, Willmot M, Dixit A, Hassan A, Quinn TJ, et al; SETICOS Collaborators. Characteristics and outcomes of COVID-19 associated stroke: a UK multicentre case-control study. *J Neurol Neurosurg Psychiatry*. 2021;92:242–248. doi: 10.1136/jnnp-2020-324927
40. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X, Merdji H, Clere-Jehl R, Schenck M, Fagot Gandet F, et al; CRICS TRIGGERSEPS Group (Clinical Research in Intensive Care and Sepsis Trial Group for Global Evaluation and Research in Sepsis). High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. *Intensive Care Med*. 2020;46:1089–1098. doi: 10.1007/s00134-020-06062-x
41. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, Bikdeli B, Ahluwalia N, Ausiello JC, Wan EY, et al. Extrapulmonary manifestations of COVID-19. *Nat Med*. 2020;26:1017–1032. doi: 10.1038/s41591-020-0968-3
42. Iba T, Connors JM, Levy JH. The coagulopathy, endotheliopathy, and vasculitis of COVID-19. *Inflamm Res*. 2020;69:1181–1189. doi: 10.1007/s00011-020-01401-6
43. Levi M, Thachil J, Iba T, Levy JH. Coagulation abnormalities and thrombosis in patients with COVID-19. *Lancet Haematol*. 2020;7:e438–e440. doi: 10.1016/S2352-3026(20)30145-9
44. Wichmann D, Sperhake JP, Lütgehetmann M, Steurer S, Edler C, Heinemann A, Heinrich F, Mushumba H, Knipf I, Schröder AS, et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med*. 2020;173:268–277. doi: 10.7326/M20-2003
45. Stark K, Massberg S. Interplay between inflammation and thrombosis in cardiovascular pathology. *Nat Rev Cardiol*. 2021;18:666–682. doi: 10.1038/s41569-021-00552-1
46. Sagris D, Papanikolaou A, Kvernland A, Korompoki E, Frontera JA, Troxel AB, Gavriatopoulou M, Milionis H, Lip GH, Michel P, et al. COVID-19 and ischemic stroke. *Eur J Neurol*. 2021;28:3826–3836. doi: 10.1111/ene.15008
47. Masi P, Hékimian G, Lejeune M, Chommeloux J, Desnos C, Pineton De Chambrun M, Martin-Toutain I, Nieszczowska A, Lebreton G, Bréchet N, et al. Systemic inflammatory response syndrome is a major contributor to COVID-19-associated coagulopathy: insights from a prospective, single-center cohort study. *Circulation*. 2020;142:611–614. doi: 10.1161/CIRCULATIONAHA.120.048925
48. Manne BK, Denorme F, Middleton EA, Portier I, Rowley JW, Stubben C, Petrey AC, Tolley ND, Guo L, Cody M, et al. Platelet gene expression and function in patients with COVID-19. *Blood*. 2020;136:1317–1329. doi: 10.1182/blood.2020007214
49. Skendros P, Mitsios A, Chrysanthopoulou A, Mastellos DC, Metallidis S, Rafailidis P, Ntinopoulou M, Sertariou E, Tsonidou V, Tsigalou C, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *J Clin Invest*. 2020;130:6151–6157. doi: 10.1172/JCI141374
50. Nicolai L, Leunig A, Brambs S, Kaiser R, Joppich M, Hoffknecht ML, Gold C, Engel A, Polewka V, Muenchhoff M, et al. Vascular neutrophilic inflammation and immunothrombosis distinguish severe COVID-19 from influenza pneumonia. *J Thromb Haemost*. 2021;19:574–581. doi: 10.1111/jth.15179
51. Ramlall V, Thangaraj PM, Meydan C, Foox J, Butler D, Kim J, May B, De Freitas JK, Glicksberg BS, Mason CE, et al. Immune complement and coagulation dysfunction in adverse outcomes of SARS-CoV-2 infection. *Nat Med*. 2020;26:1609–1615. doi: 10.1038/s41591-020-1021-2
52. Goshua G, Pine AB, Meizlish ML, Chang CH, Zhang H, Bahel P, Baluha A, Bar N, Bona RD, Burns AJ, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. *Lancet Haematol*. 2020;7:e575–e582. doi: 10.1016/S2352-3026(20)30216-7
53. Gencer S, Lacy M, Atzler D, van der Vorst EPC, Döring Y, Weber C. Immunoinflammatory, thrombohaemostatic, and cardiovascular mechanisms in COVID-19. *Thromb Haemost*. 2020;120:1629–1641. doi: 10.1055/s-0040-1718735
54. Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science*. 2005;309:1864–1868. doi: 10.1126/science.1116480
55. Liang B, Wang X, Zhang N, Yang H, Bai R, Liu M, Bian Y, Xiao C, Yang Z. Angiotensin-(1-7) attenuates angiotensin II-induced ICAM-1, VCAM-1, and MCP-1 expression via the MAS receptor through suppression of P38 and NF- κ B pathways in HUVECs. *Cell Physiol Biochem*. 2015;35:2472–2482. doi: 10.1159/000374047
56. Fraga-Silva RA, Pinheiro SV, Gonçalves AC, Alenina N, Bader M, Santos RA. The antithrombotic effect of angiotensin-(1-7) involves mas-mediated NO release from platelets. *Mol Med*. 2008;14:28–35. doi: 10.2119/2007-00073.Fraga-Silva
57. Puntmann VO, Carerj ML, Wieters I, Fahim M, Arendt C, Hoffmann J, Schendrygina A, Escher F, Vasa-Nicotera M, Zeiher AM, et al. Outcomes of cardiovascular magnetic resonance imaging in patients recently recovered from coronavirus disease 2019 (COVID-19). *JAMA Cardiol*. 2020;5:1265–1273. doi: 10.1001/jamacardio.2020.3557
58. Kelly P, Weimar C, Lemmens R, Murphy S, Purroy F, Arsovska A, Bornstein NM, Czlonkowska A, Fischer U, Fonseca AC, et al. Colchicine for prevention of vascular inflammation in non-cardioembolic stroke (CONVINCE) - study protocol for a randomised controlled trial. *Eur Stroke J*. 2021;6:222–228. doi: 10.1177/2396987320972566
59. Libby P, Everett BM. Novel antiatherosclerotic therapies. *Arterioscler Thromb Vasc Biol*. 2019;39:538–545. doi: 10.1161/ATVBAHA.118.310958
60. Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, Mam V, Hasan A, Rosenberg Y, Iturriga E, et al; CIRT Investigators. Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med*. 2019;380:752–762. doi: 10.1056/NEJMoa1809798
61. Matzinger P. Tolerance, danger, and the extended family. *Annu Rev Immunol*. 1994;12:991–1045. doi: 10.1146/annurev.12.040194.005015
62. Alexander SP, Fabbro D, Kelly E, Mathie A, Peters JA, Veale EL, Armstrong JF, Faccenda E, Harding SD, Pawson AJ, et al. THE CONCISE GUIDE

- TO PHARMACOLOGY 2021/22: catalytic receptors. *Br J Pharmacol*. 2021;178(suppl 1):S264–S312. doi: 10.1111/bph.15541
63. Gülke E, Gelderblom M, Magnus T. Danger signals in stroke and their role on microglia activation after ischemia. *Ther Adv Neurol Disord*. 2018;11:1756286418774254. doi: 10.1177/1756286418774254
 64. Schulze J, Zierath D, Tanzi P, Cain K, Shibata D, Dressel A, Becker K. Severe stroke induces long-lasting alterations of high-mobility group box 1. *Stroke*. 2013;44:246–248. doi: 10.1161/STROKEAHA.112.676072
 65. Liesz A, Dalpke A, Mracsco E, Antoine DJ, Roth S, Zhou W, Yang H, Na SY, Akhisaroglu M, Fleming T, et al. DAMP signaling is a key pathway inducing immune modulation after brain injury. *J Neurosci*. 2015;35:583–598. doi: 10.1523/JNEUROSCI.2439-14.2015
 66. Fitzgerald KA, Kagan JC. Toll-like receptors and the control of immunity. *Cell*. 2020;180:1044–1066. doi: 10.1016/j.cell.2020.02.041
 67. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol*. 2010;11:373–384. doi: 10.1038/ni.1863
 68. Caso JR, Pradillo JM, Hurtado O, Lorenzo P, Moro MA, Lizasoain I. Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke. *Circulation*. 2007;115:1599–1608. doi: 10.1161/CIRCULATIONAHA.106.603431
 69. Urra X, Cervera A, Obach V, Climent N, Planas AM, Chamorro A. Monocytes are major players in the prognosis and risk of infection after acute stroke. *Stroke*. 2009;40:1262–1268. doi: 10.1161/STROKEAHA.108.532085
 70. Fernández G, Moraga A, Cuartero MI, García-Culebras A, Peña-Martínez C, Pradillo JM, Hernández-Jiménez M, Sacristán S, Ayuso MI, Gonzalo-Gobernado R, et al. TLR4-binding DNA aptamers show a protective effect against acute stroke in animal models. *Mol Ther*. 2018;26:2047–2059. doi: 10.1016/j.ymthe.2018.05.019
 71. Zera KA, Buckwalter MS. The local and peripheral immune responses to stroke: implications for therapeutic development. *Neurotherapeutics*. 2020;17:414–435. doi: 10.1007/s13311-020-00844-3
 72. Berchtold D, Priller J, Meisel C, Meisel A. Interaction of microglia with infiltrating immune cells in the different phases of stroke. *Brain Pathol*. 2020;30:1208–1218. doi: 10.1111/bpa.12911
 73. García-Culebras A, Durán-Laforet V, Peña-Martínez C, Ballesteros I, Pradillo JM, Díaz-Guzmán J, Lizasoain I, Moro MA. Myeloid cells as therapeutic targets in neuroinflammation after stroke: specific roles of neutrophils and neutrophil-platelet interactions. *J Cereb Blood Flow Metab*. 2018;38:2150–2164. doi: 10.1177/0271678X18795789
 74. Parada E, Casas AI, Palomino-Antolin A, Gómez-Rangel V, Rubio-Navarro A, Farré-Alins V, Narros-Fernandez P, Guerrero-Hue M, Moreno JA, Rosa JM, et al. Early toll-like receptor 4 blockade reduces ROS and inflammation triggered by microglial pro-inflammatory phenotype in rodent and human brain ischaemia models. *Br J Pharmacol*. 2019;176:2764–2779. doi: 10.1111/bph.14703
 75. Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, Gao Y, Chen J. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke*. 2012;43:3063–3070. doi: 10.1161/STROKEAHA.112.659656
 76. Lyu J, Jiang X, Leak RK, Shi Y, Hu X, Chen J. Microglial responses to brain injury and disease: functional diversity and new opportunities. *Transl Stroke Res*. 2021;12:474–495. doi: 10.1007/s12975-020-00857-2
 77. Hu X, Leak RK, Shi Y, Suenaga J, Gao Y, Zheng P, Chen J. Microglial and macrophage polarization—new prospects for brain repair. *Nat Rev Neuro*. 2015;11:56–64. doi: 10.1038/nrneuro.2014.207
 78. Mastorakos P, Mihelson N, Luby M, Burks SR, Johnson K, Hsia AW, Witko J, Frank JA, Latour L, McGavern DB. Temporally distinct myeloid cell responses mediate damage and repair after cerebrovascular injury. *Nat Neurosci*. 2021;24:245–258. doi: 10.1038/s41593-020-00773-6
 79. Villa A, Klein B, Janssen B, Pedragosa J, Pepe G, Zinnhardt B, Vugts DJ, Gelosa P, Sironi L, Beaino W, et al. Identification of new molecular targets for PET imaging of the microglial anti-inflammatory activation state. *Theranostics*. 2018;8:5400–5418. doi: 10.7150/thno.25572
 80. Cai W, Dai X, Chen J, Zhao J, Xu M, Zhang L, Yang B, Zhang W, Rocha M, Nakao T, et al. Stat6/arg1 promotes microglia/macrophage efferocytosis and inflammation resolution in stroke mice. *JCI Insight*. 2019;4:e131355. doi: 10.1172/jci.insight.131355
 81. Gerhard A, Schwarz J, Myers R, Wise R, Banati RB. Evolution of microglial activation in patients after ischemic stroke: a [11C]@-PK11195 PET study. *Neuroimage*. 2005;24:591–595. doi: 10.1016/j.neuroimage.2004.09.034
 82. Morris RS, Simon Jones P, Alawneh JA, Hong YT, Fryer TD, Aigbirhio FI, Warburton EA, Baron JC. Relationships between selective neuronal loss and microglial activation after ischaemic stroke in man. *Brain*. 2018;141:2098–2111. doi: 10.1093/brain/awy121
 83. Tan YL, Yuan Y, Tian L. Microglial regional heterogeneity and its role in the brain. *Mol Psychiatry*. 2020;25:351–367. doi: 10.1038/s41380-019-0609-8
 84. Uriarte Huarte O, Richart L, Mittelbronn M, Michelucci A. Microglia in health and disease: the strength to be diverse and reactive. *Front Cell Neurosci*. 2021;15:660523. doi: 10.3389/fncel.2021.660523
 85. Young AMH, Kumasaka N, Calvert F, Hammond TR, Knights A, Panousis N, Park JS, Schwartztruber J, Liu J, Kundu K, et al. A map of transcriptional heterogeneity and regulatory variation in human microglia. *Nat Genet*. 2021;53:861–868. doi: 10.1038/s41588-021-00875-2
 86. Rubio-Ponce A, Hidalgo A, Ballesteros I. How to bridle a neutrophil. *Curr Opin Immunol*. 2021;68:41–47. doi: 10.1016/j.coi.2020.08.003
 87. Adrover JM, Del Fresno C, Crainiciuc G, Cuartero MI, Casanova-Acebes M, Weiss LA, Hueriga-Encabo H, Silvestre-Roig C, Rössaint J, Cossío I, et al. A neutrophil timer coordinates immune defense and vascular protection. *Immunity*. 2019;50:390–402.e10. doi: 10.1016/j.immuni.2019.01.002
 88. Hermann DM, Kleinschnitz C, Gunzer M. Implications of polymorphonuclear neutrophils for ischemic stroke and intracerebral hemorrhage: predictive value, pathophysiological consequences and utility as therapeutic target. *J Neuroimmunol*. 2018;321:138–143. doi: 10.1016/j.jneuroim.2018.04.015
 89. Peña-Martínez C, Durán-Laforet V, García-Culebras A, Ostos F, Hernández-Jiménez M, Bravo-Ferrer I, Pérez-Ruiz A, Ballenilla F, Díaz-Guzmán J, Pradillo JM, et al. Pharmacological modulation of neutrophil extracellular traps reverses thrombotic stroke tPA (tissue-type plasminogen activator) resistance. *Stroke*. 2019;50:3228–3237. doi: 10.1161/STROKEAHA.119.026848
 90. Kang L, Yu H, Yang X, Zhu Y, Bai X, Wang R, Cao Y, Xu H, Luo H, Lu L, et al. Neutrophil extracellular traps released by neutrophils impair revascularization and vascular remodeling after stroke. *Nat Commun*. 2020;11:2488. doi: 10.1038/s41467-020-16191-y
 91. Enzmann G, Mysiorek C, Gorina R, Cheng YJ, Ghavampour S, Hannocks MJ, Prinz V, Dirnagl U, Endres M, Prinz M, et al. The neurovascular unit as a selective barrier to polymorphonuclear granulocyte (PMN) infiltration into the brain after ischemic injury. *Acta Neuropathol*. 2013;125:395–412. doi: 10.1007/s00401-012-1076-3
 92. Perez-de-Puig I, Miro-Mur F, Ferrer-Ferrer M, Gelpi E, Pedragosa J, Justicia C, Urra X, Chamorro A, Planas AM. Neutrophil recruitment to the brain in mouse and human ischemic stroke. *Acta Neuropathol*. 2015;129:239–257. doi: 10.1007/s00401-014-1381-0
 93. Zrzavy T, Machado-Santos J, Christine S, Baumgartner C, Weiner HL, Butovsky O, Lassmann H. Dominant role of microglial and macrophage innate immune responses in human ischemic infarcts. *Brain Pathol*. 2018;28:791–805. doi: 10.1111/bpa.12583
 94. Weisenburger-Lile D, Dong Y, Yger M, Weisenburger G, Polara GF, Chaigneau T, Ochoa RZ, Marro B, Lapergue B, Alamowitch S, et al. Harmful neutrophil subsets in patients with ischemic stroke: association with disease severity. *Neural Neuroimmunol Neuroinflamm*. 2019;6:e571. doi: 10.1212/NXI.0000000000000571
 95. Ballesteros I, Rubio-Ponce A, Genua M, Lusito E, Kwok I, Fernández-Calvo G, Khojraty TE, van Grinsven E, González-Hernández S, Nicolás-Ávila JÁ, et al. Co-option of neutrophil fates by tissue environments. *Cell*. 2020;183:1282–1297.e18. doi: 10.1016/j.cell.2020.10.003
 96. Mildner A, Schmidt H, Nitsche M, Merkle D, Hanisch UK, Mack M, Heikenwalder M, Brück W, Priller J, Prinz M. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci*. 2007;10:1544–1553. doi: 10.1038/nn2015
 97. Chu HX, Broughton BR, Kim HA, Lee S, Drummond GR, Sobey CG. Evidence That Ly6C(hi) monocytes are protective in acute ischemic stroke by promoting M2 macrophage polarization. *Stroke*. 2015;46:1929–1937. doi: 10.1161/STROKEAHA.115.009426
 98. García-Bonilla L, Faraco G, Moore J, Murphy M, Racchumi G, Srinivasan J, Brea D, Iadecola C, Anrather J. Spatio-temporal profile, phenotypic diversity, and fate of recruited monocytes into the post-ischemic brain. *J Neuroinflammation*. 2016;13:285. doi: 10.1186/s12974-016-0750-0
 99. Gliem M, Mausberg AK, Lee JI, Simiantonakis I, van Rooijen N, Hartung HP, Jander S. Macrophages prevent hemorrhagic infarct transformation in murine stroke models. *Ann Neurol*. 2012;71:743–752. doi: 10.1002/ana.23529
 100. Dimitrijevic OB, Stamatovic SM, Keep RF, Andjelkovic AV. Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice. *Stroke*. 2007;38:1345–1353. doi: 10.1161/01.STR.0000259709.16654.8f
 101. Urra X, Cervera A, Villamor N, Planas AM, Chamorro A. Harms and benefits of lymphocyte subpopulations in patients with acute stroke. *Neuroscience*. 2009;158:1174–1183. doi: 10.1016/j.neuroscience.2008.06.014

102. Kim E, Yang J, Beltran CD, Cho S. Role of spleen-derived monocytes/macrophages in acute ischemic brain injury. *J Cereb Blood Flow Metab*. 2014;34:1411–1419. doi: 10.1038/jcbfm.2014.101
103. Denes A, McColl BW, Leow-Dyke SF, Chapman KZ, Humphreys NE, Grecis RK, Allan SM, Rothwell NJ. Experimental stroke-induced changes in the bone marrow reveal complex regulation of leukocyte responses. *J Cereb Blood Flow Metab*. 2011;31:1036–1050. doi: 10.1038/jcbfm.2010.198
104. Benakis C, Llovera G, Liesz A. The meningeal and choroidal infiltration routes for leukocytes in stroke. *Ther Adv Neurol Disord*. 2018;11:1756286418783708. doi: 10.1177/1756286418783708
105. Croese T, Castellani G, Schwartz M. Immune cell compartmentalization for brain surveillance and protection. *Nat Immunol*. 2021;22:1083–1092. doi: 10.1038/s41590-021-00994-2
106. Herisson F, Frodermann V, Courties G, Rohde D, Sun Y, Vandoorne K, Wojtkiewicz GR, Masson GS, Vinegoni C, Kim J, et al. Direct vascular channels connect skull bone marrow and the brain surface enabling myeloid cell migration. *Nat Neurosci*. 2018;21:1209–1217. doi: 10.1038/s41593-018-0213-2
107. Cugurra A, Mamuladze T, Rustenhoven J, Dykstra T, Beroshvili G, Greenberg ZJ, Baker W, Papadopoulos Z, Drieu A, Blackburn S, et al. Skull and vertebral bone marrow are myeloid cell reservoirs for the meninges and CNS parenchyma. *Science*. 2021;373:eabf7844. doi: 10.1126/science.abf7844
108. Brioschi S, Wang WL, Peng V, Wang M, Shchukina I, Greenberg ZJ, Bando JK, Jaeger N, Czepielewski RS, Swain A, et al. Heterogeneity of meningeal B cells reveals a lymphopoietic niche at the CNS borders. *Science*. 2021;373:eabf9277. doi: 10.1126/science.abf9277
109. Rohde D, Vandoorne K, Lee IH, Grune J, Zhang S, McAlpine CS, Schloss MJ, Nayyar R, Courties G, Frodermann V, et al. Bone marrow endothelial dysfunction promotes myeloid cell expansion in cardiovascular disease. *Nature Cardiovasc Res*. 2022;1:28–44
110. Kolabas ZI, Kuemmerle LB, Pernecky R, Förster B, Büttner M, Caliskan OS, Ali M, Rong Z, Mai H, Hummel S, et al. Multi-omics and 3d-imaging reveal bone heterogeneity and unique calvaria cells in neuroinflammation. *bioRxiv*. 2021
111. Pulous FE, Cruz-Hernández JC, Yang C, Kaya Z, Wojtkiewicz G, Capen D, Brown D, Wu JW, Vinegoni C, Yamazoe M, et al. Cerebrospinal fluid outflow through skull channels instructs cranial hematopoiesis. *bioRxiv*. 2021
112. das Neves SP, Delivanoglou N, Da Mesquita S. CNS-draining meningeal lymphatic vasculature: roles, conundrums and future challenges. *Front Pharmacol*. 2021;12:655052. doi: 10.3389/fphar.2021.655052
113. Sreeramkumar V, Adrover JM, Ballesteros I, Cuartero MI, Rossaint J, Bilbao I, Nacher M, Pitaval C, Radovanovic I, Fukui Y, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science*. 2014;346:1234–1238. doi: 10.1126/science.1256478
114. Koupenova M, Livada AC, Morrill CN. Platelet and megakaryocyte roles in innate and adaptive immunity. *Circ Res*. 2022;130:288–308. doi: 10.1161/CIRCRESAHA.121.319821
115. Yilmaz G, Granger DN. Leukocyte recruitment and ischemic brain injury. *Neuromolecular Med*. 2010;12:193–204. doi: 10.1007/s12017-009-8074-1
116. Htun P, Fateh-Moghadam S, Tomandl B, Handschu R, Klinger K, Stellos K, Garlachs C, Daniel W, Gawaz M. Course of platelet activation and platelet-leukocyte interaction in cerebrovascular ischemia. *Stroke*. 2006;37:2283–2287. doi: 10.1161/01.STR.0000236638.75591.61
117. del Zoppo GJ, Becker KJ, Hallenbeck JM. Inflammation after stroke: is it harmful? *Arch Neurol*. 2001;58:669–672. doi: 10.1001/archneur.58.4.669
118. Staessens S, François O, Brinjikji W, Doyle KM, Vanacker P, Andersson T, De Meyer SF. Studying stroke thrombus composition after thrombectomy: what can we learn? *Stroke*. 2021;52:3718–3727. doi: 10.1161/STROKEAHA.121.034289
119. Laridan E, Denorme F, Desender L, François O, Andersson T, Deckmyn H, Vanhoelbeke K, De Meyer SF. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann Neurol*. 2017;82:223–232. doi: 10.1002/ana.24993
120. Novotny J, Oberdieck P, Titova A, Pelisek J, Chandraratne S, Nicol P, Hapfelmeyer A, Joner M, Maegdeflessel L, Poppert H, et al. Thrombus NET content is associated with clinical outcome in stroke and myocardial infarction. *Neurology*. 2020;94:e2346–e2360. doi: 10.1212/WNL.0000000000009532
121. Liesz A, Hu X, Kleinschnitz C, Offner H. Functional role of regulatory lymphocytes in stroke: facts and controversies. *Stroke*. 2015;46:1422–1430. doi: 10.1161/STROKEAHA.114.008608
122. Selvaraj UM, Ujas TA, Kong X, Kumar A, Plautz EJ, Zhang S, Xing C, Sudduth TL, Wilcock DM, Turchan-Cholewo J, et al. Delayed diapedesis of CD8 T cells contributes to long-term pathology after ischemic stroke in male mice. *Brain Behav Immun*. 2021;95:502–513. doi: 10.1016/j.bbi.2021.05.001
123. Gee JM, Kalil A, Thullberg M, Becker KJ. Induction of immunologic tolerance to myelin basic protein prevents central nervous system autoimmunity and improves outcome after stroke. *Stroke*. 2008;39:1575–1582. doi: 10.1161/STROKEAHA.107.501486
124. Liesz A, Zhou W, Na SY, Hämmerling GJ, Garbi N, Karcher S, Mracsco E, Backs J, Rivest S, Veltkamp R. Boosting regulatory T cells limits neuroinflammation in permanent cortical stroke. *J Neurosci*. 2013;33:17350–17362. doi: 10.1523/JNEUROSCI.4901-12.2013
125. Kleinschnitz C, Kraft P, Dreykluft A, Hagedorn I, Göbel K, Schuhmann MK, Langhauser F, Helluy X, Schwarz T, Bittner S, et al. Regulatory T cells are strong promoters of acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature. *Blood*. 2013;121:679–691. doi: 10.1182/blood-2012-04-426734
126. Heindl S, Ricci A, Carofiglio O, Zhou Q, Arzberger T, Lenart N, Franzmeier N, Hortobagyi T, Nelson PT, Stowe AM, et al. Chronic T cell proliferation in brains after stroke could interfere with the efficacy of immunotherapies. *J Exp Med*. 2021;218:e20202411. doi: 10.1084/jem.20202411
127. Doyle KP, Quach LN, Solé M, Axtell RC, Nguyen TV, Soler-Llavina GJ, Jurado S, Han J, Steinman L, Longo FM, et al. B-lymphocyte-mediated delayed cognitive impairment following stroke. *J Neurosci*. 2015;35:2133–2145. doi: 10.1523/JNEUROSCI.4098-14.2015
128. Benakis C, Brea D, Caballero S, Faraco G, Moore J, Murphy M, Sita G, Racchumi G, Ling L, Pamer EG, et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal $\gamma\delta$ T cells. *Nat Med*. 2016;22:516–523. doi: 10.1038/nm.4068
129. Singh V, Roth S, Llovera G, Sadler R, Garzetti D, Stecher B, Dichgans M, Liesz A. Microbiota dysbiosis controls the neuroinflammatory response after stroke. *J Neurosci*. 2016;36:7428–7440. doi: 10.1523/JNEUROSCI.1114-16.2016
130. Smith CJ, Heal C, Vail A, Jeans AR, Westendorp WF, Nederkoorn PJ, van de Beek D, Kalra L, Montaner J, Woodhead M, et al. Antibiotic class and outcome in post-stroke infections: an individual participant data pooled analysis of VISTA-acute. *Front Neurol*. 2019;10:504. doi: 10.3389/fneur.2019.00504
131. Schafflick D, Wolbert J, Heming M, Thomas C, Hartlehnert M, Börsch AL, Ricci A, Martín-Salamanca S, Li X, Lu IN, et al. Single-cell profiling of CNS border compartment leukocytes reveals that B cells and their progenitors reside in non-diseased meninges. *Nat Neurosci*. 2021;24:1225–1234. doi: 10.1038/s41593-021-00880-y
132. Ren X, Akiyoshi K, Dziennis S, Vandenberg AA, Herson PS, Hurn PD, Offner H. Regulatory B cells limit CNS inflammation and neurologic deficits in murine experimental stroke. *J Neurosci*. 2011;31:8556–8563. doi: 10.1523/JNEUROSCI.1623-11.2011
133. Zbesko JC, Frye JB, Becktel DA, Gerardo DK, Stokes J, Calderon K, Nguyen TV, Bhattacharya D, Doyle KP. IgA natural antibodies are produced following T-cell independent B-cell activation following stroke. *Brain Behav Immun*. 2021;91:578–586. doi: 10.1016/j.bbi.2020.09.014
134. Weitbrecht L, Berchtold D, Zhang T, Jagdmann S, Dames C, Winek K, Meisel A. CD4+ T cells promote delayed B cell responses in the ischemic brain after experimental stroke. *Brain Behav Immun*. 2021;91:601–614. doi: 10.1016/j.bbi.2020.09.029
135. Planas AM, Gómez-Choco M, Urrea X, Gorina R, Caballero M, Chamorro Á. Brain-derived antigens in lymphoid tissue of patients with acute stroke. *J Immunol*. 2012;188:2156–2163. doi: 10.4049/jimmunol.1102289
136. Becker KJ, Kalil AJ, Tanzi P, Zierath DK, Savos AV, Gee JM, Hadwin J, Carter KT, Shibata D, Cain KC. Autoimmune responses to the brain after stroke are associated with worse outcome. *Stroke*. 2011;42:2763–2769. doi: 10.1161/STROKEAHA.111.619593
137. Ito M, Komai K, Mise-Omata S, Iizuka-Koga M, Noguchi Y, Kondo T, Sakai R, Matsuo K, Nakayama T, Yoshie O, et al. Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. *Nature*. 2019;565:246–250. doi: 10.1038/s41586-018-0824-5
138. Shi L, Sun Z, Su W, Xu F, Xie D, Zhang Q, Dai X, Iyer K, Hitchens TK, Foley LM, et al. Treg cell-derived osteopontin promotes microglia-mediated white matter repair after ischemic stroke. *Immunity*. 2021;54:1527–1542.e8. doi: 10.1016/j.immuni.2021.04.022
139. Ortega SB, Torres VO, Latchney SE, Whoolery CW, Noorbhai IZ, Poinsette K, Selvaraj UM, Benson MA, Meeuwissen AJM, Plautz EJ, et al. B cells migrate into remote brain areas and support neurogenesis and functional recovery after focal stroke in mice. *Proc Natl Acad Sci USA*. 2020;117:4983–4993. doi: 10.1073/pnas.1913292117
140. Chapman NM, Chi H. Metabolic adaptation of lymphocytes in immunity and disease. *Immunity*. 2022;55:14–30. doi: 10.1016/j.immuni.2021.12.012
141. Zhang Y, Li F, Chen C, Li Y, Xie W, Huang D, Zhai X, Yu W, Wan J, Li P. Rage-mediated t cell metabolic reprogramming shapes t cell

- inflammatory response after stroke. *J Cereb Blood Flow Metab*. 2021;27:1678X211067133. doi: 10.1177/0271678X211067133
142. Sadler R, Cramer JV, Heindl S, Kostidis S, Betz D, Zuurbier KR, Northoff BH, Heijink M, Goldberg MP, Plautz EJ, et al. Short-chain fatty acids improve poststroke recovery via immunological mechanisms. *J Neurosci*. 2020;40:1162–1173. doi: 10.1523/JNEUROSCI.1359-19.2019
 143. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: a systematic review and meta-analysis. *BMC Neurol*. 2011;11:110. doi: 10.1186/1471-2377-11-110
 144. Langhorne P, Ramachandra S; Stroke Unit Trialists' Collaboration. Organized inpatient (stroke unit) care for stroke: network meta-analysis. *Stroke*. 2020;51:e349–e350. doi: 10.1161/STROKEAHA.120.030825
 145. Eltringham SA, Kilner K, Gee M, Sage K, Bray BD, Smith CJ, Pownall S. Factors associated with risk of stroke-associated pneumonia in patients with dysphagia: a systematic review. *Dysphagia*. 2020;35:735–744. doi: 10.1007/s00455-019-10061-6
 146. Hoffmann S, Harms H, Ulm L, Nabavi DG, Mackert BM, Schmehl I, Jungehulsing GJ, Montaner J, Bustamante A, Hermans M, et al; PREDICT Investigators. Stroke-induced immunodepression and dysphagia independently predict stroke-associated pneumonia - The PREDICT study. *J Cereb Blood Flow Metab*. 2017;37:3671–3682. doi: 10.1177/0271678X16671964
 147. Meisel C, Schwab JM, Prass K, Meisel A, Dirnagl U. Central nervous system injury-induced immune deficiency syndrome. *Nat Rev Neurosci*. 2005;6:775–786. doi: 10.1038/nrn1765
 148. Ruhnau J, Schulze K, Gaida B, Langner S, Kessler C, Bröker B, Dressel A, Vogelgesang A. Stroke alters respiratory burst in neutrophils and monocytes. *Stroke*. 2014;45:794–800. doi: 10.1161/STROKEAHA.113.003342
 149. Vahidy FS, Parsha KN, Rahbar MH, Lee M, Bui TT, Nguyen C, Barreto AD, Bambhroliya AB, Sahota P, Yang B, et al. Acute splenic responses in patients with ischemic stroke and intracerebral hemorrhage. *J Cereb Blood Flow Metab*. 2016;36:1012–1021. doi: 10.1177/0271678X15607880
 150. Roth S, Cao J, Singh V, Tiedt S, Hundeshagen G, Li T, Boehme JD, Chauhan D, Zhu J, Ricci A, et al. Post-injury immunosuppression and secondary infections are caused by an AIM2 inflammasome-driven signaling cascade. *Immunity*. 2021;54:648–659.e8. doi: 10.1016/j.immuni.2021.02.004
 151. O'Connell GC, Tennant CS, Lucke-Wold N, Kabbani Y, Tarabishy AR, Chantler PD, Barr TL. Monocyte-lymphocyte cross-communication via soluble CD163 directly links innate immune system activation and adaptive immune system suppression following ischemic stroke. *Sci Rep*. 2017;7:12940. doi: 10.1038/s41598-017-13291-6
 152. Klehmet J, Harms H, Richter M, Prass K, Volk HD, Dirnagl U, Meisel A, Meisel C. Stroke-induced immunodepression and post-stroke infections: lessons from the preventive antibacterial therapy in stroke trial. *Neuroscience*. 2009;158:1184–1193. doi: 10.1016/j.neuroscience.2008.07.044
 153. Prass K, Meisel C, Höflich C, Braun J, Halle E, Wolf T, Ruscher K, Victorov IV, Priller J, Dirnagl U, et al. Stroke-induced immunodeficiency promotes spontaneous bacterial infections and is mediated by sympathetic activation reversal by poststroke T helper cell type 1-like immunostimulation. *J Exp Med*. 2003;198:725–736. doi: 10.1084/jem.20021098
 154. Wong CH, Jenne CN, Lee WY, Léger C, Kubes P. Functional innervation of hepatic iNKT cells is immunosuppressive following stroke. *Science*. 2011;334:101–105. doi: 10.1126/science.1210301
 155. Römer C, Engel O, Winek K, Hochmeister S, Zhang T, Royl G, Klehmet J, Dirnagl U, Meisel C, Meisel A. Blocking stroke-induced immunodeficiency increases CNS antigen-specific autoreactivity but does not worsen functional outcome after experimental stroke. *J Neurosci*. 2015;35:7777–7794. doi: 10.1523/JNEUROSCI.1532-14.2015
 156. Wang L, Song Q, Wang C, Wu S, Deng L, Li Y, Zheng L, Liu M. Neutrophil to lymphocyte ratio predicts poor outcomes after acute ischemic stroke: a cohort study and systematic review. *J Neurol Sci*. 2019;406:116445. doi: 10.1016/j.jns.2019.116445
 157. Zonneveld TP, Nederkoorn PJ, Westendorp WF, Brouwer MC, van de Beek D, Kruyt ND; PASS Investigators. Hyperglycemia predicts poststroke infections in acute ischemic stroke. *Neurology*. 2017;88:1415–1421. doi: 10.1212/WNL.0000000000003811
 158. Sun Y, Lu J, Zheng D, Qian J, Zhang H, Xing D, Rong F, Cao Y, Liu CF, You S. Predictive value of monocyte to hdl cholesterol ratio for stroke-associated pneumonia in patients with acute ischemic stroke. *Acta Neurol Belg*. 2021;121:1575–1581. doi: 10.1007/s13760-020-01418-y
 159. Bustamante A, Vilar-Bergua A, Guettier S, Sánchez-Poblet J, García-Berrococo T, Giralto D, Fluri F, Topakian R, Worthmann H, Hug A, et al. C-reactive protein in the detection of post-stroke infections: systematic review and individual participant data analysis. *J Neurochem*. 2017;141:305–314. doi: 10.1111/jnc.13973
 160. Schweizer J, Bustamante A, Lapierre-Fétaud V, Faura J, Scherrer N, Azurmendi Gil L, Fluri F, Schütz V, Luft A, Boned S, et al. SAA (serum amyloid A): a novel predictor of stroke-associated infections. *Stroke*. 2020;51:3523–3530. doi: 10.1161/STROKEAHA.120.030064
 161. Hotter B, Hoffmann S, Ulm L, Montaner J, Bustamante A, Meisel C, Meisel A. Inflammatory and stress markers predicting pneumonia, outcome, and etiology in patients with stroke: biomarkers for predicting pneumonia, functional outcome, and death after stroke. *Neurol Neuroimmunol Neuroinflamm*. 2020;7:e692. doi: 10.1212/NXI.0000000000000692
 162. Bustamante A, Sobrino T, Giralto D, García-Berrococo T, Llombart V, Ugarriza I, Espadaler M, Rodríguez N, Sudlow C, Castellanos M, et al. Prognostic value of blood interleukin-6 in the prediction of functional outcome after stroke: a systematic review and meta-analysis. *J Neuroimmunol*. 2014;274:215–224. doi: 10.1016/j.jneuroim.2014.07.015
 163. Lin SP, Ye S, Chen XH, Jiang HL, Mao HF, Chen MT, Ma QJ, Long Y, Fan Y, Lin PY. Increased expression of microRNA-21 in peripheral blood mediates the down-regulation of IFN- γ and increases the prevalence of stroke-associated infection. *J Neural Sci*. 2016;366:235–239. doi: 10.1016/j.jns.2016.03.034
 164. Smith CJ, Kishore AK, Vail A, Chamorro A, Garau J, Hopkins SJ, Di Napoli M, Kalra L, Langhorne P, Montaner J, et al. Diagnosis of stroke-associated pneumonia: recommendations from the Pneumonia in Stroke Consensus group. *Stroke*. 2015;46:2335–2340. doi: 10.1161/STROKEAHA.115.009617
 165. Westendorp WF, Vermeij FH, Smith CJ, Kishore AK, Hodsoll J, Kalra L, Meisel A, Chamorro A, Chang JJ, Rezaei Y, et al. Preventive antibiotic therapy in acute stroke patients: a systematic review and meta-analysis of individual patient data of randomized controlled trials. *Eur Stroke J*. 2021;6:385–394
 166. Puig J, Blasco G, Daunis-I-Estadella J, Thomalla G, Castellanos M, Figueras J, Remollo S, van Eendenburg C, Sánchez-González J, Serena J, et al. Decreased corticospinal tract fractional anisotropy predicts long-term motor outcome after stroke. *Stroke*. 2013;44:2016–2018. doi: 10.1161/STROKEAHA.111.000382
 167. Wen H, Weymann KB, Wood L, Wang QM. Inflammatory signaling in post-stroke fatigue and depression. *Eur Neurol*. 2018;80:138–148. doi: 10.1159/000494988
 168. Doyle KP, Buckwalter MS. Immunological mechanisms in poststroke dementia. *Curr Opin Neurol*. 2020;33:30–36. doi: 10.1097/WCO.0000000000000783
 169. Corraini P, Henderson VW, Ording AG, Pedersen L, Horváth-Puhó E, Sørensen HT. Long-term risk of dementia among survivors of ischemic or hemorrhagic stroke. *Stroke*. 2017;48:180–186. doi: 10.1161/STROKEAHA.116.015242
 170. Pendlebury ST, Rothwell PM; Oxford Vascular Study. Incidence and prevalence of dementia associated with transient ischaemic attack and stroke: analysis of the population-based Oxford Vascular Study. *Lancet Neurol*. 2019;18:248–258. doi: 10.1016/S1474-4422(18)30442-3
 171. Weaver NA, Kuijff HJ, Aben HP, Abrigo J, Bae HJ, Barbay M, Best JG, Bordet R, Chappell FM, Chen CPLH, et al. Strategic infarct locations for post-stroke cognitive impairment: a pooled analysis of individual patient data from 12 acute ischaemic stroke cohorts. *Lancet Neurol*. 2021;20:448–459. doi: 10.1016/S1474-4422(21)00060-0
 172. Ivan CS, Seshadri S, Beiser A, Au R, Kase CS, Kelly-Hayes M, Wolf PA. Dementia after stroke: the Framingham study. *Stroke*. 2004;35:1264–1268. doi: 10.1161/01.STR.0000127810.92616.78
 173. Cuartero MI, de la Parra J, Pérez-Ruiz A, Bravo-Ferrer I, Durán-Laforet V, García-Culebras A, García-Segura JM, Dhaliwal J, Frankland PW, Lizasoain I, et al. Abolition of aberrant neurogenesis ameliorates cognitive impairment after stroke in mice. *J Clin Invest*. 2019;129:1536–1550. doi: 10.1172/JCI120412
 174. Domínguez-Rivas E, Ávila-Muñoz E, Schwarzacher SW, Zepeda A. Adult hippocampal neurogenesis in the context of lipopolysaccharide-induced neuroinflammation: a molecular, cellular and behavioral review. *Brain Behav Immun*. 2021;97:286–302. doi: 10.1016/j.bbi.2021.06.014
 175. Becker KJ, Tanzi P, Zierath D, Buckwalter MS. Antibodies to myelin basic protein are associated with cognitive decline after stroke. *J Neuroimmunol*. 2016;295–296:9–11. doi: 10.1016/j.jneuroim.2016.04.001
 176. Tsai AS, Berry K, Beneyto MM, Gaudilliere D, Ganio EA, Culos A, Ghaemi MS, Choisy B, Djebali K, Einhaus JF, et al. A year-long immune profile of the systemic response in acute stroke survivors. *Brain*. 2019;142:978–991. doi: 10.1093/brain/awz022
 177. Cai W, Mueller C, Li YJ, Shen WD, Stewart R. Post stroke depression and risk of stroke recurrence and mortality: a systematic review and meta-analysis. *Ageing Res Rev*. 2019;50:102–109. doi: 10.1016/j.arr.2019.01.013

178. Shi Y, Yang D, Zeng Y, Wu W. Risk factors for post-stroke depression: a meta-analysis. *Front Aging Neurosci.* 2017;9:218. doi: 10.3389/fnagi.2017.00218
179. Chen Y, Pu J, Liu Y, Tian L, Chen X, Gui S, Xu S, Song X, Xie P. Pro-inflammatory cytokines are associated with the development of post-stroke depression in the acute stage of stroke: a meta-analysis. *Top Stroke Rehabil.* 2020;27:620–629. doi: 10.1080/10749357.2020.1755813
180. Tanaka T, Ihara M. Post-stroke epilepsy. *Neurochem Int.* 2017;107:219–228. doi: 10.1016/j.neuint.2017.02.002
181. Bentes C, Martins H, Peralta AR, Casimiro C, Morgado C, Franco AC, Fonseca AC, Galdes R, Canhão P, Pinho E, Melo T, et al. Post-stroke seizures are clinically underestimated. *J Neurol.* 2017;264:1978–1985. doi: 10.1007/s00415-017-8586-9
182. Castro-Apolo R, Huang JF, Vinan-Vega M, Tatum WO. Outcome and predictive factors in post-stroke seizures: a retrospective case-control study. *Seizure.* 2018;62:11–16. doi: 10.1016/j.seizure.2018.09.007
183. Shen L, Yang J, Tang Y. Predictive values of the SeLECT score and IL-1 β for Post-Stroke Epilepsy. *Neuropsychiatr Dis Treat.* 2021;17:2465–2472. doi: 10.2147/NDT.S324271
184. Naylor J, Thevathasan A, Churilov L, Guo R, Xiong Y, Koome M, Chen Z, Chen Z, Liu X, Kwan P, et al. Association between different acute stroke therapies and development of post stroke seizures. *BMC Neurol.* 2018;18:61. doi: 10.1186/s12883-018-1064-x
185. Ducruet AF, Zacharia BE, Sosunov SA, Gigante PR, Yeh ML, Gorski JW, Otten ML, Hwang RY, DeRosa PA, Hickman ZL, et al. Complement inhibition promotes endogenous neurogenesis and sustained anti-inflammatory neuroprotection following reperfused stroke. *PLoS One.* 2012;7:e38664. doi: 10.1371/journal.pone.0038664
186. Weissberg I, Wood L, Kamintsky L, Vazquez O, Milikovsky DZ, Alexander A, Oppenheim H, Ardizzone C, Becker A, Frigerio F, et al. Albumin induces excitatory synaptogenesis through astrocytic TGF- β /ALK5 signaling in a model of acquired epilepsy following blood-brain barrier dysfunction. *Neurobiol Dis.* 2015;78:115–125. doi: 10.1016/j.nbd.2015.02.029
187. Necula D, Cho FS, He A, Paz JT. Secondary thalamic neuroinflammation after focal cortical stroke and traumatic injury mirrors corticothalamic functional connectivity. *J Comp Neurol.* 2021
188. Maier IL, Becker JC, Leyhe JR, Schnieder M, Behme D, Psychogios MN, Liman J. Influence of beta-blocker therapy on the risk of infections and death in patients at high risk for stroke induced immunodepression. *PLoS One.* 2018;13:e0196174. doi: 10.1371/journal.pone.0196174
189. Westendorp WF, Vermeij JD, Brouwer MC, Roos YB, Nederkoorn PJ, van de Beek D; PASS Investigators. Pre-stroke use of beta-blockers does not lower post-stroke infection rate: an exploratory analysis of the preventive antibiotics in stroke study. *Cerebrovasc Dis.* 2016;42:506–511. doi: 10.1159/000450926
190. Starr JB, Tirschwell DL, Becker KJ. Increased infections with β -blocker use in ischemic stroke, a β 2-receptor mediated process? *Neurol Sci.* 2017;38:967–974. doi: 10.1007/s10072-017-2877-x
191. Sykora M, Siarnik P, Diedler J; VISTA Acute Collaborators. β -Blockers, pneumonia, and outcome after ischemic stroke: evidence from virtual international stroke trials archive. *Stroke.* 2015;46:1269–1274. doi: 10.1161/STROKEAHA.114.008260
192. Dames C, Winek K, Beckers Y, Engel O, Meisel A, Meisel C. Immunomodulatory treatment with systemic GM-CSF augments pulmonary immune responses and improves neurological outcome after experimental stroke. *J Neuroimmunol.* 2018;321:144–149. doi: 10.1016/j.jneuroim.2018.03.005
193. Meisel C, Meisel A. Suppressing immunosuppression after stroke. *N Engl J Med.* 2011;365:2134–2136. doi: 10.1056/NEJMcibr1112454
194. Ritzel RM, Al Mamun A, Crapser J, Verma R, Patel AR, Knight BE, Harris N, Mancini N, Roy-O'Reilly M, Ganesh BP, et al. CD200-CD200R1 inhibitory signaling prevents spontaneous bacterial infection and promotes resolution of neuroinflammation and recovery after stroke. *J Neuroinflammation.* 2019;16:40. doi: 10.1186/s12974-019-1426-3
195. McCulloch L, Harris AJ, Malbon A, Daniels MJD, Younas M, Grainger JR, Allan SM, Smith CJ, McColl BW. Treatment with igm-enriched intravenous immunoglobulins (igm-ivig) enhances clearance of stroke-associated bacterial lung infection. *bioRxiv.* 2021
196. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, et al; JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med.* 2008;359:2195–2207. doi: 10.1056/NEJMoa0807646
197. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, et al; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med.* 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914
198. Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, Pinto FJ, Ibrahim R, Gamra H, Kiwan GS, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med.* 2019;381:2497–2505. doi: 10.1056/NEJMoa1912388
199. Nidorf SM, Eikelboom JW, Budgeon CA, Thompson PL. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol.* 2013;61:404–410. doi: 10.1016/j.jacc.2012.10.027
200. Nidorf SM, Fiolet AT, Mosterd A, Eikelboom JW, Schut A, Opstal TSJ, The SHK, Xu XF, Ireland MA, Lenderink T, et al; LoDoCo2 Trial Investigators. Colchicine in patients with chronic coronary disease. *N Engl J Med.* 2020;383:1838–1847. doi: 10.1056/NEJMoa2021372
201. Zhao TX, Kostapanos M, Griffiths C, Arbon EL, Hubsch A, Kaloyirou F, Helmy J, Hoole SP, Rudd JHF, Wood G, et al. Low-dose interleukin-2 in patients with stable ischaemic heart disease and acute coronary syndromes (LILACS): protocol and study rationale for a randomised, double-blind, placebo-controlled, phase I/II clinical trial. *BMJ Open.* 2018;8:e022452. doi: 10.1136/bmjopen-2018-022452
202. Alasheev AM, Belkin AA, Leiderman IN, Ivanov RA, Isakova TM. Granulocyte-colony-stimulating factor for acute ischemic stroke: a randomized controlled trial (STEMTHER). *Transl Stroke Res.* 2011;2:358–365. doi: 10.1007/s12975-011-0091-3
203. Ringelstein EB, Thijs V, Norrving B, Chamorro A, Aichner F, Grond M, Saver J, Laage R, Schneider A, Rathgeb F, et al; AXIS 2 Investigators. Granulocyte colony-stimulating factor in patients with acute ischemic stroke: results of the AX200 for ischemic stroke trial. *Stroke.* 2013;44:2681–2687. doi: 10.1161/STROKEAHA.113.001531
204. Emsley HC, Smith CJ, Georgiou RF, Vail A, Hopkins SJ, Rothwell NJ, Tyrrell PJ; Acute Stroke Investigators. A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients. *J Neurol Neurosurg Psychiatry.* 2005;76:1366–1372. doi: 10.1136/jnnp.2004.054882
205. Smith CJ, Hulme S, Vail A, Heal C, Parry-Jones AR, Scarth S, Hopkins K, Hoadley M, Allan SM, Rothwell NJ, et al. SCIL-STROKE (Subcutaneous Interleukin-1 Receptor Antagonist in Ischemic Stroke): a randomized controlled phase 2 trial. *Stroke.* 2018;49:1210–1216. doi: 10.1161/STROKEAHA.118.020750
206. Elkins J, Veltkamp R, Montaner J, Johnston SC, Singhal AB, Becker K, Lansberg MG, Tang W, Chang I, Muralidharan K, et al. Safety and efficacy of natalizumab in patients with acute ischaemic stroke (ACTION): a randomised, placebo-controlled, double-blind phase 2 trial. *Lancet Neurol.* 2017;16:217–226. doi: 10.1016/S1474-4422(16)30357-X
207. Elkind MSV, Veltkamp R, Montaner J, Johnston SC, Singhal AB, Becker K, Lansberg MG, Tang W, Kasliwal R, Elkins J, Natalizumab in acute ischemic stroke (ACTION II): a randomized, placebo-controlled trial. *Neurology.* 2020;95:e1091–e1104. doi: 10.1212/WNL.0000000000010038
208. Zhu Z, Fu Y, Tian D, Sun N, Han W, Chang G, Dong Y, Xu X, Liu Q, Huang D, et al. Combination of the immune modulator fingolimod with alteplase in acute ischemic stroke: a pilot trial. *Circulation.* 2015;132:1104–1112. doi: 10.1161/CIRCULATIONAHA.115.016371
209. Hess DC, Wechsler LR, Clark WM, Savitz SI, Ford GA, Chiu D, Yavagal DR, Uchino K, Liebeskind DS, Auchus AP, et al. Safety and efficacy of multipotent adult progenitor cells in acute ischaemic stroke (MASTERS): a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Neurol.* 2017;16:360–368. doi: 10.1016/S1474-4422(17)30046-7
210. Gulati A, Agrawal N, Vibha D, Misra UK, Paul B, Jain D, Pandian J, Borgohain R. Safety and efficacy of sovateltide (IRL-1620) in a multicenter randomized controlled clinical trial in patients with acute cerebral ischemic stroke. *CNS Drugs.* 2021;35:85–104. doi: 10.1007/s40263-020-00783-9
211. Díez-Tejedor E, Gutiérrez-Fernández M, Martínez-Sánchez P, Rodríguez-Frutos B, Ruiz-Ares G, Lara ML, Gimeno BF. Reparative therapy for acute ischemic stroke with allogeneic mesenchymal stem cells from adipose tissue: a safety assessment: a phase II randomized, double-blind, placebo-controlled, single-center, pilot clinical trial. *J Stroke Cerebrovasc Dis.* 2014;23:2694–2700. doi: 10.1016/j.jstrokecerebrovasdis.2014.06.011