The immune system in stroke: clinical challenges and their translation to experimental research

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Abstract

Stroke represents an unresolved challenge for both developed and developing countries and has a huge socio-economic impact. Although considerable effort has been made to limit stroke incidence and improve outcome, strategies aimed at protecting injured neurons in the brain have all failed. This failure is likely to be due to both the incompleteness of modelling the disease and its causes in experimental research, and also the lack of understanding of how systemic mechanisms lead to an acute cerebrovascular event or contribute to outcome. Inflammation has been implicated in all forms of brain injury and it is now clear that immune mechanisms profoundly influence (and are responsible for the development of) risk and causation of stroke, and the outcome following the onset of cerebral ischemia. Until very recently, systemic inflammatory mechanisms, with respect to common comorbidities in stroke, have largely been ignored in experimental studies. The main aim is therefore to understand interactions between the immune system and brain injury in order to develop novel therapeutic approaches. Recent data from clinical and experimental research clearly show that systemic inflammatory diseases –such as atherosclerosis, obesity, diabetes or infection – similar to stress and advanced age, are associated with dysregulated immune responses which can profoundly contribute to cerebrovascular inflammation and injury in the central nervous system. In this review, we summarize recent advances in the field of inflammation and stroke, focusing on the challenges of translation between pre-clinical and clinical studies, and potential anti-inflammatory/immunomodulatory therapeutic approaches.

Keywords: stroke, immune system, comorbidities, inflammation, brain injury, IL-1

Introduction

Stroke is the second leading cause of death worldwide (Top Ten Causes of Death, 2011) and the leading cause of adult neurological disability (The Global Burden of Disease, 2004). The total number of stroke deaths is estimated at 508,000 per year in the 27 European Union members, with an annual cost of 27 billion € (European cardiovascular disease statistics, 2008). Ischemic stroke, accounting for approximately 80% of all strokes, is a heterogenous and multifactorial disorder with complex interplay between environmental and genetic factors contributing to its causation, and influencing its subsequent outcome. Despite well-recognised conventional vascular risk factors (e.g. increasing age, hypertension, smoking, diabetes), stroke mechanisms (e.g. cardioembolism, artery to artery embolism, hypoperfusion) and underlying aetiologies (e.g. atherotherombosis, atrial fibrillation (AF), small vessel disease), the cause of ischemic stroke remains unclear in up to 24% of patients (Hajat et al. 2011). It is increasingly recognised that novel mechanisms may contribute not only to the pathophysiology of stroke of unknown cause, but also to that associated with conventional vascular risk factors and aetiologies of ischemic stroke. Despite many years of translational research into putative treatments for acute ischemic stroke, the only pharmacological treatments remain antiplatelet therapy for the majority (Chen et al. 2000) and thrombolysis with intravenous alteplase for selected individuals (Wardlaw et al. 2012). Prevention of stroke is based largely on management of conventional vascular risk factors, yet recurrent vascular events in stroke survivors remain frequent, despite standard secondary prevention, with 21% suffering myocardial infarction, recurrent stroke, cardiovascular death or hospitalisation for atherothrombotic events by 2 years (Venketasubramanian et al. 2011).

Prevention of stroke is therefore a massive public health challenge and major priority worldwide; and safe, effective treatments for acute ischemic stroke are urgently needed. Improving our understanding of the mechanisms causing ischemic stroke, and mediating subsequent injury and repair, are therefore crucial in developing novel therapeutic targets. The failure of so many putative therapies for ischemic stroke in bench-to-bedside research has highlighted the importance of reconsidering how translational research is approached. At a time when translational research is at a “road-block”, the need to consider the realities of the clinical setting when developing experimental models of cerebral ischemia and

undertaking pre-clinical studies has become increasingly recognised. In particular, the influence of age and comorbidities on inflammatory responses preceding, or following cerebral ischemia, has been relatively neglected in pre-clinical studies.

The immune system and stroke

Stroke is primarily, although not exclusively, a disease of the elderly. Indeed, advancing age is the single most important risk factor in stroke: in both men and women the stroke rate more than doubles for each successive 10 years after age 55 (Brown et al. 1996; Wolf et al. 1992). As activity of the immune system declines with age, the causal role of immune mechanisms in stroke pathophysiology does not seem straightforward to explain. Nevertheless, aging is associated with an elevated systemic inflammatory burden and reduced ability of cell regeneration, which contributes to diverse diseases including heart disease, cancer or stroke. In addition, the incidence of chronic inflammatory diseases appears to increase in developed countries even amongst young people, indicating that lifestyle in itself could be a major contributor to these diseases. Alterations in immune regulation seen in aged individuals, but also in young people presenting with systemic inflammation, appears to be a plausible common link between increased incidence of cardio- and cerebrovascular diseases.

Evidence indicates that inflammation is a crucial contributor to the development of all known risk factors for stroke and is also implicated in mechanisms of brain injury that occur after stroke. Stroke is a vascular disease, and major comorbidities such as atherosclerosis, hyperlipidaemia, diabetes, obesity, hypertension or infection are characterized by elevated systemic inflammatory burden, including prolonged vascular inflammation (Denes et al. 2010b; Iadecola and Anrather 2011). Although much information has been accumulated over the last decades about the involvement of immune mechanisms in these diseases, experimental models of common comorbidities in stroke have been investigated in detail only recently. Current research is aiming to understand how acute and chronic inflammatory mechanisms predispose to stroke and also, how inflammation contributes to the ischemic injury itself. In experimental studies, blockade of immune processes can result in marked protection against brain injury in otherwise healthy, young animals. Inhibition of immune cells (T lymphocytes, neutrophils, etc.), proinflammatory cytokines or receptors that recognize pathogen- or damage-associated molecules can reduce infarct size in experimental rodent

models of stroke (Denes et al. 2010b; Iadecola and Anrather 2011; Denes et al. 2011b). This indicates that essential defense mechanisms, which are indispensable for protection against infectious agents, are inherent components of ischemic injury in the central nervous system (CNS). In addition, recent data show that brain injury is increased in experimental models of hypertension, diabetes, obesity, atherosclerosis or infection. Blockade of key inflammatory pathways in these models reversed brain injury induced by comorbidities after stroke, suggesting that underlying systemic inflammation indeed contributes to brain injury (Denes et al. 2010b; Denes et al. 2011b; McColl et al. 2009; Emsley et al. 2008). These results are in accordance with clinical data, showing impaired outcome in patients presenting with risk factors that involve systemic inflammation (Wolf et al. 1992; McColl et al. 2009; Emsley and Hopkins 2008a; Emsley et al. 2008; Emsley et al. 2005b). Thus, identification of major mechanisms whereby inflammation contributes to brain injury in stroke, could lead to targeted therapies in order to limit stroke incidence and improve outcome.

Inflammation preceding ischemic stroke: beyond vascular risk factors?

Advancing age and certain comorbidities, notably hypertension, stress, smoking, metabolic syndrome (including obesity) and diabetes, are themselves associated with altered or dysregulated inflammation, which may also be influenced by medications commonly used in primary and secondary prevention (e.g. antiplatelets, statins and anti-hypertensives). Inflammatory processes are considered important in the initiation and progression of atherosclerosis, plaque destabilisation and subsequent thromboembolism (Packard et al. 2009). Furthermore, emerging evidence has implicated inflammation in the pathophysiology of AF (Friedrichs et al. 2011) and cerebral small vessel disease (Sierra et al. 2011). Numerous epidemiological studies have investigated the association between peripheral inflammatory markers and incident cerebrovascular events in apparently healthy individuals or those with conventional risk factors (e.g. hypertension), without a previous clinical history of stroke. Of these, the most extensively studied is C-reactive protein (CRP). In a recent meta-analysis of individual participant data from 54 prospective studies, plasma CRP concentration was associated with development of incident ischemic stroke after adjusting for age and sex, although the strength of the association diminished after adjusting for conventional vascular risk factors and fibrinogen (The Emerging Risk Factors Collaboration 2010). These data imply

that the majority of the observed association with ischemic stroke depends on age and conventional vascular risk factors. Whether such low-grade inflammation, measured by plasma CRP, has any causal role remains controversial. For example, in the JUPITER and Heart Protection Study, statins lowered both LDL-cholesterol and CRP concentrations, but the role of baseline CRP and its reduction in determining incident vascular events appear to be conflicting (Ridker et al. 2008; Heart Protection Study Collaborative Group 2011).

Lacunar infarction secondary to cerebral small vessel disease is a distinct aetiological subtype accounting for around 27% of all ischemic strokes (Hajat et al. 2011)**.** Several studies have examined the relationships between peripheral inflammatory markers, conventional vascular risk factors and apparently asymptomatic burden and progression of cerebral small vessel disease using brain magnetic resonance imaging (MRI) white-matter hyperintensities (WMH). In the Rotterdam Scan Study, baseline plasma CRP was associated with progression of WMH and asymptomatic incident lacunar infarcts, after adjustment for conventional vascular risk factors and extent of carotid atherosclerosis (van Dijk et al. 2005). In a study of Japanese diabetics, soluble intercellular adhesion molecule-1 (ICAM-1) was associated with progression of WMH after adjustment for age and vascular risk factors (Umemura et al. 2011). However, in other studies, baseline plasma CRP was not associated with volume or progression of WMH, whilst ICAM-1 was only weakly associated with progression of WMH (Markus et al. 2005; Schmidt et al. 2006; Wright et al. 2009). Such differences may reflect underlying selection bias, racial/ethnic differences, techniques for measuring WMH and other confounding variables such as medications.

Numerous studies have explored inflammation in human carotid atherosclerosis. In individuals without a history of stroke, plasma CRP is associated with progression of carotid atherosclerosis after adjustment for conventional vascular risk factors (Schmidt et al. 2006). Molecular imaging using ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET), which accumulates in macrophages, has provided insights into carotid plaque inflammation *in vivo*. In patients with symptomatic carotid atherosclerosis, ^{18}FDG uptake correlates with macrophage-rich areas of plaque, matrix metalloproteinase-9 (MMP-9) expression (Rudd et al. 2010; Tawakol et al. 2006; Graebe et al. 2009), distal microembolic signals and early recurrence of cerebrovascular events (Moustafa et al. 2010; Marnane et al. 2012).

Participants in these studies tended to be receiving secondary prevention with antiplatelets and statins at the time of assessment, although the influence of vascular risk factors on these findings is unclear. A further study reported attenuation of carotid plaque ^{18}FDG uptake in individuals without history of stroke randomised to 3months *de-novo* treatment with simvastatin, compared with placebo (Tahara et al. 2006).

It has been proposed for many years that acute systemic infections and chronic infectious agents may play a role in the development of ischemic stroke. Several observational clinical studies have reported associations between preceding common infections (mainly respiratory tract infections) and ischemic stroke (Grau et al. 2010). The reported prevalence of preceding infection, and timing prior to index stroke varies considerably between these studies, and one study found no difference in the prevalence of recent infection between cases and controls (Paganini-Hill et al. 2003). In the largest study, based on the UK General Practice Research Database, risk of first stroke was greatest in the 3 day period following confirmed respiratory tract or urinary-tract infection (Clayton et al. 2008). Platelet activation and platelet-monocyte aggregation was exaggerated in patients presenting with stroke and recent preceding infection, compared to those without or non-stroke controls (Zeller et al. 2005). The relationship between preceding infection, conventional vascular risk factors and incident stroke subtype is unclear. One study reported no association between stroke subtype and preceding infection (Roquer et al. 2012), whilst others suggest preceding infection occurred more often in atherothromboembolic or cardioembolic infarction (Grau et al. 2010; Paganini-Hill et al. 2003). However, preceding infection was associated with more severe incident strokes in multivariate analyses (Roquer et al. 2012), which is likely to influence conclusions relating to aetiological subtype. A recent case-crossover study revealed that hospitalisation with infection was associated with incident stroke, particularly within the 14 day period following admission (Elkind et al. 2011). AF is detected more frequently in hospitalised individuals with sepsis than without, and new-onset AF during sepsis is associated with in-hospital incident stroke (Walkey et al. 2011). Taken together, allowing for differences in study design and methodology, the effects of bias and confounding factors, these studies suggest that common systemic infections occur frequently within the month preceding ischemic stroke. Whilst it is presumed that infections may precipitate atherothromboembolism or

cardioembolism, our understanding of the potentially culpable organisms or mechanisms remains unclear.

Chronic infection has also been proposed to modulate the risk of ischemic stroke. Several micro-organisms, notably *Chlamydia pneumonia,* have received particular attention. *C.pneumoniae* serology is associated with incident ischemic stroke in some observational studies, but not others, but robust relationships with specific stroke subtypes are lacking (Grau et al. 2010). *C.pneumoniae* DNA has also been identified in carotid atheromatous plaque (Prager et al. 2002). It has been proposed that risk of stroke may be higher with exposure to multiple candidate infectious agents, the so-called concept of “infectious burden”. An infectious burden index based on seropositivity of five organisms (*C. pneumoniae, Helicobacter pylori*, herpes simplex virus 1 and 2 and cytomegalovirus), was associated with carotid plaque thickness on ultrasound and incident stroke, after adjustment for vascular risk factors (Elkind et al. 2010b; Elkind et al. 2010a). Seropositivity for any of the organisms in isolation was not associated with incident stroke.

Therapeutic trials of antibiotics to eradicate *C.pneumoniae* have been disappointing. One clinical trial evaluated the effect of roxithromycin on carotid intima-to-media thickness (IMT), a marker of atherosclerosis, in individuals with a previous transient ischemic attack or minor stroke (46% with positive *C.pneumoniae* serology) (Sander et al. 2004). Treatment with roxithromycin for 30 days reduced the progression of IMT in those with positive *C.pneumoniae* serology during the first 2 years, but not during the subsequent 2 year follow-up. The absence of an effect on recurrent cardiovascular events was perhaps not surprising given the limited power of the study. To date, the precise role of *C.pneumoniae* or other specific infectious agents in the pathophysiology of atherosclerosis and causation of stroke remains obscure.

Although some controversies exist regarding the predictive value of commonly used biomarkers and results from large clinical studies, there is a clear elevation in inflammatory mediators in all major risk factors for stroke. Atherosclerotic plaques produce various inflammatory mediators such as interleukin-1 (IL-1), or IL-6 and various immune cells, like T cells and macrophages. Elevated proinflammatory cytokines IL-1, IL-6, tumor necrosis factor-alpha (TNFα) and interferon gamma (IFNγ) as well as altered activation of macrophages, T

cells, natural killer (NK) cells and other immune cell populations are associated with hypertension, diabetes, infection or obesity, and can also be affected by acute or chronic stress (Table 1).

Inflammation and outcome following ischemic stroke

It is now well-established that cerebral ischemia induces an inflammatory response in the brain and its vasculature, activation of neuro-endocrine pathways and a peripheral systemic inflammatory response which influences clinical outcome**.** Age, sex and comorbidities influence outcome following stroke and are themselves important contributors to inflammatory responses to cerebral ischemia. Advancing age and stroke severity are powerful predictors of functional outcome and survival following ischemic stroke (Weimar et al. 2004; Andersen et al. 2011). Women have a higher case fatality than men, and women survivors have worse outcomes (Di Carlo et al. 2003; Appelros et al. 2009). Diabetes and AF are predictors of mortality (Andersen et al. 2011). Infections complicating the acute phase of stroke occur in up to 30% of patients, with pneumonia and urinary tract infections accounting for up to 10% each (Westendorp et al. 2011). Pneumonia increases the risk of death three-six fold (Katzan et al. 2003; Ovbiagele and Nguyen-Huynh 2011) and likelihood of worse functional outcome or length of stay in survivors (Ovbiagele and Nguyen-Huynh 2011; Kammersgaard et al. 2001; Aslanyan et al. 2004).

In clinical studies of ischemic stroke, elucidating the complexities of CNS inflammatory responses to acute cerebral ischemia poses particular methodological challenges. Available data from *in vivo* imaging techniques, sampling of cerebrospinal fluid (CSF) and post-mortem studies are therefore relatively scant, based on relatively small studies in relatively selected patients. Expression of pro-inflammatory cytokines (e.g. TNF-α) and adhesion molecules (e.g. ICAM-1) have been demonstrated in post-mortem human cerebral infarcts (Krupinski et al. 1994; Lindsberg et al. 1996; Sairanen et al. 2001). The pro-inflammatory cytokines IL-1β, IL-6 and TNF-α are also detected in cerebrospinal fluid obtained from patients with acute ischemic stroke (Tarkowski et al. 1995; Zaremba et al. 2001). MRI based methodologies have employed systemically administered ultrasmall superparamagnetic iron oxide (USPIO) contrast to label macrophages. USPIO-enhancement of ischemic lesions in patients with

acute ischemic stroke is heterogenous and seemingly unrelated to infarct volume or blood-brain barrier (BBB) disruption (Saleh et al. 2004; Nighoghossian et al. 2007). [^{11}C]PK11195 PET studies have demonstrated evolution of microglial activation within days of cortical/subcortical ischemic stroke, persisting for several months (Gerhard et al. 2000; Pappata et al. 2000; Radlinska et al. 2009)**.** Using single-photon emission tomography (SPECT), radiolabelled peripheral neutrophils accumulate in cerebral ischemic lesions within hours of onset, and the extent of this accumulation is associated with infarct volume (Akopov et al. 1996; Price et al. 2004). Neutrophil recruitment to the ischemic hemisphere was confirmed histologically at post-mortem in the parenchyma and intravascular compartments (Price et al. 2004).

As inflammation in the CNS is known to modulate inflammation in the periphery, measuring peripheral systemic inflammatory responses is a far more practical approach in clinical research. Numerous studies have investigated the time-course and prognostic value of the acute-phase response and other peripheral inflammatory markers in ischemic stroke. As would be expected, these studies have been in relatively older individuals with significant prevalence of comorbidities (prior stroke or TIA 30%; hypertension 45-70%; diabetes 8-41%; coronary artery disease 17.8-35%) and medications at baseline (e.g. statins 56%; antiplatelets 25%-38%) (Winbeck et al. 2002; Emsley et al. 2003; Ladenvall et al. 2006; Rallidis et al. 2009; Tuttolomondo et al. 2009; Whiteley et al. 2009). Cytokines expressed locally at sites of tissue inflammation, such as IL-1 and TNF-α are not consistently detected in plasma of patients with acute ischaemic stroke compared to controls (Tarkowski et al. 1995; Emsley et al. 2007), but plasma concentrations of other cytokines (e.g. interleukin-1 receptor antagonist (IL-1RA), IL-6), adhesion molecules (e.g. (ICAM-1, E/P-selectin) and chemokines (e.g. CXCL16) are elevated early after ischemic stroke and relate to clinical outcome (Rallidis et al. 2009; Tuttolomondo et al. 2009; Emsley et al. 2007; Vila et al. 1999; Ueland et al. 2012). The acute-phase response (APR), characterized by elevated plasma concentrations of CRP and IL-6, neutrophil leukocytosis and activation of the hypothalamic-pituitary-adrenal (HPA) axis, is induced within hours of ischemic stroke (Emsley et al. 2003). Increased serum adrenocorticotropic hormone (ACTH) and plasma or urinary free cortisol have been reported in patients after acute ischaemic stroke (Emsley et al. 2003; Olsson 1990; Fassbender et al.

1994). Cortisol is elevated within 4h of onset of stroke, with maximal values in the first 24h (Emsley et al. 2003; Fassbender et al. 1994), which is likely to reflect the magnitude of the rapid stress response to cerebral ischemia. Parameters of the APR, particularly plasma CRP, cortisol and IL-6 concentrations, correlate with stroke severity, infarct volume and worse outcome (Smith et al. 2004; Di Napoli et al. 2005; Di Napoli et al. 2001). Plasma CRP is elevated in all subtypes of ischemic stroke compared with controls, but concentrations are greatest in cardioembolic and large artery embolic stroke (Ladenvall et al. 2006) and lowest in lacunar infarcts. However, it is not clear whether these differences between subtypes are independent of stroke severity.

A key question is whether peripheral systemic inflammatory markers relate to outcome by directly contributing to evolution of infarct pathogenesis, or are simply markers of acute CNS/ peripheral inflammatory injury severity. In the acute phase of ischemic stroke, the modest correlation observed between CRP with stroke severity and infarct volume reflects its induction by upstream mediators, such as IL-1, TNFα and IL-6, in response to tissue injury. However, plasma CRP concentrations remain significantly elevated compared to controls even at 3 months after index stroke, particularly in large vessel disease aetiology (Emsley et al. 2003; Ladenvall et al. 2006), which may well reflect return of the APR to abnormal baseline levels indicative of ongoing vascular risk. Plasma CRP during the acute or subacute phase is associated with worse outcomes (beyond 6 months) and recurrent vascular events, independent of confounders such as age or baseline stroke severity (Whiteley et al. 2009; Di Napoli et al. 2005). CRP might therefore contribute to adverse outcome in the acute or subacute phase by directly mediating deleterious proinflammatory/thrombotic effects (e.g. activating the classical complement pathway, inducing expression of tissue factor (TF), von-Willebrand Factor (vWF), E-selectin, ICAM-1, MMP-9) (Bisoendial et al. 2005; Bisoendial et al. 2009).

The vast majority of clinical studies evaluating systemic inflammatory markers in stroke excluded patients with pre-existing infectious or inflammatory conditions, or infections complicating the acute phase, in order to limit factors confounding interpretation of the inflammatory response to stroke. However, the impact of infections should not be underestimated, and requires consideration in translational research. Several clinical factors,

particularly stroke severity, age and dysphagia, are consistently associated with infections in stroke patients. As would be expected, the peripheral systemic inflammatory response, evidenced by plasma CRP concentrations and peripheral white-blood counts, is exaggerated in patients with infection, compared to those without (Emsley et al. 2003; Fassbender et al. 1997). In contrast to the elevated plasma concentrations of inflammatory markers in acute ischemic stroke, recent studies have demonstrated suppressed peripheral blood cellular immune responses. Induction of the cytokines IL-1, TNF-α, IL-6, IL-10 and IL-8 by whole blood cultures stimulated with lipopolysaccharide (LPS) *in vitro* was significantly lower in patients with ischemic stroke than in controls (Emsley et al. 2007; Smith et al. 2012). This suppression of cytokine induction was evident in cultures obtained within hours of stroke onset, and persisted at day 5 to 7. The magnitude of impaired cytokine induction correlated with stroke severity and with plasma cortisol concentrations, and was less pronounced in patients without infections in the acute phase (Emsley et al. 2007). Several other studies have also reported suppression of *in vitro* TNF induction and monocyte HLA-DR expression in acute ischemic stroke (Haeusler et al. 2008; Hug et al. 2009; Urra et al. 2009). Induction of TNF, monocyte HLA-DR expression, plasma normetanephrine and plasma cortisol concentrations in the acute phase were associated with stroke-associated infections independent of stroke severity and age (Urra et al. 2009). However, in another study, induction of TNF and monocytic HLA-DR expression were associated with incident infections, but these relationships were not independent of infarct volume (Hug et al. 2009). The clinical significance of these observations remains unclear. It has been proposed that stroke-associated immune-suppression represents enhanced susceptibility to infection in the setting of infectious challenge, although a causal relationship is yet to be established in clinical studies.

Modeling risk factors and inflammatory mechanisms in stroke

The role of the immune system in stroke, especially in cerebral ischaemia has been investigated in detail in animals (mostly rodents with middle cerebral artery occlusion (MCAo)) without apparent comorbidities. Various anti-inflammatory interventions have been shown to grant protection against brain injury, if administered within 3-6 h post-stroke. Centrally or peripherally expressed proinflammatory cytokines/chemokines such as IL-1, TNFα or

RANTES (CCL5), proteases such as MMP-9, toll-like receptors such as TLR4 or TLR2, various immune cell populations such as T cells, macrophages, platelets or neutrophils have all been implicated in the development of ischemic brain injury, similarly to experimental models of ischaemic injury in peripheral tissues, such as myocardial infarction, kidney- or liver ischemia (Denes et al. 2010b; Denes et al. 2011b; McColl et al. 2009; Klingenberg and Hansson 2009; Dinarello 2011; Zhai et al. 2011). Although our understanding of how basic inflammatory mechanisms contribute to ischemic injury in the absence of comorbidities is far from complete, several recent review articles have addressed this issue in detail (Denes et al. 2010b; Iadecola and Anrather 2011; Chamorro et al. 2012). Instead, we present some recent pre-clinical advances in translational stroke research below, highlighting the role of inflammation in some common stroke comorbidities and showing that blockade of key immune mechanisms could be therapeutically useful in experimental models of systemic inflammation and stroke.

Inflammatory mechanisms in major comorbidities in stroke

Aging, atherosclerosis, diabetes and hypertension

In experimental models, aging appears to impair outcome after experimental stroke, however, not all studies have come to the same result. Moreover, clear gender-differences are seen: estrogen (17*β*-estradiol) has a protective role in young females, but exacerbates brain injury in aged female rodents, possibly via interactions with insulin-like growth factor (IGF)-1 (Liu et al. 2009; Selvamani and Sohrabji 2010a, b; Dinapoli et al. 2010; DiNapoli et al. 2008; Wang et al. 2003; Dubal and Wise 2001). Male, aged rodents often show decreased gray matter injury after experimental stroke compared to young animals, however, an increase in white matter lesions has been demonstrated (Shapira et al. 2002; Correa et al. 2011). Aging in combination with other risk factors such as obesity or atherosclerosis was found to augment inflammation and BBB injury in the brain (Pradillo et al. 2012). Aged animals express altered microglial activation, enhanced expression of proinflammatory cytokines such as IL-6, TNFα and CCL2 (Dinapoli et al. 2010), although other studies reported reduced serum IL-6 levels in response to cerebral ischemia (Liu et al. 2009). The use of different rodent models (different rat or mouse strains, embolic versus filament MCAo, animals from different age groups, etc)

makes it currently difficult to compare existing studies and conclusively evaluate the impact of aging and the involvement of inflammatory processes in outcome after cerebral ischaemia.

Diabetes impairs outcome in rodent models of focal cerebral ischemia (Bomont and MacKenzie 1995). Interestingly, brain injury and inflammation in diabetic models can be reversed by angiotensin II type 1 receptor (AT(1)-R) antagonist, PPARγ agonist, administration of 17*β*-estradiol, IGF-1, aspirin and niacin (B3 vitamin) even at high blood glucose levels, highlighting the potential role of inflammatory mechanism in diabetes-induced exacerbation of brain injury (Kusaka et al. 2004; Toung et al. 2000; Tureyen et al. 2007; Rizk et al. 2007; Wang et al. 2009; Ye et al. 2011).

Brain injury and neurological outcome are exacerbated after cerebral ischemia in rodent models of hypertension, which can mostly (although not uniformly (Porritt et al. 2010)) be reversed by blood pressure lowering drugs (Elewa et al. 2007; Kozak et al. 2008). Nevertheless, administration of statins, ibuprofen, epidermal growth factor (EGF), or inhibition of TNFα can mediate protection against ischemic brain injury in hypertensive animals, indicating that some detrimental effects of prolonged hypertension might be explained by inflammatory mechanisms (Cole et al. 1993; Dawson et al. 1996; Hatashita et al. 1990; Yu et al. 2009; Mariucci et al. 2011).

In addition to plaque formation and focal injuries in the vasculature, atherosclerosis is now recognised as a chronic inflammatory condition. Compared to the number of studies concerning mechanisms of thrombus formation – a major contributor to cerebral ischemic events – relatively few data are available on mechanisms of brain injury in atherosclerotic animals after experimental stroke. Hyperlipidaemic ApoE-deficient mice display increased brain injury following cerebral ischemia (Laskowitz et al. 1997). When fed a high fat diet, ApoE KO mice show increased BBB injury and elevated MMP-9 expression (ElAli et al. 2011). However, ApoE has anti-inflammatory actions in the brain and therefore studies using other models of atherosclerosis are also needed to clarify the role of inflammation in brain injury mediated by atherosclerosis. Recent data show that brain inflammation is evident in high-fat fed, atherosclerotic ApoE KO mice, and in obese, atherosclerotic corpulent rats even in the absence of any acute cerebrovascular event. These inflammatory changes appear to be IL-1-dependent in mice (Denes et al. 2012; Drake et al. 2011). Neuroinflammatory changes are

also seen in patients at risk of stroke, who present with multiple risk factors for stroke and chronically elevated serum CRP, but no known neurological disease (Drake et al. 2011).

Altered inflammatory parameters and increased expression of proinflammatory mediators in animal models of major risk factors for stroke show good correlation with some of the markers identified in the corresponding human disease (Table 1).

Obesity/Metabolic syndrome

Metabolic syndrome increases the risk for stroke but also other diseases including coronary artery disease and type 2 diabetes. One of the key features of metabolic syndrome is central obesity. Although being obese can result in several conditions that are associated with increased stroke risk including hypertension, diabetes and hypercholesterolaemia, obesity has now been identified as an independent risk factor for stroke (Strazzullo et al. 2010). Furthermore, being obese may lead to a worse outcome after a stroke (e.g. (Chen et al. 2012; Towfighi and Ovbiagele 2009; Whitlock et al. 2009; Zhou et al. 2008; Oki et al. 2006)). However, the reports on how obesity affects stroke in humans are contradictory, as not all studies report a relationship between obesity and increased mortality/morbidity after stroke and some studies suggest that being obese actually leads to a better outcome (Vemmos et al. 2011; Ryu et al. 2011; Olsen et al. 2008; Towfighi and Ovbiagele 2009). The reasons for this 'obesity paradox' are not understood fully but are likely to be due to a complex relationship between how obesity is measured, age, type and severity of stroke and concurrent medication (Katsnelson and Rundek 2011; Towfighi and Ovbiagele 2009). Studies have also demonstrated that obesity also leads to worse outcome after experimental stroke. The extent of ischemic damage, the incidence hemorrhagic transformation (HT) and the permeability of the BBB are exacerbated in obese rodents (McColl et al. 2010b; Terao et al. 2008; Langdon et al. 2011; Kumari et al. 2011; Park et al. 2011; Nagai et al. 2007). White matter damage has also been reported to be enhanced after experimental stroke in obese mice (Chen et al. 2011). This detrimental effect of obesity has been observed in various models of cerebral ischemia, in different species (rats, mice and gerbils) and in several models of obesity including rodents that are either genetically-obese (e.g. *ob/ob* and *db/db* mice) or made obese through a high-fat diet (DIO).

Obesity is now considered an ‘inflammatory condition’ that is associated with elevated peripheral and vascular pro-inflammatory profiles (Scarpellini and Tack 2012; Sun et al. 2012) and central inflammatory changes including gliosis and activation of the cerebrovasculature in the brains of obese and high-fat fed rodents (Pistell et al. 2010; Drake et al. 2011; Thaler et al. 2012). It is generally considered that peripheral inflammation is a consequence of obesity, however, recent data suggests that central inflammation actually precedes weight gain in high-fat fed rodents (Thaler et al. 2012). Obesity-related inflammation has been shown to be responsible for some of the secondary consequences of the condition, especially insulin resistance (Dali-Youcef et al. 2012). As inflammation can strongly influence outcome following cerebral ischemia, it is possible that dysregulated inflammatory mechanisms in obesity also contribute to the poorer outcome after stroke.

In support, the central inflammatory response to stroke in obese animals appears to be compromised, and depending on the time post-reperfusion, the expression of several cytokines and activation of resident cells such as microglial is either enhanced or diminished (Zhang et al. 2004; Kumari et al. 2007; Kumari et al. 2010). Furthermore, obese mice have an exaggerated cerebrovascular inflammatory response after stroke, characterised by an increase in vascular activation and, leukocyte and platelet adhesion (Tureyen et al. 2011; Terao et al. 2008). Leukocyte adhesion to brain endothelial cells triggers endothelial signalling cascades that cause cytoskeletal alterations and tight junction disorganisation (Afonso et al. 2007). Tight junction expression (e.g. occludin) is reduced during cerebral ischaemia, an effect that is exacerbated in obese mice (Kumari et al. 2011) and could therefore contribute to the enhanced BBB breakdown after stroke observed in obesity. Cerebrovascular inflammation is associated with increased proteolytic activity, for example via the release of proteases from adherent leukocytes (Gidday et al. 2005). Substrates for these proteases include the vascular basement membrane matrix molecules (e.g. collagen-IV, laminin) and endothelial tight junction proteins (Jian and Rosenberg 2005). Endothelial cells derive mechanical and trophic support from matrix constituents via integrin receptors, and their disruption is an important contributor to BBB dysfunction (del Zoppo and Milner 2006). In particular, MMPs, especially MMP-9, have been strongly implicated in ischaemic brain pathophysiology (Zhao et al. 2007), as they contribute to the disruption of the BBB and development of HT after stroke in animals

(Romanic et al. 1998; Rosenberg et al. 1998; Heo et al. 1999; Asahi et al. 2000) and humans (Horstmann et al. 2003; Rosell et al. 2006), via degradation of vascular matrix components. MMP expression is increased in immune cells and plasma of obese humans (Ghanim et al. 2004; Laimer et al. 2005; Glowinska-Olszewska and Urban 2007; Derosa et al. 2008a), and MMP-9 immunoreactivity is markedly increased in the brain microvasculature of ischemic tissue in obese mice (McColl et al. 2010b; Kumari et al. 2011; Chen et al. 2011). MMPs are expressed by several cell types, such as endothelial cells and neutrophils, which are the principal sources of MMP-9 after experimental and human stroke (Gidday et al. 2005). Neutrophil infiltration into the brain after experimental stroke is also increased in obese mice and rats although it is not yet known if these cells contribute to the increase in MMP-9 observed in the obese ischaemic brain. Overall, these data suggest an aggravated acute inflammatory response to stroke in obesity and a potential link between obesity and increased brain microvascular disruption. Similarly to obesity, other comorbidities are also associated with altered microvascular responses in the brain before and after stroke (Figure 1).

Infection

As outlined above, acute viral or bacterial infection in patients, mainly of the respiratory tract origin, in the week preceding stroke is associated with elevated risk of cerebral ischemia, independently of other risk factors. Preceding infection is (although not uniformly) also associated with worse clinical outcome (Emsley and Hopkins 2008a; Grau et al. 1995; Macko et al. 1996). In mice, administration of LPS prior to MCAo results in worse neurological outcome, cerebral edema and increased BBB injury (McColl et al. 2008, 2007; Denes et al. 2011a). After endotoxin treatment, blood-derived leukocytes, primarily neutrophils have been shown to contribute to BBB injury via MMP-9 production that results in altered expression of endothelial tight junction proteins (McColl et al. 2008). Endotoxin administration also results in rapid systemic upregulation of pro-inflammatory cytokines and chemokines, such as IL-1β, TNFα, IL-6, CXCL1 (KC), RANTES (CCL5) and MCP-1 that is apparent in both the circulation and the brain prior to cerebral ischemia (Denes et al. 2011a). Thus, systemic inflammatory stimulus induced by bacterial cell wall products not only leads to activation and mobilisation of various leukocyte subsets, but also primes inflammatory responses in the brain.

Proinflammatory cytokines, primarily IL-1, seem to be critical drivers of systemic inflammatory responses in cerebral ischaemia: intraperitoneal administration of IL-1β exacerbates brain injury after stroke, whereas blockade of IL-1 signalling with IL-1Ra results in protection against endotoxin-induced increase in infarct size (McColl et al. 2007).

Although LPS can be used to mimic infection by gram-negative bacteria that activate toll-like receptor-4 (TLR-4), experimental studies of infection preceding stroke have been lacking until recently. One major difficulty is to investigate how infection-induced inflammatory responses interact with mechanisms of cerebral ischemia, without affecting the course of infection and being disturbed by effects caused by the infectious organism itself. We have developed a model of gut infection prior to experimental stroke, using a parasitic nematode, *Trichuris muris* (*T. muris*), which enabled polarisation of the anti-parasitic immune response towards a chronic T helper-1 (Th1) or a Th2 direction, making it possible to dissect parasite-specific effects from infection-induced inflammatory responses in cerebral ischemia. Chronic Th1-type response results in a marked exacerbation of brain injury after MCAo, which is associated with increased platelet aggregation, leukocyte recruitment and vascular injury in the brain and systemic up-regulation of a pro-inflammatory chemokine, RANTES (CCL5). Neutralisation of RANTES reversed infection-induced increase in brain injury and BBB damage without altering parasite load, indicating that inflammation in response to infection was the major contributor to brain injury. Supporting this, Th2-type polarisation of the immune response by the same parasite had no effect on stroke outcome (Denes et al. 2010a). RANTES has also been found to be increased after influenza virus infection in the circulation and the brain. Preceding influenza infection resulted in increased brain injury and BBB breakdown in a mouse permanent MCAo model (Muhammad et al. 2011), whilst MMP-9 was found to be up-regulated in recruited leukocytes and microvessels in the brain both in response to both influenza and chronic *T. muris* infection (Muhammad et al. 2011; Denes et al. 2010a). Influenza-induced brain injury after MCAo was effectively reversed by administration of GTS-21, an alpha7 nicotinic acetylcholine receptor agonists, highlighting the role of inflammatory mediators in the pathogenesis of ischemia with preceding infection (Muhammad et al. 2011). Thus, immune mechanisms induced by infection in various models appear to be the main mediators of brain injury, indicating the potential benefits of targeted anti-inflammatory

interventions in stroke. Paradoxically, brain injury itself (seen after cerebral ischemia, subarachnoid hemorrhage or brain trauma), results in long-lasting immunosuppression that is apparent in both patients and various experimental models, leading to infectious complications that compromise survival and impair recovery (Dirnagl et al. 2007; Emsley and Hopkins 2008a). As discussed above, stroke patients often present with immune cells in the blood that show a suppressed response to inflammatory stimuli (such as bacterial endotoxin) *in vitro*. It is currently unclear whether post-stroke immunosuppression exerts any beneficial effects via dampening harmful inflammatory mechanisms after acute cerebrovascular events. Nevertheless, the negative impact of infectious complications on outcome in stroke patients highlights the key role of a functional immune system in limiting the spread of opportunistic pathogens even if some of these essential inflammatory mechanisms could be causing additional injury in the CNS. Thus, deeper understanding of infection-induced inflammatory mechanisms after stroke could be crucial for targeted therapies in order to limit the side effects of any anti-inflammatory interventions. Nevertheless, animal models of infection and systemic inflammation suggest a functional role for inflammation in impaired outcome after stroke, which appears to be similar to other models of comorbidities like hypertension, diabetes, obesity or stress (Table 2).

Stress

Life time stress is generally held as a major risk- and complicating factor in various neurological diseases including stroke (Herman 2012). Exposure to acute or chronic stressors increases the vulnerability to the disease and/or exacerbates stroke damage (Surtees et al. 2008; Hankey 2006). Exposure to various stressors results in several physiological and pathophysiological changes in the periphery as well as in the CNS that may contribute to the damage. Stress-induced autonomic arousal contributes to various cardiovascular- (high blood pressure, increased heart rate) and metabolic alterations that are acknowledged independent risk factors for stroke. It has been shown recently that stress augments insulin resistance and evokes inflammation to increase coagulation factors through adipose tissue derived MCP-1 (Uchida et al. 2012).

There might be several potential cellular mechanisms through which stress pathophysiology underlie stress-stroke interaction ranging from impairment of neurogenesis

(McEwen 2000; Banasr and Duman 2007) to exacerbation of neuroinflammation (Munhoz et al. 2008) and activation of HPA axis in response to proinflammatory mediators (Ericsson et al. 1994; Kovacs and Elenkov 1995).

Similarly to LPS, cellular stressors (such as UV, osmotic, hypoxia and hypoglycemia) also converge onto MyD88/NFkB/MAPK pathways to provide the means how cellular insults aggravate neuroinflammation (Munhoz et al. 2010). However, it remains unknown how systemic challenges are translated to cellular stress effects.

Stress results in innate immune "arousal" in the brain (Fleshner et al. 2002; Fleshner 2012) by priming and activating microglia and astrocytes (Sugama et al. 2007). Stress-induced arousal of glia may result in increased synthesis and release of cytokines such as IL-1α and IL-1β, TNFα and IL-6 along with up-regulation of iNOS and COX2. At the cellular level, it is very likely that systemic stress provokes "sterile inflammation" via expression of danger-associated molecular patterns (DAMPs) especially alarmins that potentiate neuroinflammatory changes that occur in the neurovascular unit during stroke. It is becoming increasingly evident that stress- level glucocorticoids are proinflammatory in the cortex and corticosterone potentiates LPS-induced proinflammatory cytokines, IL-1β and TNFα via NFkB activation (Munhoz et al. 2010).

Chronic stress also results in endothelial dysfunction, impaired endothelium dependent vasodilatation, increased superoxide production and reduced brain endothelial NOS levels. These changes are likely to contribute to stroke vulnerability mediated by stress-induced corticosteroids, since glucocorticoid receptor antagonist RU38486 (mifepristone) completely reversed these deleterious endothelial effects (Balkaya et al. 2011).

Subchronic stress results in "leaky gut" and bacterial LPS of invading bacteria triggers the TLR-4 pathway, activates NFkB and releases proinflammatory cytokines (IL-6) and proinflammatory mediators (COX2, iNOS) (Garate et al. 2012), thus contributing to the stress-induced immune priming. Indeed, stress-induced colonic bacterial transfer has been implicated as one of the possible mechanisms through which stress worsens stroke outcome (Caso et al. 2009).

IL-1β is highly expressed within the brain in response to stress and/or injury. IL-1 acts on glia via the NFκB pathway to exacerbate local inflammatory responses and affects

hypothalamic and cortical neurons by regulating CREB activity via p38 MAPK signalling, thus coordinating adaptive neuronal responses to stress and injury (Srinivasan et al. 2004). In parallel, social stress suppresses cell protective and pro-survival mechanisms, such as Bcl-2 expression (DeVries et al. 2001) and exacerbates stroke.

Cortisol in humans, and corticosterone in rodents, are major glucocorticoid hormones released by the adrenals during systemic stress. Synthetic glucocorticoids are widely used in clinical neurology to control CNS inflammation, yet there is no evidence that exogenous corticosteroids are beneficial, and may even be harmful, in acute stroke patients (Sandercock and Soane 2011). Although corticosteroids were long held as anti-inflammatory, recent evidence (Dinkel et al. 2003) suggest that the context of glucocorticoid exposure is important in determining how corticosteroids will affect inflammation and, consequently, stroke injury. Whether glucocorticoid hormones increase or decrease CNS inflammation depends on the dose, timing and duration of the steroid exposure as well as on the type of glucocorticoids administered. The immunosuppressive effect of glucocorticoids originates from the observed leukopenia following application of synthetic hormone (dexamethasone) in pharmacological doses. In contrast, substantial evidence indicates that in an acute alarm situation, stress-induced glucocorticoids redirect leukocytes from the systemic circulation to the place of the injury and thus play a dramatic role in leukocyte trafficking that may also have an important impact in stroke-damage. While basal level of glucocorticoids are required for the immune activation (permissive effect), stress-induced corticosteroid elevation stimulates cellular immune response (acute effect), brief glucocorticoid exposure enhances immune responses to subsequent inflammatory insults (priming effect) and chronic exposure to very high steroid doses are immunosuppressive (Sorrells and Sapolsky 2007; Sorrells et al. 2009). Furthermore, microglia express glucocorticoid receptors, which provide direct means through which stress-induced corticosteroids increase microglia proliferation and induce their inflammatory phenotype (Nair and Bonneau 2006).

Finally, proinflammatory cytokines that are released in the periphery in stroke subjects, like other stressors, are able to activate the final arm of the HPA axis to start a reverberating cycle to even further worsen the outcome of the injury. Furthermore, post-stroke stress-

arousal of the sympathetic nervous system induces depression of the cell mediated immunity that may increase vulnerability to post-stroke infectious complications (Selmeczy et al. 2008).

IL-Ra as a candidate treatment for acute cerebral ischemia: experimental and clinical evidence

Only a few inflammatory mechanisms that have proved to contribute to outcome in experimental models of comorbidities in stroke have been tested in humans. As outlined above, IL-1 has been implicated in the pathogenesis of several risk factors for stroke, and is a major driver of ischemic brain injury (Allan et al. 2005). IL-Ra is a naturally expressed protein and a reversible, competitive inhibitor of IL-1 actions with high selectivity. During the last fifteen years, IL-1Ra has been tested experimentally in rodents using different models of cerebral ischemia, different routes of administration (peripherally or centrally) and at different time points: before or at the time of stroke, and at the beginning or at 3h of reperfusion (Garcia et al. 1995; Stroemer and Rothwell 1997; Mulcahy et al. 2003). IL-1Ra has been shown to be neuroprotective by reducing the number of necrotic neurons, infarct volume and improving overall outcome. Endogenous IL-1Ra is upregulated after cerebral ischemia, but it is likely to be insufficient to fully prevent IL-1 actions (Pinteaux et al. 2006; Denes et al. 2008). Subcutaneous administration of a single dose of IL-1Ra reduced brain injury and resulted in sustained high concentrations in the plasma and the CSF, penetrated into areas of BBB disruption and co-localized with viable neurons (Greenhalgh et al. 2010). In accordance with the STAIR guidelines it is imperative to test candidate drugs in aged and co-morbid animals, to use clinically relevant administration routes and techniques to facilitate the translation from '*bench to bedside*'. With this in mind, the effect of subcutaneous administration of IL-1Ra has been tested at clinically relevant time points after experimental stroke to aged and co-morbid animals. Corpulent rats that are homozygous for the autosomal recessive cp gene (cp/cp) and spontaneously develop obesity, hyperlipidemia, insulin resistance, glomerular sclerosis, and atherosclerosis with enhanced vascular contractility and reduced vascular relaxation have been used for this study (Mangat et al. 2007). Peripheral administration of IL-1Ra at the time of reperfusion or with 3h delay to aged lean and Cp rats, reduces ischemic brain injury, BBB breakdown, microglial activation, neutrophil infiltration and expression of MMP-9 and IL-6,

which provides further supporting evidence for IL-1Ra as a lead candidate for the treatment of ischemic stroke (Pradillo et al. 2012).

In a phase II randomised, placebo-controlled trial of intravenous IL-1Ra in patients presenting within 6h of onset of stroke symptoms, concentrations of plasma IL-6, CRP and neutrophil leukocytosis were markedly reduced during the first 5-7d in patients receiving IL-1Ra (Emsley et al. 2005a). No serious adverse events were attributed to test treatment and more patients randomised to IL-1Ra achieved a favourable outcome at 3 months in exploratory analyses. These data suggest IL-Ra is safe, well-tolerated and reduces parameters of peripheral inflammation which are known to correlate with stroke severity, infarct volume and poor outcome, by blocking induction by IL-1-mediated pathways. In secondary analyses, the effects of IL-Ra on *ex vivo* blood cytokine induction were evaluated (Smith et al. 2012). Induction of TNF-α, IL-1β, IL-6, IL-8 and IL-10 by LPS was significantly reduced in patients at admission, prior to receiving IL-1Ra or placebo, compared to controls without stroke. At 24h and day 5-7, cytokine induction remained suppressed in the placebo group, whilst in those treated with IL-1Ra induction of TNF-α and IL-1 was significantly greater. Plasma cortisol concentrations, elevated at admission in patients compared to controls, were substantially reduced at 24h in the patients receiving IL-1Ra and inversely correlated with either TNF-α or IL-1β induction at admission. The mechanisms underlying this apparent reversal of peripheral innate immune suppression by IL-1Ra remain unclear. It is well recognized that corticosteroids exert potent suppressive effects on monocyte cytokine induction. One plausible explanation is therefore that IL-1Ra reverses innate immune suppression by blocking IL-1-induced pathways involved in augmenting HPA axis activity. The clinical relevance of these observations on incident infections and clinical outcome in patients is also unresolved. It is possible that reversal of peripheral immune suppression by IL-Ra may reduce susceptibility to infection in the acute phase of stroke, which might therefore improve clinical outcomes. A similar number of infections occurred in the IL-Ra and placebo groups during the acute phase, but interpretation is limited by the small study size. Large-scale studies are required to determine the effects of treatment with IL-1Ra on incident infection and clinical outcome, particularly when considering the influence of other confounding factors.

Conclusions

Inflammatory mechanisms contribute to all major comorbidities in ischemic stroke, and inflammation is a key driver of ischemic brain injury and repair even in the absence of comorbidities. Modulating immune and inflammatory pathways therefore offers considerable potential for stroke prevention and improving outcome following stroke. However, many complex factors need to be considered in successfully developing such therapies from bench to bedside. A fundamental challenge to address is the balance between pro- and anti-inflammatory pathways in determining risk and outcome, in the clinical setting of advanced age, comorbidities (including infections) and medications already used in standard care. Therapies targeting immune/ inflammatory pathways have several potential advantages. Blocking deleterious pro-inflammatory pathways might enable more prolonged time-windows for administration in the acute phase of ischemic stroke, and augment beneficial effects of thrombolysis by protecting reperfused penumbra and reducing reperfusion injury/haemorrhagic transformation. As inflammation is also implicated in haemorrhagic stroke, anti-inflammatory agents may offer novel therapeutic opportunities in intracerebral and subarachnoid haemorrhage. By contrast, prolonged blockade of pro-inflammatory pathways may also have detrimental effects on outcome in different stroke subtypes. A key consideration is the effect this may have on the transition to potentially beneficial anti-inflammatory or reparative pathways. Previous clinical trials of immune-modulating therapies have already provided cautionary tales. In the Enlimomab Acute Stroke Trial, a randomised controlled trial of a murine monoclonal antibody to ICAM-1 (Enlimomab investigators, 2001), the enlimomab-treated patients had significantly worse outcomes, and a higher incidence of fever, infections and death. Whilst this has been attributed mainly to adverse immunoactivation precipitated by administration of murine antibody, this highlights the importance of considering the impact of blocking innate responses that are also crucial in host defence to infection.

Using appropriate animal models of stroke, which attempt to incorporate the complexities of the clinical setting, is an essential component of drug development acknowledged by international consensus (Fisher et al. 2009).To facilitate translation, animal models will need to account for central and systemic effects of anaesthesia and surgical stress. Experimental

settings incorporating comorbidities (aging, infection, atherosclerosis, etc) and delayed assessment of outcome with particular impact on recovery/regeneration will likely yield clinically relevant data. Even so, it is not possible to consider all aspects of the clinical setting in pre-clinical modelling (e.g. exposure of patients to a wide array of medicines, differences in life span and main physiological parameters compared with rodents). Therefore, it is vital that experimental models are continuously monitored for their translational value and their limitations are carefully considered.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure and table legends

Table 1 Comorbidities in stroke: prevalence, relative risk of stroke and inflammatory status in patients and corresponding animal models. Levels of cytokines, chemokines and amount of inflammatory cells are generally elevated in major comorbidities for stroke. Altered inflammatory cell activation is observed in all comorbidities except for stress and age, in which inflammatory cells appear to display reduced activity, although can show excessive activation in response to inflammatory or other challenges. See further explanation in the text. RR-relative risk, [a]Dependent on time of pre-stroke infection. [b]Over 45 years. [c]After the age of 55.

Table 2 Experimental models of major risk factors and their impact on stroke outcome. Note that only inflammatory mediators, or mediators that exert inflammatory/anti-inflammatory properties are shown where appropriate and only mechanisms that have been functionally linked to stroke outcome have been included. [a]Infarct size is reduced in young females due to E2. [b]E2 increases infarct size if administered to aged female animals. α7nAchR - α7 nicotinic acetylcholine receptor, BBB – blood brain barrier, E2 - 17*β*-estradiol, EGF - epidermal growth factor, HMG-CoA - 3-hydroxy-3-methyl-glutaryl-coenzyme-A, HT – haemorrhagic transformation, IGF-1 - with insulin-like growth factor -1, MCP-1 - monocyte chemoattractant protein-1, PAI-1 - plasminogen activator inhibitor-1, PAMP – pathogen-associated molecular pattern, tPA - tissue plasminogen activator. ↑ - increase, ↓-decrease, Ø – no change

Fig. 1 Vascular effects of systemic inflammation before and after stroke. Elevated systemic inflammatory burden in major comorbidities for stroke can exert several effects in the CNS, many of which manifest in the vasculature. Altered leukocyte-platelet-endothelial interactions, expression of adhesion molecules, inflammatory cytokines, proteases, endothelial deposition of lipids, inflammatory chemokines and other processes prior to stroke (orange) lead to altered microvascular and glial milieu in the brain, which is likely to impact on the occurrence and progression of acute cerebrovascular events. After stroke (black), primed inflammatory

cells, altered kinetics of cytokine expression, altered procoagulant-anticoagulant balance, leukocyte recruitment, etc., contribute to BBB injury, glial activation, haemorrhagic complications, which eventually exacerbate brain injury and compromise repair mechanisms. See more detailed explanation and mechanisms in the text.

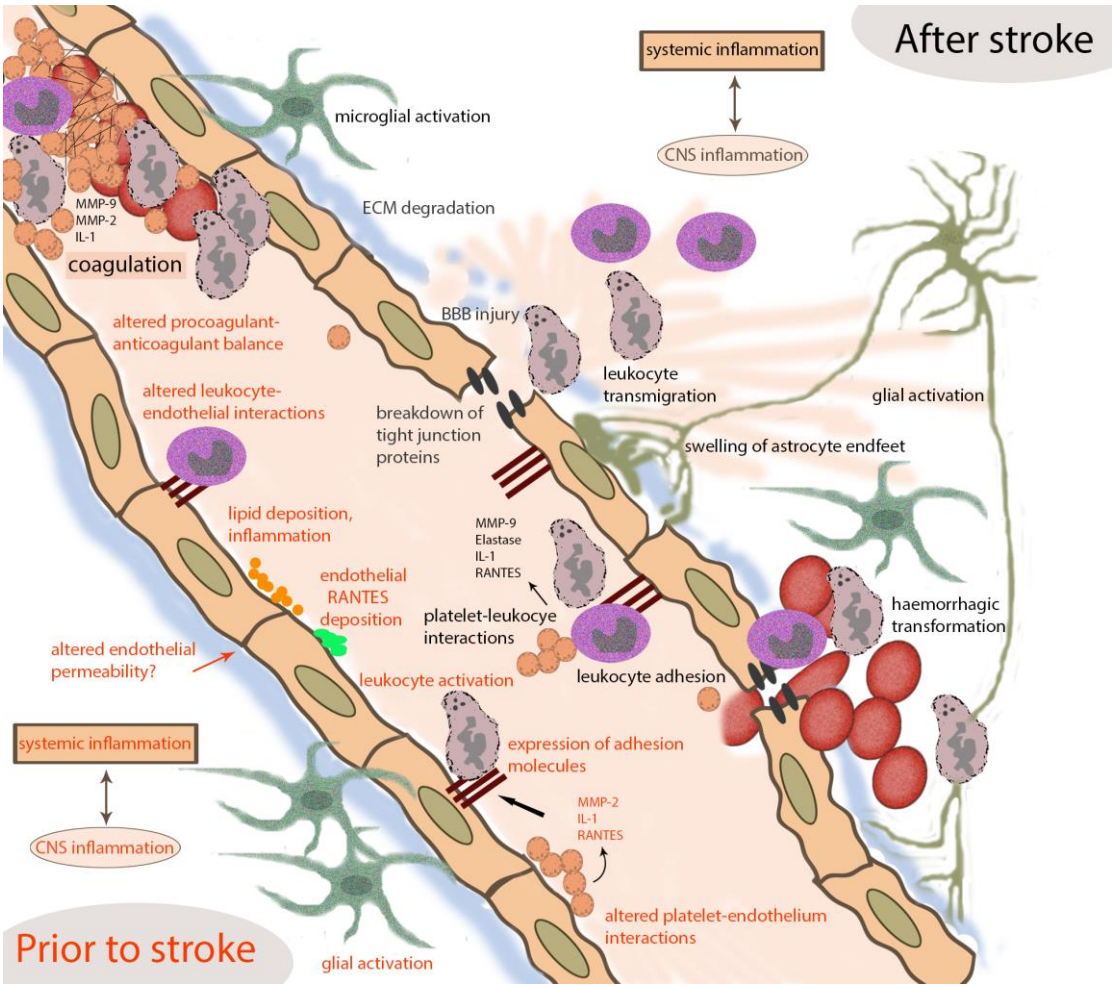
Table 1.

HUMAN					ANIMAL MODELS		
Prev. in stroke	**RR**	**Infl. markers**	**Infl. cells**	**Refs.**	**Infl. markers**	**Infl. cells**	**Refs.**
66%	>4	↑ IL-1 ↑ IL-6 ↑ sICAM ↑ TF	↑ Macrophages ↑ T cells (in atherosclerotic plaques)	(O'Donnell et al. 2010; Bousser 2012; Dalekos et al. 1997; Chae et al. 2001; Celi et al. 2010; Moreno et al. 1994)	↑ IL-1 ↑ IL-6 ↑ TNFα ↑ TF	↑ Endothelial activation ↑ Macrophages ↑ Leukocytes ↑ T cells	(Sanz-Rosa et al. 2005; Celi et al. 2010; Liu et al. 1996; Marvar et al. 2010; Schmid-Schonbein et al. 1991)
21%	2-4	↑ IL-1 ↑ IL-6 ↑ TNFα	↑ Macrophages ↑ T cells	(Moreno et al. 2000; O'Donnell et al. 2010; Bousser 2012; Purushothaman et al. 2012; Yaochite et al. 2012; Bradshaw et al. 2009)	↑ IL-1 ↑ IL-6 ↑ TNFα	↑ Endothelial activation ↑ Macrophages ↑ T-cells	(Gustavsson et al. 2010; Yang and Santamaria 2006; Calderon et al. 2006)
42%	2-3	↑ IL-1 ↑ IL-6 ↑ TNF	↑ Macrophages ↑ T cells (in atherosclerotic plaques)	(Galea et al. 1996; Barath et al. 1990; Schieffer et al. 2000; Hansson and Libby 2006; Dziewas et al. 2007; Sander et al. 2011; Gui et al. 2012)	↑ IL-1 ↑ IL-6 ↑ TNFα ↑ IL-4 ↑ IFNγ	↑ Endothelial activation ↑ Leukocytes ↑ T cells ↑ Microglial activation	(Kleemann et al. 2008; Nakashima et al. 1998; Zhou et al. 2000; Dong and Wagner 1998; Drake et al. 2011b)
43%	<2	↑ IL-1 ↑ IL-6 ↑ TNFα ↑ MMPs	↑ Macrophages ↑ T cells ↑ NK cells	(Duffaut et al. 2009; Derosa et al. 2008b; O'Donnell et al. 2010; Bousser 2012; Li et al. 2008; Stelzer et al. 2012; Weisberg et al. 2003; Ohmura et al. 2010)	↑ IL-1 ↑ IL-6 ↑ TNFα	↑ Macrophages ↑ Astrocytic activation	(Kim et al. 2012; Pan et al. 2012)
20-40%[a]	<2	↑ IL-1 ↑ IL-6 ↑ TNFα ↑ IFNγ	↑ Monocytes ↑ NK cells	(Bousser 2012; Fieren 2012; Andaluz-Ojeda et al. 2011; Leon et al. 2007; Emsley and Hopkins 2008b)	↑ IL-1 ↑ IL-6 ↑ TNFα	↑ Endothelial activation ↑ Neutrophils	(Lewis et al. 2012)
20%	2-4	↑ IL-1 ↑ TNFα ↑ IL-4 ↑ IFNγ	↓ Macrophage activation ↓ NK activitation	(Kamezaki et al. 2012; Baybutt and Holsboer 1990; Miller et al. 1999; Egido et al. 2012; O'Donnell et al. 2010)	↑ IL-1 (in the brain) ↑ CCL20 ↑ CXCL5	Endothelial disfunction ↓ Macrophage activation ↓ T cells ↓ Antibodies ↑ Glial activation	(Frank et al. 2012; Sugama et al. 2010; Balkaya et al. 2011b; Maslanik et al. 2012; Du et al. 2012; Coe et al. 1988; Fleshner et al. 1998)
86%[b]	x2[c] every 10 years	↑ IL-1 ↑ IL-6 ↑ TNFα	↑ Neutrophils ↑ NK (with ↓activity)	(Sacco et al. 1997; Ershler and Keller 2000; Solana and Mariani 2000; Lord et al. 2001; Mariotti et al. 2006)	↑ IL-1 (in infection) ↑ CXC (in infection)	↓ Endothelial function ↓ NK activity ↑ Glial activation	(Gomez et al. 2007; Hazeldine et al. 2012; Morgan et al. 1999; Brandes et al. 2005)

Table 2.

Risk factor / condition	Animal	Gender	Outcome	Known mediator /mechanism	Ref.
Old age	mouse	male	Infarct size ↓, edema ↓, BBB injury ↓, gray matter injury ↓, white matter injury ↑	tPA	(Liu et al. 2009; Shapira et al. 2002; Correa et al. 2011)
Old age	rat	male	Infarct size Ø/↑, mortality ↑, brain inflammation ↑	IL-1	(Wang et al. 2003; Pradillo et al. 2012)
Young age[a]	mouse, rat	female	Infarct size ↓, edema ↓	E2, IGF-1	(Liu et al. 2009; Selvamani and Sohrabji 2010a, b; Dubal and Wise 2001)
Old age	rat	female	Infarct size ↑, BBB injury ↑	E2[b]	(Selvamani and Sohrabji 2010a, b; Dinapoli et al. 2010; DiNapoli et al. 2008)
Obesity	mouse, rat, gerbil	male	Infarct size ↑, BBB injury ↑, HT ↑, Inflammatory cells in the brain ↑	PAI-1, MCP-1, IL-1	(McColl et al. 2010a; Langdon et al. 2011; Nagai et al. 2007; Park et al. 2011; Terao et al. 2008) (Kumari et al. 2011; Pradillo et al. 2012)
Diabetes	mouse	male, female	Infarct size ↑, BBB injury ↑, Inflammatory cells in the brain ↑, vascular injury ↑	AT(1)-R, PPARgamma, IGF-1	(Kumari et al. 2011; Bomont and MacKenzie 1995; Kusaka et al. 2004; Tureyen et al. 2007; Rizk et al. 2007; Wang et al. 2009)
Atherosclerosis, hyperlipidaemia	mouse, rat	male	Infarct size ↑, BBB injury ↑, brain inflammation ↑, brain edema ↑	IL-1	(Pradillo et al. 2012; Laskowitz et al. 1997; ElAli et al. 2011)
Hypertension	rat	male	Infarct size ↑, BBB injury ↑, mortality ↑, vascular injury ↑	PPARgamma, Cox-2, TNFα, EGF, HMG-CoA	(Tureyen et al. 2007; Hatashita et al. 1990; Cole et al. 1993; Dawson et al. 1996; Porritt et al. 2010; Yu et al. 2009; Mariucci et al. 2011)
Infection / PAMP-induced systemic inflammation	mouse	male	Infarct size ↑, BBB injury ↑, mortality ↑, vascular injury ↑, brain inflammation ↑, Inflammatory cells in the brain ↑	IL-1, RANTES (CCL5), MMP-9, neutrophils,	(McColl et al. 2007, 2008; Denes et al. 2011a; Denes et al. 2010a; Muhammad et al. 2011)
Stress	rat	male	Infarct size ↑, behavioral outcome ↓, endothelial function ↓, superoxide production ↑	TNFα, glucocorticoids	(Balkaya et al. 2011b; Caso et al. 2009; Madrigal et al. 2003; Sugo et al. 2002; Caso et al. 2006)

Figure 1.


After stroke
systemic inflammation
CNS inflammation
microglial activation
ECM degradation
MMP-9
MMP-2
IL-1
coagulation
altered procoagulant-anticoagulant balance
BBB injury
leukocyte transmigration
glial activation
altered leukocyte-endothelial interactions
breakdown of tight junction proteins
swelling of astrocyte endfeet
lipid deposition, inflammation
MMP-9
Elastase
IL-1
RANTES
endothelial RANTES deposition
platelet-leukocye interactions
haemorrhagic transformation
altered endothelial permeability?
leukocyte activation
leukocyte adhesion
systemic inflammation
expression of adhesion molecules
CNS inflammation
MMP-2
IL-1
RANTES
altered platelet-endothelium interactions
Prior to stroke
glial activation