INNATE IMMUNITY

TREM1-ors shake the brain and gut after stroke

A new report shows that upregulation of the receptor TREM1 on macrophages and neutrophils, dependent on the adrenergic nervous system, links stroke to systemic inflammation and gut barrier dysfunction, which result in bacterial translocation and exacerbation of neurological damage.

Francesco Roselli and Markus Huber-Lang

It is common for physicians involved in the treatment of patients who have suffered stroke to be confronted with many, often life-threatening infectious complications; it might well be as common to wonder where the peripheral inflammation actually originates and how it affects the prognosis of each patient. In this issue of *Nature Immunology*, Liu et al. identify a previously unknown mechanism that links the two sides of this problem: how peripheral inflammation might affect stroke-related pathogenesis, and how stroke results in penetration of the organism by bacteria, specifically at the level of the gut mucosa[1]. In a compelling set of experiments, they identify critical involvement of the receptor TREM1 (triggering receptor expressed on myeloid cells 1) in mediating the activation of peripheral immunity that leads to detrimental brain infiltration after stroke and the inflammatory damage to the gut mucosa that allows translocation of bacteria into the bloodstream.

TREM1 and TREM2 are transmembrane proteins that belong to the immunoglobulin superfamily. Receptors of the TREM family are constitutively expressed on immune cells, endothelial cells and platelets, where they signal via association with intracellular partners (such as DAP12) to activate the kinase Btk (Bruton's tyrosine kinase), Syk and PI(3)K (phosphatidylinositol-3-OH kinase) cascades. TREM1, in particular, can activate several major inflammatory transcription factors (such as NF-κB and STAT3) and is considered a master switch for inflammatory responses in innate immunity. TREM1 and TREM2 appear to be counter-regulated in terms of gene expression[2], reflective of their largely opposing roles: whereas TREM1 strongly polarizes the macrophage and neutrophil response toward all-out inflammatory responses, TREM2 seems to induce a reparative or disease-protective phenotype, in particular in microglia[3], as well as in extracerebral sites.

Liu et al. use elegant genetic and marker-based characterization of myeloid and microglial cells to identify the distinct upregulation of TREM1 on monocytes–macrophages and on neutrophils at 48 hours after occlusion of the middle cerebral artery[1]. Notably, upregulation of TREM1 in peripheral myeloid cells occurs as early as 4.5 hours after stroke, which suggests that the systemic effects of stroke appear very early. The authors use both genetic approaches and pharmacological approaches to interfere with the activation of TREM1 and thus pinpoint the biological functions of this pathway. In mice lacking TREM1 and in mice given peptides that antagonize the activation of TREM1, infarct volume and overall survival are substantially improved, relative to that of TREM1-sufficent mice and untreated mice. Interestingly, neutralization of TREM1 is associated with an increase in infiltration of the ischemic hemisphere by monocytes and macrophages and with a distinctive gene-expression pattern, in which loss of TREM1 results in the upregulation of antioxidant and lysosomal pathways. Notably, *Cd93* (which encodes the receptor for the complement component C1q) stands out among the upregulated genes, which suggests that CD93-expressing neutrophils might facilitate the clearance of ischemia-induced C1q-labeled neuronal debris[4]. Interestingly, in the absence of TREM1, expression of the chemokine receptor CCR5 is also upregulated on circulating neutrophils. How to reconcile this with the reported beneficial effects of systemic blockade of CCR5 in stroke[5] will require further investigation.

Notably, *Trem2* is among the genes most upregulated after experimental stroke in TREM1-deficient mice, which leads to the suggestion that microglia and myeloid cells activated by the engagement of TREM2 (previously described as immune cells with a protective phenotype in neurodegenerative conditions) may be instrumental in bringing about the protective effects of the loss of TREM1. Therefore, a 'TREM balance' model may be hypothesized (Fig. 1) in which external factors that tip the equilibrium of TREM1 and TREM2 may lead to radically polarized outcomes in terms of innate immune responses. Because none of the reported manipulations of TREM1 are cell specific, the relative contributions of TREM1 expressed on different subpopulations remains to be ascertained. Of note, TREM1 is expressed not only on leukocytes but also on endothelial cells and platelets[6], which raises the possibility that deletion or blockade of this protein may also substantially diminish the so-called 'thrombo-inflammatory' mechanisms in stroke.

Liu et al. very imaginatively set out to track the upregulation of TREM1 by positron-electron-tomography tracers, which not only allows non-invasive mapping of TREM1 expression in the brain but also reveals an unexpected massive increase in TREM1 in the gut, histologically assigned to macrophages in the lamina propria, as well as remarkable uptake by the spleen[1]. Upregulation of TREM1 in the spleen is not completely unexpected, given that upregulation of TREM1 occurs on peripheral myeloid cells. However, the selective uptake of the TREM1 tracer in the gut is quite a surprising and intriguing finding, given the large population of macrophages that reside there and patrol the interface with the gut microbiome. These findings are in agreement with previously published evidence of early disruption of gut (and lung) barrier integrity after cerebral ischemia, revealed by a single-photon-emission-computed-tomography permeability tracer[7]. Strikingly, the upregulation of TREM1 expression in gut macrophages appears very early, considerably before infiltration of the brain. Furthermore, the upregulation of TREM1 can be pharmacologically prevented by diminishing the adrenergic drive via beta blockers. This finding provides a notable mechanistic explanation for the previously reported effect of beta blockers in limiting the disruption of the gut-mucosa permeability[8]. Most notably, blockade of TREM1 prevents the loss of gut-mucosal-barrier integrity: after loss of TREM1, the amount of circulating lipopolysaccharide and the number of colony-forming bacteria

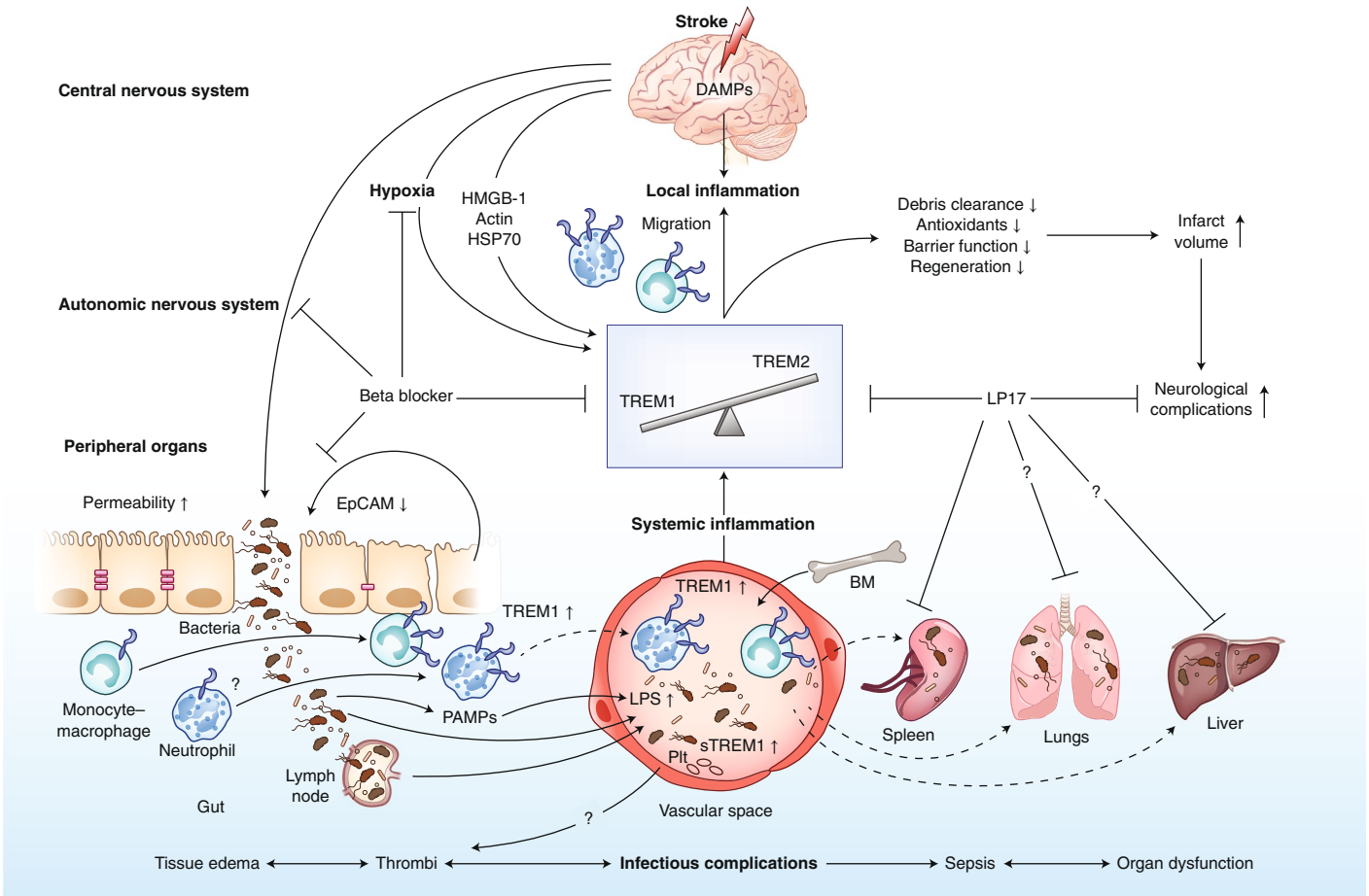

Fig. 1 | Stroke-induced alterations to the TREM1–TREM2 balance of innate immunity and associated gut-barrier dysfunction with subsequent infectious and organ complications. sTREM1, soluble TREM1; BM, bone marrow; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; EpCAM, epithelial cell adhesion molecule; HSP70, heat-shock protein 70; HMGB-1, endogenous danger signal (high-mobility-group box1 protein); Plt, platelets; LPS, lipopolysaccharide.

isolated from spleen after stroke are substantially lower. Liu et al. remain very cautious about the involvement of other organs, although a tantalizing trend toward increased TREM1 expression in bone marrow[1] may point toward a third site of peripheral involvement, which might be worthy of further scrutiny.

Therefore, TREM1 seems to be at the crossroads of two major pathways involved in the biology and clinical context of stroke: it contributes to triggering the influx of myeloid cells, with net detrimental consequences in the brain, and it elicits a net weakening of the mucosal barrier of the gut, which enables bacteremia. The findings by Liu et al.[1] thus constitute an important mechanistic piece of the puzzle of the previously reported bacterial translocation that is caused by stroke and leads to increased infectious risk in patients who have suffered a stroke[8].

The work by Liu et al.[1] lends itself to prompt translation into clinical settings, and a flurry of follow-up investigations is anticipated. The most obvious consequence is further support for the study and application of beta blockers, moving them to the forefront in the prevention of inflammatory responses in stroke, and questioning of the liberal use of catecholamine treatment in conditions in which triggering TREM1 responses may add to the detrimental inflammation (such as in traumatic injuries). Repurposing agents from the extensive catecholaminergic repertoire for modulating the induction of TREM1 may therefore result in the generation of TREM1-selective agents[9].

More directly related to the evidence provided by Liu et al.[1], the use of molecular imaging strategies based on TREM1 expression may pave the way to the study of organ-specific activation of innate immunity in several mouse models of disease. Indeed, the mechanisms that cause compartmentalization of the immune response and upregulation of TREM1 remain unknown, despite clear evidence of an organ-specific inflammatory response after systemic exposure to bacteria[10]. Furthermore, this concept may be readily extended to the study of human patients; patient stratification based on assessment of the TREM1–TREM2 balance might inform clinicians of the risk for neurological worsening and for infectious complications.

In terms of the therapeutic perspective, Liu et al. demonstrate that a peptide antagonist of TREM1 (the TREM1 decoy peptide LP17) is as good as genetic knockout for interfering with improper myeloid activation[1]. The effect of LP17 on stroke-associated dysfunction of other organs is open to further investigation. Notably, LP17 loses most of its efficacy when applied beyond the first few hours after stroke. Therefore, a TREM1-directed treatment might have a therapeutic window that overlaps that of thrombolytic approaches and might thus be administered together with thrombolytic agents.

The feasibility of this combined strategy is a major point for future translational research as more TREM1-targeting agents with acceptable pharmacokinetics become available[9].

Nevertheless, a few critical questions remain open. What are the relative contributions of macrophages and neutrophils (or other TREM1-expressing subpopulations) in generating the detrimental milieu in the central-nervous-system lesion? Can any direct role of TREM1 in microglia be discounted? What is the actual adrenergic mechanism that induces TREM1 in peripheral macrophages? The last issue appears to be particularly intriguing, because TREM1 may be modulated by hypoxia, and hypoxia itself may affect macrophage polarization[11], but locally generated catecholamines by innate immune cells[12] could also contribute to alterations in the balance of TREM1 versus TREM2.

The evidence provided by Liu et al.[1], building on the existing literature[8], makes a move toward clinical trials now compelling, aiming to eventually revisit the current standard of care. If bacterial translocation occurs after stroke and can be limited by reducing the adrenergic tone, will beta blockers diminish infectious complications? Can peripheral levels of TREM1-expressing leukocytes be used to meaningfully stratify patient risk? Finally, because TREM1-blocking agents seem to leave other branches of innate and acquired immunity untouched[13] and do not induce systemic immunosuppression, the first in-human trials of such agents aimed at TREM1 in stroke are anticipated as the pharmacological toolset expands[9]. ❐

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Competing interests

The authors declare no competing interests.