Lectin Complement Pathway and Its Bloody Interactions in Brain Ischemia

Stefano Fumagalli, PhD; Maria-Grazia De Simoni, PhD

Stroke is associated with vascular events that follow the initial insult and involve the activation of several blood-borne cascades including coagulation, contact/kinin, and complement cascade. There is growing evidence implicating the complement system as a co-ordinator of these cascades in brain ischemia.1–4 Although full complement cascade activation leads to cell lysis, complement components activated along the cascade (upstream of the final event) have active parts in inflammatory processes, implying they are in intimate cross talk with the other systems.

The complement system is a physiological component of innate immunity, which is activated on recognition of danger signals, the so-called damage-associated molecular patterns, in addition to pathogen-associated molecular patterns.5,7 Damage-associated molecular patterns include glycoproteins and proteins from the extracellular matrix, intracellular proteins, DNA or RNA fragments, and heat-shock proteins, all produced when tissue homeostasis is altered by pathological condition. Different signals lead to the activation of different complement activation pathways, namely, the classical (activated on binding antibodies, C-reactive protein, apoptotic cells, and nucleic acids), lectin (LP, activated on recognition of carbohydrate patterns), alternative (constitutively active and working as an amplifying loop of complement) and extrinsic (activated by tissue factor, and thrombin) pathways, all generating cleaved active fragments by cascade-like proteolytic reactions.5,8

The LP seems to have a vital role in brain ischemia.6 This pathway depends on initiator molecules including mannos-binding lectin (MBL), ficolin-1, -2, and -3, and collectin-11 that can recognize and bind carbohydrates exposed on the surface of altered or damaged cells, including endothelial cells.6 They normally circulate complexed with MBL-associated serine proteases (MASPs). On binding to their ligands, the complexes become activated, promoting downstream complement activation.9 LP active complexes can also cleave coagulation factors and may, thus, directly contribute to thrombosis.1,4,10

Here, we discuss recent findings on how LP is involved in stroke and its interaction with the coagulation and kinin systems in brain ischemia.

Lectin Pathway and Its Initiators in Brain Ischemia

Early studies reported that MBL, the first recognition molecule activating LP to be identified and one of the most widely studied, is involved in heart, kidney,13,14 and intestine ischemia/reperfusion injury. MBL and the LP are also involved in brain ischemic injury, as documented in patients16–19 and in experimental models.16,20,21 MBL is mainly synthesized in the liver, and its circulating levels are genetically determined. In humans, genetic variants cause MBL deficiency in ≈20% of the general population.19,22–24 Interestingly, stroke patients with MBL deficiency have smaller infarctions and better outcomes.10,18 (Table) lending support to the hypothesis of a pathogenic role of MBL in this condition.

Experimental studies have examined MBL’s role in brain ischemia. In line with the clinical data, in MBL knock-out mice (double-knock out for the 2 murine MBL isoforms: MBL-A and MBL-C), the brain ischemic injury is attenuated.16 In addition, pharmacological targeting of MBL, by intravenous injection of anti–MBL-A antibody or Polyman2, a mannosylated molecule acting as MBL inhibitor,21,29 improves neurological deficits and reduces the cerebral lesion in ischemic rats and mice, respectively. Importantly, these treatments were effective when given ≤18–24 hours after injury, indicating a wide therapeutic window.21

Immunohistochemical studies show that MBL-A and -C are selectively deposited on the ischemic endothelium as early as 30 minutes and up to at least 48 hours after injury. This seems to be a crucial pathogenic event whose consequences are still largely unexplored.21 On injury or in stressful conditions, new epitopes are exposed and the molecular profile of endothelial cells changes and consequently their ability to attract soluble proteins.2,30 MBL deposition on the damaged endothelium may conceivably be because of recognition of carbohydrates exposed on the surface of ischemic endothelial cells. The surface of the endothelial cells also presents membrane-anchored proteins such as heparin sulfate, thrombomodulin, tissue factor inhibitors, and tissue-type plasminogen activator, oriented on the vessel luminal side to regulate blood flow and prevent clotting.30 The ischemic endothelium should, therefore, be regarded as a site where the complement, kinin, and coagulation systems—all active on the endothelial...
Table. The Lectin Pathway Studies in Ischemic Stroke Patients

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
<th>Outcome</th>
<th>Blood Sampling</th>
<th>Protein Studied</th>
<th>Main Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>Age &gt;18 y, symptoms &gt;3 h, not hemorrhagic</td>
<td>TIA &lt;24 h, AMI &lt;6 mo, autoimmune diseases, sepsis</td>
<td>NIHSS, mRS, and Barthel index at 6 d</td>
<td>Admission</td>
<td>C1r, C1s, C1-inh, C4d, C3a, C5a, and SC5b-9</td>
<td>Positive correlation between SC5b-9 serum levels (mainly because of LP activation) and stroke severity</td>
<td>Széplaki et al25</td>
</tr>
<tr>
<td>135</td>
<td>Age &gt;18 y, NIHSS ≥5 (including 19.3% hemorrhagic stroke)</td>
<td>Infection &lt;3 mo, axillary temperature &gt;37.7 °C, allergy to fluoroquinolones, epilepsy history, use of antibodies, immunosuppressants, or steroids &lt;3 mo</td>
<td>NIHSS, mRS, Barthel index at 3 mo, and infections at 1 wk</td>
<td>From admission to 7 d</td>
<td>MBL and MASP2</td>
<td>MBL deficiency is associated with better outcome</td>
<td>Cervera et al16</td>
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<tr>
<td>359</td>
<td>Ischemic stroke with onset &lt;72 h</td>
<td>…</td>
<td>NIHSS at admission, MRI ischemic volume during initial work out, mRS, Barthel index, and mortality at 3 mo</td>
<td>Admission</td>
<td>MBL</td>
<td>MBL deficiency is associated with less severe stroke, better functional outcome, and smaller infarct size in patients receiving conservative treatment (no thrombolysis)</td>
<td>Osthoff et al16</td>
</tr>
<tr>
<td>188</td>
<td>Type 2 diabetic patients with first acute ischemic stroke &lt;48 h</td>
<td>Malignant tumor, intracerebral hemorrhage, surgery or trauma &lt;2 mo, renal insufficiency, febrile disorders, systemic infections at study enrollment, and autoimmune diseases</td>
<td>NIHSS at admission and at 1 y, MRI ischemic volume at 24–48 h, and mortality at 1 y</td>
<td>Admission</td>
<td>MBL</td>
<td>MBL serum levels correlate with stroke severity and infarct volume and are associated with increased 1-y mortality</td>
<td>Song et al24</td>
</tr>
<tr>
<td>220</td>
<td>Ischemic stroke with onset &lt;24 h</td>
<td>Malignant tumor, intracerebral hemorrhage, renal insufficiency, febrile disorders, acute/chronic inflammatory on enrollment, and autoimmune diseases</td>
<td>NIHSS at admission, mRS and mortality at 3 mo</td>
<td>Admission</td>
<td>MBL</td>
<td>MBL is an independent prognostic marker of 3-mo functional outcome and mortality</td>
<td>Zhang et al27</td>
</tr>
<tr>
<td>158</td>
<td>First ischemic stroke with onset &lt;6 h, Age 16–80 y</td>
<td>Known complement deficiency</td>
<td>NIHSS at admission, mRS at 3 mo</td>
<td>6 h, 48 h, 3–5 d, and 1 mo</td>
<td>Ficolin-1, 2, 3 and MBL</td>
<td>Ficolins are consumed at 6 h. Ficolin-1 is an independent early (6 h) marker of unfavorable outcome 3 mo after stroke</td>
<td>Zangari et al19</td>
</tr>
<tr>
<td>65</td>
<td>First ischemic stroke with onset &lt;12 h</td>
<td>Hemorrhagic stroke, infectious disease, fever &lt;4 wk, high leukocytes, erythrocyte segmentation rate, C-reactive protein and procalcitonin at admission, and positive chest x-ray</td>
<td>NIHSS at admission and mRS at 3–4 d</td>
<td>Admission and 3–4 d</td>
<td>Ficolin-2 and -3</td>
<td>Ficolin-2 and -3 are rapidly consumed after stroke. Ficolin-3 consumption correlates with NIHSS at admission and S100β index of cerebral infarction at 3–4 d</td>
<td>Füst et al17</td>
</tr>
<tr>
<td>66</td>
<td>Ischemic stroke confirmed by MRI</td>
<td>…</td>
<td>NIHSS at admission</td>
<td>admission and 1 and 2 d</td>
<td>MASP1, 2, 3, and MAp44</td>
<td>At admission, no protein change compared with healthy controls. In stroke patients MASP3 and MAp44 decrease at days 1 and 2 compared with admission (no. of patients=22)</td>
<td>Frauenknecht et al23</td>
</tr>
</tbody>
</table>

AMI indicates acute myocardial infarction; LP, lectin pathway; MASP, MBL-associated serine protease; MBL, mannose-binding lectin; MRI, magnetic resonance imaging; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; and TIA, transient ischemic attack.
cell surface—interact. Although most of these interactions are still not understood, recent works report direct or indirect communication between these systems, as discussed below.

Similar to MBL, ficolins too can activate the LP on binding with their targets, thus promoting downstream complement activation. Emerging clinical evidence implicates ficolins in the progression of brain damage in stroke, as shown by reports of lower ficolin levels early (within 6 hours from onset) after stroke because of consumption exceeding production/release in the acute phases (Table). Ficolin-1 and ficolin-3 seem to be independent predictors of outcome after ischemic stroke, suggesting that they might be sensitive prognostic markers.17,19

Ficolins share with MBL proinflammatory, procoagulant, and prothrombotic properties and thus contribute to vascular events. Ficolin-1 is primarily synthesized and presented on the surface of peripheral monocytes and neutrophils, promoting neutrophil adhesion, aggregation, and migration,9,31–34 all vascular events driving clot formation5,56 and atherosclerosis.37 Ficolin-2 and -3 are mainly produced by the liver and liver/lung and circulate in the bloodstream. Evidence now links these proteins to prothrombotic conditions such as atherosclerosis,17 but direct mechanistic information on their prothrombotic roles is still needed.

Less data are available on collectin-11 (CL-11), the last LP initiator molecule to be discovered, in ischemic injury. It is mainly produced by the kidneys, and its structure is similar to MBL, including a carbohydrate-binding motif.58 High CL-11 plasma levels have been found in patients with disseminated intravascular coagulation because of derangement of the coagulation system after substantial activation of the innate immunity.39 A recent article reports that CL-11 contributes to renal ischemia.40 These observations link CL-11 to coagulation and ischemic conditions, but to date there is no evidence of CL-11’s involvement in brain ischemia and specifically in prothrombotic functions.

Other molecules belonging to the collectin family and sharing structural similarities with MBL are the surfactant proteins SP-A and SP-D, mainly from the lungs. These proteins bind to different receptors including complement receptors such as C1qR and can activate the complement system.41,42 The importance of surfactant proteins in vascular conditions has been investigated in biomaterial science because they are involved in host-versus-graft reactions. Advanced health technologies involve devices such as stents, hemodialysis tubes, artificial implants, bypass circuits, etc, all expose the bloodstream to exogenous materials.43 So many studies have focused on biomaterial toxicity and new biomaterials functionalized to evade the host reaction, such as contact activation, coagulation, and complement activation.44,45 Interestingly, high serum SP-D levels have been associated with atherosclerosis in patients on long-term hemodialysis.46 SP-D positively correlates with carotid artery intima-media thickness and plaque calcification, 2 risk factors for plaque instability and thrombi.

Molecular Complement–Coagulation–Kinin System Interactions

The complement, coagulation, and kinin systems are major blood-borne proteolytic pathways. They have common evolutionary origins and their interaction is warranted by their sharing the same compartment (blood) and activation time (first line of defense). The molecular interactions among these 3 pathways support vessel clotting and inflammatory properties.10 Many components are common to the 3 cascades: there is MASP1, which has thrombin-like activity47 and can cleave high-molecular-weight kininogen into kallikrein, and serine protease inhibitors that may inhibit all the pathways at different levels. C1 inhibitor regulates the classical pathway and LP by neutralizing C1s, C1r, and MASPs and also regulates coagulation by neutralizing factors XIIa, Xla, and kallikrein. Antithrombin neutralizes thrombin and other coagulation enzymes, for example, factor Xa, and also inhibits the classical pathway and LP by neutralizing C1s and MASPs2. The connections among complement–coagulation–kinin systems are intricate as activated proteins from one cascade can either dampen or enhance the others.7 Moreover, these interactions are not one way and often work on feedback loops. Thus, MASPs appear at the crossroads of several events that control complement, coagulation, and kinin systems (Figure).

However, some thrombotic events activated by the LP may not directly involve MASPs. After cerebral ischemia, MBL-deficient mice have reduced fibrin deposition, suggesting that MBL has a direct role in thrombin activation.55 Because MBL and ficolins and collectins have a recognition site for fibrinogen,56 they can directly promote coagulation, inducing fibrin deposits. In line with this, mouse MBL isoforms and mouse ficolin A (closely related to human ficolin-2) bind to fibrinogen, and this binding enhances the LP activation in vitro.57 The tetrapeptide Gly-Pro-Arg-Pro mimicking the N-terminal sequences of fibrin α and β chains inhibits the binding of human ficolin-1 to its substrate N-acetylglucosamine,58 suggesting a direct ficolin-1–fibrinogen interaction.

Hypotheses on other important vascular interactions in ischemic conditions can be drawn from studies in thrombotic conditions. Thrombosis mediated by antiphospholipid (aPL) antibodies is a common complication of patients with antiphospholipid syndrome. High serum levels of aPL are the main nonconventional risk factor for stroke in young people.59 Complement system activation seems to be required for aPL-mediated thrombosis, as mice deficient in complement components or receptors are protected from its thrombogenic effects.60,61 The pathogenic clotting events mediated by aPL require a 2-step mechanism: a first hit provided by the presence of aPL and a second hit involving a procoagulant condition requiring complement factors, cytokines, β2 glycoprotein I, prostaglandin E2, platelets, and erythrocytes.59 The exact molecular interactions among these components are far from completely known, and although they are likely, their involvement after brain ischemia has not yet been reported.

Complement System and Atherosclerosis

The prothrombotic mechanisms driven by complement after the initial ischemic event offer potential pharmacological targets to limit the damage progression. However, complement may also be implicated in thrombotic processes that are risk factors for stroke, as documented in the few studies on atherosclerotic patients. Atherosclerosis involves chronic and
progressive inflammation leading to vascular alterations and plaque formation. 37 The plaques may cause critical narrowing or become unstable and embolize, changing the hemodynamics or inducing partial/complete occlusion (atherothrombosis). 62 Atherosclerosis, therefore, raises the risk of transient ischemic attack and stroke. 63–65 

The atherosclerotic process provides danger stressors that activate several components of innate inflammation, 66 including the complement system. 67,68 The classical pathways and alternative pathways have a dual function in atherosclerosis, counteracting plaque formation by clearing debris or favoring atherogenesis. 66,68–70 Similarly, LP may have either antiatherogenic 71–73 or proatherogenic 74–76 and prothrombotic functions. 1,55 In support of these latter functions, Füst et al 17 reported higher ficolin-2 and ficolin-3 serum levels in asymptomatic patients with severe carotid atherosclerosis than in healthy controls, suggesting that they both accumulate in the atherosclerotic process. This is different from an acute ischemic event where circulating ficolin levels fall early after injury as a result of consumption. 17,19

In line with the proatherogenic action of the LP, high MBL serum levels are associated with an increased risk of coronary artery disease, as reported in a cohort of European men 75 and diabetic patients. 74 Moreover, patients with the normal MBL genotype are more likely to develop new vessel narrowing after surgical plaque removal. 76 In contrast, a few studies report an antiatherogenic role for MBL, given that variant genotypes causing MBL deficiency are associated with an increased risk of coronary artery disease. 71 These discordant observations may depend on the specific features of patients enrolled in the studies, for example, age, sex, and clinical evaluation, suggesting that MBL may have different effects in atherosclerosis depending on the clinical setting. 74,77

Conclusions

Clinical and experimental evidence implicates the LP in the progression of brain damage in stroke. Its rapid activation in response to danger signals expressed early after injury and its ability to control and co-ordinate multiple pathogenic cascades make it a hub in vascular injury. The LP also seems to

Figure. The documented connections between the lectin complement pathway, the coagulation system, and the kinin system through mannose-binding lectin (MBL)–associated serine proteases (MASPs). MASPs complexed with lectin pathway (LP) initiators (MBL, ficolins, and collectins) have active proteolytic functions driving complement, coagulation, and LP activation. 48–50 Only the pathways involved in complement–coagulation–kinin systems interaction and driven by MASPs are reported. 1. MASP1 has similar structure and substrate specificity to thrombin. 51,52 MASP1 favors the generation of fibrin from fibrinogen by its thrombin-like function or by cleaving factor II into IIa, leading to clot formation. 47 In a negative feedback loop, factor IIa co-operates with thrombomodulin to activate thrombin-activatable fibrinolysis inhibitor (TAFI), a negative regulator of thrombin 53 and complement. 2. MASP1 cleaves high-molecular-weight kininogen (HMWK) into kallikrein that is needed for the production of (1) the active fragments of C3 (C3a, C3b, C5 (C5a, C5b), and factor B (FBa, FBb), leading to platelet activation and complement terminal pathway; and (2) bradykinin, leading to vascular leakage, NO production, inflammation, and clot formation. TAFI negatively regulates C3 and C5 active fragments dampening platelet activation. 3. MASP2 promotes the generation of complement fragments C3b and C4b2a, resulting in full complement and platelet activation. C4bBP (C4b binding protein) targets these fragments acting as negative regulator and induces anticoagulant protein S, therefore lowering coagulation. 4. All the proteolytic pathways downstream to MASPs can drive platelet activation. Platelets express many complement proteins including complement receptors (eg, C3aR and C1qR) and regulatory molecules (eg, C1-inh and CD55), making them closely controlled by complement. On activation, platelets induce coagulation and boost complement by cleaving C3 into its active fragments C3a and C3b. Serine protease inhibitors such as C1-inh and antithrombin act at different levels in the above-reported cascades.
play a role in chronic conditions such as atherosclerosis. It may, therefore, offer a promising therapeutic opportunity in stroke therapy and prevention.

In addition, this pathway seems to contribute significantly to other acute brain injury conditions. The LP is activated in subarachnoid hemorrhage patients, and the actual plasma concentrations of ficolin-3 reflect the severity of brain injury, as evaluated by clinical and structural parameters. This pathway is also activated in traumatic brain injury patients, and its functional inhibition by gene deletion or pharmacological targeting is protective in murine traumatic brain injury.

Being activated within the vessels, the LP can be accessed easily, for example, with no need to cross the blood–brain barrier. A few compounds able to target the LP, such as human recombinant C1 inhibitor, Polyman, and Polyman have been successfully used when systemically administered in preclinical models of acute brain injury, showing that this is a druggable pathway. Acute pharmacological LP inhibition confers lasting protection against acute brain injury and has a minimal impact on LP in subacute phases, when its activation seems to be required for tissue repair and regenerative mechanisms.

The multiple lines of evidence documenting the LP’s contribution to brain injury, its role as a hub in several pathogenic vascular events, and the proof of its druggability make it an attractive target for the development of therapeutic tools for acute brain conditions.

Acknowledgments
We thank J. Baggott for English editing.

Sources of Funding
S. Fumagalli is funded by Fondazione Cariplo (grant number: 2015-1003).

Disclosures
None.

References


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Stroke. 2016;47:3067-3073; originally published online November 3, 2016;
doi: 10.1161/STROKEAHA.116.012407
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
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