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Mannose binding lectin and complement pathway in brain ischemic injury: studies in mouse models and patients

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Preface

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Lectin pathway of complement activation after subarachnoid hemorrage. *This paper is a work in progress*

List of main abbreviations

ARDS	acute respiratory distress syndrome
C1-INH	C1-inhibitor
CAMs	cell adhesion molecules
CCA	common carotid artery
CI	cerebral ischemia
CNS	Central Nervous System
CR1	complement receptor 1
CRD	carbohydrate recognition domains
CRP	C-reactive protein
CSF	cerebrospinal fluid
CT	computer tomography
DCI	delayed cerebral ischemia
ECA	external carotid artery
ECI	early cerebral ischemia
ELISA	enzyme-linked immunoassorbent assay
GOS	Glasgow outcome scale
Hgb	hemoglobin
Hp-Hgb	HaptoglobinHb protein
I/R	ischemia/riperfusion
ICA	internal carotid artery
ICP	intracranial pressure
ICU	Intensive Care Unit
MAC	membrane attack complex

MASPs	MBL-associated serine proteases
MBL	Mannose Binding Lectin
MBL-/-	MBL-A and MBL-C double knockout mice
MCP	and membrane cofactor protein
MRI	magnetic resonance imaging
OD	optical density
PAMPs	pathogen- associated molecular patterns
pMCAo	permanent middle cerebral artery occlusion
PWI	Perfusion-weighted Imaging
RBCs	Red blood cells
rhC1-INH	recombinant human C1 inhibitor
SAH	subarachnoid hemorrhage
SC5b-9	plasma levels of terminal complement complex
Sham	sham-operated
SNP	single nucleotide polymorphisms
sPRM	small pattern recognition molecule
TCC	terminal complement complex
tMCAo	transient middle cerebral artery occlusion
VSP	clinical vasospasm
WFNS	World Federation of Neurological Surgeons

Abstract

Background. The involvement of the complement system in brain injury has been scarcely investigated. Here we document the pivotal role of lectin pathway, initiated by mannose binding lectin (MBL) and ficolin-3, in brain ischemic injury in mice and humans.

We first evaluated the role of the lectin pathway in brain ischemic mice.

In order to investigate the clinical relevance of these experimental observations, the second aim was to evaluate the relevance of the lectin pathway in subarachnoid hemorrhage (SAH) patients. Brain ischemia is a main determinant of unfavorable outcome in SAH patients. It can play a role in the acute phase as a consequence of the initial intracranial bleeding and/or at delayed stages due to cerebral vasospasm.

Methods and results: brain ischemic injury in mice. Focal ischemia was induced in C57Bl/6 (WT) or in MBL-A and MBL-C double knockout mice (MBL-/-), by permanent or transient middle cerebral artery occlusion (pMCAo and tMCAo, respectively). Neurological deficits and infarct volume were measured 48 h after ischemia. MBL presence on cerebral vessels was assessed by immunostaining and confocal microscopy. The activation of lectin pathway after ischemia was analyzed by measuring circulating functional MBL/MASP-2 complexes by ELISA. Complement activation was assessed by western blot analysis of C3 fragments in plasma samples.

We first observed that MBL is deposited on ischemic vessels up to 48h after injury and that functional MBL/MASP-2 complexes and C3 complement fragments were significantly increased after tMCAo and pMCAo, indicating that the lectin pathway is activated in the ischemic injury.

Next, we demonstrated that MBL-/- mice were protected from anatomical and functional ischemic injury, showing 28% and 41% lesion reduction after tMCAo and pMCAo, respectively, compared to WT, thus suggesting a deleterious role of MBL and suggesting that inhibition of this protein could lead to neuroprotection.

Methods and results: subarachnoid hemorrhage patients.

Thirty-nine patients with SAH were enrolled. Clinical vasospasm (VSP) was defined as neuro-worsening with angiographic confirmation of vessel narrowing. Cerebral ischemia was defined as a hypodense lesion on computer tomography (CT) scan performed before Intensive Care Unit (ICU) discharge. Early cerebral ischemia was defined as a hypodense lesion on CT performed in acute phase (ECI), while delayed ischemia (DCI) was defined as a new hypodense lesion on CT scan performed in delayed phase. Six-month outcome was assessed using Glasgow outcome scale (GOS). Clinical severity, radiological status and outcome were studied in relation to lectin concentrations. Plasma levels of MBL, MBL/MASP-2 functional complexes, ficolin-3 and of complement factors (C3 and C5b-9) from patients and 20 healthy subjects, were determined in acute (1-3 day) and post acute phase (4-14 day) through western blot analysis and ELISA.

The plasma concentrations of MBL in SAH patients, however, was not significantly different from healthy subjects. Despite unaffected MBL levels, we have observed an acute and persistent reduction of MBL/MASP-2 levels.

On the contrary, a persistent increase in ficolin-3 was detected in SAH patients. Furthermore, ficolin-3 was related to brain injury severity. Namely, significantly lower levels of ficolin-3 were found in: severe patients, patients with VSP and patients with CT cerebral ischemia.

Conclusion: Our data show an important role for the lectin pathway in the pathogenesis of acute brain injury and provide a strong support to the concept that lectin pathway may be a relevant therapeutic target in humans with a wide therapeutic window of application.

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