Conclusion: We developed and characterized a closed head repetitive injury model and demonstrated a role for complement in cognitive decline and the upregulation of multiple neurodegenerative markers/pathways post rmCHI. Targeting the complement system as a therapeutic approach in repetitive brain injuries requires further investigation.

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Proteolytic activity of secreted proteases from pathogenic leptospires and effects on phagocytosis by murine macrophages

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Leptospirosis is a zoonosis caused by spirochete bacteria that belong to the genus Leptospira. This disease represents a serious public health problem, especially in developing countries with tropical and subtropical temperatures. Pathogenic leptospires escape from the Complement System, a property that permits them to survive in vitro when in contact with normal human serum (NHS). In a previous study carried out by our group, it was observed that culture supernatants from different pathogenic species of leptospires (SPL) contain proteases that cleave many Complement proteins, including the central molecule C3 and its fragments C3b and iC3b. Our hypothesis is that these proteases, could decrease the phagocytic clearance of leptospires. Using flow cytometry, we observed decreased amounts of CR3 and CR4 in murine peritoneal macrophages treated with SPL for 24 h. By confocal microscopy, we observed reduction in TLR2, CD11b and CD206 levels when these cells were treated with SPL and recombinant thermolysin for 24 h. Furthermore, opsonins such as C3b/iC3b deposited on the surface of pathogenic leptospires were observed to be completely degraded in the presence of SPL or recombinant thermolysin. Finally, we decided to investigate the phagocytosis of pathogenic leptospires by macrophages in the presence of these proteases. We observed an increase of phagocytosis of leptospires opsonized with normal mouse serum even when macrophages were treated with the proteases. However, when opsonized bacteria were also incubated with SPL, recombinant thermolysin and recombinant leptolysin., there was a decline in leptospires phagocytosis. This suggests that the proteolytic activity can affect phagocytosis by peritoneal macrophages mainly through the degradation of opsonins deposited in the membrane of leptospires. These observations lead us to suggest that proteases secreted by pathogenic leptospires could degrade opsonins present in normal serum or deposited in the bacterial membrane as well as cleave or inhibit macrophage surface molecules. Therefore, these proteases could interfere with the recognition and internalization by murine macrophages, favoring the spread of leptospires in the host.

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Distinctive dosage requirements between C3 and Factor D (FD) in the activation of the alternative complement pathway

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Background: Alternative pathway (AP) contributes substantially to complement activation through its powerful amplification loop. Factor D (FD) is the smallest in size and lowest in concentration among AP components. We and others have established that a relatively small amount of FD is required to activate the AP. The role of FD function in combination with other AP components is further investigated in this study.

Methods: Crry/FD double knockout (DKO) and Crry/FD/C3 triple knockout (TKO) mice were used to study AP function. Serum or purified FD was transferred into DKO or TKO and Western blots were employed to quantitate C3 consumption. A rabbit erythrocyte hemolysis assay was utilized to monitor AP functional activity.

Results: Using hemolysis assay, 180 ng/ml of purified FD (approximately 1/100 of normal) rescues the AP defect in FD KO mice while 360 μg/ml of C3 (about 1/3 of normal) is required to rescue the AP in C3 KO mice. C3 consumption occurs in the Crry KO mouse due to a lack of AP control but Crry/FD DKO mice have a normal level of C3. Injection of purified FD into the Crry/FD DKO mouse leads to a rapid decrease (within 15 minutes) of C3 in blood. Transferring 200 μl of WT serum into the Crry/FD/C3 TKO mouse results in the virtual disappearance of blood C3, being consumed in an environment lacking the membrane regulator Crry. Infusion of FB KO serum [without initial C3 (H2O) Bb convertase] versus WT serum into Crry/FD/C3 TKO leads to equivalent C3 consumption. The defect in hemolysis of Crry/FD/C3 TKO serum was not rescued by adding a higher concentration of FD or C3. A low dose of FD at 100 ng/ml and doses of C3 from 40 to 360 μg/ml were added to the serum of Crry/FD/C3 TKO mice. C3 at 360 μg/ml was again required to achieve about 90% of WT hemolysis.

Conclusion: Our results demonstrate that a low amount of FD but C3 at more physiological levels are required to activate the AP. These experimental data have implications for treating diseases with anti-FD therapeutics.

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Complement System and leptospirosis: understanding the role of C3 during the renal fibrosis caused during chronic infection by pathogenic leptospires in murine experimental model

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Leptospirosis is a neglected zoonosis affecting approximately 1 million people each year worldwide and causing near 5% deaths.