could antagonize binding of each other to CR3. The full-length FH but not the fragments of FH, Aβ1–42 or BSA inhibited binding of apoE2 to activated CR3, indicating competitive binding between apoE2 and FH to CR3. We also observed that FH forms stable complement-resistant oligomers with apoE2-amyloid-β (1–42) complexes but not with the apoE4-amyloid-β (1–42) complexes. Flow cytometry and transcriptomic analysis revealed that apoE and FH reduced phagocytosis of amyloid-β (1–42) by microglial cells, which increased expression of microglial genes related to amyloid-β clearance. These results show that binding between FH and apoE is isotype-specific suggesting that this interaction reduces neurotoxic effects of amyloid-β (1–42), thus revealing novel roles for FH and apoE interaction in the pathogenesis of AD.

Complement Crosstalk

152

Role of the complement protein C1q in the regulation of hyaluronic acid cleavage in malignant pleural mesothelioma

Andrea Baldui1, Romana Vidergar1, Paola Zacchi1, Alessandro Mangogna, Chiara Agostinis2, Micaela Grandolfo3, Fabrizio Zanconati4, Marco Confalonieri, Roberta Bulla1

1 Department of Life Sciences, University of Trieste, Trieste, Italy
2 Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy
3 International School for Advanced Studies (SISSA), Trieste, Italy
4 Department of Medical, Surgical and Health Science, University of Trieste, Trieste, Italy

Background: The component of the classical pathway C1q and hyaluronic acid (HA) play a pivotal role in malignant pleural mesothelioma(MPM) tumour microenvironment and their interaction has been demonstrated to favour pro-tumorigenic behaviours of MPM cells by enhancing adhesion, migration and proliferation (Agostinis et al., 2017), as well as by upregulating the hyaluronan synthase HAS3 (Vidergar et al., 2021), increasing the production of short pro-invasive and pro-metastatic HA fragments. Here, we aimed to determine whether HA-bound C1q can exert its modulation also on hyaluronidase (HYAL1, HYAL2, HYAL3) expression, the enzymes responsible for HA degradation, and which receptors may be involved in this process.

Methods: Real-time qPCR, flow cytometry and Western blot analysis were performed on primary MPM cells to evaluate HYAL expression, upon treatment with C1q-HA matrix. Enzyme distribution was inquired by immunofluorescence, surface biotinylation assay and proximity ligation assay (PLA). The bioinformatics tool GEPIA was used for TCGA-based survival analysis.

Results: Real-time qPCR in MPM cells highlighted a downregulation of HYAL1 and an upregulation of HYAL2 expression, upon treatment with C1q-HA matrix. Enzyme distribution was inquired by immunofluorescence, surface biotinylation assay and proximity ligation assay (PLA). The bioinformatics tool GEPIA was used for TCGA-based survival analysis.

Conclusion: The study provides novel insights on complement activation and regulation in space and time against malaria infection.

References


Conclusion: C1q-HA interaction can act as a signaling complex by enhancing HYAL2 expression, suggesting a consequent higher rate of HA catabolism and the release of shorter HA fragments, confirming an overall pro-tumorigenic effect promoted by C1q interaction with HA. The co-localization and interaction between HYAL2 and gC1qR, being a receptor of both C1q globular head and HA, led us to hypothesize a potential involvement of gC1qR within this macromolecular complex, most likely requiring the presence of the preferential HA receptor CD44.

References

COVID-19

SARS-CoV-2 causes delayed complement activation in an ex vivo whole blood model

Martin Lo1, Alberto Ortiz2, John Lee1, Eduardo Albornoz1, Alexander Khromykh2,3, Daniel Watterson2,3, Trent Woodruff1

1 School of Biomedical Sciences, Faculty of Medicine, University of Queensland, St Lucia, Brisbane 4072, Australia
2 School of Chemistry and Molecular Biology, University of Queensland, St Lucia, Brisbane 4072, Australia
3 Australian Infectious Diseases Research Centre, Global Virus Network Centre of Excellence, Brisbane, Australia

Background: In severe cases, COVID-19 is associated with a hyperinflammatory response that manifests as an acute respiratory distress syndrome. Emerging evidence suggests that the complement system plays a key role in this condition, but the relationship between complement and SARS-CoV-2 remains incompletely understood. Herein, we show that SARS-CoV-2 directly activates the complement system in human blood in a uniquely delayed fashion.

Materials and methods: Whole blood was anti-coagulated with lepirudin and inoculated with SARS-CoV-2 at MOI 0.1 and 1.0 or LPS. Plasma samples were collected at 30 min and 24 h, which were then analysed for C5a production with an ELISA. Whole blood was also processed for flow cytometry at 3 and 24 h, in which samples were stained for granulocyte and monocyte lineage markers and complement receptors C5aR1 and CR3, as a functional measure of complement activation. Samples pre-treated with complement inhibitors for C3, C5, and C5aR1 were also assayed to confirm these effects.

Results: Compared to LPS, SARS-CoV-2 inoculation of whole blood caused a relatively small increase in plasma C5a levels (5–10 ng/ml) at 30 min and a modest increase in plasma C5a levels (10–25 ng/ml) after 24 h. C5aR1 engagement on granulocytes and monocytes, as determined by C5aR1 cell-surface internalisation and CR3 upregulation, was only detectable after 24 h post-SARS-CoV-2 inoculation. Pre-treatment with C3, C5, and C5aR1 inhibitors blocked these effects.

Conclusion: Herein we show that SARS-CoV-2 causes a delayed form of complement activation in an ex vivo whole blood model, which is consistent with the protracted clinical progression and complement activation that is seen in COVID-19. However, the viral doses that were required for complement activation are most consistent with the viral titres found in sputum and bronchoalveolar lavage fluid and not blood from patients with severe COVID-19. Thus, SARS-CoV-2 is most likely a tissue-based complement activator in vivo, especially given that other studies have shown its capacity to drive local complement production and activation with deleterious effects. This may also explain the limited ability of parenterally administered anti-complement drugs to curtail severe COVID-19 and suggests that strategies that provide localised and tissue-based complement inhibition are required to improve efficacy.