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 apoE**2**-amyloid-β (1-42) complexes but not with the apoE**4**-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 in Infectious Diseases

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Role of pentraxin 3 and interaction with complement in immune defence against opportunistic infections

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Background: The Long pentraxin 3, PTX3, an octameric protein, is part of the humoral arm of innate immunity (Garlanda et al., 2013; Haapasalo and Meri, 2019). PTX3 is a soluble pattern recognition molecule that binds to Fc receptors and phagocytes, promoting phagocytosis and acting as an ancestral antibody. Its functions include regulating inflammatory responses and tissue remodeling, and modulating complement-dependent inflammation. The complement system confers protection for malaria patients acting in innate and acquired immunity. Complement binding and opsonization impedes invasion, and mediates lysis and phagocytosis, while its regulation prevents excessive hemolysis and inflammatory responses. However, the exact mechanism on how it balances protection, regulation, variations and the parasite's survival tactics remains to be a myth. PTX3 is known to modulate C1q, Factor H and ficolin-1 binding. On the other hand, elevated PTX3 level is associated with more severe malaria.

Methods: *Plasmodium falciparum* 3D7 human malaria strain was the experimental model. The study compared the deposition of PTX3 and different complement proteins/complex on iRBCs versus normal RBCs, using techniques including immunofluorescence, Western blot, electron microscopy and flow cytometry, to provide novel perspectives on the interplay between PTX3 and complement on combating malaria.

Results: The results confirmed C1q, C3 and C5b-9 depositions on free merozoites and iRBCs. During parasite cycle, complement proteins (e.g. C1q) first appeared associated to the apicoplast structure, then were distributed step-by-step onto parasite plasma membrane (PPM), parasitophorous vacuolar membrane (PVN), tubulovesicular network (TVN), and eventually to the RBC membrane. MAC was activated, despite the presence of the regulatory CD59 or Factor H, and treatment with normal human serum led to a reduction of knob-like structures, rich in parasitic proteins such as PfEMP1, central for increased cytoadherence. PTX3 was found associated with RBC membrane, suggesting it may play regulatory roles during the process.

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

References

Garlanda, C., Bottazzi, B., Magrini, E., Inforzato, A., Mantovani, A., 2018. PTX3, a Humoral pattern recognition molecule, in innate immunity, tissue repair, and cancer. Physiol. Rev. 98 (2), 623–639, doi: 10.1152/physrev.00016.2017.

Haapasalo, K., Meri, S., 2019. Regulation of the complement system by pentraxins. Front. Immunol. 10, 1750, doi: 10.3389/fimmu.2019.01750.

Complement Crosstalk

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

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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, as compared to HA alone, then confirmed at protein level by Western blot and flow cytometry. No significant modulation was observed on HYAL3. Since only HYAL2 mRNA expression levels found a correlation with MPM patient survival by bioinformatics analysis, being associated with poorer outcome, we proceeded with the characterization of HYAL2 distribution and potential interaction with HA or C1q receptors to better understand their interplay: we detected a striking overlap between HYAL2 and globular C1q receptor (gC1qR) localization both at the cell membrane and intracellularly, by immunofluorescence co-labelling, surface biotinylation assay and PLA. PLA confirmed also HYAL2-CD44 interaction.

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

Agostinis, C., Vidergar, R., Belmonte, B., et al., 2017. Complement protein C1q binds to hyaluronic acid in the malignant pleural mesothelioma microenvironment and promotes tumor growth. Front. Immunol. 8, 1559, doi: 10.3389/fimmu.2017.01559.

Vidergar, R., Balduit, A., Zacchi, P., et al., 2021. C1q-HA matrix regulates the local synthesis of hyaluronan in malignant pleural mesothelioma by modulating HAS3 expression. Cancers (Basel) 13 (3), 416, doi:10.3390/cancers13030416.

COVID-19

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

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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.