9.03 Inhibition of C3d signalling downregulates NLRP3 inflammasome activation and IL-1β production in monocytes of individuals with alpha-1 antitrypsin deficiency

Debananda Gogoi[1], Michelle Casey[1,2], Azeez Yusuf[1], Daniel Fraughen[1], Malcolm Herron[1], Lameese Alhaddah[1], Tomás P. Carroll[1], Noel G. McElvaney[1,2] & Emer P. Reeves[1]

[1] Royal College of Surgeons in Ireland, Dublin,[2] Cystic Fibrosis Unit, Beaumont Hospital, Dublin

Alpha-1 antitrypsin (AAT) deficiency (AATD) is characterised by sustained inflammation. Elevated levels of the complement activation product C3d were previously detected in plasma and airway samples, and correlated with airway obstruction. The aim of this study was to investigate C3d as a trigger of monocyte activation that drives inflammation in AATD.Blood was collected from patients with AATD (n=9, mean FEV1: 45.16 % ± 22.88) or healthy control donors (HC) (n=16). Purified monocytes were challenged with C3d and key markers of monocyte activation, including phosphorylated p85, AKT, as well as caspase-1 and NLRP3 required for IL-1β activation, were measured by qPCR, ELISA or western blot analyses. In AATD, increased monocyte membrane expression levels of C3d (P=0.006) and cognate receptor CR3 (P=0.003), triggers IL-1β secretion (P<0.001). Mechanistically, C3d, but not C5, increased IL-1β expression and secretion through the PI3/AKT/NF-κβ pathway, activating the NLRP3 inflammasome (P<0.001). In corroboration, AATD monocytes demonstrated increased cytosolic calcium levels (P<0.001) and caspase-1 activity (P<0.0001), effecting increased plasma IL-1β levels (P<0.001). *In vitro*, exogenous, glycosylated AAT, binds C3d (P=0.0008) and modulates inflammasome activation (P=0.001).These results demonstrate that the C3d:CR3-inflammasome axis may represent a key target in regulating the inflammatory response in AATD monocytes, and is significantly downregulated by exogenous AAT. This is of particular interest as intravenous AAT to treat AATD-related lung disease, remains unavailable for people with AATD. This project was funded by the US Alpha-1 Foundation (Grant # 615848) and HRB/HRCI Ireland (Grant # HRB /MRCG-2018-04).

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