IMMUNOLOGY

TIME: 15.25 – 16.05

LOCATION: BERWICK ROOM

MECHANISMS OF MACROPHAGE SECRETION OF SIRT2, A NOVEL CANDIDATE BIOMARKER FOR PERIODONTITIS.

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OBJECTIVES: SIRT2 is an NAD-dependent deacetylase that removes acetyl groups from histones and from other proteins such as α-tubulin and NF-κβ. SIRT2 is strongly linked to cellular catabolism and potentially links metabolism, inflammation and susceptibility to chronic disorders such as periodontitis. We have determined that SIRT2 is elevated in the saliva of patients with periodontitis, but there is currently no published information regarding how this may link to pathogenesis. Our aim is to investigate the relationship of TLR2 signalling to SIRT2 secretion and to substantiate this novel proinflammatory pathway.
METHODS: qPCR and western blotting were used to analyse *SIRT2* mRNA and protein expression in THP1 monocyte-derived macrophages at basal levels and after stimulation with toll-like receptor (TLR) 2 and 4 agonists. ELISA analysis of culture supernatants of macrophages stimulated with TLR 2 and 4 agonists was used to quantify extracellular SIRT2.
RESULTS: We found no evidence that that *SIRT2* is regulated at the mRNA or protein level by TLR2 or TLR4 signalling. We have identified a novel pathway which leads to secretion of SIRT2 from macrophages stimulated with the TLR2 agonists Pam2CSK4 and lipoteichoic acid, but not with the TLR4 agonist lipopolysaccharide, despite all 3 molecules eliciting a powerful pro-inflammatory response in macrophages.
CONCLUSIONS: SIRT2 is secreted from THP1 monocyte-derived macrophages in response to activation of TLR2, but not TLR4. This finding may represent a novel pathway relevant to periodontitis and provides a possible pathway to explain the elevated levels of SIRT2 in the saliva of patients with periodontitis.

THE ROLE OF DNA DAMAGE IN THE FORMATION OF LANGHANS-TYPE MULTINUCLEATED GIANT CELLS

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Background: Giant cell arteritis (GCA) is distinguished histologically by the presence of Langhans-type multinucleated giant cells (LMGCs). Characterisation of these cells is primarily morphological and their origin and role in disease remains unclear. Investigating how they develop may help us understand the pathogenesis of GCA and distinguish patients with GCA from those with other forms of inflammation.
Methods: Human CD14+ monocytes were isolated from healthy donor peripheral blood and cultured in GM-CSF and IFNγ. Morphology was assessed by confocal microscopy. LMGCs were identified using DRAQ5 or DAPI. Time-lapse confocal microscopy was performed with SiR-DNA. Sequencing libraries were generated using SmartSeq2.
Results: Time-lapse microscopy revealed multinucleation occurred in this in vitro model through failed cytokinesis. Immunofluorescence and electron microscopy confirmed this with chromatin bridges linking nuclei in large LMGCs but absent in osteoclasts. Electron microscopy identified multiple chromatin bridges in GCA-affected tissue LMGCs. Single cell RNA sequencing of in vitro LMGCs revealed enrichment of gene modules associated with autophagy, inhibition of cell cycle progression and DNA damage response.
Conclusion: These data suggest LMGCs form in conditions of cellular stress with DNA damage

inhibiting proliferation. The effect of DNA damage occurring in-situ in GCA affected arteries is unclear and needs further investigation.

MAPPING THE ROAD TO PERIPHERAL TOLERANCE

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Background: Dendritic cells (DCs) are responsible for eliciting and determining the fate of T cell responses. Manipulating DCs to induce regulatory T cells (Tregs) and re-establish tolerance where there has been a breach in regulation has provided an attractive avenue for cellular therapies. A variety of protocols detailing in-vitro generation of tolerogenic DCs (tolDCs) are available, however methodological differences have resulted in heterogeneity between tolDC products; which will affect which regulatory pathways are deployed. Further investigation to establish which aspects of tolDC function induce which Treg pathway is required. To this aim, we have compared different methods of tolDC generation; building a referencing tool mapping specific phenotypical changes to functionally distinct Treg subtypes.
Methods: Monocyte derived dendritic cells (moDC) from healthy donors were tolerised with the addition of Vitamin D_3 alone, Dexamethasone alone or a combination of both. After 7 days, moDCs were assessed morphologically (light microscopy), harvested and phenotypically analysed using an optimised flow cytometry panel.
Results: Preliminary data shows changes in the expression of key functional molecules such as PDL-1 and ILT-3 as well as changes in maturation state, differentiation and morphology of differentially generated moDCs. Moreover, novel findings confirming surface expression of CD_{32} and chemokine receptor $ChemR_{23}$ on tolDCs generated with dexamethasone provide new markers to distinguish tolDC types.
Conclusion: The differences identified highlight substantial heterogeneity between the three tolDC types investigated thus far; warranting further examination to establish a library of tolDC types and whether their defining characteristics translate to induction of distinct Treg subtypes.

A NOVEL ROLE FOR C1Q UPON BINDING TO C1Q-FIXING ANTIBODY ON ENDOTHELIAL CELL ACTIVATION

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Objectives: Donor specific antibodies (DSAs) are increasingly recognised as important mediators of allograft injury. Despite increasing interest in complement-fixing DSAs, the contribution of complement in inducing allograft inflammation in the presence of DSAs is not fully understood. We have investigated the role of C1q in inducing cell activation on endothelial cells in the presence of DSAs.
Methods: Human microvascular endothelial cells (HMEC-1) were stimulated with either anti-human leukocyte antigen (HLA) class I antibody (W6/32) in the form of chimeric IgG1 and IgG_4 or DSAs from sensitised patients in the presence and absence of C1q. The activation of cell signalling, expression of adhesion molecules, cytokine secretion, and monocytes adhesion were examined using Western Blotting, flow cytometry, ELISA, and static based adhesion assay, respectively.
Results: HMEC-1 stimulated either with anti-HLA class I antibodies (chimeric IgG1 and IgG_4) or DSAs from sensitised patients showed significant HMEC-1 activation, including expression of ICAM-1, phosphorylation of Akt and ERK, IL-8 secretion, and monocytes adhesion compared to control. Chimeric IgG1 and DSAs bound C1q. Moreover, incubation of HMEC-1 with either chimeric IgG1 or DSAs and C1q led to significantly greater HMEC-1 activation compared to either chimeric IgG1 or DSAs alone. The presence of C1q did not increase the effect of IgG_4 (non-C1q binding) on HMEC-1.
Conclusion: We describe a novel mechanism by which complement can contribute to antibody mediated rejection. Clustering of HLA by antibody and further by C1q lowers the amount of antibody required to activate endothelial cells, increasing their potential to mediate graft injury.