JSLE and the NLRP3 Inflammasome – A Novel Therapeutic Target

J. Gamble 1 M.W Beresford 1,2

1. Department of Women’s and Children’s Health, Institute of Translational Medicine, University of Liverpool, Liverpool, UK

2. Department of Paediatric Rheumatology, Alder Hey Children’s NHS Foundation Trust, Liverpool, UK

Introduction:

Juvenile-onset Systemic Lupus Erythematosus (JSLE) is a severe autoimmune disease causing organ damage and long-term morbidity. Impaired clearance of nuclear debris from cells and upregulated circulating damage-associated molecular patterns (DAMPs) and cytokines are thought to trigger cell death and thus, further increase release of pro-inflammatory molecules following the cytosolic assembly of inflammasomes.

Objectives:

To investigate the role of pyroptotic cell death in JSLE, which occurs following the assembly and activation of the NLRP3 inflammasome, which may be a promising target in the treatment of JSLE and other inflammatory diseases.

Methods:

THP-1 cell line-derived macrophages (Mφs) were primed using lipopolysaccharide (LPS; 10ng/ml) and interferon (IFN) γ (20ng/ml) in complete media at 37°C for 24 hours, and subsequently treated with 10mM Adenosine triphosphate (ATP) for 30 min. Primed Mφs not treated with ATP were used as a negative control. Some Mφs were incubated with 10% JSLE patient sera or NETosis-derived material (10ng/ml) from PMA-treated neutrophils. Primed Mφs were tested for cell surface markers, HLA-DR, CD282 (TLR2), and CD68 using flow cytometry. ATP-treated Mφs were collected and either assayed for pyroptosis marker, lactate dehydrogenase (LDH) activity, cleaved caspase-1 with immunofluorescence (IF), or lysed for cleaved caspase-1 using western blotting.

Results:

Primed Mφs showed an M1 phenotype with geometric means (+/-SEM) of HLA-DR, TLR2, and CD68 expression respectively of: 1177+/-±1.15, 613 (+/-0.9), and 1549 (+/-0.9) gMFI, respectively compared with un-primed Mφs of 597 (+/-1.0), 122 (+/-0.88), and 1225 (+/-0.9) gMFI, respectively (n=3; p<0.05). ATP-treated Mφs showed increased LDH activity compared to controls (3.4x10-3 (+/-0.12x10-3) compared to 0.00 milliunits/mL, respectively; n=3; p<0.05). Furthermore, a greater increase in LDH activity was observed in Mφs that were incubated with JSLE serum and NET material; 3.7x10-3 (+/-0.5x10-3) and 7.6x10-3 (+/-0.5x10-3) compared to 0.00 milliunits/mL, respectively; n=3-6; p<0.05). IF was positive for cleaved caspase-1 in ATP treated Mφs; and this was confirmed in lysed cells, using western blotting.

Conclusion:

Overall, the results indicate that Mφs undergo pyroptosis via the NLRP3 inflammasome when challenged with a two-signal approach of priming and ATP, and that cytokines, nuclear debris and DAMPs may not only trigger, but amplify the inflammatory response of this pathway. The NLRP3 inflammasome is thought to be an important mediator in the pathogenesis of certain inflammatory diseases, and flare episodes associated with JSLE. Further work is planned to investigate the role of the inflammasome in JSLE, using pharmacological interventions with specific known and novel inhibitors of the NLRP3 inflammasome. This work could prove the NLRP3 inflammasome to be a promising target for future therapy for JSLE.