**Investigating the neutrophil-macrophage interplay in juvenile lupus nephritis pathogenesis**

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**Background:** Juvenile systemic lupus erythematosus (JSLE) is a multifactorial systemic autoimmune disease that can cause severe kidney damage. In JSLE increased neutrophil apoptosis and NETosis combined with impaired phagocytosis1,2 result in prolonged autoantigen exposure and enhanced autoimmunity. We hypothesized that in lupus nephritis (LN) apoptotic and/or NETotic neutrophils infiltrate the kidneys in response to pro-inflammatory stimuli. Monocytes are then recruited to the kidney where they induce native kidney cell damage leading to proteinuria and potentiated inflammation. Studies have shown that monocytes isolated from SLE patients produce high levels of IL-1Ra and IL-1β has been detected in the glomerular biopsies from LN patients3. Macrophages may significantly contribute to LN development by regulating the local IL-1β/IL-1Ra balance in the kidney.

**Objectives:** To investigate the mechanisms and outcomes of macrophage activation by viable and apoptotic neutrophils and determine how this affects the secretion of pro-inflammatory IL-1β and anti-inflammatory IL-1Ra.

**Methods:** Paired monocytes and neutrophils were isolated from healthy adult donors (n=7), neutrophil culture medium was collected at 2h and 24h after incubation at 37°C. Monocytes were cultured for 6 days in RPMI (+10% FCS + 50ng/mL M-CSF) and their differentiation into macrophages was determined by light microscopy. Macrophages were then treated for 6h with supernatants from the 2h viable (mean apoptosis - 9.5%) and 24h apoptotic neutrophils (mean apoptosis - 70%), with LPS as a positive control. Following 6h the macrophage culture media were collected and IL-1β/IL-1Ra were assessed via ELISA.

**Results:** IL-1β levels did not significantly differ within treatment groups with levels being below the lower limit of detection for most samples. IL-1Ra expression however was significantly increased in LPS-treated macrophages (4374pg/ml ± 910.3, P = 0.0012) compared to control (845.6pg/ml ± 240) while no significant difference was seen with 2h (1379pg/ml ± 344.4) or 24h (1696pg/ml ± 371.1) neutrophil culture medium.

**Conclusions:** Macrophages treated with culture medium from both apoptotic and viable neutrophils produce high levels of IL-1Ra, however this does not differ from levels seen in control macrophages. IL-1β levels are below the level of detection under all conditions. This suggests that secreted factors from apoptotic neutrophils do not induce an IL-1 response in macrophages but does not exclude a role for direct cell-cell contact. Other immune cells may also be playing a role in promoting renal cell damage through IL-1β secretion.

1Ren, Y. et al. Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. Arthritis and Rheumatism 48, 2888–2897 (2003).

2Suzuki, H., et al. Interleukin-1 receptor antagonist in patients with active systemic lupus-erythematosus-enhanced production by monocytes and correlation with disease-activity. Arthritis and Rheumatism 38, 1055–1059 (1995).

3Takemura, T. et al. Cellular-localization of inflammatory cytokines in human glomerulonephritis. Virchows Archiv – An International Journal Of Pathology 424, 459–464 (1994).