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**INTERLEUKIN-1 ALPHA ACTIVITY IN NECROTIC
ENDOTHELIAL CELLS IS DYNAMICALLY CONTROLLED
BY INTRACELLULAR INTERLEUKIN-1 RECEPTOR 2**L Burzynski, M Humphry, M Bennett, M Clarke *University of Cambridge*

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Inflammation is a key driver of both atherosclerosis and graft rejection. Interleukin-1 alpha (IL-1 α), a powerful cytokine that activates both innate and adaptive immunity, induces vessel inflammation after release from necrotic vascular smooth muscle cells (VSMCs). Similarly, IL-1 α released from damaged endothelial cells (ECs) during vessel transplant drives allogeneic graft rejection. Therefore it is important to understand how IL-1 α activity is controlled after EC necrosis, and how this affects atherosclerosis and graft rejection.

We investigated IL-1 α activity in control and TNF α or IL-1 β stimulated ECs. Necrotic ECs have much lower IL-1 α activity than VSMCs, but show comparable levels to other cell-types, even though they contain a tenth of the IL-1 α . Following TNF α or IL-1 β stimulation IL-1 α activity in necrotic ECs is increased up to 8-fold without alteration to IL-1 α protein level. Together, these data imply that IL-1 α activity is controlled independently of protein level in necrotic ECs.

Immunofluorescence and proximity ligation assays show a cytosolic association between IL-1 α and IL-1R2, which is known to inhibit IL-1 α activity. Following TNF α or IL-1 β stimulation necrotic EC lysates contain more calpain cleaved IL-1 α , which shows increased activity. In addition, IL-1R2 in stimulated lysates is less able to protect exogenously added pro-IL-1 α , suggesting TNF α or IL-1 β dissociates IL-1 α from IL-1R2, subsequently allowing calpain to cleave and increase IL-1 α activity upon necrosis.

We conclude that necrotic EC-derived IL-1 α is regulated by binding to cytosolic IL-1R2, and that TNF α and IL-1 modulate this protective mechanism to license IL-1 α after necrosis. These and previous data suggest that necrotic ECs may play an important role in vessel wall inflammation during graft rejection, and may also drive atherosclerosis.