173 **INTERLEUKIN-1 ALPHA ACTIVITY IN NECROTIC ENDOTHELIAL CELLS IS DYNAMICALLY CONTROLLED BY INTRACELLULAR INTERLEUKIN-1 RECEPTOR 2**

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doi:10.1136/heartjnl-2013-304019.173

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

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

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

We conclude that necrotic EC-derived IL-1α is regulated by binding to cytosolic IL-1R2, and that TNFα and IL-1 modulate this protective mechanism to license IL-1α 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.