

Department of Chemistry and Molecular Biology
Biochemistry Candidate Seminar
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“TNF-induced activation of a novel Nox1 NADPH Oxidase complex and its role in the induction of necrotic cell death”

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Abstract

Tumor necrosis factor (TNF α) is a crucial cytokine involved in immunity and inflammation, as well as other biological processes. The main receptor for TNF α , TNF-R1, is ubiquitously expressed and thus influences signaling in most cell types, though the downstream effects of TNF α may be cell type-specific. TNF α highly influences the production of superoxide in monocytes, macrophages, and neutrophils, where it is important in the immune response to pathogens. In contrast to these cell types, where TNF α primes the activation of NOX2, we have discovered that TNF α activates the NOX1 NADPH oxidase in L929 cells and p65 $^{-/-}$ MEFs through a more direct mechanism. Upon TNF α treatment, a novel signaling complex is formed that contains the TNF Receptor adapter proteins TRADD and RIP, along with NOX1 and Rac1. TRADD and RIP bind to the NOX organizer protein 1 (NOXO1), providing a potential mechanism for Nox1 activation. In support of the potential roles for TRADD and RIP in oxidase activation, we have shown that a polyproline region in TRADD is required for the TRADD-NOXO1 interaction, and mutation of this region creates a dominant-negative molecule with respect to superoxide generation. In addition, NOX1 is not recruited to the complex in RIP-deficient MEFs, suggesting RIP is essential for complex formation. Dominant negative TRADD and RAC1 molecules, as well as knockdown of NOX1 using siRNA, inhibit superoxide formation and cell death under caspase inhibitory conditions. Thus, the formation of the TNF α -induced Nox1 complex contributes to its ability to induce necrotic cell death, and may therefore play a role in the clearance of infected cells from the body.