

Genome wide RNAi screen for components of an innate immune response

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The events required to initiate host defenses involve a complex signaling cascade involving numerous adaptor molecules, kinases, and transcriptional elements. Identification of factors involved in the regulation of an inflammatory response has been greatly aided through gene disruption techniques. To further our understanding of the signaling pathways involved in the initiation of a proinflammatory response, we have taken a forward genetics approach using a completely random short hairpin RNA (shRNA) expression library. The shRNA expression library is based on the human U6 small nuclear RNA promoter to transcribe a random shRNA sequence. The induction of TNF- α production was used as a readout to screen for genes necessary to initiate a proinflammatory response. We have developed a reporter system in which the TNF- α promoter drives the expression of diphtheria toxin A (DT-A). Stable expression of the construct into the RAW264.7 macrophage-like cell line allowed negative selection of TNF- α expressing clones when stimulated with the appropriate ligands. For example, stimulation of with the TLR4 ligand, lipopolysaccharide, resulted in cell death in a dose dependent manner. As a proof-of-principle we have inhibited MyD88, an adaptor molecule critical for LPS induced TNF- α production, with a specific shRNA sequence. Inhibition of MyD88 gene expression results in rescue from LPS induced cytotoxicity, whereas a nonspecific shRNA sequence was ineffective. Large scale screening of the shRNA library is currently underway. It is expected that cells expressing a shRNA sequence complementary to genes necessary for TNF- α production will be positively selected, as DT-A expression will be inhibited and cells will survive. Retrieval of the shRNA sequence from the genomic DNA of the surviving clones will allow us to identify the genes associated with the initiation of a proinflammatory response.