A unique mode of immune response activation by the extracellular matrix: how tenascin-C activates toll-like receptor 4.

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Introduction

Extracellular matrix (ECM) molecules generated upon tissue damage can directly promote an immune response via the activation of toll-like receptors (TLRs). Tenascin-C, an ECM glycoprotein transiently induced upon injury and persistently expressed during chronic inflammatory diseases, stimulates cytokine synthesis via activation of TLR4 by its C-terminus fibrinogen-like globe (FBG-C) domain. However, how FBG-C binds to and activates TLR4 is not known. Unlike tenascin-C, other tenascin family members (tenascin-R, -W and –X) are constitutively expressed in adults but also contain a highly homologous FBG domain. Comparative analysis of the tenascin family FBG domains was used to elucidate the molecular mechanisms by which matrix molecules activate innate immune responses.

Materials and Methods

The ability of the tenascin FBG domains to activate NF-κB in ThP1 cells and cytokine synthesis in primary human macrophages was assessed. Direct binding to TLR4 was evaluated *in vitro* using a solid phase binding assay. Peptide mapping and site-directed mutagenesis were used to elucidate specific epitopes within FBG-C involved in binding and activation of TLR4. Molecular modelling and docking simulations were performed to analyse the structural basis of the FBG-TLR4 interaction.

Results

In addition to FBG-C, we found that FBG-R and -W can activate TLR4, where FBG–X cannot. We identified positively charged residues in one site of the FBG domains necessary for receptor activation and two other regions involved in stabilizing FBG-TLR4 binding. These residues were conserved in the active tenascin FBG domains but not present in the inactive FBG-X. Introducing these sites in FBG-X created a TLR4 activator that could bind to the receptor and induce an inflammatory response.

Discussion

This is the first report showing how ECM proteins can activate TLR4. Our data indicates a different mechanism of receptor activation compared to that described for pathogenic TLR4 stimuli. Together these data reveal how endogenous molecules can induce an inflammatory response and may help in the design of specific inhibitors of FBG domain containing TLR stimuli.