ImmunoTools *special* Award 2014

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Determine if altered TLR4 signaling strength modulates development of AAM in human monocyted-derived macrophages (MDM)

Respiratory Syncytial Virus (RSV) is the most significant cause of serious lower respiratory tract infections in children <3 years old, for which there is no vaccine. Infants and young children who contract severe RSV disease often develop asthma later in life. The RSV fusion (F) protein mediates RSV entry into cells, and is the target of Synagis™, a monoclonal antibody administered prophylactically to infants at high risk for RSV infection in the USA and Western Europe.

Macrophages are abundant in the lungs and airways, and are among the first cells exposed to both viruses and aeroallergens. Macrophages respond to environmental cues to adopt different phenotypes that, in turn, promote different immune environments. Strong inflammatory stimuli, *e.g.*, IFN-γ and bacterial lipopolysaccharide (LPS), result in highly microbicidal, classically activated macrophages (CAM) that produce a highly proinflammatory cytokine profile. Conversely, exposure to IL-4 or IL-13 leads to differentiation into alternatively activated macrophages (AAM) that counter the strong proinflammatory CAMs and are associated with wound healing and allergic responses. We previously reported that AAM mediate resolution of RSV-induced lung pathology, and that this mechanism was IL-4Rα-, TLR4-, and IFN-β-dependent.

Despite these advances, the cellular mechanism(s) underlying development of asthma after RSV infection is/are still not well-understood, yet both TLR4 signaling and AAM have been implicated. A low, but not high, dose of LPS is required to sensitize mice to ovalbumin (OVA)-induced asthma, consistent with the observation that in farming communities, exposure to high LPS levels correlated with reduced asthma/atopy. TLR4 expression in mice is required for allergic lung inflammation using OVA or house dust mite (HDM) allergen. Our own work indicates that AAM differentiation induced by RSV is TLR4-dependent, and that RSV F protein is a weak TLR4 agonist compared to LPS. The inheritance of the TLR4 $Asp^{299}Gly$ single nucleotide polymorphism (SNP), that has been shown to respond more weakly to TLR4 agonists than wild-type TLR4, is associated with severe RSV. We hypothesize

that this TLR4 SNP may weaken TLR4 dimer interactions and thereby reduce signaling. This hypothesis is supported by the observation that LPS-induced recruitment of MyD88 and TRIF adapters to the human TLR4 receptor complex was decreased in cells expressing human $Asp^{299}Gly$ TLR4, resulting in a significant inhibition of LPS-induced TNFα and a reduced, but significant, level of IFN-β. This suggests that the $Asp^{299}Gly$ SNP, through weakened TLR4 signaling, alters the balance of cytokines produced by TLR4-mediated MyD88- and TRIF-dependent signaling pathways. Of note, two cytokines known to favor Th2 differentiation, IL-33 and TSLP, are IRF-3- (i.e., TRIF-) dependent. A novel hypothesis emerges from these seemingly unrelated studies: reduced TLR4 signaling strength, as seen in cells stimulated with weak TLR4 agonists, *e.g*., RSV F protein, or low-dose LPS exposure could alter the balance between the MyD88- and TRIF-dependent signaling pathways, thereby favoring a "Th2-like" allergic milieu.

The aim of this project is to determine if altered TLR4 signaling strength modulates development of AAM in human monocyte-derived macrophages (MDM). To test this, human MDM derived from elutriated monocytes of healthy, anonymous adult donors will be treated with a broad range of highly purified *E. coli* K235 LPS doses (0.05 ng/ml to 100 ng/ml) to determine if varying the stimulating dose of LPS alters the ratio of MyD88 to TRIF signaling. We will also test the purified RSV F protein, which is a weak TLR4 agonist. RNA from cell cultures will be analyzed by quantitative real-time PCR for gene expression associated with both CAM and AAMs. In addition, ELISAs for cytokine production will be carried out using ImmunoTools human ELISA sets (IFN-γ, IL-4, IL-6, IL-10, TNFα, TSLP, IL-12 p40 total, and IL-12 p40 differential). MDMs will also be analyzed by flow cytometry for expression of CD14 and CD11b, markers for mature human macrophages, as well as specific AAM markers (*e.g.,* MRC1, Alox15, YKL-40), again using ImmunoTools reagents. At the conclusion of this study, we expect to have determined if decreased TLR4 signaling strength alters the balance of cytokines such that AAM differentiation is favored. Having a better understanding of the role that TLR4 signaling strength plays in the development of AAM could provide a window of opportunity to therapeutically manipulate macrophages to improve the resolution phase of RSV, while mitigating long-term effects of AAM on the development of asthma.