A Biomimetic Membrane System to Dissect the Role of TNF-α in Inflammatory Disease

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The tumor necrosis factor alpha (TNF-α) and its receptor play a critical role in the pathogenesis of autoimmune and/or systemic inflammatory diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis, and Chron's disease. As a result, TNF-α blockade is currently being explored as a treatment strategy to alter the course of these disease states. Despite some promising results, there is considerable variation in regards to efficacy and side effects. For example, only 60% of RA patients respond to TNF-α blockers. Likely, this variability is related to the many unanswered fundamental questions regarding the mechanism of TNF-α signaling. One of which is the relationship between the presentation of TNF-α and the corresponding cellular response. The particular questions that this experimental project tries to answer in the context of the response of RA synovial fibroblasts (Figure 1), the so-called conductors of joint destruction, are: How is cellular response such as cytokine secretion and mRNA expression dependent on the concentration of membrane bound TNF-α? How is this response dependent on the ratio of soluble to membrane bound TNF-α? What kind of dynamic reorganization does the TNF-α receptor undergo upon binding? What effects do specific kind and quantities of TNF-α blocker have on the cellular responses?

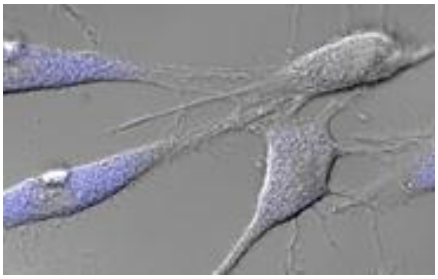

Figure 1: Confocal image of synovial fibroblasts - often called "the conductors of joint destruction" in rheumatoid arthritis.

The design of these experiments draws from the toolbox of several disciplines. For one there is the site specific lipidation of proteins recently developed by the Forstner and Bader Lab. This technique that is based on the insertion of a non-standard amino acid, allows for the creation of large membrane surfaces that present to cells proteins that are membrane bound but yet mobile within the plane of the interface (Figure 2). Since these artificial membranes are supported on planar substrates such as glass, they are ideally suited for investigations involving light based techniques such as quantitative light and fluorescence microscopy. Since this cell-artificial membrane hybrid allows to adjust the content and concentration of the artificial membrane this model for cell-cell contact is perfect for quantitative studies of cellular interactions in a controlled and quantitative way. While strong responses such as apoptosis can be directly assessed under the microscope, the more complex patterns of cytokine secretion and mRNA expression can be readily assessed using multiplex immunoassay on the Luminex Platform.

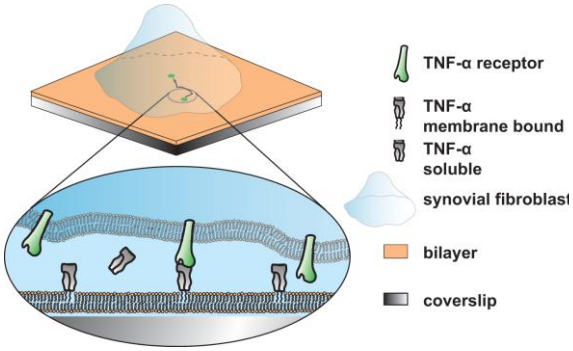

Figure 2: Schematic of the live-cell/supported membrane hybrid. Cells can be exposed to controlled amounts of surface bound and soluble TNF-α

After the successful completion of this research project several milestones and insights will have been reached. On the engineering side a new live cell-membrane hybrid for the quantitative study of RA synovial fibroblast interactions will have been developed and put to use, giving added knowledge of RA pathogenesis. We will have learned about the details of the competitive interactions of soluble and membrane bound TNF-α and the resulting cellular response. In a broad context, these insights can be expected to bring about a new understanding of the role of TNF-α in diseases that are characterized by chronic inflammation.