ImmunoTools *special* Award 2014

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The effects of extracellular calreticulin on peripheral blood immune cells

Calreticulin is an endoplasmic reticulum (ER)-resident protein that is involved in many aspects of normal cellular physiology and pathology. In the ER, calreticulin maintains calcium homeostasis and as a molecular chaperone, ensures correct folding of glycoproteins. Although typically residing in the lumen of the ER, calreticulin can also be found on the surface of cells and in the serum of both cancer and autoimmune patients.

Extracellular calreticulin affects diverse immune processes. Binding of extracellular calreticulin to mannose-binding lectin and complement C1q alters immune complex formation and regulates innate immunity. Further, surface calreticulin acts as the dominant pro-phagocytic signal in several types of human cancer. On the surface of viable cells, the pro-phagocytic signal of calreticulin is masked by its association with the ‘don’t eat me’ signal CD47. However, in apoptotic cells, calreticulin disassociates from CD47 and moves into distinct patches enriched in phosphatidylserine (PS) after which calreticulin-mediated phagocytosis can proceed. In apoptotic cells, intracellular stores of calreticulin are further co-transported to the cell surface with PS.

In recent years, extracellular calreticulin was identified as a crucial component of the so-called immunogenic cell death (ICD) pathway. ICD is a type of apoptosis in which a ‘danger signal’ appears on the surface of dying cancer cells. Consequently, cancer cells are engulfed by dendritic cells (DC) which may result in the presentation of tumor-derived peptides to T-cells and the initiation of adaptive anti-tumour T-cell response. Previously, it was demonstrated that pre-apoptotic cell surface exposure of calreticulin is critical for the pro-immunogenic uptake and processing of dying cancer cells by DCs.

Interestingly, several recent reports indicate that calreticulin has direct immunostimulatory effects on peripheral blood immune cells. Recombinant calreticulin appears to trigger maturation of DCs and to activate murine macrophages. In contrast, no direct effects of calreticulin on immune cells were observed in the ICD studies. Thus, the exact mechanism of the pro-immunogenic activity of calreticulin towards human immune cells remains unresolved.

Currently, we are investigating the effects of calreticulin on peripheral blood immune cell subsets. To this end, freshly-isolated blood cells are treated with increasing concentrations of calreticulin after which up regulation of various immune activation markers are evaluated by flow cytometry. To be able to distinguish the different immune cell populations, we will use combinations of fluorescently labelled antibodies. To study T-cell activation we will use antibodies directed against CD3, CD4, CD8, and CD25. To study activation of the various myeloid effector cells we will use antibody combinations to detect expression of CD14/HLA-DR, CD16/CD11b, CD16/CD66acde and CD56/CD25.

We will also investigate the capacity of calreticulin to induce apoptosis using Annexin-V combined with appropriate antibodies to identify the various immune cell population involved. Moreover, ELISA will be used to determine production of various cytokines including TNF-alpha.

Finally, we will perform experiments to study ICD. In short, IL-4 and GM-CSF will be used to generate dendritic cells from $CD14^+$ blood cells. After several days of culture, maturation will be assessed using fluorescently labelled antibodies directed against CD14, CD86 and HLA-DR. Mature DCs are then used to induce T-cell activation and proliferation in a co-culture to which IL-6, IL-12, IL-7, IL-2 and IL-15 are added. During the co-culture, T-cell activation will be assessed by evaluating up regulation of CD69 and CD25 in combination with antibodies directed to CD3, CD4 and CD8.

Our research efforts will lead to more insight into the mechanism via which calreticulin can activate various immune cells. This knowledge may be of use to improve cancer immunotherapy.