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Characterizing the inflammatory and immunological effects of nucleosome release from dead cells by Factor VII-activating protease

One of the key processes in the maintenance of tissue homeostasis is the removal of dying or dead cells. A range of molecular and cellular mechanisms such as phosphatidylserine exposure by cells undergoing apoptosis, opsonisation of apoptotic cells by complement components and phagocytosis of opsonized dying cells by macrophages are intricately connected and involved in executing this removal process. Insufficient clearance of apoptotic cells could lead to the formation of late apoptotic or secondary necrotic cells. These cells have lost the integrity of their plasma membrane and can leak intracellular constituents into the extracellular milieu. Some of these constituents such as dsDNA, histones, HMGB1 and heat shock proteins are known as Damage-Associated Molecular Patterns (DAMPs). DAMP's have been recognized to be potent immunostimulatory molecules and are increasingly being investigated for their roles in driving inflammation and autoimmunity. However, how these DAMPs are released into the extracellular space and how the body gets rid of them are questions that remain to be answered.

Our group has described in the past that nearly all DNA of late apoptotic cells is released in the form of nucleosomes into the extracellular space upon incubation with healthy donor plasma. The plasma serine protease Factor VII-activating protease (FSAP) has been recognized to be responsible for this release. Strengthening this observation, a monoclonal inhibitory antibody for FSAP is able to completely abrogate the release of nucleosomes by plasma, indicating FSAP is essential in this process. Furthermore, plasma-purified FSAP similarly to plasma is able to release nucleosomes. FSAP is activated in plasma upon contact with late apoptotic and necrotic cells and it's activity is regulated by the serpins C1-esterase inhibitor and alpha-2-antiplasmin. Interestingly, in inflammatory conditions such as sepsis, FSAP activation has been shown to correlate with circulating nucleosome levels as well as with severity of the disease and mortality.

As nucleosomes consist of dsDNA and histones, they could play a vital role in driving inflammation. Our research therefore focuses on the mechanism of nucleosome release by FSAP and the immunological effects this release may have during inflammation. The questions that we aim to answer in this project are:

1. Are late apoptotic cells of which nucleosomes have been released by FSAP more efficiently phagocytosed by human primary M1 and M2 macrophages?
2. Do late apoptotic cells of which nucleosomes have been released by FSAP induce different immunological responses in above-mentioned macrophages?
3. Do nucleosomes released by FSAP have different immunostimulatory capacities than nucleosomes passively leaked by late apoptotic cells?

Answering these questions will allow for a better understanding of the role of nucleosome release from dead cells by FSAP in both tissue homeostasis as well as during inflammation. Furthermore, improved understanding of the immunostimulatory potential of nucleosomes may help characterize their role in driving inflammation.

In order to answer above questions, CD14-positive human primary monocytes will be differentiated to M1 and M2 macrophages using recombinant M-CSF and GM-CSF. Subsequently, (FSAP-treated) late apoptotic Jurkat cells will be incubated with these macrophages and phagocytosis efficiency will be determined by flow cytometry using (among others) anti-CD3, anti-CD16 and anti-CD32 antibodies to distinguish between the different cell populations present. Immunological responses of these cells will be measured by flow cytometry (CD80/CD86 upregulation) and by characterizing the cytokine response (IL-6, IL-10 and TNF-alpha ELISA).