

# Annexinabhängige Modulation der Immunantwort und Hämostase

Author: Sandra Kreft

## Abstract

Most annexins bind positively charged calcium ions via type II calcium binding sites and thereby interact with negatively charged phosphatidylserine. By binding to phosphatidylserine on the cell surface of apoptotic cells the annexins may modulate the recognition and removal of such cells and alter the immune response towards dying cells. Moreover, annexins could interact with phosphatidylserine on the surface of activated thrombocytes to regulate the binding and activation of coagulation factors. Due to the high structural similarity of the twelve vertebrate annexins it is thought that several members of the protein family could fulfill redundant functions in phosphatidylserine dependent immune and coagulation reactions. However, for most members of the annexin family decisive *in vitro* and *in vivo* evidence for such functions is still pending and the molecular mechanisms of the annexin-phosphatidylserine interaction are not yet well described.

In this dissertation the essential amino acids in the type II calcium binding sites that mediate the interaction of annexins with phosphatidylserine were determined by site directed mutagenesis. Recombinant fusion proteins of different AnxA8 mutants were expressed in bacteria and purified. Their binding to phosphatidylserine was characterized in liposome sedimentation experiments and in flow cytometry assays with apoptotic cells exposing phosphatidylserine. Two aspartate residues at positions 112 and 272 located in the type II calcium binding sites were found to be essential for the interaction with phosphatidylserine. Quantitative RT-PCR demonstrated that the annexins AnxA1-AnxA7, AnxA9 and AnxA11 are expressed in professional phagocytes and that their expression is modulated upon pro- and anti-inflammatory stimulation. In exudates of chronic inflamed wounds, high amounts of an N-terminally truncated AnxA1 could be detected. However, *in vivo* no alterations in skin wound healing were detected in Anxa1- or Anxa5-deficient mice. To the contrary, the intravenous injection of AnxA5 resulted in massive bleeding of skin wounds.

*In vitro* coagulation experiments in the presence of AnxA1, AnxA3, AnxA4, AnxA5 and the phosphatidylserine binding AnxA8 mutant demonstrated that the annexins can inhibit the intrinsic and extrinsic coagulation pathways as well as thrombin generation. In contrast, AnxA8, which does not bind to phosphatidylserine, could not inhibit coagulation. Hence, binding of annexins to phosphatidylserine is needed to modulate coagulation but additional mechanisms like multimerization and phosphatidylserine independent interactions may account for the inhibitory effect of the annexins on coagulation. *In vivo* murine thrombosis triggered by damage to mesenteric arteries by ferric chloride revealed a strong accumulation of injected fluorescently labeled AnxA5 in the area of damaged vessel walls, whereas AnxA5

could not modulate the blood cell extravasation, inflammation, kidney damage or thrombocyte accumulation upon LPS-induced sepsis *in vivo*.

In conclusion, annexins are expressed in professional phagocytes and specific amino acids were identified that mediate the binding of annexins to phosphatidylserine containing liposomes and phosphatidylserine exposing apoptotic cells. This binding can inhibit the phagocytosis of cells exposing phosphatidylserine and in addition delays phosphatidylserine dependent coagulation processes. The results point to novel aspects of phosphatidylserine dependent annexin functions within the immune system and hemostasis.