

Calpains and microparticles : from sepsis to angiogenesis

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Calpains and inflammation

Calpains are calcium-activated neutral cysteine proteases [1]. Two ubiquitously expressed isoforms, calpain μ and m , are activated by micromolar and millimolar calcium concentrations, respectively, whereas other isoforms have tissue-specific distributions. Calpastatin is a specific endogenous inhibitor of calpain activity through four equivalent inhibitory domains [2]. First, calpains play an important role in inflammatory process. They are involved in the activation of nuclear factor (NF)- κ B, and thereby in the NF- κ B dependent expression of proinflammatory cytokines and adhesion molecules [3]. Calpains are critical for inflammatory cell adhesion and chemotaxis, and inflammatory mediator processing [4]. We have previously demonstrated that calpains participate in the development of inflammatory lesions in an acute model of antglomerular basement membrane nephritis [5]. Here, we focused on the role of calpains during sepsis and angiogenesis.

Calpains and sepsis

Sepsis develops when the host response to an infection, initially appropriate, becomes amplified and then dysregulated. This response leads to the activation of a number of host mediator systems, including leukocyte, cytokine, and hemostatic/thrombotic process, which contribute to the development of hypoperfusion, intravascular thrombosis, and subsequent multiple organ failure. To study the role of calpains during sepsis, we induced polymicrobial sepsis by cecal ligation and puncture in wild type (WT) mice and transgenic (TG) mice expressing high levels of calpastatin. Calpastatin overexpression improved survival and organ dysfunction (including lung, kidney and liver damage). It decreased the sepsis induced systemic proinflammatory response (cytokines IL-1, IL-6, TNF α) and disseminated intravascular coagulation, by reducing the rate of procoagulant circulating microparticles and therefore delaying thrombin generation [6].

Figure 1. Calpastatin Overexpression Improves Survival during sepsis. We measured the survival rates after CLP in TG and WT mice. There was a statistically significant improvement in the survival of TG mice: 50% of the TG mice survived until the end of day 7, and 16% of the WT mice until the end of day 7.

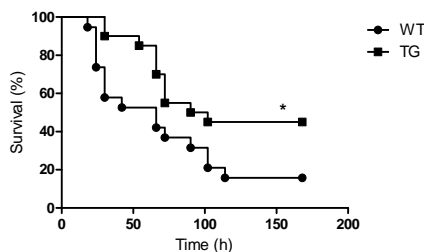


Figure 2. Sepsis-induced release of circulating microparticles is prevented in calpastatin transgenic mice

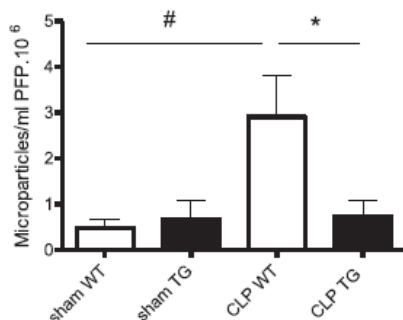
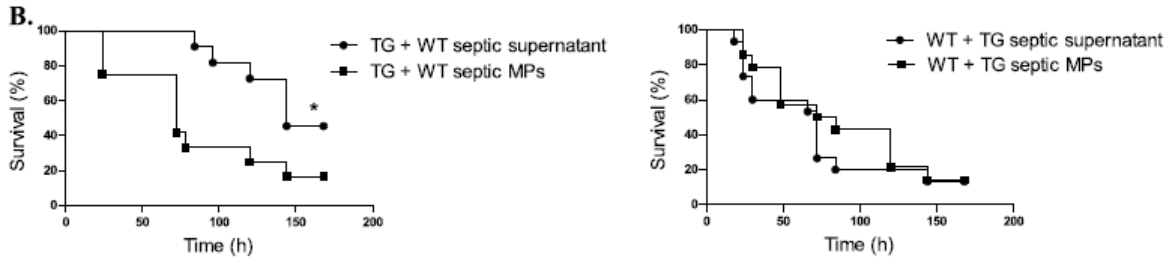


Figure 3. Sepsis Survival benefit in calpastatin transgenic mice can be alleviated by transfer of wild-type septic microparticles . Injection of micoparticles from WT septic mice worsened the prognosis of TG mice during sepsis, as assessed by survival curves. Conversely, supplementation of WT septic mice with MPs from TG mice did not change the survival outcome.



Extracellular calpains and angiogenesis

In addition, calpains can be externalized. Interestingly, intra- and extracellular calpains have differential effects. We demonstrated in vitro and in vivo in 2 different animal models (glomerulonephritis and cutaneous wound models) that externalized calpains participate in angiogenesis and vascular repair, likely by promoting fibronectin cleavage. Moreover, we showed that extracellular calpains can be conveyed in microparticles [7].

Figure 3. Extracellular calpains induce both formation of new blood vessels and vascular repair in vivo

- Using a Matrigel plug assay, we showed that hemoglobin content and number of capillary cross-sections per high power field were significantly reduced after addition of calpastatin to the plugs (Figure 4A).
- Similarly, during wound healing process, local delivery of calpastatin significantly limited the formation of both blood and lymphatic vessels (Figure 4B).

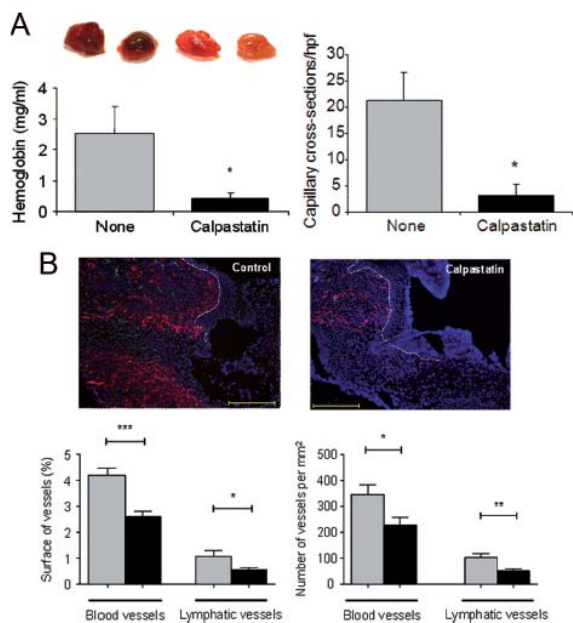
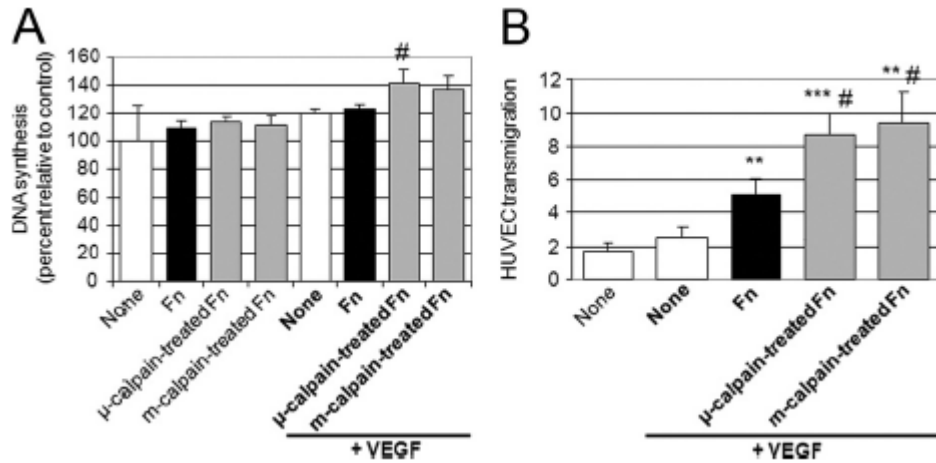


Figure 4. Proangiogenic function of extracellular calpains involves fibronectin cleavage

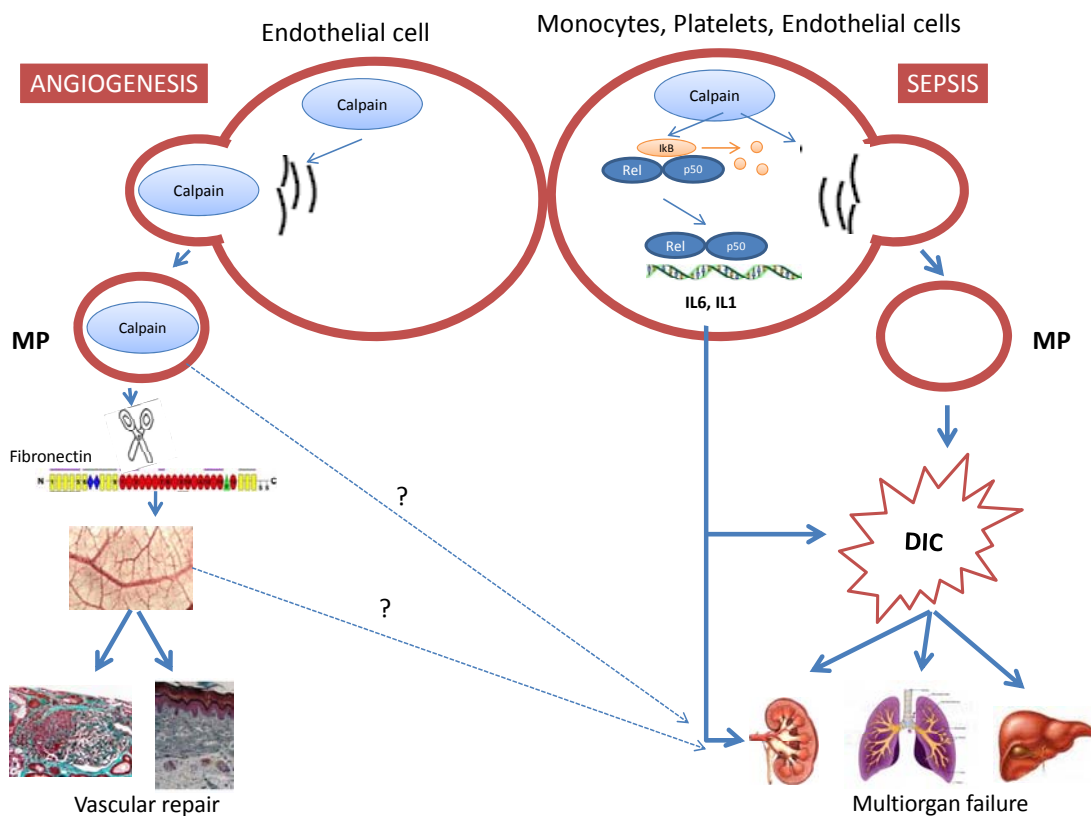
Calpain dependent fragmentation of fibronectin in extracellular matrix contributes to the induction of endothelial cell proliferation and migration in vitro. Calpain-treated fibronectin increased significantly VEGF-induced endothelial cell proliferation while native fibronectin did not (A). Moreover, calpains dramatically increased endothelial cell migration (B).



Conclusion

There is an intimate connection between calpains and microparticles for two reasons: first, calpain can modulate microparticle release into the circulation. Second, calpain can be conveyed in microparticles. The pharmacological modulation of circulating levels of microparticles via the modulation of calpain activity can be of particular interest in a therapeutic perspective.

Figure 5. Calpains and microparticles



- **Sepsis** : intracellular calpains have a central role in microparticle release from cells (especially platelets) as calcium-activated calpains degrade cytoskeletal proteins such as talin or vinculin, directly allowing microparticle release into the extracellular fluid. Microparticles participate to the coagulation activation during sepsis leading to disseminated intravascular coagulation and subsequent multiorgan failure. Intracellular calpains have also been shown to degrade the NF- κ B inhibitor I- κ B and thereby to induce the nuclear translocation of NF- κ B and the production of pro-inflammatory cytokines (IL-1, IL-6). These cytokines can also enhance the coagulation activation during sepsis.

- **Angiogenesis** : By increasing microparticle release, calpains enhance their own externalization. Extracellular calpains can be conveyed in microparticles. Externalized calpains participate in angiogenesis and vascular repair, by promoting fibronectin cleavage

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