Inflammatory pathways in the anaerobic peritonitis: modulation of coagulation and kallikrein-kinin system by commensal bacteria *Bacteroides fragilis*

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Objectives: Although *Bacteroides fragilis* is a commensal microbe present at low concentrations in the gut lumen, this opportunistic bacteria has been frequently implicated in cases of sepsis in hospitalized patients. Conditions leading to the contamination of the peritoneal cavity with *B. fragilis* associated to fecal contents induce abdominal sepsis and abscesses formation. Using a mouse model of peritonitis induced by *B. fragilis* in association to a sterile cecal content (SCC), we have recently observed that abscess formation is critically dependent on IL-1β produced via NLRP3 inflammasome. Since *B. fragilis* has been previously reported to activate the Kallikrein-Kinin System (KKS) in vitro, we wished to determine whether the formation of abscesses was modulated by the activation of the KKS.

Material and methods: To this end, *B. fragilis* and SCC were injected into C57BL/6 WT versus mice deficient of high molecular weight kininogen - ($Kng1^{-/-}$) or bradykinin B2R receptor ($B2R^{-/-}$).

Results: Strikingly, there was a drastic reduction in abscess score, suggesting that infection-driven pathology is worsened due to activation of the KKS/B2R pathway. Pharmacological studies linked *B. fragilis*-evoked swelling (Blue Evans dye/dorsal tissues) to the activation of the B2R pathway, since HOE-140 abolishes plasma leakage induced by bacteria. Motivated by these in vivo findings, we performed in vitro assays to determine whether *B. fragilis* could directly generate kinins. First, we observed that *B. fragilis* cleaved a synthetic substrate that spans the C-terminal bradykinin flanking site of the kininogen. Interestingly, the slow kinetics induced by the putative "kininogenase" was accelerated upon addition of human plasma to the bacterial suspension. This amplification was blocked by the plasma kallikrein inhibitor, suggesting that prekallikrein is converted to PKa by a uncharacterized microbial protease or, alternatively, PKa was generated due the activation of the contact pathway. However, FXII cleavage was not detected in human plasma incubated with *B. fragilis*.

Conclusions: These studies suggest that FXII might be dispensable for efficient PKa generation in human plasma. Work in progress may clarify whether PKa generated by *B. fragilis* might activate the fibrinolytic pathway, perhaps enabling microbial escape from the fibrin-rich networks associated to inflammatory abscesses. In addition, our studies may provide an opportunity to investigate the functional interplay between KKS/bradykinin receptors and inflammassome pathway in *B. fragilis*-induced peritonitis.