Akkermansia muciniphila derived tripeptide jams the gear of sepsis, inflammation and mortality

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Sepsis constitutes a global burden on medical care and accounts for nearly 20% of reported global mortality.[1] The development of sepsis is complex, though the triggering of a disproportional systemic proinflammatory response is considered pivotal in driving organ dysfunction and subsequent mortality. As functional pattern recognition receptors, toll-like receptors (TLRs) play a predominant role in this context. Hence, approaches aimed at limiting their activation are considered valuable strategies for mitigating the organ damage and mortality occurring during the advancement of sepsis. The gut microbiota's central importance in human health and its influence on sepsis progression, prompted a closer examination of specific bacterial strains.[2] *Akkermansia muciniphila* (*Akkermansia*) holds particular potential as a next-generation beneficial bacteria in this context as we and others showed promising effects of this bacterium in preventing the progression of various pathological conditions linked to disruptions in immune system regulation and metabolic imbalances (ie, type 2 diabetes, obesity, hepatic steatosis, cancer therapies) (for review[3]). *Akkermansia* has the capability to stimulate appropriate immune responses and produces biologically active compounds that can influence the functioning of nearly every system in the body. We recently discovered that live *Akkermansia* protects against anastomotic leakage-induced peritonitis/sepsis and mortality by improving wound healing during colonic wall defect through mechanisms possibly involving interleukin (IL)-22 signalling and requiring TLR signalling through myeloid differentiation primary response 88 (MyD88) in the intestinal cells (figure 1).[4] Among the suggested mechanisms, it was previously shown that *Akkermansia* produces several active compounds such as specific proteins (eg, Amuc_1100, P9), bioactive lipids (eg, diacyl phosphatidylethanolamine) or microvesicles (for review[3]).

In GUT, Xie *et al*[5] used a large set of in vitro and in vivo experiments exploiting human samples, several mouse models of sepsis, knock-out mice, germ free mice and pigs to unequivocally shown that *Akkermansia* protects against lethal sepsis by a mechanism involving a novel tripeptide (Arg-Lys-His (RKH)) that binds to TLR-4, inhibiting its activation and thereby reducing systemic inflammation during sepsis (figure 1).

They started by analysing the faeces from sham-operated and CLP (caecal-ligature-puncture) mice, a classic sepsis model mimicking polymicrobial infection. Using qPCR and species-resolved sequencing (2bRAD-M), they discovered a decreased abundance of *Akkermansia muciniphila* in the CLP group compared with the sham group. To confirm this in humans, they examined 61 faecal samples from septic patients, which also showed decreased *Akkermansia* levels compared with control subjects (figure 1). To investigate *Akkermansia*'s role, they transferred microbiota from low and high *Akkermansia* individuals to antibiotic-treated mice. Mice receiving high-*Akkermansia* samples showed slightly better survival and less inflammation. However,

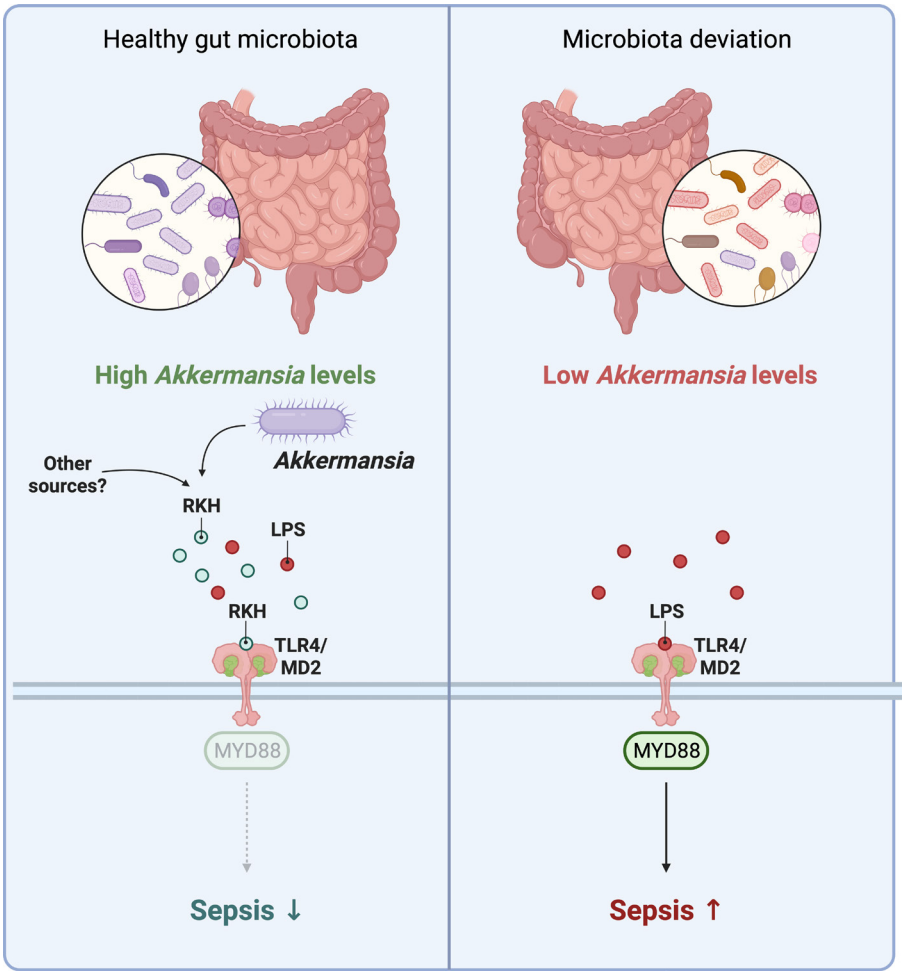

Figure 1 *Akkermansia muciniphila* and Arg-Lys-His (RKH) reduces sepsis. *Akkermansia muciniphila* or RKH reduces sepsis by blocking the action of lipopolysaccharides (LPS) on the toll-like receptor-4 (TLR-4) receptor. Subject with sepsis have low *Akkermansia* levels than healthy subjects. Figure generated with BioRender.

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this does not definitively establish *Akkermansia*'s protective role in sepsis.

To further demonstrate the direct effect of *Akkermansia* on sepsis they gavaged mice with *Akkermansia* or phosphate buffer saline (PBS) followed by sham or CLP surgery. The survival time was slightly improved over that of PBS-pretreated mice and both pulmonary and hepatic inflammatory factors were decreased. Strikingly, gavaging mice with pasteurised *Akkermansia* failed to improve these pathological changes, suggesting that *Akkermansia* must be alive to exert its effects. The culture supernatant from live *Akkermansia*, on the other hand, could replicate the effects of the live bacteria, significantly reducing mortality and pulmonary damage, while lowering the expression of inflammatory factors in both the lungs and liver. Importantly, all these effects were reproduced in a second model of lethal sepsis, the lipopolysaccharides (LPS)-induced sepsis model. An untargeted metabolomics analysis of the culture medium of live *Akkermansia* revealed distinct metabolic patterns (principal component analysis and volcano plot) compared with control medium, with a notable enrichment of a novel tripeptide called RKH. While fractionating the medium based on molecular weight and test the efficacy of the different fractions could have been a useful strategic approach, it would have likely been time-consuming. The role of *Akkermansia* in the production of RKH was next validated in germ-free mice colonised with live *Akkermansia*. Interestingly, although not discussed in the paper, RKH was also detected in non-fermented medium and in the faeces and cecum of germ-free mice, suggesting it may not be exclusive to *Akkermansia*. However, faecal RKH levels of septic patients were significantly lower compared with healthy control, aligning with the observed *Akkermansia* levels in these samples (figure 1).

To explore the potential therapeutic effect of RKH in lethal sepsis, they administered RKH to mice prior to CLP surgery. Similar to the results observed with *Akkermansia*, RKH pretreatment significantly extended survival compared with mice receiving vehicle.

The authors employed sophisticated methodologies to unravel the mode of action of RKH. Their investigation stemmed from RKH's significant reduction in the expression of proinflammatory factors triggered by the TLR-4 agonist LPS, leading to the hypothesis that RKH interfered with the TLR-4 pathway (figure 1). Through a series of experiments, they found that RKH was indeed able to inhibit TLR-4 activation by LPS by fitting to a pocket of the TLR-4/MD2 complex and compete with the binding of LPS by interfering with several amino acids (figure 1). To ascertain the role of TLR-4 in vivo, they used TLR-4 knock out mice and found that they were not protected against septic lethality when using RKH.

While these in vitro and in vivo data are highly compelling, the putative translational application of these findings has yet to be realised at this stage of the research. To bridge this gap, the authors employed a septic piglet model and showed that RKH could effectively reduce sepsis-induced mortality while improving systemic inflammation and organ damage. Furthermore, they tested the effects of RKH on human immune cells extracted from a septic patient and confirmed the anti-inflammatory effects of RKH on human macrophages.

In conclusion, the study holds paramount significance for the field and unveils novel potential applications of *Akkermansia* and its putative metabolites. Previous research has demonstrated that *Akkermansia* exerts numerous effects on the immune system and overall health, such as via interaction with TLR-2 (eg, via Amuc_1100 protein or specific phospholipids)[6 7] or by secreting specific proteins (eg, P9).[8] While the present study contributes to this knowledge, several pivotal questions are left unanswered. It remains unclear whether the dose of RKH used in vivo (50 mg/kg in mice) reflects a physiological dose found in the gut of mice. Equally important is understanding the levels of RKH produced in the gut by 4×10^8 *Akkermansia* cells, the dose used in the experiments. Also, does *Akkermansia* require RKH production to exert its effect in all physiological circumstances? To address this question, a comprehensive understanding of RKH production in *Akkermansia* is necessary in order to design genetic deletion experiments. However, such knockout approaches pose potential challenges as they may jeopardise the physiological role of RKH in the health of the bacteria itself. Moreover, is *Akkermansia* the unique source of RKH in the gut? It would be interesting to measure the levels of circulating RKH in mice treated with either *Akkermansia* or RKH and follow the RKH pharmacokinetics. Clearly, addressing these key questions is essential if RKH is to progress further in its pharmacological development.

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Competing interests PDC is inventor on patent applications dealing with the use of specific bacteria and components in the treatment of different diseases. PDC was cofounder of The Akkermansia Company SA and Enterosys S.A.

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