AIEC-derived LPS and Outer Membrane Vesicles differently drive the dysregulated immunological pathways linked to Crohn's Disease.

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Introduction: CD is a chronic inflammatory bowel disease linked to a mucosal enrichment of the adherent-invasive *E. coli* (AIEC) pathotype and a strong expansion of pathogenic Th17 (pTh17) cells. We previously proved that the deletion of the LPS-core heptose kinase RfaP, significantly impairs the IL-23 secretion by dendritic cells (DCs) and hampers pTh17 cell generation. Given the pivotal role of LPS and Outer Membrane Vesicles (OMVs) in triggering several proinflammatory processes, we aimed to deeply characterize the AIEC virulence determinants specifically implicated in the detrimental activation of the immune system in CD.

Materials and Methods: LPS and OMVs were purified from AIEC-LF82 strain, from its isogenic deletion mutants in the Type 1 fimbria adhesin (Δ*fimH),* and in specific genes of the LPS core biosynthesis operon (e.g. Δ*rfaP*), respectively involved in the invasion of intestinal epithelial cells (IECs), persistence within DCs, and pTh17 cell generation. Intracellular bacterial load and host inflammatory responses, upon infection with live bacteria or purified LPS/OMVs, were quantified in HT29 IECs, human macrophages (MDMs) and DCs.

Results: The double mutant Δ*rfaP*/Δ*fimH* showed an impaired ability to invade IECs and a complete loss of intracellular persistence. Notably, IL-8 and CCL-20 secretion resulted strongly diminished in response to Δ*rfaP*/Δ*fimH* double mutant, compared to single LPS mutants or Δ*fimH*. Similarly, all LPS mutants, Δ*rfaP*/Δ*fimH,* but not Δ*fimH*, displayed defective intracellular persistence within MDMs and DCs. Of note, none of tested mutants displayed a reduced TNFα secretion by MDMs, whereas all the LPS mutants and Δ*rfaP*/Δ*fimH* strongly impaired the IL-23/IL-1β secretion by DCs. Interestingly, LPS induced a comparable inflammatory response to that triggered by live bacterial strains exclusively in IECs, while it induced only weak cytokines release in MDMs and DCs. In contrast, OMV promoted a selective higher inflammatory response in DCs compared to LPS. Intriguingly, LPS mutants and Δ*rfaP*/Δ*fimH* secreted significantly higher OMV compared to LF82 but induced a lower proinflammatory response.

Discussion and Conclusions:

The AIEC-host cells interplay involves different virulence determinants, with LPS and Type 1 fimbriae promoting intracellular persistence and pro-inflammatory response in IECs and MDMs, while OMVs selectively drives polarizing cytokine secretion by DCs. Particularly, simultaneous targeting of RfaP and FimH in AIEC showed an impaired persistence within host cells and hamper the immunological pathways highly dysregulated in CD. In conclusion, RfaP represent a paramount bacterial target that, together with FimH inhibitors, could be pivotal for the development of novel and more effective therapeutic strategies for CD.