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Paper Poster Session II

Gram-positive bacterial pathogenesis and virulence

Streptococcus gallolyticus subsp. *gallolyticus* and *Enterococcus faecium* gut translocation: virulence or opportunism?

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Objective: *Streptococcus gallolyticus* subsp. *gallolyticus* (*S. gallolyticus*) and *Enterococcus faecium* are recognized microorganisms in endocarditis/bacteremia in colorectal cancer and immunocompromised patients, respectively. The **aim of this work** was to investigate the differences of clinical relevant isolates in translocation across the intestinal epithelium, their cellular adhesion/invasion and their ability to form biofilms in in-vitro models.

Methods: Six *E. faecium* isolates genetically characterized (4 from oncohematologic patients with endocarditis/bacteremia and 2 from feaces of healthy volunteers) and 4 *S. gallolyticus* isolates (2 causing bacteremia in patients with colon cancer and 2 from animal faecal colonization) were selected for the study. For translocation assay, differentiated monolayer of Caco-2 cells grown on transwell inserts were apically inoculated with 10E7 colony forming units (CFU)/ml of each isolate. Transepithelial resistance (TER) and translocated bacteria (CFU/ml) were measured at six time point along 8 hours. A *Lactobacillus reuteri* isolate was used as negative translocation control. To analyse the adherence of the isolates to the intestine epithelium, the same type of cells were incubated with 10E7 CFU/ml of each isolate for 2 h at 37ºC, then washed with PBS, treated with 0,1% triton X-100 and seeded onto agar plates for CFU counts. The invasion ability was determined after a second incubation (1h) with bactericidal antibiotics and a final treatment with 1% Triton and CFU counts. Additionally, biofilm formation on polystyrene and collagen-I covered microplates was quantified by cristal violet method (cutoff: OD_{600}>0.1). All experiments were performed in triplicate in at least three independent experiments and a multilevel statistical model was applied for translocation data analysis.

Results: Translocation ability was demonstrated in 6/6 *E. faecium* and 1/4 *S. gallolyticus* isolates, although non-significant differences in translocation speed (CFU/ml/h) were found. Two *E. faecium* isolates from gut colonization and bacteremia, translocated significantly more efficiently ($p<0.01$) than those from healthy volunteers colonization. TER increments of 1% were correlated with translocation drops of 0,04 logs. *S. gallolyticus* and *E. faecium* isolates exhibited similar adhesion ability (<1.5 % of the inoculum, range: 10E4-10E5 CFU/ml) without remarkable epithelial cells invasion. Finally, biofilm formation was observed in all *E. faecium* (6/6) and *S. gallolyticus* isolates (3/4) but the translocating *S. gallolyticus* from animal origin, evidencing a particular predilection for collagen rich surfaces.

Conclusions: Globally, both species form biofilms efficiently on collagen-rich surfaces, present good adhesiveness to intestinal epithelial cells and do not exhibited high invasion ability. Under physiological conditions, *E. faecium* isolates are able to efficiently translocate whereas intestinal epithelium alteration as it happens in colorectal cancer seems to be required for *S. gallolyticus* translocation.