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Title: Intestinal membrane vesicles – A novel innate defense mechanism?

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Sponsor: Bowel and Liver Trust

Introduction:

Crohn's disease is a non-curable inflammatory condition of the intestinal tract. It is thought to be caused by an inappropriate and self-perpetuating reaction to intestinal bacteria. The severity of symptoms often fluctuates, with cycles of 'flares' and periods of remission. Treatment for most individuals involves high doses of drugs that suppress the immune system, but often these have significant side effects such as increased susceptibility to infection and hormone imbalances. Liquid diets are often used in children for this reason, and they have been shown to be as effective as corticosteroid drugs in inducing remission. Polymeric formula is one type of liquid diet used frequently for children with Crohn's disease. The rationale for its use is that it reduces the work required to digest and absorb food, as well as being hypoallergenic and containing all the necessary components of a healthy diet. However, our research suggests that polymeric formula may also have additional effects on the innate immune response that include dampening the expression of inflammatory messengers and inhibition of bacterial binding to the surface of gut epithelial cells. It is currently unclear whether this effect of PF relates to direct antibacterial activity and/or an indirect effect. Indirect mechanisms include enhanced cell differentiation that is associated with increased production of membrane vesicles from the cell surface. These membrane vesicles have the potential to act as releasable decoys, thereby limiting bacterial binding to the intestinal epithelium.

Aim:

(1) To determine if polymeric formula has a direct effect on bacterial cell growth

(2) To investigate whether membrane vesicles limit bacterial binding to the gut wall.

Method:

Three bacterial species (adherent-invasive *Escherichia coli* (AIEC), *Campylobacter jejuni*, *Salmonella typhimurium*) were each cultured with and without increasing concentrations of polymeric formula, up to and including the likely physiological concentration (around 20%). Bacterial growth was measured over time as a change in absorbance.

In separate experiments, membrane vesicles (MVs) were harvested from the supernatant of Caco-2 cells following long-term culture. Negative staining Transmission Election Microscopy (TEM) was used to confirm the presence of vesicles, and fractions were dot blotted for CEACAM-6 (a protein found in MVs) following sucrose density gradient centrifugation to confirm that the vesicles have a similar density.

AIEC and *S. typhimurium* were incubated with MVs (200 µg/mL protein) for 30 minutes before being added to Caco-2 cells for 3 h at 37°C. Unbound bacteria were removed by extensive washing and remaining adherent bacteria were enumerated by serial dilution of cell lysates.

Results:

Polymeric formula was found to have a dose-dependent effect on the growth of bacteria, with 20% concentration consistently resulting in at least a 75% reduction in growth across all three species.

Membrane vesicles shed from the surface of Caco-2 cells and concentrated by ultracentrifugation were shown to have a ring structure, which is typical of the morphology of MVs. Moreover, the size of the MVs was consistent with previous measurements of MV size.

Fraction 5 (of 10) of the sucrose gradient showed high affinity for CEACAM-6 antibodies, suggesting that MVs are concentrated in this fraction.

MVs were shown to limit the association of bacteria with Caco-2 cells over 3 hours. This ranged from a 64% reduction in bacterial binding when MVs were incubated with *S. typhimurium*, to an 84% reduction when the same concentration of MVs were added to AIEC.

Conclusion:

These results indicate that inhibition of bacterial growth and/or inhibition of bacterial binding to the intestinal wall may play a significant role in the success of polymeric formula in managing Crohn's disease. Better understanding of the mechanisms that underlie the host innate immune response to infection will allow exploitation of these in the prevention and/or management of gastrointestinal disease.