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OLD HERBORN UNIVERSITY SEMINAR ON TRANSLOCATION: MINUTES AND REVIEW OF THE DISCUSSION

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INTRODUCTION

The discussion was organised around different topics concerning with translocation. The used definition for translocation was "Passage of viable bacteria from the gastro-intestinal tract to the mesenteric lymph nodes and other possible organs" (*Berg* et al, 1979). However, also dead particulate matter may "translocate", even particles of up to 150 mm.

The discussion was centred on the following topics (Figure 1):

1. Intestinal content: Gut flora/ inert particles
2. Site of translocation: structured elements or anywhere along the enterocytes?
3. After translocation: innate and specific immune system in the lamina propria
4. Systemic factors influencing translocation

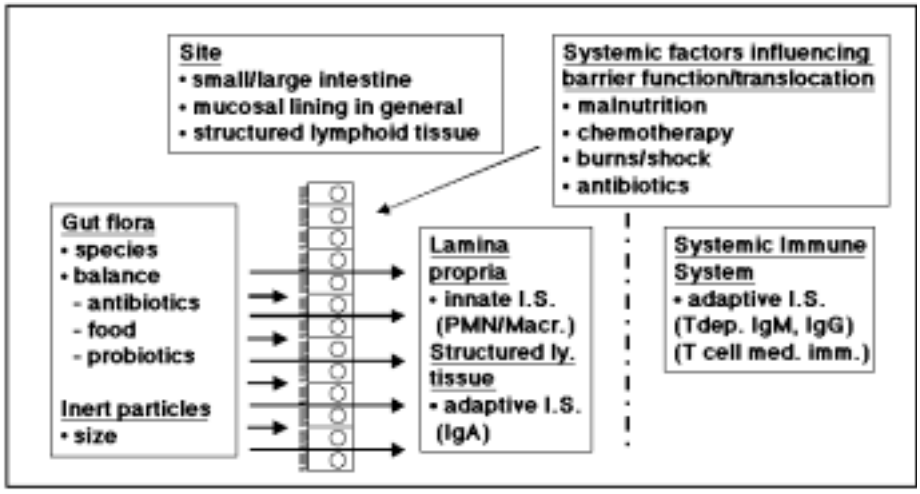

Figure 1: Factors involved in intestinal translocation

INTESTINAL CONTENT

The discussion first focused on the observations done by Dr. Volkheimer, that large particles (7-150 micrometer) such as starch can be transported very quickly from the gut lumen to different parts of the body. Most observations were done in humans and some in dogs. Next to the presence in lymph, the bloodstream, particles were found in kidney, in the urine, bile and within the peritoneal cavity. Passage to the peritoneal cavity was discussed and lymph vessels that end into the peritoneal cavity was suggested (Alexander), but denied by others (Pabst).

The site of passage can vary from the stomach to the large intestine. In gastric translocation the site of entry might be related to the mucus excretion sites. Within the small intestine the tips of the villi seem to be mostly involved, which might be related to turnover of the epithelium.

Drugs influencing the peristaltic movement, such as nicotine and adrenaline clearly have an effect on the passage frequency, which is about 1: 50,000 particles under normal conditions.

Another surprising observation discussed was that such particles are not caught within the mesenteric lymph nodes, but proceed beyond that. This contradicts the general opinion of the filter function of the (mesenteric) lymph nodes. Currently we have no explanation for these observations.

In addition, Hussain discussed possibilities to use this process for drug delivery. There is a clear distinction between particles <500 nm which are processed through M-cells, in contrast to the very large particles. Surface properties of particles might be manipulated to direct their uptake in Peyer's patches or within the villi. Lectins can promote particles to stick to the surface of different parts of the gut. In addition to lectins, bacterial ligands such as invasin promote inert nanoparticle uptake. More importantly, these ligand-conjugated nanoparticles were more readily absorbed through normal enterocytes rather than M-cells. Lactose that binds to rat mammalian intestinal lectins was found not to promote the uptake of polystyrene particles (Hussain), probably because this might render the surface hydrophilic. A balance should be found between hydrophilicity for quick passage through the mucus layer, versus hydrophobicity for good uptake into cells. Delivery of gut-labile molecules, such as peptides, vaccines and DNA might also be delivered by the oral route via encapsulation using biodegradeable particles. The relevance of *in vitro* models such as the Caco-2 cell lines to this process was discussed. Surface properties of Caco-2 cells are very different from intestinal epithelia *in vivo* (Jepson). Mucus-secreting intestinal epithelial cell lines also exist (HT-29), but the mucus produced by this cell line may also be very different from the mucus of the *in vivo* situation. It is also possible that epithelial cells themselves change the uptake of certain particles by producing factors upon binding of particles.

Studies concerning uptake of small particles have been done from 45 minutes and on, while passage of starch particles was within five minutes. Experiments should be done with earlier time points.

Otherwise, collecting of lymph through thoraxic duct canulation might give insight into the route of particles (Pabst).

Other factors like total parenteral nutrition (TPN) might be important in uptake by changing the amount of mucus present. This might also be one of the

reasons why TPN enhances translocation of bacteria. Another explanation might be the increase in the number of M-cells after TPN.

D. van der Waaij presented some data by *L. van der Waaij* et al. (1996) which show that patients with inflammatory bowel disease (IBD) have a significantly increased percentage of coating by immunoglobulins; IgA (range 25-80%) and IgG (range 10-40%); the percentage of *in vivo* IgG coating of endogenous faecal bacteria is normally much lower (range 2-10%). This difference in coating incidence could be the result of enhanced translocation in IBD. The only direct evidence for enhanced translocation, however, comes from patients where mesenteric lymph nodes have been removed during operation, which show an increase in enteric bacteria in the MLN.

SITE OF TRANSLOCATION

Berg opened the discussion with presenting some data on the site of translocation. In the experiment with *E. Coli* C25 the ileum and caecum seem to be most prominent sites for translocation. Removal of the caecal patches did not change the rate of translocation. Also ligation of the ileal Peyer's patches did not influence the number of *E. Coli* observed in the MLN. These experiments suggest that the site of translocation is not limited to the lymphoid areas, but can occur with the same efficiency along the enterocytes. Its was suggested to repeat the experiments with the currently available knock-out mice such as the LTB knockout, that are not able to make lymphoid follicles (Pabst).

Another issue is the possibility of bacteria to survive inside an intestinal tract. In general the more bacteria are sensitive to oxygen, the less they translocate. In a comparative study where SPF animals were antibiotic treated and then given 10^9 bacteria, Berg and colleagues showed that obligate anaerobes almost do not translocate, Gram-positive bacteria on average are intermediate (~20 bacteria/MLN), while *Enterobacteriaceae* translocate to about 50 bacteria/MLN. The initial rate of translocation of bacteria as observed by radioactive labelling of the bacteria was, however, the same for the different kinds of bacteria (Alexander). Of course, anaerobic bacteria will have a disadvantage in the aerobic environment of the host.

Then the role of virulence factors of bacteria was discussed. Berg stated experiments were Entero-pathogenic *E. coli* (EPEC) and Entero-invasive *E. Coli* (EIEC) showed low penetration in the MLN translocation assay. This suggested that virulence factors not necessarily cause enhanced translocation. Salmonella does have specific factors involved in enhanced translocation and survival inside the host. Salmonella for instance encodes specific invasion machinery, recognises matrix proteins and is able to survive inside macrophages. Type 1 fimbriae on *E. Coli* can be involved by turning off the production of the fimbriae while passing through the mucus and turn it on again for epithelium attachment (Herias).

The composition of the mucus in different layers can also be of influence. Rodents for instance have a more hydrophobic mucus layer. Alexander referred to experiments by *Katouli* et al. (1994) in intensive care patients in which translocated vancomycin resistant bacteria were compared with the caecal bacteria by biotyping. In these experiments there was no clear correlation between the biotypes observed in caecal

isolates and the translocated species. This suggests that there is a selective advantage of some species over others in their ability to translocate (Alexander) and also within the same species (*E. coli*) there are some strains differing in certain traits that facilitate translocation (Herias, referring to studies by Bark and Katouli (1993), where they found different biochemical phenotypes present among translocating *E. coli* strains).

AFTER TRANSLOCATION

Berg presented data about the effect that a *Propionibacterium acnes* vaccine had on translocation of *E. Coli* after decontamination. In conventional mice this vaccination reduced the rate of translocation. When, however germfree (GF) mice that were recently conventionalised were used, the vaccination had no effect on the rate of translocation, as it was similar to GF mice. When the same experiments were repeated in neonatal GF mice that were conventionalised at less than 1 week after birth, the ex-GF mice reacted as conventional mice and responded to the vaccination. These results suggested that the indigenous flora had to be established before 1 week after birth.

Also in tolerance experiments the same observations were done. A conventional animal can be tolerised to ovalbumine by oral administration, while this is not possible in germfree mice. When germfree mice are neonatally conventionalised they can be tolerised as adults, while conventionalisation at adult age does not result in facilitating tolerisation (Nieuwenhuis). Other examples of the importance of the gut flora in the early shaping of the immune system come from experiments comparing the occurrence of allergies in different countries with similar ethnic background but differing in economic and hygienic conditions. For example, comparing Swedish children with children form Estonia showed that children of Estonia had a lower intestinal worm infestation than Swedish children (Waldström, Herias). Herias, referring to the studies by *Björksten* et al. (1999), suggested that this might be related to the higher incidence of asthma and allergy among Swedish children. Sweden has a higher index of allergic disease than Estonia, and these differences could depend on a reduced microbial stimulation during early life that could result in a slower postnatal maturation of the immune system, especially the TH1/TH2 balance (shift from TH2 to TH1).

The role of the structured lymphoid tissue was next discussed. There is an intimate contact between lymphocytes and the epithelium. M-cells that overlay the dome of structured lymphoid tissue such as Peyer's patches contain some lymphocytes of which the function is unknown. Also the role of IEL's that are in close contact with the epithelium elsewhere still remains unclear. The origin of M-cells was discussed by Jepson. There seems to be an early predetermination, since early M-cell markers are already present within the crypts where the M-cells differentiate (*Gebert* and *Posselt*, 1999; *Gebert* et al., 1999; *Giannasca* et al., 1994). The number of M-cells can be regulated. Cytokines derived from B-cells might be involved, since IgM-KO mice have less M-cells (*Debard* et al., 1999; *Golovkina* et al., 1999; *Niedergang* and *Kraehenbuhl*, 2000) and co-culture of Caco-2 cells with B-lymphocytes induces formation of cells with some properties of M-cells (*Kerneis* et al., 1997). Also *Salmonella*

typhimurium and Streptococcus pneunomiae have been reported to increase the number of M-cells (*Borghesi* et al., 1999; *Meynell* et al., 1999; *Savidge* et al., 1991). The fate of M-cells is unclear. They may die or differentiate into enterocytes (*Borghesi* et al., 1999; *Debard* et al., 1999; *Niedergang* and *Kraehenbuhl*, 2000). M-cells are also found in the epithelium covering isolated follicles. The functional relevance of the great heterogeneity of markers for M-cells in different species is still unknown.

The role of IgA was discussed by Bos. In general sIgA within the gut lumen is thought to enhance exclusion of bacteria from the gastro-intestinal tract by prevention of attachment. Otherwise, Lamm and colleagues have shown that excretion of IgA-immune complexes via the poly-Ig receptor might also be part of the natural clearance of bacteria. Although commensal bacteria are also very often coated with IgA, they are maintained at steady levels. Pathogenic bacteria, however, are completely excluded from the gut. The affinities and specificities of the involved IgA molecules might be important in explaining such different effects of IgA. Bos showed experiments where there is a clear IgA response after mono-association of GF mice with the commensal, strictly anaerobic Segmented Filamentous Bacterium (SFB) (*Talham* et al., 1999). The numbers of SFB in the small intestine show a sharp rise directly after weaning, which disappears after some weeks. In breeding experiments with SFB mono-associated mice, where the mother and/or the pups were impaired in IgA production, this temporary localisation does not occur. This example illustrates the effect that IgA can have on the composition of the gut flora.

The different methods of detection of translocation were next discussed. According to the definition of Berg et al., the culturing of live bacteria from mesenteric lymph node is still the standard assay. Labelling of micro-organisms is very useful for tracing the bacterial load within different organs, but the viability of these micro-organisms within the host is unknown. The detection of NOx within the urine, is a measurement of macrophage activation. In some infection models there is a good correlation between the NOx levels and the level of translocation, but in situations where the infectious agent is unknown, this might not be true. Detection of micro-organisms by PCR in blood samples is very sensitive, but very often inhibitors of the PCR reaction results in false negative results. The endotoxin level within the blood is also a good indicator for Gram-negative sepsis.

SYSTEMIC FACTORS INVOLVED IN TRANSLOCATION.

Alexander discussed the role of nutrition in burn patients. Good results were obtained by feeding patients immediately an "immune enhancing diet" containing extra arginine, glutamine and fish oil. These additions seem to down-regulate the inflammatory response. Arginine influences the level of factors such as Insulin-like growth factor 1. Starvation and malnutrition alone do not increase the chance of translocation. In combination with hypovolaemic shock there is an increased translocation. Lack of critical factors such as Vitamin-A might lead to increased translocation.

Total parenteral nutrition (TPN) also leads to increased translocation. Addition of solid particles or fibres to an oral diet decreases translocation. Addition of dried food does not help. Fresh fruits

might be very beneficial because of the amount of the amino acid taurin present, which fills up macrophages.

The balance between natural clearance and translocation can be disturbed by local malnutrition of the colon. Bacterial digestion allows LPS to be released and absorbed onto particles derived from the environment. This might lead to products that might influence cytokine levels, opsonisation and anti-oxidants. LPS in solution is relatively innocuous to the epithelium when compared to LPS absorbed onto a solid surface (Hussain). Also the composition of the mucus is influenced by the food intake.

In patients that where implanted with biomaterials, such as Teflon, most implant related infections were not blood borne. This suggests that in those cases most infections originate from the time of operation.

In critically ill patients there is some evidence for gut-derived infections. Selective decontamination with non-absorbable antibiotics against Gram-negative bacteria has obtained variable success rates. To determine how successful the selective decontamination has been, one should culture the stools for Gram-negative bacteria. The disturbance of the flora by this method might lead to overgrowth of certain species, such as enterococci and an enhanced risk for translocation.

The mechanisms of a Ca^{++}-rich diet to prevent translocation were discussed by Bovee-Oudenhoven.

Dietary Ca^{++} decreases the soluble concentration of intestinal bile acids and fatty acids. Precipitation of these surfactants prevents damage to the intestinal epithelium, decreases proliferation of epithelial cells (risk marker for colon carcinogenesis) and strengthen the mucosal barrier function (*Bovee-Oudenhoven* et al., 1999*; van der Meer* et al., 1997).

Calcium carbonates are also used for the reflux-syndrome. The long-term effects of the increased intake of these kinds of Ca-salts are unknown. Possibly, a high intake of carbonate neutralises gastric acid, which is important for host defence against food borne infections.

Furthermore, Ca^{++} seems to decrease the number of Gram-negative bacteria, while the number of Gram-positive bacteria such as Lactobacilli can be increased. However, also other Gram-positive bacteria such as Listeria monocytogenes may increase in number, which could be regarded as a negative side effect. When the daily human intake of Ca^{++} is less then 500 milligram, addition of Ca^{++} might be beneficial to improve resistance.

Bengmark stressed the importance of the fermentation by intestinal bacteria. Micro-organisms ferment 20-25% of our food, which results in release of a great number of nutrients. The processing of food (cooking, drying, and freezing) can lead to a great loss of nutrients.

Lactobacillus plantarum is very active in these fermentation processes and has been used as probiotic.

The modern food style does not favour the presence of this bacterium within the digestive tracts since only 5% of Swedish students have this bacterium in their stools, while in Africa 100% of the autochthonous population delivered positive cultures from their stools. Also people eating vegetarian diets have an increased presence of *L. plantarum*.

Preliminary data in liver transplantation patients, show that addition of 10^{10} live *L. plantarum* to their diet in combination with selective bowel decontamination leads to less patients with infections (2/15) compared to SDD alone (6/15) or SDD + dead bacteria (4/15) (*Rayes*, 1999).

Since with the modern food style

lactobacilli very often are only transient members of the gut flora, alterations of food habits might ultimately lead to a better establishment of such fermenting bacteria

Food additives such as galactose, fructose and mannose-oligosaccharides might lead to better fermentable carbohydrates for such bacteria.

CONCLUSION

Translocation can be regarded as an illustration of the close interaction between the gut microbiota and the host. Many aspects of these interactions are still poorly understood. A better understanding is needed of both the physiology of this dynamic equilibrium and the pathological consequences when the dynamic balance between the gut microbiota and the host is disturbed. Factors influencing this balance can be of crucial importance for managing general health conditions.

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