3rd International Immunonutrition Workshop

Session 9: Food ingredients, immunity and inflammation: animal and in vitro models

A rat model of mild intestinal inflammation induced by Staphylococcus aureus enterotoxin B

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The epithelial barrier of the intestine and the gut-associated lymphoid tissue (GALT) protects the host against luminal pathogenic micro-organisms. This is important at weaning, when animals are exposed to infectious agents and stresses. We have developed a rat model of intestinal inflammation post weaning, based on the systemic administration of Staphylococcus aureus enterotoxin B (SEB). Since the inflammatory response obtained is mild, the food intake pattern is not affected, which makes this model useful for studies of nutritional therapies for intestinal inflammatory disease. SEB increased T-lymphocytes in Peyer’s patches and the number of activated T-lymphocytes in mesenteric lymph nodes (organized GALT). In the lamina propria, SEB increased activated T-lymphocytes as well as cytotoxic and natural killer-cell populations of the diffuse GALT. It also increased pro-inflammatory cytokines and inflammatory mediators in both Peyer’s patches and mucosa. Rats given SEB had higher paracellular permeability to macromolecules, which was associated with a reduction in epithelial tightness. This model was used to examine whether dietary supplementation with spray-dried animal plasma proteins affects intestinal inflammation. Results showed that dietary plasma proteins can attenuate the mucosal immune response in both organized and diffuse GALT and that these effects are mediated by a reduction in the production of pro-inflammatory cytokines.


Intestinal physiology

The intestinal epithelium has two functions. First, it acts as a barrier to the passage of harmful intraluminal agents including foreign antigens, micro-organisms and their toxins. Second, it acts as a selective filter, allowing the absorption of essential dietary nutrients, electrolytes and water from the intestinal lumen to the circulation. The contact between neighbouring intestinal epithelial cells involves desmosomes, adherent junctions and tight junctions which regulate paracellular permeability. This is accomplished by interlocking proteins called claudins that bind scaffolding proteins, such as ZO-1, which in turn link them to the cellular cytoskeleton (1). An alteration of the intestinal barrier function contributes to disease, especially when the intestine is challenged by luminal antigens.

The intestine is a large surface in contact with the external environment. In a healthy intestine, mucosal immune cells must distinguish between beneficial antigens such as those present in food, innocuous antigens present in the commensal flora and potentially harmful antigens mainly involving pathogenic bacteria. The gut constitutes

Abbreviations: GALT, gut-associated lymphoid tissue; IC, Ig fraction concentrate; PP, Peyer’s patches; SDP, spray dried plasma; SEB, Staphylococcus aureus enterotoxin B.

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the largest lymphoid organ in the body. It contains a broad network of secondary lymphoid organs and a large number of lymphocytes including several intestine-specific subpopulations. Upon activation, the intestinal immune system coordinates a strong inflammatory response against invasive pathogenic bacteria, while providing inhibitory mechanisms to prevent an excessive response against commensal bacteria. However, if the immune system is stimulated and the response is not controlled, the tissue may be damaged; there may be extracellular matrix destruction due to metalloproteinase release and increased pass of neutrophils and other immune cells across the epithelium due to increased epithelial permeability. In addition, stimulation of the immune system diverts energy and nutrients from growth and other productive functions.

Gut-associated lymphoid tissue (GALT) accounts for up to 80% of the mucosal immune system and is distributed along the intestine in two forms: as organized GALT, which includes Peyer’s patches (PP), isolated follicles and mesenteric lymph nodes, and as diffuse GALT, consisting of lymphocytes scattered in the epithelium and the lamina propria. Both compartments are part of a regulatory system with specific roles; organized GALT is the inductor site of the immune response and diffuse GALT is the effector site.

GALT has a role in both innate and acquired immune responses. Innate immunity is non-specific and acts as the first line of defence by preventing the entry of infectious agents or eliminating invading pathogens. It comprises physical barriers, such as skin or mucous membranes; cells in blood and tissues, such as phagocytes, neutrophils and monocytes; and soluble mediators, such as complement proteins, cytokines and defensins, which are antimicrobial peptides secreted by Paneth cells. While protection by innate immunity is effective, some pathogens escape detection or clearance by this system, which leads to the activation of the acquired immune system. This system consists of two major cell types, the T- and B-lymphocytes, which enable the specific recognition of and response to invaders. T-lymphocytes mediate mainly cellular acquired immunity while B-lymphocytes are involved in humoral acquired immunity.

Another component of the mucosal immune system consists of inducible regulatory T-cells, which maintain immunological unresponsiveness to self-antigens and suppress excessive immune responses that are deleterious to the host. Regulatory T-cells mediate peripheral T-cell tolerance to antigens derived from the dietary origin or from the commensal flora.

### Physiology of the gastrointestinal tract at weaning

In mammals, weaning is associated with intestinal maturation and changes in diet. Many changes in the intestinal physiology occur in the first 2 weeks after weaning, affecting villous and crypt development as well as the activity of many brush-border digestive enzymes. Weaning increases antigenic exposure, resulting in physiological inflammation mediated by the mucosal immune system. The number of mucosal mast cells and intraepithelial lymphocytes in the jejunum increases. In rodents, weaning is associated with changes in the activation status and numbers of leucocytes in the intestinal mucosa.

The permeability of the immature intestine is higher than in adults and in weaning permeability depends on the integrity and stability of the tight junctions, as they control the diffusion of ions, water and macromolecules as well as luminal antigens. At weaning, dietary manipulation can affect the properties of the intestinal mucosa, such as permeability and nutrient absorption. For example, young pigs are highly susceptible to enteric challenge, particularly as passive immunity declines. At weaning, the digestive system of pigs adapts to a dry diet. Consequently, weaning pigs often acquire enteric infections that may cause intestinal inflammation, villous atrophy and malabsorption, which contribute to high rates of mortality.

### Models of intestinal inflammation to study the role of drugs and diets

There are several models to study intestinal inflammation, most of them based on the administration of microorganisms such as enteropathogenic *Escherichia coli*, *Clostridium difficile* or *Cryptosporidium parvum*. *E. coli* is the most abundant facultative anaerobe in the normal microflora of the mammalian colon. In physiological conditions, the relationship between the luminal bacteria and the host is mutually beneficial; however, certain strains of *E. coli* (e.g. enteropathogenic *E. coli*) have acquired virulence and may contribute to the development of acute gastroenteritis. *C. difficile* has been implicated as the main cause of antibiotic-associated diarrhoea in adult human subjects and similar clinical conditions in a variety of other mammals. *C. parvum* is a protozoan parasite that causes diarrhoea and gastroenteritis in human subjects and animals, which may become a chronic, life-threatening disease in immunocompromised patients.

A common feature of the intestinal inflammation induced by these micro-organisms is the increased permeability of the intestinal epithelium, which is associated with secretory diarrhoea. Toxins from *Clostridium* change the localization of several tight-junction proteins and enteropathogenic *E. coli* infection induces redistribution of occludin which decreases the barrier function. Moreover, enterotoxins can also induce the release of pro-inflammatory cytokines, such as interferon-γ and TNF-α. Both cytokines increase the epithelial permeability by reducing the expression of zona occludens-1 and occludin.

#### The *S. aureus* Enterotoxin B model

Rodent models are frequently used to analyse the immune mediated pathways of intestinal inflammation, and most of them are currently applied in studies of the pathophysiology of colitis. However, few of these models reproduce mild intestinal inflammation. Mild or transient inflammation can be induced by low doses of chemical agents used to induce colitis (e.g.: dextran sodium sulfate), parasite infections, such as the *C. parvum*...
Exposure to staphylococcal enterotoxins induces a range of clinical abnormalities from gastrointestinal upset to lethal toxic shock syndrome (27). They can induce nausea, abdominal pain and diarrhoea in human subjects (28) and cause severe pathologies in farm animals (29). In mice, intraperitoneal administration of *S. aureus* enterotoxin B (SEB) evokes a self-limiting enteropathy characterized by various degrees of histopathology, increased MHCII expression and increase in CD3$^+$ T cells (30).

Our objective is to study the functional properties of dietary supplements for use in nutritional therapy of intestinal inflammatory diseases, especially after weaning. At this stage, the gastrointestinal tract, still in transition to its adult characteristics, is exposed to a wide range of new bacterial populations and food antigens. We hypothesized that SEB would be a good candidate to reproduce a mild inflammatory condition in the laboratory, in young rats. The rats received an intraperitoneal injection of SEB (0.5 mg/kg) on days 30 and 33 after birth. This resulted in an intestinal inflammation syndrome, characterized by the activation of T helper lymphocytes and an increase in several of the cell populations involved in inflammation (31,32) (Fig. 1). SEB induced the recruitment of immune cells belonging to the innate immune system like neutrophils (31) and eosinophils (33). It also increased the gd-T cell subset modulates the inflammatory response by promoting the influx of lymphocytes and monocytes to mucosal surfaces (34).

Administration of SEB also increased the transmural permeability of the small intestine, tested with tracers like dextran, with relatively low molecular weight (4 kDa) and horseradish peroxidase with a molecular weight similar to that of food antigens (35). These results indicate that the...
paracellular route can increase the protein flux across the intestine in animals challenged with the enterotoxin and they are consistent with the facilitated flux of antigen across the paracellular space observed by Toivola et al. (18). SEB also reduced the expression of junctional proteins, such as ZO-1 and β-catenin, indicating a relation between the reduced epithelial tightness and the higher mucosal permeability (35) (Fig. 2). Furthermore, the increased luminal water content observed after SEB administration indicates that the effects of SEB on intestinal permeability can alter the absorptive/secretory water balance (31). SEB also reduced the expression of the apical sodium-glucose transporter 1 glucose and galactose transporter, especially at the villous apex where its expression is maximal, indicating that the inflammatory syndrome also impairs nutrient uptake (36).

The obvious candidates to mediate the SEB effects are the mucosal pro-inflammatory cytokines. SEB stimulates lymphocytes to secrete interferon-γ and TNFα (37) and this is correlated with reduced tight-junction protein expression (21) and increased permeability (28). In our model, SEB administration also increased the release of TNFα and interferon-γ, IL-6 and leukotriene B₄ in both mucosa and PP (38). In addition, the stimulation of mucosal GALT by SEB led the mucosal immune response to a T-helper 1 response, since IFN-γ and TNFα enhanced activation and proliferation of T-helper 1 cells (39).

In human explants, SEB modulates the expression of both constitutive and inducible defensins (40). In the rat model, SEB reduced the expression of α-defensin cryptdin 4 and β-defensin 1 (41). Since cryptdin 4 can block IL-1β release from lipopolysaccharide-activated monocytes (42), a decrease in its expression may increase intestinal IL-1β production. This would make the intestine more susceptible to SEB-induced damage and contribute to inflammatory bowel diseases (41).

**Fig. 2.** Effects of *Staphylococcus aureus* enterotoxin B (SEB) on the epithelial barrier. (A,B): Immunolocalization of β-catenin, present at the adherens junctions (A) and ZO-1, present at the tight junctions (B) in control rats and in rats administered with SEB. The expression of both proteins was decreased by SEB, consistent with a reduction in epithelial tightness. (C) Shows the distribution of horseradish peroxidase in the intercellular space in Control and SEB treated rats. The mucosa of the jejunum was mounted in using chambers and incubated with horseradish peroxidase added to the mucosal side (35). The results are consistent with an increase in paracellular permeability in the rats challenged with SEB.

**Fig. 3.** Cytokine release in the *Staphylococcus aureus* enterotoxin B (SEB) model. Release of pro-inflammatory TNFα and anti-inflammatory IL-10 in intestinal mucosa (A), peyer’s patches (B) and serum (C). Experimental groups were: Control group; rats administered with SEB (SEB group) and rats challenged with SEB and supplemented with spray-dried plasma (SEB-SDP group) or with Ig concentrate (SEB-IC group). The statistical analysis (one-way ANOVA followed by Bonferroni post-hoc test) showed that the SEB group has a higher TNFα concentration in the three tissues than controls and that both dietary supplements can reduce the expression of TNFα and stimulate the release of IL-10 (38).
The use of the *S. aureus* entero toxin B model to study the properties of plasma supplements in the prevention of intestinal inflammation

Spray dried plasma (SDP) and its Ig fraction concentrate (IC) from either porcine or bovine origin are widely used to feed farm animals. They have also been used in human trials to reduce the clinical effects of cryptosporidiosis associated with AIDS. The protein composition of plasma is: approximately 50% albumin, 25% globulin (including α-, β- and γ-globulin), 5% fibrin and 20% other proteins, such as haptoglobin, transferrin, growth factors and other proteins and peptides. There is evidence that Ig and other functional components present in SDP and IC are well conserved and are highly functional, providing immunological benefits. For example, they reduce the intestinal inflammation produced by pathogenic bacteria and viruses or by protozoa.

The results of our studies on the effects of animal plasma preparations on intestinal inflammation indicate that these supplements can reduce, and in some cases prevent, the inflammatory syndrome induced by SEB. In summary, rats challenged with SEB fed dietary SDP and IC supplements showed reduced activation of CD4 cells (i.e. T-helper lymphocytes) in the intestine (in PP, lamina propria and intraepithelial compartments) and a lower increase in the γδ-T-lymphocytes population in PP and in lamina propria. These data support the hypothesis that diets supplemented with animal plasma can attenuate the immune response. In pigs challenged with *E. coli* K88, Bosi *et al.* observed that the expression of pro-inflammatory cytokines was lower in animals fed SDP. Results from our laboratory indicate that plasma supplements reduce the expression of mucosal pro-inflammatory cytokines and the activation of T-helper subsets in the lamina propria and in the epithelium. These effects on mucosal cytokine profile can explain the reduction of mucosal inflammation by plasma supplements and the preventive effects of SDP and IC on changes in the mucosal permeability, tight-junctional protein expression and nutrient absorption following SEB administration. This reduction of the toxin-induced increase in mucosal permeability may prevent the passage of microbial and food antigens to the interstitial space, thereby blocking local inflammation. Recent results, summarized in Fig. 3, show that both SDP and IC reduce the SEB-induced release of pro-inflammatory cytokines, while increasing IL-10 concentration in GALT as well as at the systemic level. Interestingly, SDP can increase mucosal IL-10 concentration either in the presence or absence of an SEB challenge.

There are several mechanisms by which SDP and IC can modulate the intestinal immune response. Luminal effects were suggested by van Dijk *et al.* who reported a reduction in mucosal binding of luminal antigens by plasma glycoproteins. Moreover, Ig present in SDP may bind to potential antigens in the lumen of the small intestine and prevent their attachment to the mucosa. These Ig may also cause changes in intestinal microbiota, increasing the richness of the ecosystem, as suggested by Martin-Orúe *et al.* The full plasma supplement is a complex mixture of growth factors, cytokines and biologically active compounds. Therefore, a role for these proteins interacting with immune cells present in the mucosa, thus changing the mucosal cytokine profile, should also be considered.

In conclusion, the rat model based on the systemic administration of SEB induces a mild inflammatory syndrome, which does not affect the animal’s welfare or feeding behaviour. These characteristics make this model appropriate for studies on the use of nutrition therapy in inflammatory diseases. It has contributed to the discovery of the anti-inflammatory properties of SDP supplements.

Acknowledgements

We thank J. Campbell, J. Crenshaw, J. Polo, L. Russell and E. Weaver for their comments and suggestions. This study was supported by the Eureka Program Euroagri (E!2452) and by funds from APC Inc., IA and APC Europe. Data presented within and figures of this paper have been recreated with permission from the primary authors. M.M. declares no conflicts of interest; A.P.-B. is employed part-time by APC-Europe supported by a Beatriu de Pinós grant (Generalitat de Catalunya, Spain). A.P.-B. and M.M. analysed the data and wrote the paper. Both authors have read and approved the final manuscript.

References


