MICROBIAL TRANSLOCATION IN THERMAL INJURY

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SUMMARY

Cutaneous injury by thermal burns in experimental animals produces a highly reproducible acute model for studying the barrier function of the intestine wherein the magnitude of microbial translocation is directly associated with the extent of injury. Translocation after burn occurs throughout the intestinal tract with extreme rapidity, reaches an early peak within hours, and then has a less intense prolonged phase (for up to 20 days). Blood flow and oxygenation in individual villi and oxygenation appear to play important roles. Translocation occurs primarily through intact epithelial cells and M-cells but passage through mucosal ulcerations may also be important. The loss of barrier function to microbial products may be even more important than translocation of intact microbes since it appears that the products from approximately 100 microbes pass across the intestinal barrier for every one microbe that can be detected by culture. Loss of the barrier function is clearly associated with the hypermetabolic response in experimental animals. Importantly, a number of therapeutic interventions can improve gut barrier function against bacteria. These include lumenal nutrients, especially arginine, glutamine and the omega-3 fatty acids, growth factors such as G-CSF, GM-CSF, sucralfate and basic fibroblast growth factor, prostaglandin analogues, interferon-gamma, heparan sulphate and enalapril. Adrenocortical steroids will increase translocation but this can be counteracted by dietary arginine or glutamine. Thus, burn injury has provided a highly reproducible model for studying the mechanisms and pathological events following translocation as well as the development of treatments that may have useful clinical application in improving outcome following injury.

INTRODUCTION

Thermal injury provides a multifactorial clinically relevant model for studying barrier functions of the intestine in diseased states since there is altered nutritional intake by the afflicted subject, reduced blood flow to the intestine, and altered intestinal flora because of treatment, especially with antibiotics. Furthermore, the size and depth of the burn as well as its location can be varied at will in experimental animals, and all of the commonly used experimental animals can be used for such studies. The magnitude of translocation is directly related to the extent of burn with the maximum effect achieved with a 30% full thickness injury (*Gianotti* et al., 1993). However, larger burns further

impair the ability to kill the translocated organisms. The extent of translocation is also related to a variety of other variables, including the types and numbers of organisms within the intestinal tract, the nutritional status, the immunological status of the host, and the genetic background of the individual. Translocation of organisms occurs with similar intensity throughout the small and large intestine but there is better killing of the translocated organisms in the lower intestine (*Fukushima* et al., 1994). Translocation occurs with extreme rapidity after burn, with large numbers translocating to the mesenteric lymph nodes as early as five minutes (*Eaves-Pyles* and *Alexander*, 1998). Significant but delayed translocation to the liver occurs over a four-hour period whereas translocation to the spleen occurs at an intermediate rate. This is consistent with the primary route of translocation occurring by the lymphatics rather than the portal venous system as has also recently been suggested by Deitch's group who have shown that ligation of the main lymphatics draining the intestine decreases the extent of lung injury associated with translocation (*Sambol* et al., 2000). After 24 hours, the rate of translocation across the intestinal tract progressively diminishes for up to 5 days after which there is a lower but persistently elevated number of organisms that translocate across the intestine compared to control animals for up to at least 20 days (*Eaves-Pyles* and *Alexander*, 1998). The rapid translocation of organisms from the intestinal tract is consistent with the observations of Arnold and his colleagues in the 1920s in a non-burned canine model (*Arnold* and *Brody*, 1928).

Burn injury increases the translocation of all organisms we have studied. However, the pattern of translocation in organs and tissues is different. *Staphylococcus epidermidis* and *Escherichia coli* preferentially translocate to the mesenteric lymph nodes whereas *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Serratia marcescens* preferentially translocate to the liver and spleen (*Eaves-Pyles* and *Alexander*, submitted).

Early translocation appears to correlate with the relative blood flow to individual villi with an increased translocation occurring in villi that have reduced perfusion (*Gianotti* et al., 1993). This suggests that blood flow, and therefore oxygenation, to the individual villi may be an important factor determining the extent of translocation. This hypothesis is supported by experiments which show that hyperoxia will decrease translocation from the intestine after burn injury (*Gennari* and *Alexander*, 1996) and that progressive lowering of the pH using *in vitro* models involving cultured cacao 2 cells is associated with an increased extent of internalisation of *E. coli* (unpublished data). Vaso-active substances undoubtedly play a role, and it has recently been shown that elimination of neutrophils will improve intestinal barrier function (*Fazal* et al., 2000) suggesting that cytokines such as IL-8 may play an important role.

MODELS AND QUANTIFICATION OF TRANSLOCATION AFTER BURN INJURY

The most commonly used method for detection and quantification of translocating organisms is by culture of the organisms from normally sterile tissues and organs (*Maejima* et al., 1984). However, the values obtained depend upon several important factors that may not be easily controlled. These include

1) the barrier function of the intestine itself, 2) the numbers of organisms within the lumen that are exposed to the mucosal surface and 3) the ability of the host to kill the translocated organisms, making interpretation of the results somewhat difficult if only viable organisms in the tissues are counted. We have tried to circumvent this problem by using micro-organisms labelled with radioisotopes and also by infusing a quantified number of viable organisms into the stomach at the time of burn injury or at other selected intervals. Two isotopes have been used by our laboratory for these studies. ^{14}C glucose in the culture medium becomes incorporated widely throughout the organism. It has the disadvantage that it may be excreted via metabolic by-products as well as being released whenever the organism dies. In contrast, ^{111}In oxide becomes attached to macromolecules within the organisms and is not released until the organism is killed. Macrophages, which take up the bacteria, then incorporate the isotope. Our experiments suggest that for every viable organism that is detected by culture from normally sterile tissues and organs, there are 10 intact organisms that translocate to these tissues (90% kill rate) but the metabolic products of approximately 100 organisms reach the tissues as measured by ^{14}C radioactivity (*Alexander* et al., 1991). It is perhaps of great importance that the toxic products from 100 organisms reach the tissues for every 1 organism that is detected by viable counts since toxins may be central to inducing clinical illness. Obviously, consistent relationships do not always exist and such estimates may be changed markedly by the type of organism and the innate ability of the host to kill the microbes. Nevertheless, it would appear that the extent of "translocation" far exceeds that which can be estimated by bacterial cultures. The biological effects of these products from dead organisms may be a much more important factor than the physiological and pathological effects of failure of gut barrier function. Even more sensitive methods of detection of translocation include measurement of ß-glucuronidase in tissues (unique to *E. coli*) and the use of PCR for genes unique to different bacteria. With this technique, it has been shown that the genetic markers for bacteria appear much more frequently than viable organisms in the subjects who have received a thermal injury (*Kane* et al., 1996).

HOW DO ORGANISMS TRANSLOCATE?

There are four potential sites where organisms can cross the epithelial barrier. These are through M-cells, through epithelial cells, through ulcerations, or through the tight junctions. For several years, we have studied this process by using electron microscopy and have established clear relationships for the first three pathways but have never observed either bacteria or *Candida albicans* translocating through tight junctions. *Candida albicans* has been the easiest organism to study, and this has been primarily in Thiry-Vella loops so that the effects of local nutrients could be avoided (*Alexander* et al., 1990). In loops that are infused with a suspension of *C. albicans*, attachment occurs early after injury to M-cells, but more commonly to epithelial cells, as shown by scanning electron microscopy. After contact with the microvilli of epithelial cells, the microvilli appear to undergo disruption and lysis with movement of the candidal body into the microvillous layer. Some of the disrupted villi be-

come attached to the cell wall of the *Candida* and can remain there during passage of the *Candida* through the epithelial cell. The candidal body rapidly crosses the cell membrane with repair of the membrane. Then it passes through the cell that appears to be undisturbed and without lysosomal degranulation. Finally, when the candidal body reaches the basement membrane, it is released into the lamina propria still surrounded by the cytoplasm of the cell. There, they are taken up by macrophages or may enter the lymphatics or venules without prior phagocytosis. Interestingly, Flory, the discoverer of penicillin, made similar observations using a light microscope in 1933 (*Flory*, 1933). Since the extent of translocation is measured by determining the number of microbes and/or their products that pass through the epithelium, it cannot be determined how many actually pass through the intact intestinal cell and how many enter through ulcerations. However, numerous studies have shown that ulcerations may occur at the villous tips and that these become a site for the attachment of the microbes and undoubtedly their penetration.

That *Candida* can penetrate the intact mucosa of a healthy individual was clearly shown by the striking experiment of *Krause* and his colleagues (1969) who demonstrated the existence of fungaemia and funguria after oral administration of a suspension of *Candida albicans* by a normal human volunteer. Blood cultures were positive for *Candida* between 3 and 6 hours after ingestion, and the subject was symptomatic between 2 and 9 hours.

THE RELATIONSHIP OF TRANSLOCATION TO THE HYPERMETABOLIC RESPONSE AFTER BURN INJURY

In one study (*Gianotti* et al., 1994), guinea pigs were given a gastrostomy and then subjected to a 40% total body surface thermal injury one week later. They were then randomly assigned to receive complete enteral diets by gastrostomy or lactated Ringer's in an equal volume. Both were started immediately post-burn and continued for 48 hours. The animals were then gavaged with 10^{10} ^{14}C *E. coli* at 48 hours post-feeding. Resting metabolic expenditure was determined four hours post-gavage and the animals were killed for measurement of radioactivity in the portal blood and ileal mucosal, intestinal mucosal weight, plasma cortisol and urinary vanillylmandelic acid (VMA). There was an inverse relationship between the numbers of bacteria in the intestinal mucosa and the mucosal weight (R=0.676) with the animals given enteral nutrition having a higher intestinal weight and lower numbers of bacteria in the mucosa. Furthermore, the numbers of bacteria in the mucosa, as measured by radioactivity, correlated directly with the numbers of bacteria in the portal blood (R=0.88). There was also a direct relationship between the numbers of bacteria in the blood and plasma cortisol. Both plasma cortisol and the urinary VMA were related to the degree of hypermetabolism (R=0.84 and 0.73 respectively). Not unexpectedly, there was also a strong relationship between the radioactivity in the blood and the portal blood and the increase in the metabolic rate compared to baseline (R=0.86). All of these relationships were statistically significant and showed that starvation increased translocation which then triggered a hypermetabolic response. These studies also support the clinical benefit of early and aggressive enteral feeding following burn injury (*Chiarelli*, 1990).

It has been difficult to demonstrate

that the degree of translocation has an effect on mortality. However, *Fukushima* et al (1992) were able to determine that the radioactivity from ^{14}C *E. coli* infused into the stomach at the time of burn injury in a mouse was detectable by sampling of the blood from the retroorbital plexus four hours after injury. The amount of radioactivity (trans-location) that occurred at 4 hours related to both the overall survival and the length of time to death, with larger amounts of translocation being associated with earlier death. *Inoue* et al. (1991) also made similar associations using *Candida albicans* in burned guinea pigs.

EFFECTS OF TREATMENT ON TRANSLOCATION AND OUTCOME FOLLOWING BURN INJURY

Numerous therapeutic agents have been studied for their ability to improve survival in burned animals that have been made susceptible to intestinal translocation by burn injury. Many successful agents have different effects. Sucralfate and basic fibroblast growth factor seem to improve survival by improving the barrier function and decreasing translocation without an effect on killing (*Gianotti* et al., 1993). Prostaglandin E_1 analogues, misoprostal and einosoprost also improve the barrier function but inhibit killing of the translocated bacteria (*Gianotti* et al, 1993). Interferon gamma (*Gennari* et al., 1994), G-CSF (*Eaves-Pyles* and *Alexander*, 1996) and heparan sulphate (*Gennari* et al., 1994) all improve survival, primarily by improving the ability of the host to kill the translocated bacteria. GM-CSF improves both barrier function and killing of translocated bacteria (*Gennari* et al., 1994). Enala-pril, an ACE inhibitor, decreases translocation, possibly by improving blood flow to the intestine (*Gennari* et al, 1996). IL-6 increases translocation and is associated with a higher mortality and this can be blocked by an anti-IL-6 antibody (*Gennari* et al., 1994).

Importantly, glutamine, arginine and the omega-3 fatty acids all improved survival in animals having translocation associated with burn injury. The effect of glutamine is primarily by improving the barrier function although it may improve the killing of bacteria (*Gianotti* et al., 1995) whereas the effect of arginine is primarily on clearance mechanisms of the translocated bacteria via a NO associated mechanism (*Gianotti* et al., 1993). The omega-3 fatty acids improve both barrier function and bacterial killing (*Gianotti* et al., 1996). Glutamine, arginine and DHEA have positive effects in burned animals additionally receiving steroids (*Gennari* et al., 1997).

CLINICAL OBSERVATIONS

Complete enteral diets containing the immunonutrients, arginine and the omega-3 fatty acids found in fish oil, have been shown to improve outcome in burn patients by decreasing the incidence of infections and shortening hospital stay, compared to diets not containing these (*Gottschlich* et al., 1990). In non-burn subjects, the immunonutrient diets which would decrease the rate of translocation have also been beneficial in shortening hospital stay, decreasing wound infections, and decreasing the incidence of multiple organ

failure (*Alexander*, 1998). The probable mechanisms are by inhibition of over-exuberant inflammatory responses that are at least partly related to translocation of microbes and their products associated with injury and/or malnutrition.

CONCLUSIONS

Burn injury has provided a suitable model for studying gut barrier function. Studies in burned animals have led to an understanding of how microbes translocate, the pathological consequences of translocation, the development of treatments that decrease translocation and concepts which have had successful clinical application such as the improvement in outcome from using enteral nutrition to improve barrier functions of the intestine.

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