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EFFECTIVE AND INEFFECTIVE DEFENCE MECHANISMS IN THE GASTROINTESTINAL TRACT

HANS COTTIER, ALFRED HÄSSIG, and KURT STAMPFLI

University of Bern, and Central Laboratory,
Swiss Red Cross Blood Transfusion Service, Bern, Switzerland.

SUMMARY

Evolutionary processes have led to a mutual relationship among the hundreds of microbial species in the lower intestinal tract and between this microflora and the host. Under physiological conditions, the macro-organism benefits from this situation, however, a number of defence mechanisms is needed to contain this mass of bacteria. Success or failure of the highly complex machinery which exerts these protective functions depends not only on the force of potentially aggressive/damaging agents/events but also on the - in part genetically determined - ability of the individual to confront them. The present short review focuses on some aspects of host defence against dangerous complications that might arise from the bacterial mass in the gut. Particular topics discussed are: help and danger from the intestinal microflora; the intestinal barrier and its breakdown; effective and ineffective immune functions of the gut-associated lymphoid tissue (GALT); harmonious and protective versus excessive and hazardous inflammatory reactions; success and failure of antibacterial defence; and containment and release of bacterial endotoxin, the triggering substance that may elicit uncontrolled pro-inflammatory cascades. The latter are the hallmark in the pathogenesis of septic shock and multiple organ failure caused by severe infections with Gram-negative bacteria, a condition which often defies conventional therapy.

INTRODUCTION

The preceding Herborn Seminar Monographs have centred around three main topics:

1) microbial ecology of the human digestive tract;

2) interactions between the indigenous microflora and the host immune system; and

3) consequences of antimicrobial therapy for the composition of the intestinal microflora.

In this monograph, we shall shift our interest more to the host's side and place the emphasis on certain aspects of effective and ineffective defence mechanisms in the gastrointestinal tract.

The term "defence", in a biomedical sense, encompasses all cell or tissue structures and processes that serve to maintain, or in case of injury to restore, the integrity of the organism. Success or failure of the highly complex machinery which exerts these protective functions depends not only on the force of the aggressive/damaging agents/events but also on the ability of the host to confront them. Beside age, the nutritional status and the presence or absence of disease,

the capacity of the organism to cope with untoward influences is largely determined by genetic factors. This may best be exemplified by the numerous hereditary disorders, including those affecting the immune apparatus, which may interfere in one way or another with defensive capabilities. In view of the innumerable peptidic molecules involved in protective mechanisms and the existence of allelic polymorphism, it should also come as no surprise that each individual has its own way of reacting to potentially hazardous influences, be they exogenous or endogenous. This may manifest itself in many situations, for instance in some degree of selective ineffective immune responsiveness (*Hässig* and *Cottier*, 1986) or the reverse of it.

In the dynamic interactions between the organism and its environment, the gastrointestinal tract, with - in the adult human - an inner surface of about 250 m^2 , takes a leading position. This is not only the site where water and a vast variety of dietary substances enter the body, it also represents by far the most important area of contact between the organism and a multitude of micro-organisms, not to speak of potentially toxic chemicals. It has once been said that "the gut contains sufficient bacteria and toxins to kill the host millions of times over" (*Border*, 1989). Although this forceful sentence, expressed by a glorious surgeon, defies logic, it helps to focus our attention on the intestinal microflora and its medical importance. It has become increasingly clear that this enormous mass of microbes, if not contained, can endanger the host's life within a short time. It should be recalled at this point that septic shock, and in particular its gut-derived infectious-toxic variant (*Cottier* and *Kraft*, 1991), remains the most feared complication of major surgery, traumata and burns. It is estimated that alone in the United States more than 130,000 deaths per year are associated with bacteraemia, and a large proportion of these results from infection by micro-organisms that normally reside in the gut (*McCabe*, 1974; *Parillo*, 1985).

HELP AND DANGER FROM THE INTESTINAL MICROFLORA

Since this topic has been discussed extensively in previous Herborn Seminar Monographs, this chapter is restricted to a few remarks that relate to the general theme of this monograph. The mutual relationships among the hundreds of enteric microbial species, and between the intestinal microflora and the host, are the result of an evolutionary process of long duration. Under physiological conditions, the macro-organism benefits from the presence of this mass of micro-organisms in various ways. This may best be exemplified by axenic ("germ-free") animals who have a poorly developed immune apparatus and in addition to other deficiencies - are highly susceptible to severe infections when exposed to pathogenic microbes (*Luckey*, 1963). "Protective colonisation" by specialised micro-organisms (*Savage*, 1984) seems to contribute markedly to host defence in small rodents and probably also plays an important role in humans. Bacterial endotoxin in small amounts is able to enhance host resistance (*Urbascheck* et al., 1984), however, this effect of LPS in humans remains to be studied. Furthermore, the intestinal microflora is well known to participate in enzymatic degradation of gut content, delivering, e.g., substrates for enterocyte metabolism and other use; to be instrumental in the transformation

of bile components; to contribute to supplement of vitamins; to catabolise certain exogenous toxins; and to help maintaining the host's homeostasis in many other ways. In all these processes, the gut microflora exhibits a high degree of flexibility, for instance in the sense of adaptive enzyme induction (*Schlegel*, 1985).

Conversely, the enormous mass of bacteria in the lumen of the lower intestinal tract constitutes a permanent threat to the macro-organism. If the more or less exponential growth of these micro-organisms is not counterbalanced by continuous propulsion through the digestive tract and ultimate elimination with the faeces, a dangerous situation can develop within a short time. In immunocompromised individuals, opportunistic infections may originate from the gut content. Enteric bacteria also play a predominant role in hospitalisation; represent a hazard in catheterism; may settle on anomalous cardiac valves or protheses; are apt to damage the host by a variety of metabolic products; and enter the tissue in case of intestinal barrier failure (see below). Furthermore, they may collaborate in the metabolic transformation of various chemicals into carcinogens. The interspecies equilibrium in the intestinal microflora, which is based, among others, on mutual tolerance and optimal antagonisms and in which obligate anaerobes appear to play an important role, can be disrupted by oral antibiotic treatment. As a result, certain species, such as *Clostridium difficile*, with its natural multiple resistance, may overgrow and damage the host via toxin production (*Bartlett* and *Laughon*, 1984). It should be recalled in the present context that pathogenic properties can be transferred, even across different species, from one micro-organism to another with the help of bacterial viruses, in particular plasmids and phages, and via transduction (*Taylor*, 1983; *Luria* and *Suit*, 1987; *Finlay* and *Fulkow*, 1989). In other words, previously non-pathogenic microbes can acquire pathogenicity, and commence, for instance, to produce exotoxins, and this process is apt to expand among the microbial population within a rather short period of time. Conversely, bacterial endotoxin, chemically a lipopolysaccharide (LPS), is a constitutive component of the outer membrane of Gram-negative bacteria. It may be released as a consequence of microbial death, to some extent also during rapid proliferation of bacteria and/or by the action of activated complement (*Doran*, this fascicle).

THE INTESTINAL BARRIER AND ITS BREAKDOWN

Under physiological conditions, the mass of intestinal micro-organisms is contained within the lumen by an intricate system of structural and functional obstacles, commonly known as "intestinal barrier" or "mucosal block" (*Gebbers* and *Laissue*, 1990). In brief, it comprises - among other from inside to outside: secretory IgA and lysozyme, which are in part associated with mucus (*Clamp*, 1980); the enterocyte layer, covered by the glycocalyx, tightened by the intercellular junction complex, primarily by the *zonulae occludentes*, and equipped with protective molecules such as interferon and enzymes; and the mucosal stroma which harbours, e.g., antibodies, complement, granulocytes, macrophages, lymphocytes, plasma cells and natural killer cells as well as lymphatics and small blood vessels. Lymphoid follicles in the intestinal wall

function primarily as immunological contact structures in as much as they are covered by a specialised epithelium with so-called "M" cells that are permeable for macromolecules and small particles (*Owen*, 1977). Taken together, these structures provide for both a rather tight seclusion of, and a constant but restricted contact with, the intestinal microflora and/or its antigens. The integrity of the intestinal barrier, in particular of the epithelial layer, depends on a sufficient supply of oxygen and nutrients (*Page*, 1989), especially also of glutamine (*Souba* et al., 1990).

Intestinal barrier failure can occur in various ways. Trivial causes are, for instance, physical disruption, ulceration/perforation and suture insufficiency in the gut wall. More complex causative mechanisms include severe disturbances of the ecological balance in the intestinal microflora and endotoxin effects. Cytotoxic chemicals and ionising radiation represent well established noxious agents attacking, among other, the intestinal epithelium. Of predominant medical importance, however, are splanchnic ischaemia (*Fiddian-Green*, 1988) and consecutive reperfusion damage, involving oxygen-derived radicals (*Deitch* et al., l990a). The lesions produced by this type of injury may range from a break-up of tight junctions (*Deitch* et al., l990b) to bland epithelial necrosis and mucosal denudation. Regeneration of the enterocyte layer seems to require 4 or more days (*Bragg* and *Thompson*, 1989).

EFFECTIVE AND INEFFECTIVE IMMUNE FUNCTIONS OF THE GUT ASSOCIATED LYMPHOID TISSUE (GALT)

Since it was shown that the follicle associated intestinal epithelium is preferentially permeable to macromolecules and microparticles (*Joel* et al., 1970), it became increasingly clear that the gut associated lymphoid tissue acts also as an immunological contact organ. The outstanding magnitude of the antigenic stimulation originating from the intestinal microflora may, for example, be exemplified by the postnatal development, in mice, of the proliferative activity in regional, i.e. mesenteric, lymph nodes, which is manifold greater than in lymph nodes of other locations (*Schwander* et al., 1980). In this species, a massive influx of thymus derived lymphocytes sets in shortly after birth (*Joel* et al., 1971). Primary humoral immune responses result in the production mainly of IgM antibodies, and germinal centres form to generate great numbers of memory B cells (*Grobler* et al., 1974). In the GALT, and under physiological conditions, the newly produced memory B cells belong predominantly to the IgA class. They enter the circulation and tend to "home" back to the intestinal or other mucosal layers (*Hall*, 1979). The mechanisms responsible for this type of homing are still disputed, however, they seem to involve adhesion molecules on the cell surfaces (for references, see *Möller* et al., 1991). Dimeric IgA is coupled by epithelial cells with the secretory component and released into the lumen as secretory IgA, which is quite resistant to proteolysis and represents the most important humoral defence instrument in the gut of the healthy adult mammalian organism (*Bienenstock* and *Befus*, 1980). In humans, it takes - after an appropriate antigenic challenge - about one week until, e.g., anti-O antibody titres rise in the circulating blood (*Stuttmann* et al., 1989). In chronic ulcerative colitis, IgG antibodies seem to take the lead over IgA antibodies

Table 1: Selective list of risk factors for the development of gut derived infectious-toxic complications, e.g., following major surgery*

Age:	Less than 1 year, more than 65 years
Nutritional status:	Malnutrition Obesity
Abuse:	Alcoholism Possibly smoking Drugs
Disease:	Metabolic disorders, e.g., diabetes mellitus Arterial atherosclerosis, with regional hypoperfusion, in particular also coronary heart disease Other cardiovascular disorders Chronic obstructive bronchitis Severe infections (e.g., HIV infection /AIDS, measles, chronic tuberculosis, parasitoses and others) Malignant neoplasia
Severe trauma	
Severe burns	
Therapy:	Cytostatic chemotherapy, including immunosuppression Glucocorticosteroids Catecholamines Possibly antibiotics Catheterism (intravascular, intraluminal) Ionising radiation, especially in the abdominal region Preceding major surgery
Preceding hospitalisation	

*Excluding primary, i.e. genetically determined, immunodeficiency syndromes.

(*Brandtzaeg*, 1985). The role of cell-mediated immunity in gastrointestinal defence systems is still not adequately understood, so are the functions of intra-epithelial T cells, which usually carry a γ/δ type T cell receptor (*Goodman* and *Lefrançois*, 1988). Quite obviously, however, and despite some uncertainties about mechanisms involved, GALT is functioning effectively most of the time and in the vast majority of individuals.

Conversely, there are many possible causes of ineffective defence by the immune apparatus associated with the digestive tract. More and more genetically determined, so-called "primary" immunodeficiency syndromes are being identified and many of these - mostly hereditary - defects manifest themselves also in gastrointestinal disorders (*Cottier* et al., 1991). If one considers the innumerable peptidic molecules engaged in immune reactions, each of which may be subject to a genetic defect, this field of research and knowledge is certain to expand in the years to come. Of even greater medical importance is the multitude of acquired conditions that interfere with successful immune reactions in the gastrointestinal tract. The individual's general condition plays an essential role since it has been shown that the risk of developing severe infectious complications following major abdominal surgery is a function of age, cell-mediated

immune reactivity, examined by skin tests, and albumin concentration in the circulating blood (*Christou*, 1989). Furthermore, a great number of diseases/disorders of infectious, toxic, metabolic and/or iatrogenic nature can also cause acquired immunodeficiency (Table 1). AIDS is just one example, albeit the most dramatic, of such conditions. The defensive capabilities of GALT may also be overcome, even in otherwise healthy individuals, by an excessive microbial attack, the causative micro-organisms being pathogenic or - as often occurs in the gut derived infectious-toxic shock (GITS) - normal constituents of the intestinal microflora: we may regard this type of events as the consequence of a "relative" immunodeficiency. In particular, the primary - or even anamnestic - immune response may come too late to be able to cope with a sudden massive bacterial attack, as it can occur, e.g., after acute intestinal barrier failure. In addition, major trauma, surgery and burns are known to be followed by marked immunosuppression. The mechanisms responsible for this "Post-TSB" immunodeficiency syndrome (*Grob* et al., 1987) are still incompletely understood. They are complex and seem to involve, among other, phagocyte dysfunction, enhanced suppressor activity of cells, release of suppressor peptides, lymphocyte sequestration, and hormonal effects (*Goodwin* and *Behrens*, 1990).

INFLAMMATORY REACTIONS: HARMONIOUS AND PROTECTIVE VERSUS EXCESSIVE AND HAZARDOUS

Inflammation can be regarded as the sum of reactions originating from soluble blood plasma constituents, blood cells, the microvascular system, mast cells, and mesenchymal elements to injury (*Iversen*, 1989). This highly complex process involves complement (*Mollnes* and *Lachmann*, 1988); the coagulation, fibrinolytic (*Kaplan* and *Silverberg*, 1987) and anticoagulant (*High*, 1988) systems; immunoglobulins (see below); granulocytes, especially neutrophils (*Benestad* and *Laerum*, 1989); monocytes/macrophages (*van Furth*, 1989); mast cells (*Enerbäck* and *Norrby*, 1989); and small vessels which may interact, e.g., via adhesion molecules, with blood cells (*Möller* et al., 1991), and soluble plasma components. Most often, inflammatory reactions fulfil their *protective* function in as much as they succeed in overcoming invading microbes and/or in restoring - at least to the possible extent - tissue integrity, and then calm down.

If, however, the causative agent (e.g., bacteria, endotoxin and others) persists or increases in amount, inflammation may build up and reach a level where it gets out of antagonistic control. In such situations, the powerful forces of defence are apt to direct themselves against the host and put its life in acute *danger*, mainly via the action of pro-inflammatory mediators. Among the latter, certain cytokines, especially tumour necrosis factor (*Fong* and *Lowry*, 1990), interferon-γ (*Billiau*, 1988), interleukin-1 (*Offner* et al., 1990) and others, play a predominant role. But platelet-activating factor (*Braquet* et al., 1987), certain eicosanoids (*Hechtman* et al., 1990), oxygen derived free radicals (*Taylor* et al., 1986), proteinases liberated from phagocytes (*Neuhof*, 1990), and others also participate in this deleterious cascade of events. This complex, progressive process is the hallmark of septic shock, which in medicine has remained an unresolved crux.

ANTIBACTERIAL DEFENCE: SUCCESS AND FAILURE

Phagocytes, in particular neutrophils, are the host's most powerful weapons against bacteria and fungi. These cells kill micro-organisms in various ways, the most important being the formation of highly reactive oxygen derived radicals in the course of a respiratory burst and their secondary products (*Taylor* et al., 1986); the release of myeloperoxidase with consecutive chlorination of endogenous amines (*Grisham* et al., 1984); and oxygen independent mechanisms. The latter encompass the elaboration of cytotoxic peptides, some of which appear to be specific for Gram-negative bacteria (*Elsbach* and *Weiss*, 1985). One prerequisite for neutrophils to enter into action in time is their rapid accumulation on site, in which complement components, especially C5a, certain cytokines, endotoxin, and adhesion molecules play their part. Another important condition for successful killing of microbes is their adequate opsonisation, in preparation of endocytosis by phagocytes. Micro-organism specific antibodies are the best promoters of this process because they can, with the constant region of the molecule, also interact with Fc receptors on cells and with complement components, in particular with C3b. Depending on the bacterial species/strains involved, C3 cleavage products bind with complement receptors CRl and/or CR3 on the phagocyte surface (*Späth*, 1991). It has been shown that C3b-IgG-heterodimers are especially good opsonisers for micro-organisms to be taken up by neutrophils (*Malbran* et al., 1987). Conversely, certain microbes, such as mycobacteria, are preferentially killed by macrophages activated by T cells.

Antibacterial defence may fail for a number of reasons. Neutropenia is, in rare instances, hereditary, but most often this type of insufficient cell production occurs in the course of an acquired disorder. The same pertains to phagocyte dysfunction (*van der Valk* and *Herman*, 1987), although primary defects of this category are well known, e.g., chronic granulomatous disease of childhood (*Curnutte* et al., 1989), the Chédiak-Higashi syndrome (*White* and *Gallin*, 1986), myeloperoxidase deficiency, and others. It is not surprising that novel types of deficient phagocyte functions are reported in increasing numbers (*Cottier* et al., 1991). It must be emphasised, however, that genetically determined disorders of this sort are far less frequently encountered than acquired forms of phagocyte dysfunction. They occur in a vast variety of infectious, toxic, metabolic, and other disorders. One important cause of ineffective microbicidal activity of phagocytes seems to be inadequate opsonisation due, for instance, to local antibody consumption. Understandably, immunosuppression (see above) can lead to similar insufficiencies of the microbicidal machinery.

CONTAINMENT AND RELEASE OF BACTERIAL ENDOTOXIN

In considering the threat posed by the presence of enormous amounts of endotoxin (a constituent of the outer membrane of Gram-negative bacteria) in the gut, a few remarks seem appropriate. *In vitro* it is difficult - if at all possible - to demonstrate a direct toxicity of LPS. Rather, this substance triggers cells, especially also macrophages, to release a multitude of pro-inflammatory media-

tors which, when produced in excess, endanger the host's life. Thus, endotoxin threatens the integrity of the macro-organism as soon as it comes - in critical amounts - into contact with responsive cells, i.e. in the tissues. The question, therefore, arises as to how this may happen. It is generally assumed that the gut mucosa is "impervious" and "resistant" to this material (*Bayston* and *Cohen*, 1990). However, information on the amount of free endotoxin in the intestinal lumen of healthy adults is scarce. We also know little about intraluminal death rates for Gram-negative micro-organisms under physiological conditions. Conversely, substantial release of endotoxin has been observed following the action of certain antibiotics (*Rokke* et al., 1987) and - probably of great importance after engulfment of Gram-negative bacteria by phagocytes. These may liberate fragments of this material that are many times more active than commercially available LPS (*Duncan* et al., 1986). Killing of micro-organisms solely by complement and/or specific antibodies does not seem to be a major source of free endotoxin (*Roantree* and *Rantz*, 1960). Disregarding antibiotic treatment, which is not without hazards, we may thus theorise that endotoxin is predominantly released from Gram-negative bacteria within the tissues, via the action of phagocytes. Quite possibly, this release may be particularly important if neutrophils and macrophages die before endocytosed micro-organisms are fully degraded. The fate of liberated endotoxin in the tissues remains debatable. It may be neutralised by naturally occurring anti-LPS antibodies, although these could rapidly be consumed (*Barclay* et al., 1989). Or it may bind to LPS-binding protein (LBP), lipoproteins, anti-thrombin III, α-2-macroglobulin and/or other, poorly characterised plasma proteins (*Bayston* and *Cohen*, 1990). Elimination of such endotoxin-protein compounds seems to be achieved primarily by the liver.

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