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SPECIAL FEATURE
SELECTIVE GASTROINTESTINAL DECONTAMINATION

History of recognition and measurement of colonization resistance of the digestive tract as an introduction to selective gastrointestinal decontamination

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INTRODUCTION

Selective decontamination of the digestive tract was developed following the observation that the digestive tract normally has considerable resistance to colonization by newly ingested bacteria. The research that eventually led to selective decontamination was performed because in the late 1960s and early 1970s, the need for prophylaxis against Gram-negative infections in immunocompromized patients became evident. At that time, the relatively small number of antibiotics available for therapy of serious infections often lead to treatment failure. To introduce the subject of selective decontamination, this paper, therefore, starts with a short historical overview of the kind of infectious agents as well as the antibiotics available in the 1960s; particularly regarding the type and treatment of infections in severely compromised patients. The fact that the possibilities in infectious diseases were limited was the reason for our experimental search for ways of successful prophylactic treatment with minimal risk of development of resistance.

The existence of a resistance in the digestive tract to colonization by newly ingested bacteria and yeasts as well as its impact for clinical medicine in regard to antibiotic use, was first reviewed by Smith in 1952 [1]. His paper started with the following still relevant sentence: 'Evidence is accumulating to show that the complex balance which exists among microorganisms constituting the normal flora of the body is disturbed by prolonged administration of the newer antibiotics'. The only difference with later, more specific, studies based on this and other early conclusions [2, 3] is that Smith did not give the phenomenon a name and did not recognize the necessity of measuring and expressing its quality.

Later on, in the 1950s, Rogers [4] reported a study which was a logical follow-up of Smith's conclusions. Rogers compared the incidence and type of infections in the late 1950s with a comparable historical group of patients treated in the late 1930s. He came to the conclusion that the use of antimicrobials was not only associated with more infections, but in addition by a changing pattern of infections. In patients treated in the late 1930s, in the 'pre-antimicrobial period', Gram-positive bacteria such as pneumococci, streptococci and staphylococci were mostly responsible for infections associated with death. In patients treated in the

'antibiotic era' in the late 1950s however it was predominantly the Gram-negative bacilli which caused severe infections, more than half of which were associated with death of the patient. In 1967 Kessner and Lepper [5] confirmed this observation when they reviewed 107 cases, 73 of which had a serious Gram-negative infection, either single or mixed with Gram-positives, mostly located in the urinary tract. This incidence of 70% was much greater than was recorded in the previous decade. In the 1940s and 1950s, as well as later on in the early 1960s, the influence of antibiotic treatment on the type, the occurrence, and the severity of infections had in fact repeatedly been reported [6–9]. All these reports however, have apparently been neglected or were not understood. Regarding hospital epidemiology, the importance of the endogenous enteric flora as a reservoir for hospital infections had been recognised by Vosti and colleagues [10] who serotyped *Escherichia coli* strains isolated from urinary tract infections as well as from faeces of the corresponding patients and found the same serotypes in both samples. This observation was later confirmed by others [11, 12]. Meanwhile, increased transmission between patients of resistant Gram-positive bacteria had also been reported [13]. Our conclusions from this historical overview given below formed the basis for our studies started in the 1960s. The conclusions were that:

1. Most life-threatening infections are caused by Gram-negative bacilli.
2. Antibiotic use apparently enhanced the occurrence of (resistant) Gram-negative and staphylococcal infections.
3. Antibiotic use apparently promoted spread of infections by resistant Gram-negatives and Gram-positives between patients in hospitals.
4. Antibiotic use was associated with the development and/or selection of resistance among previously susceptible bacteria.

These conclusions led to our working hypothesis: 'antimicrobials may suppress, or at least modify, the endogenous microflora in such a way that they enhance the occurrence of Gram-negative infections'. It was therefore decided to try to answer the following three basic questions:

1. Do severely immunocompromised (granulocytopenic) individuals suffer predominantly from Gram-negative infections acquired from the environment, or infections by Gram-negatives which were already colonizing their intestinal tract before the onset of their granulocytopenia?
2. To what extent can systematic antibiotic therapy cure infections in severely compromised subjects and, in addition, to what degree do potentially dangerous changes occur in the microflora of their patients?
3. Is there a way to prevent Gram-negative infections in severely compromised subjects?

EXPERIMENTAL STUDIES

Effect of prevention and/or effective therapy of infections in irradiated experimental animals

Place of study and required facilities

At the University Hospital of Leyden where I qualified in the early 1960s, the type and the incidence of infections was predominantly Gram-negative. The situation regarding the incidence of infections and the type of patients involved, was comparable with that reported 10 years later by Myerowitz and co-workers in

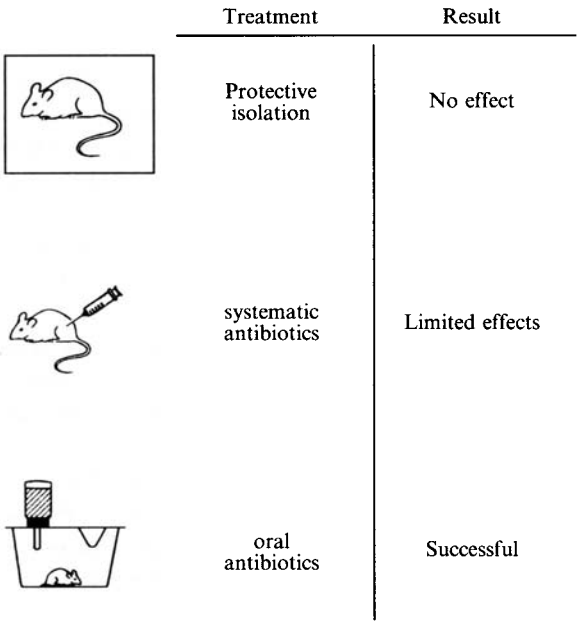

Fig. 1. Schematic presentation of three approaches to the study of infection prophylaxis in severely immunocompromised mice.

1971 [14]: the majority of serious infections, particularly in granulocytopenic patients, were caused by Gram-negative bacilli which were often difficult to treat. A study in patients to determine ways for improvement was at that time, however, difficult to visualise. Yet, we considered it essential to investigate the effect of antibiotics on the endogenous microflora and on its role in the development and spread of infections in general and of Gram-negative infections in particular. An experimental study might help to answer the questions outlined above and so provide a basis for subsequent clinical studies.

At the Radiological Institute in Rijswijk (near Leyden), this kind of study was possible. This research institute had, in addition to radiation facilities, excellent facilities for bacteriological experiments in animals, including germ-free and specific pathogen-free (SPF) animal housing. The germ-free facilities permitted a study of the influence of the composition of the intestinal microflora in greater detail. Irradiation of experimental animals would provide subjects with any desired degree of decreased resistance to infection.

The points which we aimed to study are depicted schematically in Figure 1.

The study of ways for safe infection prophylaxis

To minimize infections in animals during radiation experiments, we studied strict protective isolation as a first step. Fifty percent of a large group of irradiated mice was maintained in a plastic germ-free isolator directly after lethal irradiation, the other half of the group was housed conventionally. All animals were autopsied for culturing. This experiment was repeated several times. Strict isolation in germ-free plastic isolators had no effect on the occurrence and type of infections. The results in both the isolated and in the control group were invariably comparable.

The mean survival time was identical in both isolated and non-isolated animals. Furthermore, the type of bacteria isolated from the heart, blood as well as from their intestines at necropsy was identical in both groups [16]. Predominantly Gram-negative entero-bacilli or mixed Gram-negative/Gram-positive infections were found. This made it likely that the gastro-intestinal tract microflora had been the source of the septicaemia in both isolated mice and in the control group. Later, more specifically directed, studies [18] confirmed our conclusion that endogenous potentially pathogenic bacteria were as pathogenic for irradiated mice as exogenous (environmentally acquired) bacteria of the same kind. This might also be the case in human patients.

Finally, it was concluded from this experiment that in one way or another, the host was involved in the maintenance of the endogenous flora in a stable and optimal condition. Irradiation or anti-tumour chemotherapy appeared to affect this host contribution to the balance of the endogenous flora. Irradiation might accordingly affect the quality of the endogenous flora by decreasing its control on the proliferation of potentially pathogenic bacteria and yeasts inside the digestive tract. Histological sections of the gastro-intestinal tract samples revealed that the mucous membrane as well as the immune system of the gut may play a role in the stability and maintenance of the endogenous flora. Because this has been reviewed recently [15] and because this information may confuse the main issue of this review, this subject will not be further discussed.

Systemic antibiotic treatment

As indicated in Figure 1, our second approach was to study the effect of systemic antibiotic therapy on imminent infections in irradiated mice as well as on their microflora. The results were again rather disappointing in relation to decreasing mortality in this way. Although the susceptibility of the Gram-negative bacteria in the faeces of the mice was determined before each experiment and showed good antibiotic susceptibility, the twice daily subcutaneous injections with appropriate antibiotics caused local haemorrhages. These haemorrhagic areas often appeared to accommodate the growth of resistant bacteria. Secondly, the intestinal aerobic microflora of these animals appeared to be modified by the systemic treatment; particularly when broad-spectrum antibiotics were used such as penicillin-streptomycin, chloramphenicol, oxytetracycline or, later, cephaloridine-kanamycin.

Antibiotic decontamination of the digestive tract

The third approach to treatment/prevention of bacterial infection in immunocompromised mice was the application of oral treatment, starting before irradiation, with non-absorbable antibiotics [16] according to directions outlined by an American surgeon Poth [17]. We gave extra attention to prevention, because it might not only become clinically applicable, but successful infection prevention might be beneficial to the radiobiologists in the institue. If it became possible for the radiobiologists to perform their experiments without untimely death in their animals due to infection, their results would no longer be potentially biased in this way.

Oral administration of a sufficient daily dose of a combination of broad-

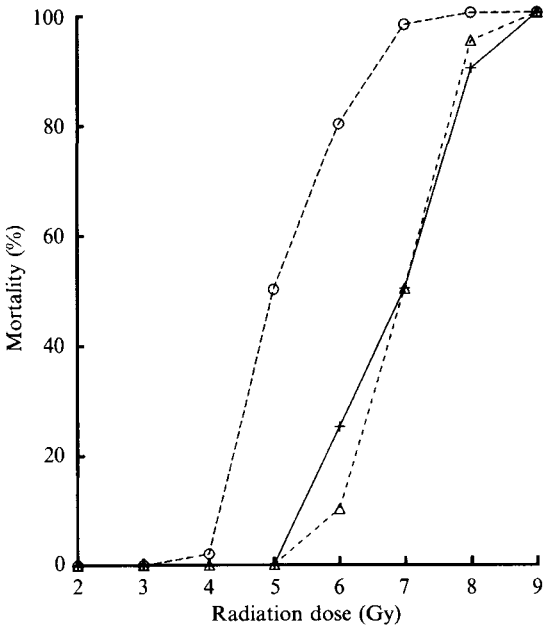

Fig. 2. Mortality after various irradiation doses in germ-free (△), oral broad-spectrum non-absorbable antibiotic-treated (+) and conventional (○) mice.

spectrum, non-absorbable, antibiotics in the drinking water, appeared a great success. In properly (completely) decontaminated mice, the median lethal irradiation dose as well as the mean survival time after irradiation with various lethal doses, were found identical to those in germ-free mice of the same strain and age (Fig. 2). This equated with the fact that all aerobic as well as anaerobic faecal and necropsy cultures were negative [18, 19].

Enhanced acquisition of resistant bacteria from the environment

When in 1968 it was found that antibiotic decontamination of the digestive tract of mice by oral administration of broad-spectrum antibiotics [20] increased their LD50 for X-irradiation by more than 1 Gy (Fig. 2), antibiotic decontamination was performed on a rather large scale in several radiation experiments in the institute. However, within the first months of large-scale application of antibiotic decontamination, all mice of a particular radiation experiment died within 24 h. All animals were autopsied and all cultures revealed a resistant *Klebsiella pneumoniae*. These animals were housed in adjacent cages. An epidemiologic search suggested that the contamination originated from the environment. A possible source of these resistant strains was the adjacent experiment rooms for monkeys. By the end of allogeneic bone marrow transplantation experiments, monkeys were frequently colonized with a number of resistant Gram-negative strains. Experimenters often performed experiments in monkeys as well as in mice; they themselves may have transmitted the bacteria from the monkeys to their mice by neglecting some of the basic rules for prevention of transmission of microorganisms. The klebsiella strain had spread rapidly from cage to cage and so had caused untimely death in all animals.

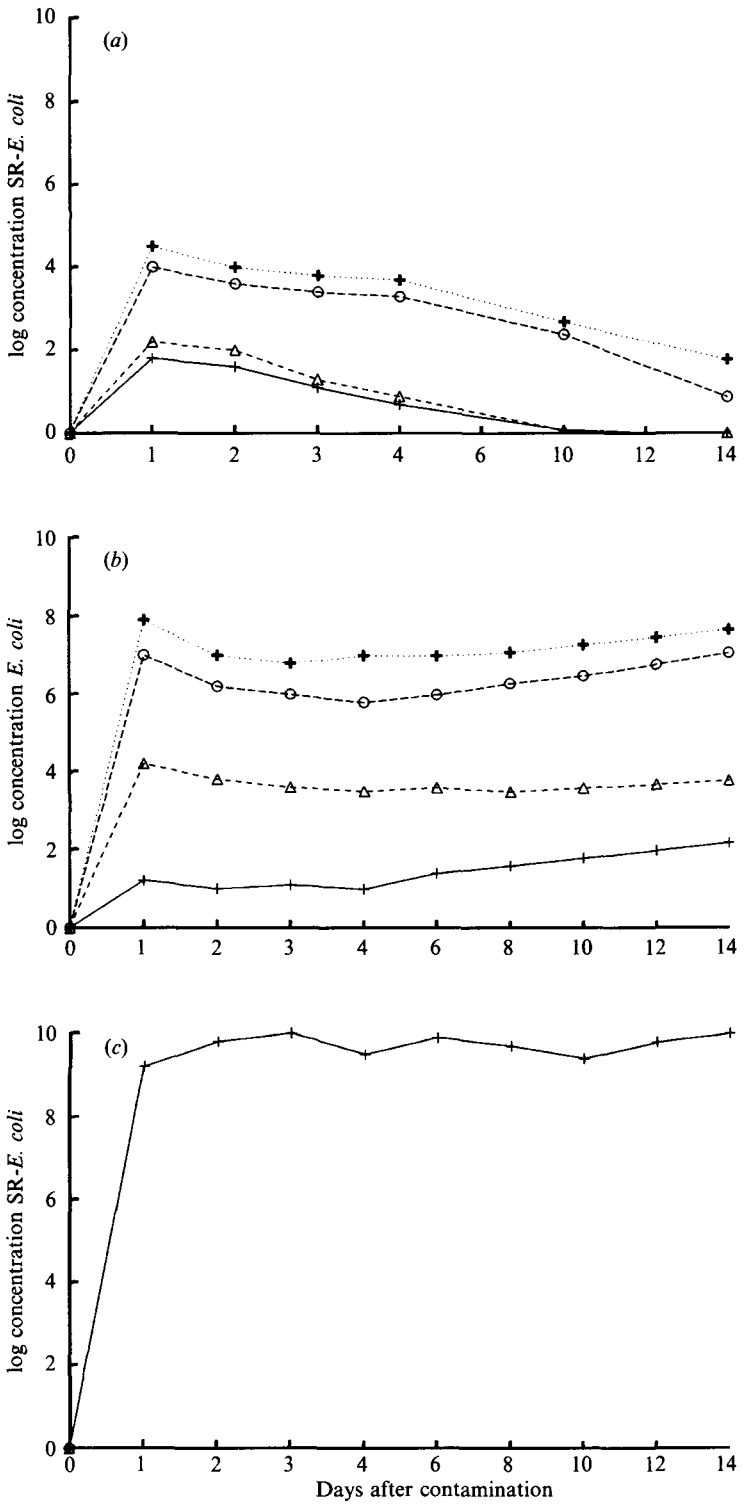

Fig. 3. For legend see opposite.

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In this, but also in subsequent unfortunate experiments, resistant bacteria appeared to spread very rapidly between cages with mice treated with the same oral antibiotic regimen. These contaminants appeared to be predominantly Gram-negative enterobacilli, which all had in common resistance to the antibiotics used.

To summarize, in antibiotic treated subjects these infections appeared highly contagious. Spread of resistant bacteria from remote potential sources as well as between cages, could only be explained by contamination with numbers of bacteria that could be neglected in conventional mice. This implied that only small numbers of resistant bacteria were required for colonization and subsequent infection. It was considered unlikely that the enhanced spread was due to a special virulence factor which was associated with antibiotic resistance genes. The same bacteria did not spread and enhance mortality in untreated irradiated mice (with a normal flora). In decontaminated mice without intestinal microflora therefore, **the spread might be strongly facilitated by the absence of an endogenous enteric microflora.**

Study of the role of intestinal flora in the control of infections

A logical consequence of the foregoing studies was, to investigate our suspicion that the intestinal microflora might be essential for the control of growth of potentially pathogenic bacteria in the gut. In addition, the endogenous flora might control invasion by potentially pathogenic microorganisms of the intestinal mucosa and subsequent systemic disease. To study this, we contaminated groups of 30 conventional mice orally with four different doses (ranging between 10^4 to 10^{11}) of Gram-negative bacteria (Fig. 3*a*). This experiment was repeated with successfully decontaminated mice (Fig. 3*c*) as well as in sublethally irradiated (LD10) animals (Fig. 3*b*). Identical oral doses of a streptomycin-resistant strain of *Escherichia coli* (SR-*E. coli*) gave completely different results in each group of mice.

In the conventional (control) group, quite high oral doses were required for intestinal colonization at average concentrations of $> 10^3$ bacteria per gram of faeces, a week after oral contamination. Peak concentrations of over 10^5 bacteria per gram of faeces were only seen about 2 days following the highest contamination dose of 10^{11} SR-*E. coli* cells (Fig. 3*a*).

In the irradiated animals, considerable higher average SR-*E. coli* concentrations were seen in faeces upon oral challenge with the same doses (Fig. 3*b*). The mice which died in this experiment, all died with the contaminant in their blood when cultured at necropsy.

In the antibiotic decontaminated mice there was a high degree of overgrowth. All contamination doses, even the lowest, resulted in persistent, extremely high, faecal concentrations of the resistant contaminant over a period of weeks (Fig. 3*c*).

On the basis of this and subsequent very similar studies [21, 22], we concluded that the endogenous microflora did apparently play a major role in the control and exclusion of newly ingested bacteria.

Fig. 3. Mean faecal concentrations of SR-*E. coli* following four different oral doses in conventional non-treated (a), in sublethally (6 Gy) irradiated (b) and in mice treated orally with broad-spectrum non-absorbable antibiotics to remove their gi-tract microflora (c). *E. coli* doses: 10^4 (+), 10^7 (△), 10^9 (□), 10^{11} (✚).

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Intestinal bacteria involved in resistance to colonization

Oral association of ex-germfree mice with single strains of aerobic bacteria or with different combinations of aerobic bacteria failed to provide these animals with 'protection against colonization' such as that achieved following oral association of ex-germfree mice with the anaerobic part of the mouse intestinal flora [23]. The latter state was obtained by combined oral and parenteral treatment of conventional mice with kanamycin. When maintained under some degree of protective isolation, this treatment rendered mice persistently free of Enterobacteriaceae species [24]. Later Koopman and co-workers [25] proved that the anaerobic intestinal flora becomes more protective, the greater the number of different anaerobic bacteria isolated from mice that are used in its composition.

The strong protective effect of colonization of the digestive tract with the endogenous anaerobic fraction of the intestinal microflora was called **colonization resistance** (CR) of the digestive tract [21]. The anaerobic flora that appeared responsible was then called colonization resistance factor (CRF) [23].

Combined treatment with oral antibiotics and strict isolation

The experiments described in the previous paragraphs showed that, if the endogenous intestinal microflora was completely removed or largely suppressed by antibiotics, infections with resistant microorganisms could apparently only be prevented, if this deficit in colonization resistance factor was replaced by an artificial barrier like that provided by strict isolation (barrier nursing). Indeed, when total antibiotic decontamination of the digestive tract was combined with strict protective isolation, complete suppression of the intestinal flora could provide animals comparable with germ-free mice in irradiation experiments.

Enhancement of septicaemia by 'intestinal overgrowth'

In a subsequent series of experiments, the occurrence of invasion by Gram-negative enterobacilli and enterococci was studied in all three types of animals; conventional, antibiotic treated and irradiated mice [23]. It was found that in conventional non-irradiated mice, oral doses as high as 10^9 bacteria or more were required to obtain positive (contaminant) cultures from their cervical and mesenteric lymph nodes as well as from their liver and spleen [26]. In lethally irradiated mice, somewhat lower contamination doses were sufficient to render these cultures positive [16]. However, in antibiotic treated mice, spread to lymphatic organs occurred following doses which were 6–8 logs lower than in untreated mice [27]. The results of a representative experiment are depicted schematically in Figure 4. Spread to lymphatic organs were several years later called *translocation* by Berg [28].

In both the oral antibiotic-treated animals and in the systemically-treated mice, the resistant bacteria involved in translocation to the lymphatic organs, were found in abnormally high numbers in the oropharynx as well as in the intestines [27]. This provided us with another clue to the clinical observations regarding the changing pattern of infections in (immunocompromised) hospitalized patients. Overgrowth in man may also be associated with translocation and thus in enhancement of clinically apparent infections.

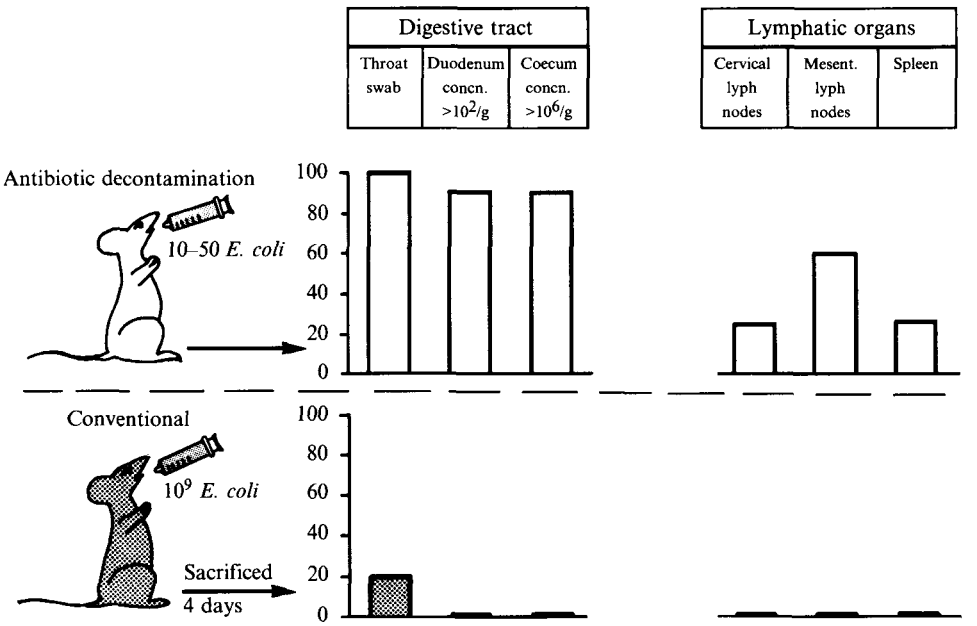

Fig. 4. Influence of oral treatment of mice with non-absorbable antibiotics on their susceptibility (dose required for gi-tract colonization) to oral challenge with *Escherichia coli* resistant to the antibiotics used: the occurrence of overgrowth and translocation in the antibiotic treated group. In mice, concentrations of $> 10^{-2}$ *E. coli*/g in the duodenum indicate a significant increase; this is also the case with caecal concentrations of $> 10^{-6}$/g.

Whether 'bacterial overgrowth' and 'translocation' occurs in patients as observed in mice and could thus be responsible for enhanced susceptibility during treatment with antibiotics in immunocompromised patients, has been studied by Tancrede [29]. Indeed, he found evidence of translocation of intestinal Gram-negatives in antibiotic treated immunocompromised patients.

The results of these experimental and clinical observations can be summarized as follows. Antibiotic treatment may enhance the risk for Gram-negative infections apparently along two lines:

1. Oral non-absorbable as well as a number of systematically applied antibiotics may strongly affect the predominantly anaerobic endogenous (for most part anaerobic) gastro-intestinal microflora and thus decrease colonization resistance. In this way antibiotics which reach the intestinal lumen, may considerably increase the susceptibility to contamination by small numbers of resistant bacteria.

2. Decreased colonization resistance is associated with 'overgrowth' by resistant microorganisms. 'Overgrowth' may be associated with translocation to internal organs as they can be isolated from lymphatic organs especially the liver and lungs, organs normally involved in the clearance of bacteria from the blood stream.

Selective decontamination of the digestive tract

A logical consequence of the finding of the importance of a colonization resistance associated anaerobic intestinal flora (CRF) in the control of infections

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and the effect of certain antibiotics on the CR was, in the early 1970s, to search for antimicrobial drugs that do not affect the CRF. The aim of this new approach was threefold:

1. Reduction, or better, prevention of suppression of CRF during treatment and if possible, the retention of antibiotics for the prevention of infections.

2. Strict isolation as a consequence of antibiotic decontamination was clinically not applicable and unrealistic so that another method was urgently needed.

3. The use of antimicrobials which did not affect CRF might have positive epidemiological consequences, as it might limit accumulation and spread of resistant microorganisms between hospitalized, antibiotic-treated, patients.

For these reasons, we studied whether such antimicrobial drugs were available for clinical use and if so, whether they could be used to 'selectively' suppress or eliminate Gram-negative bacilli in the digestive tract of compromised individuals as a means of preventing of infections. Disappearance of Gram-negatives by the treatment was monitored by culturing oral swabs and faeces. The maintenance of CRF during treatment was measured by experimental oral contamination with resistant Gram-negative enterobacilli. The results of the first successful experimental studies in which selective suppression resulted in stool cultures, negative for Gram-negatives in mice and monkeys, was reported in 1974 [30]. In these experiments it appeared that an antimicrobial drug was suitable when it did not affect the Gram-positive fraction of the endogenous intestinal flora, including aerobically growing Gram-positives. This could be accomplished by, mostly oral, treatment with a narrow, exclusively anti-Gram-negative, spectrum such as polymyxins, nalidixic acid and related compounds as well as aztreonam later on. Another mechanism by which selective suppression of Gram-negative enterobacilli could be accomplished was found to occur as a result of drug secretion and activity in the mucus layer; the drug being inactivated in the intestinal contents. This included antimicrobials with broad-spectrum activity such as co-trimoxazole and quinolones [31–33]. Later, when more theoretically suitable drugs became available, additional experiments were performed [34–39]. This information was reviewed in 1982 [40]. In clinical use, the treatment aimed at selectively suppressing Gram-negative enterobacilli in the digestive tract to prevent infection was called **selective decontamination** [41]. This treatment was also found to fulfil epidemiological requirements, as it did not cause resistance [42, 43]. The resistant strains isolated during co-trimoxazole treatment for selective decontamination in some of the patients could be ascribed to acquisition of new strains rather than to induction of resistance. It occurred in patients treated on an open ward who had received remission-induction chemotherapy for acute leukaemia [44]. The chemotherapy may have affected the quality and quantity of mucus production, the feeder layer of the endogenous flora, and therewith decreased their CR. In a hospital environment with co-trimoxazole resistant strains, acquisition is likely. The appearance of co-trimoxazole resistant Gram-negatives in the stools of selectively decontaminated (SD) patients, could be prevented by adding polymyxin to the oral regimen for SD [45]. Also the use of SD in patients who were colonized by multi-resistant Gram-negatives has been reported to be successful at freeing the patients of these organisms [46].

Further application of these principles in severely compromized patients; e.g.

selective decontamination of their digestive tract, will be well illustrated in the other papers on the subject in this special issue.

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