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COLONISATION RESISTANCE AND ANTIMICROBIAL DEFENCE OF THE DIGESTIVE TRACT; TWO POTENT PHYSIOLOGICAL DEFENCE MECHANISMS TO INFECTIONS

DIRK VAN DER WAAIJ

Laboratory for Medical Microbiology, University of Groningen,
Groningen, The Netherlands

SUMMARY

It is to be expected that the successful use of antibiotics for treatment of infections by opportunistic bacteria will soon (in several decades) come to an end. As was recently reported at the Third Western Pacific Congress on Chemotherapy and Infectious Diseases, in some countries antibiotics are already useless. Not only for the treatment of infections by potentially pathogenic microorganisms but also for treatment of infections by pathogens. Therefore, we must look for other solutions for the treatment - if possible prevention - of infectious diseases. An obvious approach could be to (artificially) maintain the physiologic antimicrobial defence capacity.

The normal physiologic clearance mechanism is given the name 'antimicrobial defence'. Hence, a deficiency of this mechanism could be indicated with 'defence deficiency'. If the immune system plays a role in the sequence of opsonisation, phagocytosis and destruction of penetrating opportunistic microorganisms, they may - one stronger than another - be suppressive to the immune system; i.e. they may induce a status of specific and perhaps even a general suppression of inflammation. This contrasts what occurs in infections by pathogenic microorganisms.

An experimental model in mice presents evidence that bacteraemias may occur clinically unnoticed. These bacteraemias could possibly be regarded as evidence of an overflow of the normal physiologic clearance capacity of the antimicrobial defence. Because opportunistic bacteria may occasionally reach along this route the central (systemic) immune system, a need was felt to be able to measure the interaction between the immune system and the intestinal flora in greater detail. Therefore, we have recently developed techniques in our laboratory. This enables us to study in man and animals the specific (and the aspecific?) influence of opportunistic bacteria on the immune system in a direct way. These techniques also permit measurement of the effect of the immune system on bacteria in the intestines of the subject.

The *Enterococcus faecalis* preparation Symbioflor 1® provided by Symbiofarm, was selected as a next best to *Enterococcus faecalis* strains isolated from the endogenous flora of each subject (healthy volunteer).

Regarding the possibility of successful application of autovaccins and/or selected pure cultures of potentially pathogenic strains, we con

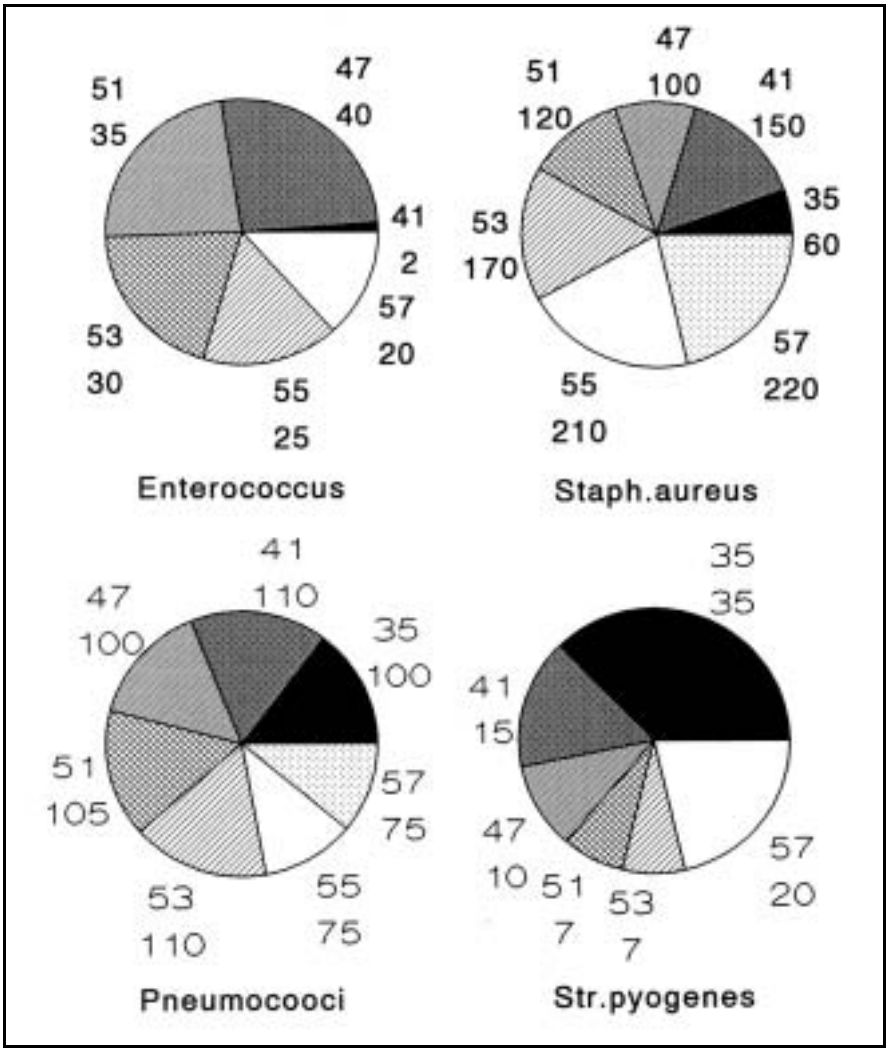

Figure 1: Bacteraemias by Gram-positive bacteria in the years 1935, 1941, 1947, 1951, 1953, 1955, and 1957. The numbers around the pie-diagrams represent these years of study (top numbers) as well as the number of cases involved (undermost numbers) (*Finland* et al., 1959).

clude from evidence in the literature and from our own recent observations, that oral preparations like Symbioflor 1® may really work in patients; i.e. may either stimulate or suppress the immune system. Preparations of this kind applied in electively hospital admitted subjects may enhance the clearance of translocating bacteria and/or prevent inflammatory responses to these microorganisms in patients with some degree of 'defence deficiency'. This implies that preparations of this kind, made for oral use, urgently require further detailed evaluation.

The results obtained by objective measurements in ten healthy volunteers indicate clearly the development of a broad-spectrum of immune suppression during and for some time after Symbioflor 1® treatment.

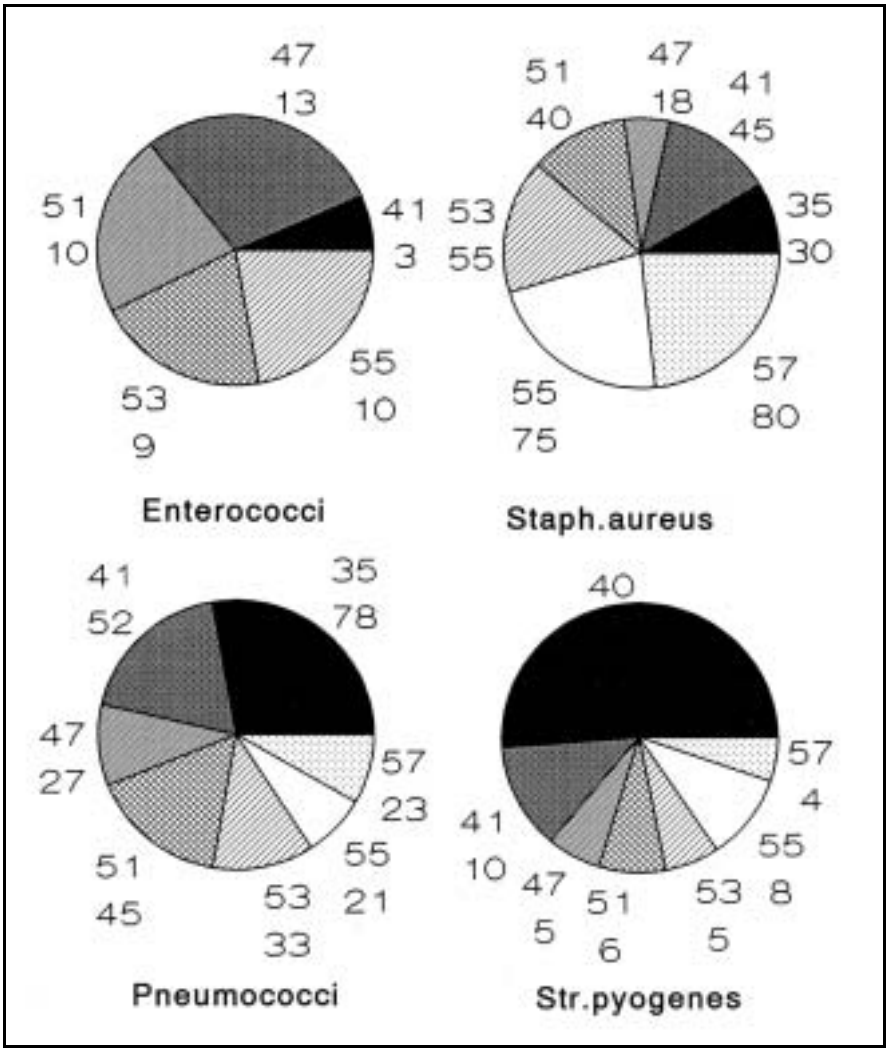

Figure 2: Deaths in patients associated with Gram-positive bacteria. The same years of study and the same way of presentation of the data is used as in Figure 1 (*Finland* et al., 1959).

INTRODUCTION

Change of the spectrum of bacteria associated with hospital infections

Since the introduction of antimicrobial drugs such as sulpha-preparations and later antibiotics, the pattern of 'hospital infections' and the bacteria involved, have changed dramatically. Before 1935 when sulpha-drugs were for the first time taken in use, the majority of infections in hospitalised patients concerned primary pathogens such as *Salmonella* spp., *Corynebacterium diphtheriae*, *Mycobacterium tuberculosis*, *Pertussis* spp., pneumococci and the like (*Finland* et al., 1959; *Julianelle* and *Siegel*, 1945). However, as Finland reported and the end of the fifties, after 1935 and after 1941 in particular, when penicillin and streptomycin be-

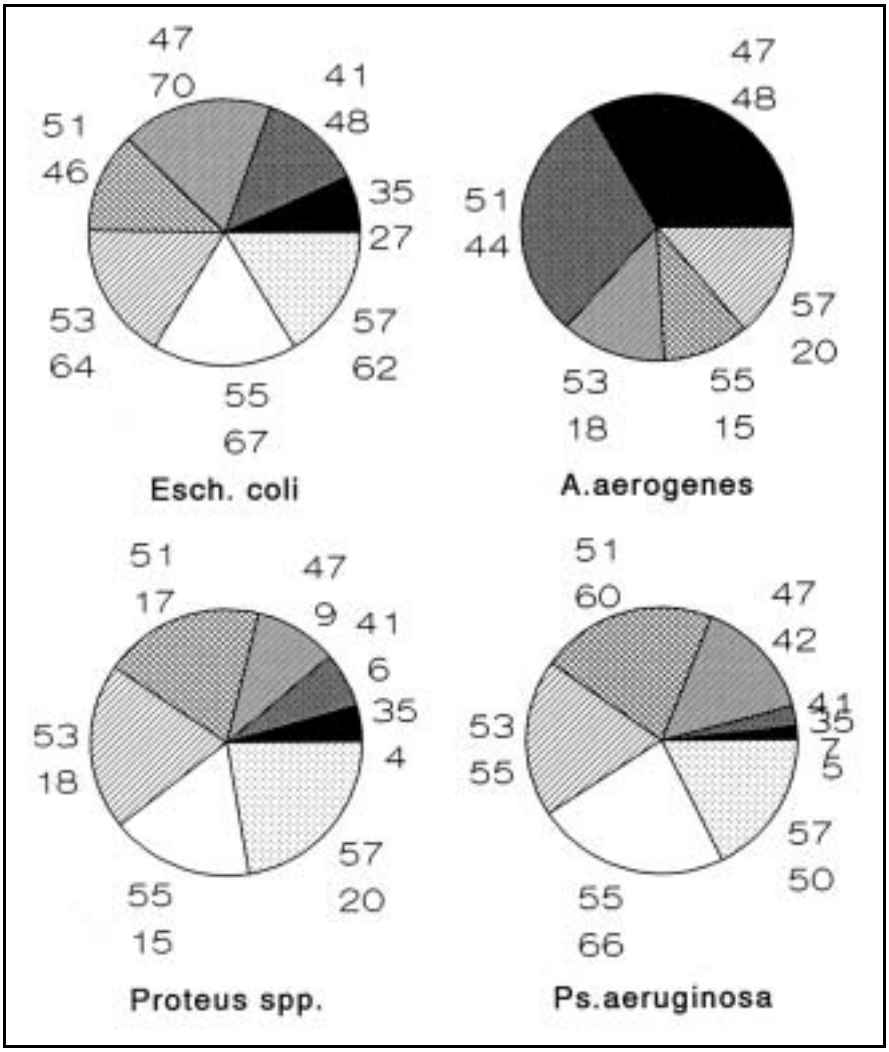

Figure 3: Bacteraemias by Gram-negative bacteria in the years 1935, 1941, 1947, 1951, 1953, 1955 and 1957. The numbers around the pie-diagram represent these years of study (top numbers) as well as the number of cases involved (undermost numbers) (*Finland* et al., 1959).

came available, the pattern of infections in hospitals changed rapidly into infections caused by potentially pathogenic (opportunistic) bacteria such as *Staphylococcus aureus* (Figures 1 and 2). When penicillin and streptomycin were taken in use, opportunistic Gram-negative enterobacteria and *Pseudomonas* spp. became predominant in hospital infections (Figures 3 and 4). With the introduction of broad-spectrum antibiotics such as tetracyclines and chloramphenicol, also fungi - such as *Candida* species - became more common as infection causative microorganisms (*Rogers*, 1959; *McGovern* et al., 1953).

Development of antibiotic resistance

The excitement about the therapeutic possibilities provided by antibiotics, did

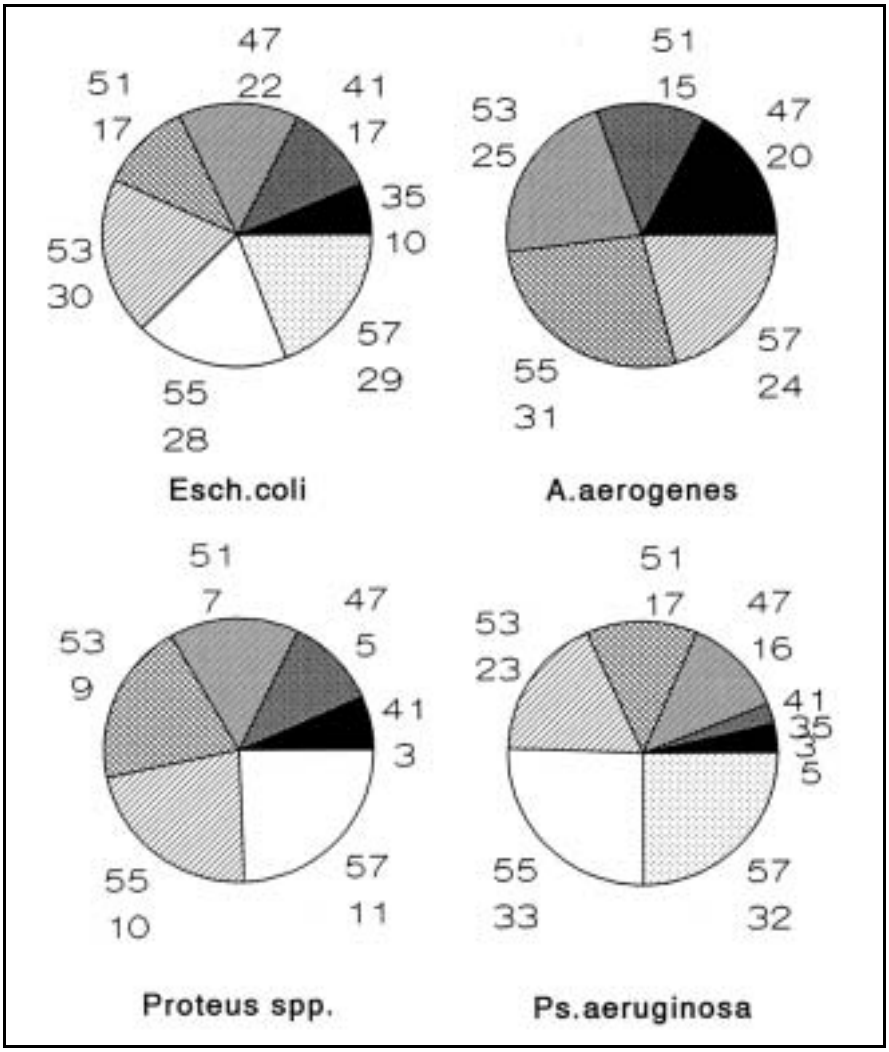

Figure 4: Deaths in patients associated with Gram-negative bacteria. The same years of study and the same way of presentation of the data is used as in Figure 3 (*Finland* et al., 1959).

unfortunately stop further studies on endogenous and exogenous bacteria (vaccines) and their specific and aspecific stimulation/suppression of the immune system. Although in comparison with our present facilities under relative primitive conditions, such studies were in progress in the forties. The fact that development of resistance to antimicrobial agents was observed soon when penicillin and streptomycin were taken in use, was generally regarded as a minor problem. It was believed that the problem of resistance development could be overcome quite easily, as it would be a matter of further technical improvement only.

The influence of antimicrobials on the endogenous microflora

Already in the forties, it was recognised that antimicrobial drugs may adversely influence the oropharyngeal and intestinal microflora (*Julianelle* and *Siegel*, 1945). It was found that resistant potentially pathogenic bacteria and

fungi got growth preference during treatment (*Mangiaracine*, 1951). Later in the late sixties, this mechanism was studied in greater detail by myself and was named colonisation resistance of the digestive tract (*van der Waaij* et al., 1971).

LESSONS BY THE EVOLUTION OF LIFE

Ecosystems with a static defence

On the basis of what has occurred during the evolution, it is conceivable that resistance to antimicrobial drugs would develop. Not only antibiotics would evoke resistance but also to primary manmade antimicrobials like sulpha preparations, trimethoprim and quinolones. This is plausible on the basis of the course of developments in the evolution. The first bacteria that developed on earth were autotrophic. Later on, bacteria developed with a more complex metabolism. At different places, different ecosystems may have developed from these bacterial combinations (*Gould*, 1989). Cross-contamination between ecosystems may have occurred by an airborne route; i.e. bacteria may have been transported by wind. This may have forced some bacteria in each ecosystem to produce an antibiotic substance to which co-colonisers of the endogenous ecosystem were obviously essentially resistant. Newly arriving bacteria on the other hand may mostly have become killed by these antibiotic substances. Only if the newcomers came in sufficiently high numbers and if they could make use of locally available nutrients, newcoming bacteria may have been in the position to adapt, i.e. to mutate in time and develop resistance. Then these bacteria could perhaps settle and multiply in the niche in which they had landed.

After bacteria, first monocellular eucaryotes and later multicellular organisms may have developed from these microorganisms (*Margulis*, 1993). When much later in the history of life on earth, multicellular organisms (animals) developed and when these animals became more complex, they got a digestive tract with a bacterial ecosystem. This ecosystem of the digestive tract may have developed - just like in the open outside world - on the basis of the principle that these communities should defend themselves - and therewith their host - to foreign bacteria. This is at least what the intestinal flora appears to do these days (*van der Waaij*, 1990).

On the basis of the foregoing hypothesis, adaptation to antimicrobial substances present in foreign ecosystems by mutation and selection may therefore be as old as the gradually developed more complex bacterial communities exist on our planet since the beginning of autotrophic life.

The need of a dynamic (rapidly adjustable) and specific defence in animals

Because the higher organisms developed means to move themselves by feet, wings or by swimming in the water they could actively and quite rapidly move from one place (environmental bacteria) to another (other bacteria in the environment). This caused contamination of their digestive tract with many different bacteria which were so far foreign and which might be dangerously invasive. Therefore, a more complex defence system - the successor of the monocellular organism that may have protected itself by phagocytosis - was required than just phagocytosis and the static anti-microbial substances (antibiotics) produced by the intestinal ecosystem. The defence of higher animals

to 'antibiotic' resistant (the antibiotic-like of the ecosystem) foreign bacteria, had to become fast and readily adjustable. In addition, in higher animals the defence had to act specific to invasive newcomers.

In the digestive tract, a static defence provided by a bacterial ecosystem (among else by antibiotic-like substances) may have been responsible for the prevention of colonisation by these newcomers as still is the case. Because of the protective function of the intestinal ecosystem to the host organism regarding pathogenic (foreign) microorganisms, the bacteria indigenous to the digestive tract in fact form an essential part of the host organism. However, this protection may have been insufficient to cope with many foreign bacteria. A dynamic defence system of the tissues of the host organism was required for survival of the species. The defence system however, had to be specific, as it should leave the indigenous ecosystem unaffected. To this end, a specific defence system may have developed in animals. Would bacteria, which colonise the digestive tract, get across the epithelial lining of the intestines, they had to be cleared without notice (without ensuing inflammation) to the host. Foreign, rapidly multiplying and invasive bacteria on the other hand, had to be rapidly killed and cleared. The 'specific part of the defence system' that developed in animals is presently known as the immune system.

Regarding the evolution of the immune system, the defence to pathogenic microbes had to be rapidly readjustable, because otherwise 'immune-resistance' might develop. Strains of bacteria, fungi and viruses may change rapidly their major antigenic composition (antigenic mimicry) and thus (try to) escape from the immune activity by developing this type of resistance.

Practical consequences of the course of the development of life on earth

The practical value of these 'lessons of the evolution' for the clinical use of antibiotics, have unfortunately not been taken in consideration in the fifties. The usefulness of antibiotics therefore became unfortunately overestimated. At a much earlier stage, it should have been realised that: Firstly, antibiotics represent a static defence. The only flexibility in the system is provided by the pharmaceutical industry; i.e. when it introduces a new modified antibiotic for practically no resistance exists. Secondly, the opportunistic microorganisms involved in hospital infections could only become massively involved in infections because of the introduction of antimicrobial drugs:

- In the first place, antibiotics have strongly (positively) influenced the progress in development of almost every specialism of medicine. This, however, has resulted in a strong increase of the percentage of compromised patients in our hospitals.
- Secondly, antibiotics with their unspecific activity to a wide range of different bacteria, often affected the intestinal ecosystem of the patients treated and thus permitted colonisation by resistant strains.

GROUPING OF BACTERIA ACCORDING TO THEIR 'DEGREE OF PATHOGENICITY'

For practical (medical) purposes, all bacteria presently known could be grouped in three major groups with increasing 'pathogenicity' for man and

animals. The first two of these groups may differ in particular between animal species:

1. A huge group, which is by far the largest, is not pathogenic at all. Bacteria belonging to this group can be found in the digestive tract and on the skin of all healthy human subjects as well as on the skin of animals and plants. They live in one way or another, in peaceful co-existence with the immune system.
2. A much smaller group, which is well-maintained under control by the immune system of the digestive tract (the so-called gut associated lymphoid tissue). This control occurs - as we all daily experience - without causing any sign or symptom of disease. Representatives of this group, which is called potentially pathogenic or opportunistic, can be found in practically every healthy human subject, in every animal and on plants as well.
3. A (fortunately) small group which is pathogenic; e.g. can cause disease upon contamination with sufficient numbers. These bacteria are often not readily controlled by the immune system of man and animals. This group therefore causes mostly disease upon contamination of a susceptible subject and, depending on its pathogenicity in relation to the condition of the host, even death. In contrast to representatives of group one and two, bacteria of group three are normally not found in healthy subjects. If, however, they are isolated from excreta of healthy subjects, the individual should be regarded as a potential transmitter and a source of infection.

As mentioned in the introduction, microbes which cause hospital infections are in general potentially pathogenic (opportunistic) and differ of primary pathogenic microorganisms by their capacity to normally colonise healthy subjects (man as well as animals) for extended periods of time. Yet, albeit in low numbers, opportunistic bacteria may translocate from time to time from the intestines into the gut associated lymphoid tissues (GALT). This occurs without evoking signs or symptoms of infection (*van der Waaij* et al., 1972; *Wells* et al., 1988), which contrasts to what occurs normally upon invasion of primary pathogenic bacteria and viruses. Macrophages - both tissue macrophages and monocytes in the circulation - may play a key role in the clearance of translocating microorganisms as well as of necrotic tissues and cells (*Border*, 1988). Macrophages could be regarded as the successors of the most primitive defence system that developed during evolution in the most primitive multicellular organisms.

A commonly known representative of a serious primary pathogen in this third group is the bacterium that causes Plague. Several other examples of pathogens, which are nowadays still causing disease, are bacteria that cause tuberculosis, typhoid fever, diphtheria or pertussis. These bacteria are pathogenic because of different properties:

- They may fool the immune system or the may produce an agent which is toxic to cells of the defence (immune?) system. The defence system may for these reasons not respond sufficiently fast therewith giving free way to the pathogen for some time. If this period of 'retarded response' is too long, the patient may not survive.
- Another reason for grouping a bacterium pathogenic to man, is based on adverse (tissue destructive) effects of the immune response itself may have. The host individual becomes largely ill due to tissue destruction like in tuberculosis or typhoid fever. Tissue destruction in these cases is partly the result of intracellular bacterial multipli-

cation, but also the result of complement binding and agents released by cells of the immune system; i.e. a consequence of the immune response to the infecting microorganism.

HOSPITAL INFECTIONS IN RELATION TO THE FOREGOING

Patients, who suffer of infections during their stay in a hospital by potentially pathogenic microorganisms, clearly have a deficit in their defence capacity. They may less rapidly and less efficiently clear translocating bacteria because they all have to some degree a 'defence deficiency'. Healthy persons with normal defence capacity on the other hand, being exposed to same extend to these bacteria because they work in the hospital, may become colonised by several different opportunistic species (*Chambers* et al., 1987) without signs or symptoms. They therefore, do not need to take any precautions to protect themselves to contamination by potentially pathogenic bacteria excreted by patients. However, in the Intensive Treatment (IT) wards, most patients have a serious defence deficiency. Therefore, if isolation precautions are taken in IT-units, they are taken because of hospital hygienic considerations. The precautions are necessary to prevent spread of resistant opportunistic microorganisms among these (often antibiotic treated) patients. Nowadays, the deficit in our knowledge concerning the pathogenesis of infections by opportunistic bacteria indeed is most experienced in IT-wards. In these high care stations, the infection rate (practically 100% caused by opportunistic microorganisms) may be as high as 31% (*Craven* et al., 1988).

URGENTLY REQUIRED RESEARCH

General

The basic questions in infectious diseases by opportunistic microorganisms have unfortunately not been studied in greater detail since the forties. The central issue in infectious diseases in hospitals should have been a study of factors involved in antimicrobial defence. By now, it should be known which defence factors are affected in severely ill patients with increased risk of infections.

Regardless our detailed knowledge of the functioning of the immune system (*Kagnoff*, 1987; *Lee*, 1985), it is still an open question how the immune system interacts with the bacteria of the ecosystem of the digestive tract in the first place. Secondly, research is necessary to elucidate all factors which play a role in the defence mechanism that enable healthy individuals to live unaffected in daily contact with residing as well as with newly ingested opportunistic microorganisms (potential pathogens).

If we had undertaken studies of this kind in the previous decades, we might at the present be able to prevent many infections. We would never have reached the frightening situation of the present with often multiply bacterial resistance to antibiotics. Instead we would perhaps be able to monitor the condition of the defence capacity of our patients at risk during their hospital stay. If indicated, we would be able to either boost 'antimicrobial defence' (possibly with so-called 'auto-vaccines' or with selected pure cultures such as for exam-

ple Symbioflor 1® (*Rusch*, 1985). This treatment would be given in advance to (elective) admission of patients to the hospital. In acute (non-elective) patients, we might be able to supply them with those factors required to prevent 'defence deficiency'. In compromised patients therefore, we may become able to prevent along these lines most infections by opportunistic microbes in the future.

Personal view

In Groningen in the Netherlands we are of opinion, that we should perform as many investigations in a systematic way as we can, in order to fill the gap of our knowledge concerning normal interactions between the immune system and intestinal microflora as soon as possible. It is concluded that in the initial studies, we could best start with monitoring the normal function and responses of the 'antimicrobial defence' to microbial stimuli from the digestive tract. This means studies in healthy volunteers on the basis of a model of opportunistic infections in mice. Such studies may elucidate how we should make practical use of measurements concerning the clearance of potentially pathogenic bacteria after translocation.

RESEARCH DEVELOPMENT IN GRONINGEN SO FAR

On the basis of the foregoing arguments, we have realised that we should try to develop an animal model in which we could study the antimicrobial defence capacity. In addition a need was felt to be able to measure - and thereby to monitor - the interaction(s) between the gastro-intestinal microflora and the immune system (both the 'gut associated' and the 'systemic') as well.

Experiments in animals to study antimicrobial defence capacity

In patients, so far the culturing of blood is the only way to determine - albeit at a late stage - evidence of translocation of bacteria. A bacteraemia in a healthy subject could perhaps be regarded as an 'overflow' of the system. The system would normally clear all translocating bacteria. Therefore, we have made use of the classical method of experimentally evoking translocation in mice, namely by oral contamination with an opportunistic bacterium (*van der Waaij* et al., 1971). In this system, we have collected 10 ml of tail blood at regular intervals for culturing, to investigate whether mice have a 'measurable overflow' of their antimicrobial defence system for bacteria following oral contamination with high numbers of opportunistic bacteria. In our study, a bacterial strain was used of which we knew by experience that translocation would occur to a considerable degree.

Animal model to study the 'normal' defence capacity in mice

Eight weeks old female C3H mice were orally contaminated in groups of five with various doses of a streptomycin and neomycin resistant (SMR) strain of *Escherichia coli*. The endogenous *E. coli* strains were all sensitive to streptomycin and neomycin. In total five groups of otherwise untreated animals were contaminated with respectively 10^2, 10^4, 10^6, 10^8 and 10^{10} SMR *E. coli*. At six hours, 24 hours and thereafter daily for five days, ten ml of tail blood was collected with a calibrated heparinised capillary under aseptical conditions. To this end, the very end of the tail was cut. Quantitative culturing on MacConkey agar (OXOID) made SR-*E. coli* selective with streptomycin (50 mg/l). After incubation overnight,

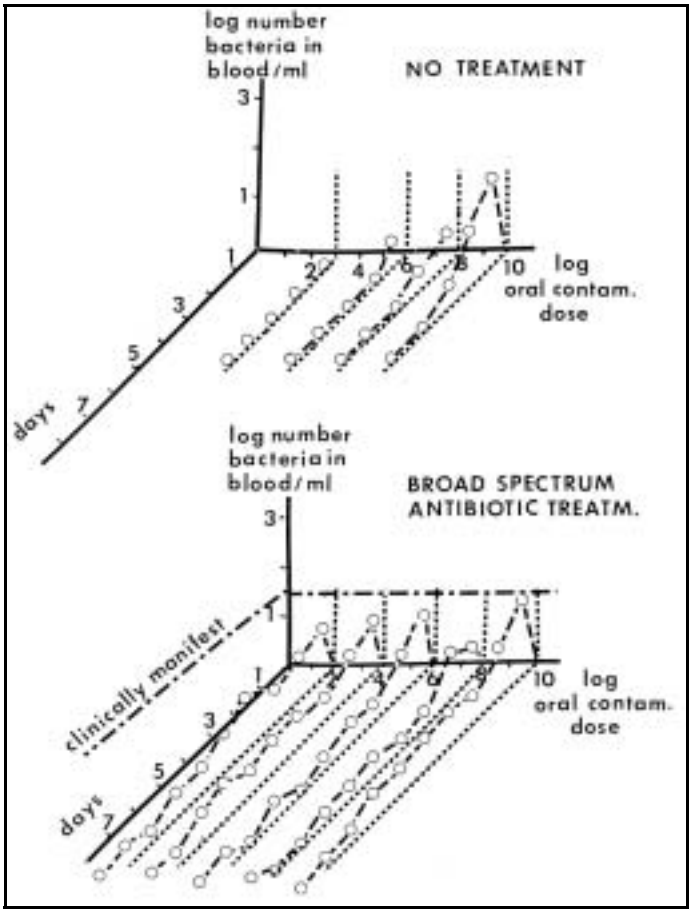

Figure 5: Translocation of SMR-*E. coli* in conventional and in antibiotic treated mice (5 mice per contamination dose).

the number of colonies was counted and the number of bacteria per ml of blood was thus estimated. Log median values are presented in Figure 5.

This experiment was repeated with mice which had been treated orally with streptomycin and neomycin in the drinking water (*van der Waaij* et al., 1971) for seven to ten days before oral contamination. Antibiotic treatment was continued during the eight days of the experiment. During the experimental period, tail blood was sampled for culturing twice daily.

The results of this study are depicted as mean log values per day in Figure 5. Firstly, it is important to read from this figure, that indeed healthy mice may experience a bacteraemia for some time after oral contamination. However, this only occurred following oral contamination with very high numbers of opportunistic bacteria. This may have caused abnormally high numbers of these bacteria in the intestines (*van der Waaij* and *Berghuis*, 1974). Clinically, the animals remained healthy: They did not show evidence of diarrhoea, and they showed normal activity. Secondly, the results also clearly show the influence of antibiotic treatment. The antibiotics suppressed the autochthonous microflora (decreased colonisation resistance). Yet, these mice did also not

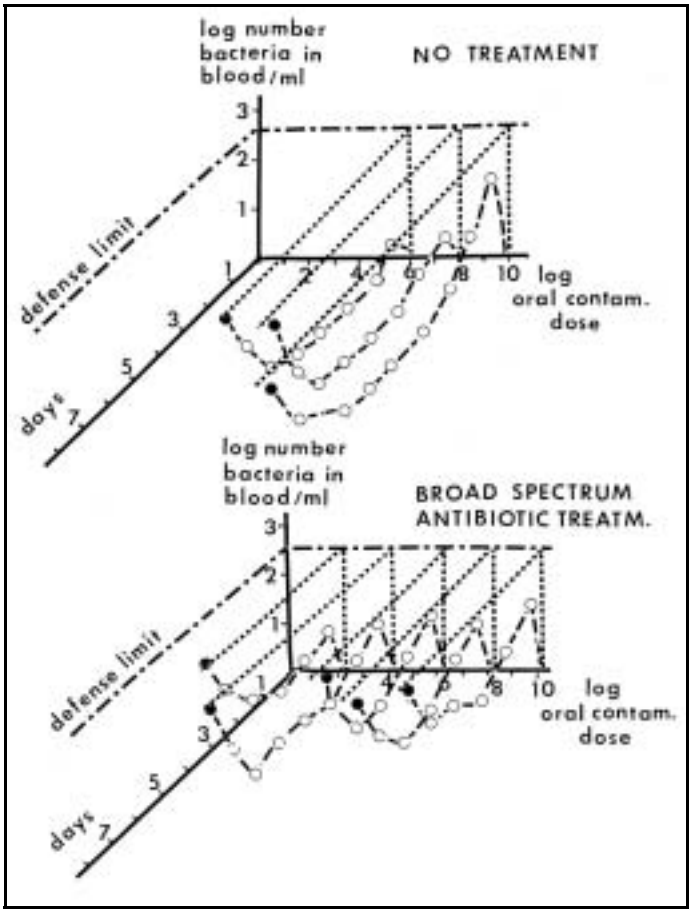

Figure 6: Translocation of SMR-*E. coli* in lethally irradiated (7 Gy) conventional and in irradiated antibiotic treated mice (5 mice per contamination dose).

show clinical signs of infection. However, they obviously had soft faeces since the onset of antibiotic treatment.

Animal model to study the effect of decreased defence capacity in antibiotic treated subjects

Two groups of 25 mice were irradiated with 7 Gy four days before oral contamination. One group had been antibiotic decontaminated with streptomycin and neomycin like in the previous experiment since seven to ten days before irradiation. Antibiotic treatment was continued after SMR-*E. coli* contamination in the antibiotic decontaminated mice. The animals in both experimental groups were contaminated in groups of five, again with respectively 10^2, 10^4, 10^6, 10^8 and 10^{10} SMR-*E. coli*. Tail blood was collected and cultured at the same intervals as in the previous two experiments. However, in the antibiotic treated mice after day three following contamination and after day four in the untreated mice, collection of tail blood was not always successful. Means of the number of bacteria per ml of blood are therefore at days three respectively day four calculated of less than four mice. Moribund animals were killed and their hearts blood was taken for quantitative culturing.

The results of these experiments are

depicted in Figure 6. The data of the two lowest contamination doses are omitted in the not antibiotic treated group for the sake of simplicity. These results did not differ from what was seen in the unirradiated mice, their curves might only confuse the presentation.

Conclusion

The first conclusion is that in mice with a strongly suppressed defence capacity, low contamination doses may - like in untreated mice - also not result in intestinal concentrations adequate to cause bacteraemia in the present model. However, from oral contamination dosages of 10^6 on, following an early peak the contaminant may reach for the second time an intestinal concentration associated with a quite strong translocation or decreased clearance as evidenced by bacteraemia.

Secondly, the results depicted in Figures 5 and 6 clearly show that the higher the numbers which the SMR-*E. coli* may have reached in the intestines, the shorter was the interval for which bacteraemia disappeared following the initial peak. All animals died with a rather equal number of approximately 5.10^2 SMR-*E. coli* per ml of blood.

From the results of this experimental study it is tentatively concluded that lethal irradiation may cause destruction of the defence system and therewith a rapid exhaustion of the clearance capacity. If under such circumstances opportunistic bacteria colonise the intestines in high numbers, they may (still) translocate and - not being normally cleared - they may soon reach lethal numbers in the blood stream and perhaps other organ systems.

Mice which had the opportunistic bacterium in very high (overgrowth) numbers in their digestive tract because of broadspectrum antibiotic treatment, survived significantly shorter than comparable animals in the untreated but lethally irradiated control group. Because of the decreased colonisation resistance, in the antibiotic treated subjects, the size of the contamination dose did not longer play a role. Oral doses of 10^2 SMR-*E. coli* had the same (rapidly lethal) effect as the highest doses of 10^{10}. These experiments make likely, that the defence capacity is formed by two mechanisms:

1. a mechanism based on bacterial interactivity, and
2. a radiosensitive mechanism typical for organisms of eucaryotic origin. The latter being primarily designed to clear the tissues of invading microorganisms (foreign as well as indigenous).

Studies aiming to obtain insight in the interaction between the autochthonous microflora and the immune system in man

Because it is very likely that the immune system is involved in the complex clearance mechanism for translocating bacteria, we decided to study the physiologic interactions between the immune system and the intestinal microflora as a first step. To this end, it was realised that we should develop techniques, which would permit us to study humoral and cellular reactivity to indigenous intestinal bacteria in greater detail. Furthermore, it was realised that in these studies washed (uncultured) intestinal bacteria had to be used in stead of pure cultures, since the bacteria might change their antigenic composition because of culturing, isolation and typing. It is known that bacteria may change outer-membrane antigens during pure culturing steps.

As will be discussed by three coworkers in greater detail during this Old Herborn University Seminar, two techniques have been set up to study humoral responses to intestinal bacteria

and to bacteria-like particles in washed faeces:

- The first technique involves 'fluorescence immuno-micromorphometry'. Briefly, this involves a microscope equipped with UV-light and phase-contrast optics. A high-resolution video camera reads the microscopic fields sequentially with normal light and with UV. Computer software especially developed for this purpose, sorts these bacteria on the basis of shape and seize, counts them and measures the titres of isotypes of antibodies which may 'coat' the bacteria. Evaluation of a sample may take a day; an experienced technician can study five samples per day. Antibody coating may take place in the gut *in vivo*. However, *in vitro* incubation of bacteria with either autologous or allogenous sera is also being performed to study possible differences in response of the systemic immune system to autologous indigenous bacteria and those of other (allogenous) persons (*Apperloo-Renkema* et al., 1992; *Jansen* et al., 1993a).
- The second approach investigated makes use of FAX-techniques. The FAX-analysis of faecal suspensions permits rapid evaluation of ten times as many bacterial particles (events) per sample. However, the FAX gives less specific information than the fluorescence immuno-morphometry does. An experienced technician can study forty samples per day (*van der Waaij*, 1994).
- Finally, techniques have been developed to study cellular responses to (washed) indigenous and allogenous intestinal microflora. This technique is now operational. However, these cellular studies are much more laborious and may in fact require experimental pre-studies in animals to clarify their outcome.

ONGOING STUDIES ON HUMORAL (IMMUNE) INTERACTION WITH INTESTINAL BACTERIA IN MAN

The results of these studies are presented by my co-workers during this Old Herborn University Seminar. Briefly: the first opportunistic bacterium we have studied in human volunteers, was *Enterococcus faecalis*. We selected this bacterium because two to three days after admission of patients to IT-wards, it usually becomes predominant in the patient's oropharyngeal microflora. This occurs in a high percentage of multi-trauma patients. It was assumed therefore, that the *Enterococcus* might be - post or propter - be associated with the defence deficiency seen in these patients. In addition, in several IT-units, *Enterococcus* is being used as an indicator organism to 'monitor' the 'defence capacity' by culturing throat washings sequentially.

A study of the influence of enterocci on the humoral immune response(s) following oral application should obviously start in healthy subjects because healthy persons have a normal 'defence capacity'. They therefore do not run a risk of infection by orally given strains of opportunistic microbes.

For a study in healthy volunteers the best approach would have been the use of an auto-vaccine containing endogenous *Enterococcus faecalis* strains in each subject. However, we had no experience in preparing auto-vaccines. A standard pure culture was therefore considered a next best. This assumption is based on the fact that many different foreign *Enterococcus faecalis* strains are being ingested frequently and thus may provide multiply antigenic information

about the species to the gut associated immune system. We were therefore very pleased by the offer of Symbiopharm to use their strains of *Enterococcus faecalis* called Symbioflor 1®.

Jansen and co-authors (1993b) recently reported in a letter to the medical journal Infection about our first findings with Symbioflor 1®. Nine persons of a group of ten volunteers who took Symbioflor 1® daily for three subsequent weeks showed a significant decrease of their antibody titre to *Enterococcus faecalis*. This titre continued to decrease furthermore in the subsequent three weeks. The one volunteer in who the titre did not decrease significantly had an abnormal low titre already in the five study weeks previous to Symbioflor 1® treatment. This could possibly be ascribed to the spastic colon this volunteer was known to suffer of.

Because not only the IgG antibody titre against *Enterococcus faecalis* appeared to decrease significantly, but also most IgG titres to other indigenous bacteria, it is tentatively concluded that *Enterococcus faecalis* treatment may also suppress the immune response to other potentially pathogenic microbes and therewith may suppress inflammatory response would they translocate (*Jansen* et al., 1993b).

Conclusion

These data indicate that there is a suppressive effect of oral treatment with Symbioflor 1® on the humoral immune response to not only *Enterococcus faecalis*, but evidently also to a wide range of other bacterial antigens. An autovaccine may have a similar or even a stronger effect than selected pure cultures of this kind, because the immune system may have more experience with bacteria in autovaccines than with orally applied immunologically foreign strains.

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