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HYPOTHESIS ON THE PHYSIOLOGICAL CONTROL OF INFECTIONS BY OPPORTUNISTIC (POTENTIALLY PATHOGENIC) MICRO-ORGANISMS

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SUMMARY

A hypothesis is set up regarding the extend at which in vertebrate animals the (four) 'layers' of the defence system, which sequentially have developed in evolution and co-operate in the clearance of translocated micro-organisms. On the basis of data from several previously published studies, it is proposed that co-operation is likely to be *additive* and in most cases perhaps *synergistic*. A mouse model for *in vivo* study of intestinal translocation is proposed. This model permits sequential (tail) blood sampling for microbiological and immunological analysis after an experimental oral contamination.

INTRODUCTION

To treat/prevent opportunistic infections in the compromised patient when multi-resistance to antibiotics becomes unsurpassable, artificial maintenance of the capacity of the defence (which clears off opportunistic bacteria) at a normal level, may provide a solution, as proposed by the ISGNAS-group (*Araneo* et al., 1996). For such a new approach for preventive treatment, however, comprehensive insight of the normal functioning of the physiologic (innate and adaptive) defence is required.

In vertebrates, the host defence to opportunistic micro-organisms consists of several 'layers'; each of them may act in the defence system more or less in the sequence in which they developed in evolution (*Medzhitov* and *Janeway*, 1998). Between these defence layers, interactions occur between the defence factors to control potentially pathogenic microbes along their route from the outside world into a host organism. The layers meant are respectively:

1. Gastro-intestinal tract microflora
2. Mucosal layer of the gastro-intestinal tract
3. Innate immune system
4. Adaptive immune system of the gut as well as the systemic immune system

In order to 'survive' the selective forces in evolution these steps in evolution of the defence system *must have evolved in optimal mutual co-operation* to provide the host organism a more effective clearance system for micro-organisms. This principle of a better outcome following concerted action is known from the clinical use of antimicrobials: In case two antibiotics are used for treatment of an infection they should work *additive*[i] or better *synergistically*[ii]. *Antagonism*[iii] may occur during use of combinations of certain antibiotics in treatment.

Synergism is of great advantage when one of the antimicrobial drugs, in

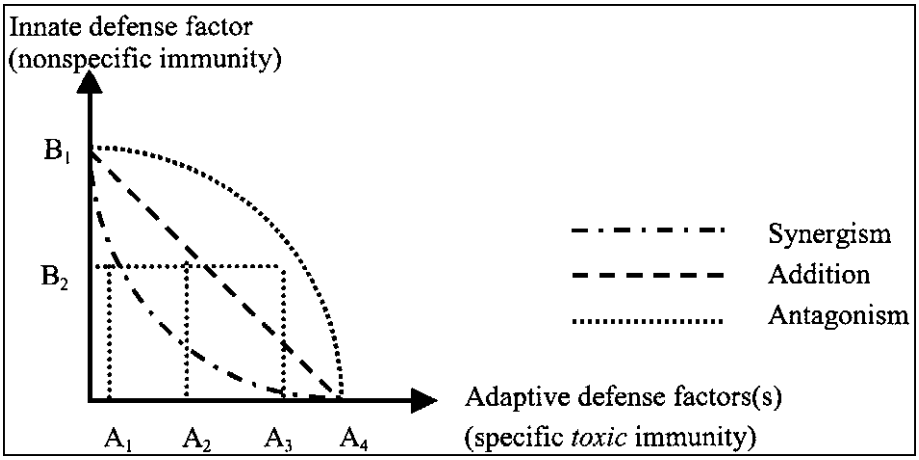

Figure 1: Graphic presentation of the influence of *synergism*, *addition* or *antagonism* between innate and adaptive immunity may theoretically have in the clearance of a certain number of micro-organisms.

a combination of two selected for treatment, is strictly indicated but *toxic*. Because of *synergism*, the toxic antibiotic can be effective in the combination in a much lower dosage than even half of the dose required when used as a single drug for treatment of the same infection. This is shown schematically in Figure 1 (dosages A_1 and B_1).

In opsonisation for clearance, complement activating antibodies such as IgG, may cause an *inflammatory reaction* and thus should be regarded as 'toxic'. These antibodies may normally not participate in the clearance of bacteria from tissues or (because of synergism with non-specific opsonins) only in low (non-toxic) concentrations.

In conclusion: If induced and in the circulation, complement activating antibodies may co-operate efficiently in low titres in the process of opsonisation and clearance with non-specific opsonins (glycoproteins) of the innate defence. It is thus hypothesised, that:

1. *In vivo* the dose ratio of opsonising adaptive- and opsonising innate defence molecules seems comparable with the dose ratio in the above given example of toxic and non-toxic antibiotics for treatment mentioned.
2. The physiologic clearance of micro-organisms from the lamina propria, mesenteric lymph nodes and spleen occurs without inflammation because of synergism between innate and adaptive immunity. Would the production of sufficient innate opsonising molecules decrease, higher antibody titres and thus inflammatory reactions are to be expected.

Because proper understanding of these basic principles is considered important for understanding of the functioning of the physiologic defence, a schematic presentation is shown graphically in Figure 1.

In this theoretical example, three ways of co-operation between innate and adaptive immunity regarding the clearance of a particular translocated micro-organism are shown. Absence of adaptive immunity would require a strong innate immune capacity B_1; conversely in the absence of innate defence a strong adaptive immune reaction (A_4) is necessary to clear the same number of trans-

locating microbes. In the presence of both innate and adaptive immunity and when their co-operation is *synergistic*, less innate immune capacity (B_2) and a very low level of adaptive immune support (A_1) may provide total clearance of the translocating micro-organisms. However, in case co-operation is *additive*, stronger specific immune support (A_2) is required for clearance, while a much stronger adaptive immunity is necessary when *antagonism* exists between the co-operating antimicrobial forces.

Intestinal microflora

In maturing and adult animals with an established intestinal microflora, the first act of defence may occur either inside the intestinal tract by resident bacteria or indirectly by host immune factors. The host immune system is modulated by molecules released by intestinal bacteria which stimulate/inhibit. Resident bacteria may in addition have advantages in competition for nutrients while some may release substances which are directly active to newcomers in the gut; i.e. substances which block attachment receptors for opportunistic bacteria on mucosal cells as well as ingested particles which would enhance proliferation of the newly ingested microbes. The mechanism which controls colonisation by newly ingested bacteria and fungi is called *colonisation resistance* (*van der Waaij* et al., 1971).

Mucosal layer of the intestinal tract

From time to time bacteria which, regardless the colonisation resistance (CR) reach the mucosal layer and adhere to it, may penetrate the epithelial cells to reach the underlying lamina propria. This penetration into the lamina propria, a process named *translocation* (*van der Waaij* et al., 1972; *Berg* and *Garlington*, 1979; *Berg*, 1981; *Wells* et al, 1988), is often followed by a further transfer to the lymphatic organs and the liver. High concentrations of an opportunistic bacterial species in the intestines as well as certain virulence factors promote translocation (*Christensen* and *Beachey*, 1984; *Miller* and *Cossart*, 1999). Some pathogens can encapsulate which hinders phagocytosis, express that capacity often only after they have reached host tissues (*Bassler*, 1999).

Immune system

The immune system consists phylogenetically of two systems:

1. *Innate* immunity, which has developed in evolution in lower animal species, and
2. *Adaptive immunity*, which is confined to phylogenetically younger groups of animal species and becomes only highly specific (T-cell controlled) in the vertebrates.

Because, the adaptive part of the immune system has developed relatively late in evolution, it is conceivable that this 'new system' had to optimally cooperate with the phylogenetic older non-specific innate part. In animal species with a thymus-controlled immune system, a previous contact of a micro-organism with the adaptive immune system, can have resulted in either *defensive immunity*, like by antibody formation (or cytotoxic T-cells) induced in the lymphnodes or/and spleen, or a negative feedback i.e. *oral tolerance* (specific non-responsiveness) predominantly induced in the Peyer's patches.

THE CONTRIBUTION OF EACH OF THE FOUR DEFENCE LAYERS

Gastrointestinal ecosystem

The gastro-intestinal ecosystem represents a very potent defence system; forming the first barrier to the outside world, it could be regarded as the most important part of the defence. Phylogenetically it is the oldest part of the defence system of man and animals. Although rather constant in composition, the intestinal microflora may vary during life and therewith in efficiency as well. The composition of the gastro-intestinal-tract microflora does not only differ between animal species, as it may also differ significantly between individuals of a species and even between individual animals of an inbred strains (*van der Waaij and van der Waaij*, 1990). The way the intestinal microflora exerts a protective role is still largely unclear and so is the composition of the microflora; let alone the composition of an ideal ('guaranteed protective') microflora.

In vertebrates, the host organism appears to co-operate in several ways with the intestinal ecosystem improving its protective effect. The host organism provides nutrients by secretion of mucus and other secretory products as well as immunologically in a (T-cell dependent) as yet unclear way. Specific antibodies may play a decisive role. The outcome of the grand total of these very complex (*synergistic*) interactions between host and resident microflora is named *colonisation resistance of the digestive tract* (CR) (*van der Waaij* et al., 1971*; van der Waaij* and *Berghuis*, 1974; *van der Waaij* et al., 1977).

In a *conventional environment*, many if not most of the daily ingested opportunistic micro-organisms may generally not reach the gut mucosa to colonise it as a result of CR-activity. If colonisation of the gastro-intestinal tract mucosa nevertheless occurs, it will mostly not result in sufficient numbers (intestinal concentration (IC) for noticeable *translocation*[iv].

Translocation (TL) is related to the intestinal concentration of the bacterial strain involved (*Medzhitov* and *Janeway*, 1998; *van der Waaij* et al., 1971; *van der Waaij* et al., 1972; *Berg* and *Garlington*, 1979). Furthermore, TL may differ between strains of the same microbial species (difference of "invasiveness") (*Berg* and *Garlington*, 1979; *Berg*, 1981). The experimental design and technique used permitted the study of the faecal (intestinal) concentration and the CR following various different oral doses, as well as the occurrence of TL of the strain at daily intervals after contamination *in vivo*. In addition, mice were before contamination daily treated orally with non-absorbable antibiotics in order to very strongly lower their CR. In the presently reported study, techniques were unfortunately not yet available to monitor the NSO and SO.

The immune system (innate and adaptive)

There is strong evidence, that certain non-specific ligands between bacteria and phagocytic cells may play a role in the control of translocation of micro-organisms (*Russell*, 1995). Recent studies show that the adaptive immune system is normally not, or only with low antibody titres, involved in the response to intestinal non-pathogenic and opportunistic micro-organisms. The various non-specific ligands, active in innate defence, are therefore likely to co-operate with each other and when necessary, with specific antibodies in a *synergistic* way. *Synergism* between innate components and (if developed) specific immunity may make a clearance of trans-

located bacteria by the non-specific system more efficient; a point which requires confirmation by *in vivo* studies (see later ISGNAS research priorities).

In serum samples of healthy human subjects, specific IgG (and IgM) antibodies to, on the average, 72% (median range 10-73%) of their own endogenous faecal microflora have been found. Many of these circulating antibodies may find their way out into the intestinal tract lumen as they have also been found on many bacteria in washed fresh faeces where as many as 30% is *in vivo* IgG coated and 45% is coated with IgA (*van der Waaij* et al., 1994; *Jansen* et al., 1993; *Apperloo-Renkema* et al., 1993). It seems likely that these antibodies are in the circulation (in low titres) to support innate clearance of translocating bacteria; secretion of antibodies with the mucus into the intestines may play an important direct role in the intestinal CR. The circulating anti-bacterial antibodies are not only binding to endogenous faecal bacteria of a host organism, since a fraction appears active against endogenous microflora components of other (unrelated) subjects (*Apperloo-Renkema* et al., 1993).

Criticism to the role of specific antibodies in the clearance of translocating bacteria is based on the fact that certain bacteria may bind antibodies non-specifically, by binding the Fc-part of antibodies subjects (*Apperloo-Renkema* et al., 1993). Such non-specific binding, however, concerns predominantly a few gram-positive bacterial species (staphylococci) and as this binding is non-specific; consequently, it does not *selectively eliminate antibodies specific to this small subset of non-specific binding bacteria*. The effect of non-specific binding of antibodies *in vivo* is unclear.

HYPOTHESIS

To my knowledge, so far no prospective study on the role of non-specific opsonising (NSO) factors in clearance of bacteria with or without specific opsonisation (antibodies) has been undertaken and published. The present hypothesis is consequently based on previously published observations. It is conceivable, that in the lamina propria *non-specific opsonisation and in some cases in combination with antibodies enhance phagocytosis for clearance of translocating intestinal bacteria.* A wide variety of bacterial products can trigger inflammation (*Henderson* et al., 1996), yet the clearance of small numbers of translocating micro-organisms by NSO is likely to occur mostly without inflammation. Translocation may be a daily occurring event in individuals who live in a conventional environment.

Depending on their concentration in the gut, bacteria may pass the mucosal lining in numbers related to their capacity to adhere and penetrate (a virulence factor). Another port of entry of the host organism in the gut is the M-cell layer overlaying a Peyer's patches. There, they may induce either IgA or (occasionally) T-suppressor cells for tolerance (or both?) (*Brandtzaeg* et al., 1999). Both may represent a (feedback?) mechanism which controls 'pathogenic immunity' (i.e. inflammation). It is likely that the proposed *synergism* between NSO factors and *specific opsonisation* (SO) represents a more important mechanism in the non-pathogenic clearance of bacteria (without inflammation) than *intestinal tolerance*.

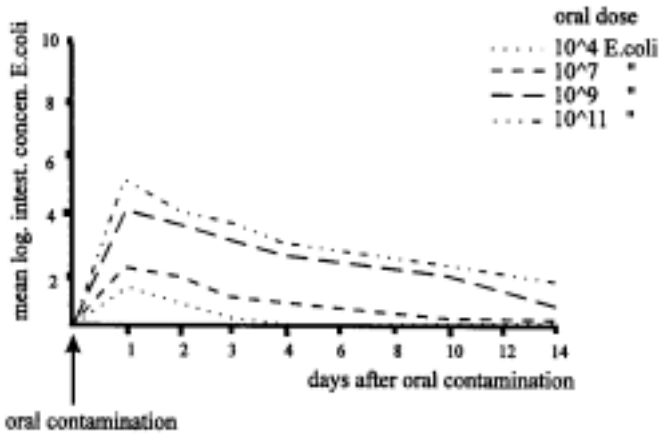

Figure 2: Relation between oral dose and mean log. intestinal (faecal) microbial concentration in mice (standard deviations of the mean were not calculated because of samples with 'zero' counts in each set of samples) (*van der Waaij* and *van der Waaij*, 1990).

Following ingestion of high(er) numbers or ingestion of more virulent bacteria, equipped with special tools to penetrate the mucosal lining, sufficient antigenic material may arrive in the lymphatic organs to induce specific immunity, either by stimulating existing (innate?) poly-reactive IgM isotype antibodies or by induction of circulating antibodies of the IgG isotype. Would the number or the pathogenicity of translocating bacteria be higher than can be managed by the 'synergistic clearance system' a certain 'threshold' may become passed which results in a stronger specific antibody production; their clearance will be predominantly due to adaptive (specific) immunity. Inflammation due to activation of complement factors must in such cases be expected. Interacting mechanisms in *the normal control of opportunistic micro-organisms* are:

1. Oral dose **(0D)** of opportunistic microbes
2. Colonisation resistance **(CR)** of the digestive tract
3. Intestinal concentration **(IC)** of opportunistic microbes reflecting the numbers which may reach the mucosa
4. Translocation **(TL)**
5. Non-specific opsonisation **(NSO)** and specific opsonisation **(SO)** to enhance phagocytosis and killing; i.e. *clearance* of TL micro-organisms.

A RETROSPECTIVE ANALYSIS OF MOUSE DATA REPRESENTING MOST OF THE FLOW OF EVENTS AFTER INGESTION OF AN OPPORTUNISTIC MICRO-ORGANISM

As a first step to test our hypothesis, evidence was sought for the assumed co-operative interactions between 'defence layers'. The following brief review of data provides certain indications for 'inter-layer co-operation':

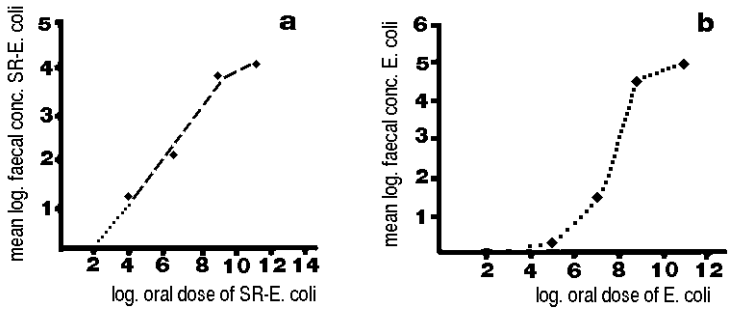

Figure 3: Relation between oral dose and faecal concentration (data from *van der Waaij and van der Waaij*, 1990 [a]; and from *van der Waaij* and *Berghuis*, 1974 [b])

The relation between an oral dose of opportunistic microbes, the colonisation resistance and the intestinal concentration reached by contaminants

The relation between the oral dose of opportunistic bacteria (a strain of *SR-E. coli* as an example) and the resulting intestinal concentration in mice during the next days, is quoted from previous publications (*van der Waaij* and *Berghuis*, 1974; *van der Waaij* et al., 1977) and shown in Figure 2.

The curves in Figure 2 show, that during two weeks after contamination, the daily relation between an oral dose of *E. coli* and their mean log concentration in faeces, is not linear. This is most clear in the first days after contamination. In Figures 3a and 3b data from two different series of experiments in mice are shown. However, on the basis of these data, it cannot be concluded, that the curves are really S-shaped is seems likely by their appearance. Furthermore, it should be made clear that the curves in these figures are derived from experiments with only one *E. coli* strain in one particular mouse strain (ND2 mice). Results may differ when other *E. coli* strains are used or other *Enterobacteriaceae* species and other (gram-positive) opportunistic bacteria such as *Enterococcus faecalis* (*van der Waaij* et al., 1972). However, these differences may not be essential, as the general (S-shaped) trend in the first days is the same in all.

The non-linear shape (S-shape?) of the curve, which may represent the relation between dose and effect, could be the result of a switch in the mechanism involved in the obtaining that effect. In the curve shown in Figures 3a and 3b, the lower oral doses could have been controlled by predominantly the intestinal microbial ecosystem, whereas following higher numbers of ingested bacteria, the immune system of the host organism may have been involved in the outcome. No experiments have been performed as yet to further investigate this prospectively.

Passing of the aforementioned 'innate-adaptive-immunity threshold' -inviting the adaptive immune system to action- could either be due to a *deficient defence system*, unable to clear translocating bacteria which normally would have been cleared optimally (like may be the case in the compromised patient), or to certain *virulence factors* of the translocating bacteria. These virulence factors permit them to escape NSO so that their clearance relies completely on SO.

The switch in the clearing system from predominantly innate to predominantly specific immunity could be made likely, by plotting the *"infective dose"*

and its *"effect"* graphically. The 'threshold', which determines the shift, from predominantly innate to predominantly specific immunity, may show it self by a change in the slope of such a curve from gradually increasing to gradually decreasing; the curve then has more or less an S-shape. On the basis of observations made in our reviewed previous studies (*van der Waaij* and *Berghuis*, 1974; *van der Waaij* et al., 1977), it is concluded that indeed antimicrobial defence factors may co-operate intensely in the process of controlling colonisation by newly ingested micro-organisms. In this S-shaped relation between oral dose and the intestinal concentration of the 'contaminant', the *E. coli*, there is evidence of a contribution of another additional mechanism from above the oral dose level of 10^8 *E. coli* on. This additional defence mechanism prevents (or ads to the control) a rise of the intestinal concentration to above 10^6 *E. coli*/g. This additional mechanism which may act in the CR, could be specific immunity as a control mechanism which maintains the intestinal concentration at 10^6 bact./g is absent in congenitally thymus-less mice once the reach an age of about 6 weeks when passive maternal antibodies have disappeared (*van der Waaij* and *Heidt*, 1990).

AN *IN VIVO* MODEL FOR MICROBIAL INFECTION, COLONISATION, TRANSLOCATION, AND CLEARANCE

Because the effect of CR on various doses appears to be non-linear but perhaps S-shaped, it was aimed to determine experimentally the graphical correlation between dose and effect, by determining the clearance of translocated bacteria *in vivo*. A graphic relation between invading numbers and their clearance might provide further insight in the physiologic clearance process of TL bacteria. To measure factors involved in the clearance *in vivo*, however, the clearance had to be defective (incomplete) for some time after oral infection, permitting a low degree of bacteraemia for several hours, in order to have some 'prove' for the occurrence of TL. Incomplete clearance furthermore, would provide some insight in the flow of events in a relatively compromised individual. In addition, blood sampling in the course of the critical first days after oral challenge would, in addition to culturing, permit the use of blood for chemical analysis of NSO and study of SO factors (antibodies). A study of this kind has been performed in the seventies as a pilot study for a grant application, unfortunately the grant was not awarded and further investigations had to wait.

Materials and Methods

Selection of E. coli strain for TL

For our experiments, a 'rather invasive' neomycin, streptomycin and ampicillin resistant *E. coli* strain was selected. Selection of this strain was carried out with a technique described by *Schabinsky* (1965). Schabinsky developed this technique in fact for another application namely to study the effect of antibiotics on i.p.-injected *E coli* by determining the number of colony forming units in the tail tip blood at several intervals.

For our purpose we selected an *E. coli* strain, which appeared in the tail blood of mice after the lowest infective dose (by *i.p. injection),* for further use. To minimise the chance that immunologic contact between the strain and the experimental animals had ever occurred, only strains of human (patient)

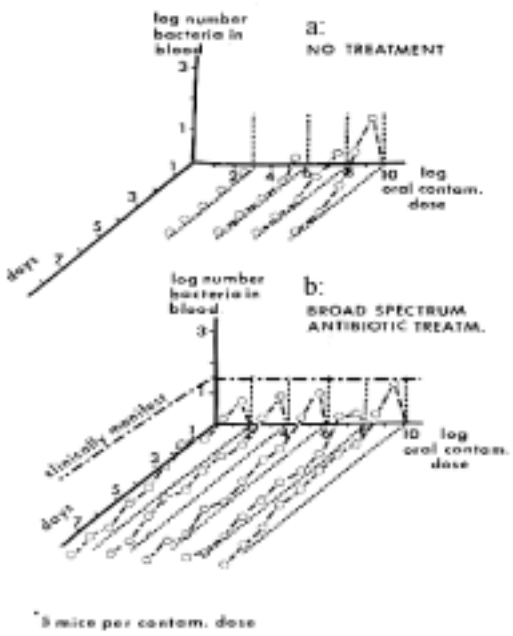

Figure 4: Mean number of E. coli colony forming units cultured from the blood of non treated mice (a) and antibiotic treated mice (b).

origin from the hospital were tested. Of the strain we selected, 10^3 *E. coli* i.p. per mouse; the strain was resistant to neomycin, streptomycin and ampicillin (NSA). The strain may have developed resistance to innate defence factors common to man and mice (*Rhen* et al., 2000).

In a pre-study with this *neomycin/streptomycin/ampicillin resistant strain* (*NSA-E. coli*) in germfree mice, it was found that following *oral* doses of 10^3 or higher, this *NSA-E. coli* strain could be cultured in varying numbers (several samples per day were positive; some were negative) from the tail blood for several days (possibly longer; not studied) after oral contamination. These oral doses of 10^3 of these rather invasive bacteria as well as higher contamination doses did not cause clinical signs and symptoms in these mice. This finding could be reproduced so that it was decided to perform a study with this strain in conventional mice. It can not be excluded that the relatively high virulence of the strain, causing its appearance in the tail blood, was due to an escape from efficient clearance as a result of resistance (developed in the patient?) to one or more innate defence factors (*Schabinsky*, 1965).

Translocation studies in conventional (control) and antibiotic decontaminated mice

In the experiments summarised hereafter, groups of five conventional or decontaminated female ND2 mice (12 weeks of age) were orally contaminated with various doses (Figures 4a and 4b) of the *NSA-E. coli* strain. Tail blood was collected *thrice* daily after disinfection of the tail tip. For the first collection of blood, the very end (small piece) of the very end of the tip of the tail was cut off. Each time a subsequent blood sample was required, bleeding started soon after some massage of the tail (automatically involved in the tail disin-

fection procedure). The drip of blood collected after tail disinfection was smeared (plated out) onto a neomycin/streptomycin-McConkey agar plate and incubated overnight at 37°C. Counting of the colonies grown at the site of the blood streak was supposed to give information about the number of organisms that were present in the blood sample. The highest count per mouse/day was used to calculate the mean number of *E. coli* colonies of each group of five mice.

In experiments in non-treated *control* mice, a fraction, proportional to the oral dose of the *NSA-E. coli,* may have reached sufficient high numbers in the gut contents to translocate. Following the highest doses, this *E. coli* strain may in the first 3 days have been able to 'bypass' (overload?) the normal sequence of events which lead to efficient clearance which may have caused the positive blood cultures. The initial 'escape' from efficient clearance may have been due to *the virulence (invasiveness and resistance to subsequent clearance) of the E. coli strain.*

Results

Oral dose -TL relation in control mice

In Figure 4a, the mean number of *E. coli* colony forming units (CFU) cultured from the blood of control mice contaminated with different oral doses are presented. Standard deviations of the mean are not calculated because of several negative cultures on most days; particularly in the control group of mice.

Oral dose -TL relation in 'antibiotic decontaminated' mice

In Figure 4b, the same results are presented obtained in mice which were 'antibiotic decontaminated' by oral treatment with neomycin and bacitracin-pimafucin respectively (1 g/l and 0.1 g/l drinking water).

Discussion

The results show a dose related mean number of colony forming units (CFU's) of *E. coli* in the tail blood of the mice at daily intervals after oral contamination in the control group. Following comparable oral doses, the numbers of CFU's in tail blood were significantly higher after the lower oral doses in the decontaminated mice (low CR). In addition in the week of observation all groups had mice with (low) positive blood cultures, which were not found in the non-treated mice. None of the mice in both group clinical signs of illness (hunched back ruffled fur or clear loss of appetite).

A *tentative conclusion* of the findings of this *TL-study* could be that, on the log-log scale in which the results are shown, a non-linear relation appear to exist between the oral dose and the occurrence of TL in the first days after oral contamination, both in non-treated as well as in the antibiotic decontaminated mice. This relation showed **no** S-shaped form, suggesting that following the highest oral doses (above 10^6 bacteria) no other (additional) mechanism came into action to control the situation as was above concluded to exist between oral dose and CR.

In the non-treated (control) group of mice in the present experiment, a logarithmic curve could perhaps be drawn through the mean number of *NSA-E. coli* CFU's in the blood collected at day one. In the antibiotic decontaminated group on the other hand, no relation between oral dose and number of *NSA-E. coli* CFU's existed. Resistance to innate defence factors, essential for the clearance of the *NSA-E. coli* after translocation makes the outcome of the experiment the resultant of the action in first barrier the CR. Specific immunity may have taken care of the intra-intestinal concentration of the contaminant as

well as after translocation. In the first (about four) days after contamination, however, no specific immunity may have existed in these mice, since the *NSA-E. coli* strain, with which they were contaminated, was of patient origin so that it is unlikely that a previous contact with the immune system of the mice in this experiment had occurred.

Several days after oral contamination, either TL decreased or the clearance efficiency increased significantly:

a. In the non-treated control group, it is likely that a gradual decrease of TL with elapsing time was responsible for this finding. The TL decrease may have been the consequence of a decrease of the *NSA-E. coli* concentration in the gut perhaps due to an increase of the CR by specific immunity.
b. In the decontaminated group however, even a week after contamination TL appeared to occur albeit at a strongly reduced level. As the intestinal concentration of the *NSA-E. coli* may not have decreased as strongly as in the control group, as the decontaminated group lacked concerted action of an intestinal microflora. Development of specific immunity may also have developed in these mice. Specific immunity (antibodies?) may have had some lowering effect on the intestinal numbers as well as have enhanced clearance after TL. Yet, a combination of both effects of specific immunity (not investigated) may explain the reduced numbers of *E. coli* in the tail blood after the first days.

RESEARCH PRIORITIES FOR THE STUDY OF EXISTENCE OF CO-OPERATIVE INTERACTIONS BETWEEN DEFENCE FACTORS

Multiply-resistance to antibiotics is increasingly causing failures in the treatment of infectious diseases. There is no doubt about the point that ways must be searched to stop this increase of resistance before it gets completely out of control. Assuming that new antibiotics may provide the ultimate answer, an international study group (International Study Group on New Antimicrobial Strategies (ISGNAS-foundation) was founded 1992 and seeks a solution(s) for the resistance problem. ISGNAS recognises the potential importance of several new avenues of research and strongly stimulates research on these subjects (*Araneo* et al., 1996). A major avenue is seen in research that leads to treatment modalities to maintain in immunocompromised patients, the defence to opportunistic bacteria and fungi at a normal (physiologic) level. Maintenance of the defence normal by either stimulation or supplementation should go along physiologic lines. To this end, precise knowledge of the mechanism(s) and factors involved in the normal physiologic control of opportunistic bacteria. The ISGNAS-group strongly stresses the importance of the urgent need of comprehensive studies on the mechanisms that lead to TL as well as those involved in the physiologic clearance of TL micro-organisms. It is felt that the *in vivo* mouse experiments reported in this paper, may provide a model for a detailed study of factors in the microflora as well in the host organism. The model permits microflora modulation as well as blood sampling, albeit in micro-amounts, for chemical and microbiological analysis.

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[i] *Additive* activity means that the outcome is the sum of the effects of each of the factors. This means that for example 50% of both doses provide the same effect as 100% of each of them.

[ii] *Synergism* means that smaller doses - in the example less than 50% - of each compound are required to obtain that 100% effect.

[iii] In *antagonism* the outcome of a combination is smaller than one would expect by summing up the effects of each of them alone.

[iv] In this paper translocation is defined as: *the penetration of the mucosal epithelial lining into the underlaying lamina propria by living micro-organisms.*