Old Herborn University Seminar Monograph 8:
Probiotics: prospects of use in opportunistic infections.
Editors: Roy Fuller, Peter J. Heidt, Volker Rusch, and Dirk van der Waaij.
Institute for Microbiology and Biochemistry, Herborn-Dill, Germany: 173-184 (1995).

OLD HERBORN UNIVERSITY SEMINAR ON PROBIOTICS: PROSPECTS OF USE IN OPPORTUNISTIC INFECTIONS

REVIEW OF THE INTERNAL DISCUSSION

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INTRODUCTION

Before probiotics can be discussed in a rational way, it is necessary to define the concept exactly. Furthermore, the mode(s) of action of different probiotics should be clarified and, finally, the potential fields of application of probiotics will have to be defined.

During the days for discussion the following topics were dealt with:

1. Definition of the concept
2. Enhancement of the colonisation resistance of the digestive tract.
3. Nutritional effects of probiotics, especially reduction of lactose intolerance.
4. Therapeutic effects of probiotics, with emphasis on carcinogenesis in the colon.
5. Biochemical functioning of different microbial ecosystems and the recognition of mechanisms which may allow flora control at this level.
6. Humoral and cellular immunomodulation of the host both at the mucosal and at the systemic level
7. Safety aspects of probiotics.

DEFINITION OF THE CONCEPT

Previous definitions

Several attempts have been made to define the concept of "probiosis". The following definitions have been proposed:

Parker (1974) : Organisms and substances which contribute to intestinal microbial balance.

Fuller (1992) : A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.

Havenaar (1992) : A mono- or mixed culture of live microorganisms which, when applied to animal or man, beneficially affects the host by improving the properties of the intestinal microbial balance.

Fuller (1994)*: A preparation of live microorganisms or microbial stimulants

* Proposed during the discussion.

which affects the indigenous microflora of the recipient animal, plant or food in a beneficial way.

Because of the paucity of information about the way in which probiotics work, the definitions already proposed are often too inexact to accurately define what is meant by the term probiotic. The discussion which followed sought to offer a better, more precise description of what is meant by probiosis.

Considerations leading to a new definition

A possible new definition obviously should not be biased by any of the shortcomings its predecessors suffered from. It should, therefore, not contain a statement concerning possible mode(s) of action and should not contain any ambiguities.

Although a possible new definition may be based on several different criteria, it should describe (i) the composition and (ii) the function of the probiotic. However, a definition of the desired function of a probiotic makes it quite difficult to distinguish the probiotic from food-additives (e.g. starter cultures) on the one side and genuine drugs (e.g. vaccines) on the other side. For this reason it was decided that the composition of a probiotic be regarded as the most important factor in the construction of a possible new definition.

Considering the composition, it was realised that a probiotic may exert different beneficial functions, both at the cellular and subcellular level. Examples of different probiotic functions at different levels were listed as: (i) living whole cells which are able to compete for substrate with potential pathogens and to produce antimicrobial substances against this latter group of bacteria (ii) dead whole cells which can contain enzymes like lactases (iii) peptidoglycan cell wall fragments which may be able to trigger a humoral or a cellular immune response. Therefore, because probiotic activity does not result from one substance or a defined mixture of substances, a possible definition should describe all components of a probiotic which are supposed to exert a beneficial function. General opinion was reached on: "A probiotic is a microbial preparation which contains live and/or dead bacteria including their components and products".*

The problem with this new definition is that it does not unambiguously discriminate between preparations like vaccines and starter cultures. It was suggested by Dr. Freter that the inclusion of a statement on the functioning of the probiotic should take care of the problem. A possible alternative then becomes: A probiotic is a microbial preparation which contains live and/or dead bacteria including their components and products, which is administered orally or to other mucosal surfaces, and which is intended to improve the microbial or enzymatic balance at mucosal surfaces or to stimulate specific or non-specific immune mechanisms". Because this suggestion was made after the meeting, there was no discussion on this alternative.

ENHANCEMENT OF COLONISATION RESISTANCE

During the third decade of this century Lotke and Volterra observed that an ecosystem is more stable when more niches are occupied. Consequently, the

* Dr. Fuller, Dr. Wallace, Dr. De Simone, Dr. Tannock, and Dr. Rowland do not accept this as a definition of probiotics.

more diverse an ecosystem, the more difficulty an invading organism will experience upon colonisation. However, it was not until 1956 that Freter and co-workers demonstrated that this mechanism was also applicable to the microbial ecosystem of the digestive tract, using pathogens like *Salmonella* spp. and *Shigella* spp. In 1971, van der Waaij and co-workers found that this rule extended to *potential* pathogens as well.

Before the active fraction of a microbiological preparation (i.e. the probiotic) can exert its function on the host it has to reach the digestive tract. Thereafter, it will have to stay biologically active in the digestive tract for some time. This latter property requires either adherence to the mucosal lining or a high division rate of the probiotic. Factors like "host-specificity" and "adaptation" may influence the colonisation of the probiotic to a considerable degree and were discussed separately. Finally, the function of the probiotic on the stability of the gut ecosystem and some specific examples will be treated.

Gastric acid kill

The acidity of the gastric environment amounts to about pH 2.0 under normal conditions. The main acidifier in gastric juice is hydrochloric acid, which is easily dissociated. Consequently, there is only a limited amount of organic material needed for strong buffering of the pH in the stomach. Selection of microbial strains for acid resistance is probably of minor importance, provided the probiotic is ingested with food or a storage medium with sufficient buffering capacity.

Neutralisation of the gastric pH by antacid drugs could facilitate gastric passage of a probiotic strain but has the disadvantage of allowing microbial overgrowth of the stomach. This phenomenon has two major disadvantages. Firstly, the increasing amount of (potentially pathogenic) bacteria in the stomach may result in a higher risk of translocation of the stomach flora to the gut and the lungs. Secondly, the increased number of bacteria in the stomach may antagonise the probiotic strain. During the discussion on this topic it was suggested by Dr. van der Waaij that antacid therapy (e.g. in gastric ulcer prophylaxis) could be accompanied by probiotic treatment. If a niche for the probiotic in the stomach ecosystem exists, the development of a potential pathogenic flora of the stomach could then be suppressed by the probiotic, while at the same time the probiotic will continuously be seeded from the stomach into the gut.

Adhesion and association

The microbial ecosystem of the lower part of the digestive tract can be divided in at least two subecosystems: the "luminal flora" and "mucosal flora". The composition of the "luminal flora" is mainly determined by the nutrients available and the effect of antimicrobial substances; the composition of the "mucosal flora" is mainly determined by the host's expression of specific adhesion sites in the enterocyte membrane, the mucus production rate, the production of secretory immunoglobulins and the extrusion of cellular material from the membrane into the mucus. Experimental data reveal that prolonged colonisation of the mucus ecosystem with a probiotic strain (after administration has ended) is difficult, if not impossible. For a newcomer it is only possible to remain in the digestive tract for a prolonged period when it is able to colonise the mucus blanket. Colonisation of the mucus blanket firstly requires the occupation of a ecological niche and secondly, multiplication with a division rate which is equal to, or higher than, the mucus-to-lumen transition rate. Occupation of a

niche in the mucus can be achieved by association with the mucosa. This may manifest itself either by cell-to-cell adhesion or by entrapment in the mucus. An adhering microorganism contains a ligand against which a receptor is present on the enterocyte membrane of the gut. This ligand can be made up of several macromolecular types (e.g. glycosaccharides or glycoproteins) and may have antigenic properties. In order to stay in the mucus it is necessary for the adhering microorganism to multiply. From the work of Freter and co-workers it has become clear, that an adhered microorganism has the selective advantage of a lower substrate turnover. In other words, the adhered microorganism does not need to multiply at high rate to remain present in the mucus. Consequently, because it can withstand mucosal wash-out while maintaining a slower metabolic rate, it can occupy niches which are too low on substrate for most other non-adhering microorganisms. Closer to the lumen, associated microorganisms withstand wash-out from the mucus by rapid multiplication without adherence. This group of microorganisms needs to have access to an excess of substrate and needs an efficient, probably oxidative, type of metabolism. The colonising ability of a probiotic strain depends, partly, on its ability to associate. Adherence may be tested *in vitro* but the results of these assays sometimes fail to predict the adhering (and thus colonising) properties of a bacterial strain. Reasons for this inconsistency could be (i) *in vitro* monolayers of enterocytes do not produce mucus (ii) enterocytes may *in vivo* express receptors other than those found *in vitro* and (iii) a monolayer does not produce secretory immunoglobulins. In the future there will probably be an increase in the use of human volunteers in research projects concerned with probiosis.

Host-specificity and adaptation

The current bibliography on the host-specificity of probiotic strains is limited. Apart from some anecdotal knowledge (e.g. *Lactobacillus* spp. have a higher host specificity than *Enterococcus* spp.) most hypotheses are based on indirect evidence obtained from experiments with germfree animals. Experiments with animals which have a very simplified gut microflora cannot give decisive results on the interaction between the host and its entire microflora on the one hand and with a probiotic strain on the other. Even when host-specificity for a certain probiotic-host combination can be demonstrated, it is still possible that the effect of the probiotic on the host differs from one host to another because interindividual differences in immunological history of the host may result in a different repertoire of secretory immunoglobulins in the mucus. At this moment it is not certain whether host-specificity does exist and, if it does, whether it results in a comparable effect in a wide range of different host animals.

There is more literature available on "adaptation" than on the issue of the "host-specificity". Experimental data on this topic comes mostly from gutflora transplantation experiments in decontaminated or germfree mice. A striking example of this phenomenon can be extracted from the work of van der Waaij and co-workers who inoculated decontaminated mice with samples of luminal flora from different species: chicken, cow, human, rat and sheep. After two weeks of incubation all mice had retained the flora they harboured before inoculation, except for the mice given the gut-flora of cows. The complex mixture of bacteria present in the lumen of the colorectal part for the bovine-gut had adapted to the intestinal environment of the mouse. Breeding with the individuals of this group of mice did re-

veal that the adaptation of a cow-flora to a mouse environment had detrimental effect on the offspring of these mice. All baby-mice died within three weeks showing symptoms like: growth retardation, severe diarrhoea, and severe loss of weight (Geertsma et al.: Microecology and Therapy 20, 447-452, 1990).

Probiotic influence on the gut ecosystem

The internal discussions on the influence of probiotics on the gut ecosystem concentrated on two intestinal disorders: diarrhoea and constipation. A third non-intestinal disorder, vaginosis, was also discussed.

Diarrhoea

At the moment many types of diarrhoea are known. They may differ with respect to progress (acute or chronic) or etiologic agent (virus, bacterium, parasite, fungus, microbial metabolites). Most of the research which has been performed in the field of probiosis and diarrhoea suffers from a black-box design. During the discussions some of the previously obtained results were mentioned:

1. 10^{10} cells of a lactobacillus starterculture per day can cure *Campylobacter cholerae*, *Yersinia enterocolitica*, *Salmonella* spp. or *Shigella* spp.-induced diarrhoea (Olukoya et al.: Journal of Tropical Pediatrics 40, 108-113, 1994).
2. *Bifidobacterium breve* 10^{10} cells per day can cure infantile *Campylobacter* enteritis (mentioned by Dr. Tanaka during the meeting).
3. There is little published evidence that Lactobacilli (i.e. *Lactobacillus* GG) can be used in the treatment of *Clostridium difficile*-induced diarrhoea (Gorbach et al.: Lancet ii, 1519, 1987).
4. *Saccharomyces boulardii* can be used in the treatment of *Clostridium difficile*-induced diarrhoea.

On the basis of the current experimental data no definite conclusions can be drawn. however, the following assumptions seem reasonable:

1. Probiotics will probably be more effective in preventing diarrhoea than in treating it.
2. Because of the self-limiting character of acute diarrhoea, probiotics are most likely to be employed in chronic diarrhoea.
3. The type of diarrhoea needs to be fully specified before probiotic treatment can be considered.
4. Because large differences exist between the effects that different bacterial species (even within the same genus) may exert on diarrhoea the probiotic needs to be fully identified.
5. In antibiotic-induced diarrhoea, it may be sensible to accompany treatment with antibiotics which have a high potential for inducing diarrhoea with a prophylactic probiotic treatment.
6. At this moment research in this field is limited by the absence of proper animal models for all the different types of diarrhoea.

Constipation

Two types of constipation are recognised: (i) constipation by blockade of the gut lumen (e.g. a tumour) while motility is still intact and (ii) decreased motility (especially in the elderly). Probiotic laxation is only rational in the second type of constipation. Traditional laxatives like bivalent salts or polyvalent alcohols have a non-specific mode of action. Therefore, pronged use of these substances may result in dehydration or disturbance of the electrolyte balance of the host. Though valid data on the underlying mechanism is absent, it may be possible that probiotic laxatives do not suffer from this side effect. This as-

sumption is supported by empirical studies of Dr. Tanaka and co-workers who found that oral administration yoghurts containing 10^{10} viable cells of *Bifidobacterium breve*, 10^{10} viable cells of *Bifidobacterium bifidum* or 10^{9} viable cells of *Lactobacillus acidophilus* per 100 ml to a group of bedridden patients on a low-fibre diet resulted in a significant increase of the bowel movement and a significant decrease of the constipation incidence (Tanaka: Japanese Journal of Geriatrics 19, 577-582, 1982). These beneficial effects were not accompanied by side-effects like dehydration or disturbance of the electrolyte balance.

Vaginosis

Production of hydrogen peroxide (H_2O_2) by lactobacilli seems to be an important factor in the maintenance of the ecological environment of the vagina. From the work of Hilier and coworkers (Obstetrics and Gynaecology 79, 369-373 (1992) it can be concluded that a stable population of H_2O_2-producing lactobacilli protects the vagina against bacterial invasion by pathogens of the female urogenital tract like *Gardnerella vaginalis*, *Candida albicans*, *Bacteroides* spp. *Peptostreptococcus* spp. and *Chlamydia* spp. The effect may even extend to viral pathogens like the human immunodeficiency virus type I (Seymour et al.: Journal of Experimental Medicine 174, 289-292, 1991). This protective function is of special importance when the normal vaginal ecosystem has been disturbed by e.g. pregnancy, antibiotic treatment or sexual intercourse. Local administration of H_2O_2-producing *Lactobacillus acidophilus* to women who suffered from bacterial vaginosis was found to be an effective therapeutic treatment (Hallen et al.: Sexually Transmitted Diseases 19, 146-148, 1992).

Lactobacillus acidophilus administered orally may also be employed in the prophylaxis of candidal vaginosis. (Hilton et al.: Annals of Internal Medicine 116, 353-357,1992). Curiously in this study the lactobacilli were ingested orally. However, the mechanism by which the lactobacilli enter the vaginal ecosystem remains unclear. These bacteria may enter the vagina after passage through the digestive tract. This seems plausible since the vaginal flora has many species in common with the rectal flora.

NUTRITIONAL EFFECTS OF PROBIOTICS

Several nutritional effects of probiotics have been investigated. In animal studies (McDonough: Journal of Food Science 47, 1463-1465, 1982) an increased growth rate has been observed after dietary supplementation with lactobacilli. Increased mineral absorption from the gut and the production of riboflavin and niacin, resulting from the temporary colonisation of the gut by lactobacilli, may partly explain the observation. However, the most convincing claims have been made in the probiotic treatment of lactose intolerance. Increased levels of intestinal lactase (i.e. ß-galactosidase) activity may help in the metabolism of lactose before bacterial enzymes can convert it to lactate. This latter substance may cause osmotic diarrhoea due to its relative slow absorption from the large intestine compared to the rapid absorption of short chain fatty acids, which, in turn, are able to enhance the absorption of water and electrolytes. In order to increase the levels of intestinal lactase activity, at least two strategies may be employed: (i) oral feeding of ß-galactosidase-producing

bacteria and (ii) oral feeding of a substrate specific for these strains. There are many studies in humans measuring the effect of lactobacillus fermented milks on lactose digestion. Some examples of these studies are (Gilliland and Kim: Journal of Dairy Sciences 67, 1-6, 1984; Hitchins and McDonough: Americam Journal of Clinical Nutrition 49, 675-684,1989) and, as part of a review on probiotics, (Gorbach: Annals of Medicine 22, 37-41 1994).

THERAPEUTIC EFFECTS

Colonic carcinogenesis

Epidemiological studies have shown that the incidence of colon cancer is higher among populations who have a low intake of fermented milks. One of the first reports of mutagenic activity in human faeces came from Bruce and coworkers (In: Hiat, H.H.; Ed.: Origins of human cancer, 1641-1646, 1977). Furthermore, it has been shown that the intestinal flora has the ability to metabolise endogenous and exogenous compounds (Bakke: Biochemical Pharmacology 30, 1641-1646, 1981). Usually conjugation by the liver leads to detoxification and inactivation of the compound. Deconjugation in the intestine by (bacterial) enzymes may regenerate the compound in the intestine. If the inactivated compound is a potential carcinogen then its reactivation in the intestine may enhance the risk of colon cancer of the host.

Biological markers

Measurement of the activity of some specified set of biological markers which are supposed to be indicative for the carcinogenic properties of a gut microflora may be useful in assessing the chance of the emergence of colon cancer. Biological markers fall in at least two functionally different categories: (i) the biological activity of mutagens in the intestines and (ii) the biological activity of (bacterial) enzymes which are supposed to catalyse the deconjugation of procarcinogens to carcinogens in the intestines. Biological markers of the first category include N-nitroso compounds, heterocyclic amines and secondary bile salts. Possible biological markers of the second category include azoreductases, nitroreductases, ß-glucuronidase and bile acid dehydroxylase. The activity of biological markers of the first category may be quantified by means of Ames' Salmonella/mammalian microsome test (Maron: Mutation Research 113, 173-215, 1983). However, these test methods do not give any information on the molecular composition of the mutagen(s) involved. The activity of biological markers of the second category can be quantified by measuring the speed at which specific substrates are metabolised by the gut microflora.

Effect on the host

Once the activity of a wide array of mutagens in the intestines is quantified, the next logical step is to assess the effect on the host organism. During the first day of the 8th Old Herborn University Seminar, Dr. Rowland presented an elegant method (the "Comet" assay) to measure the influence of potential genotoxins in the gut on the intestinal mucosal cells of the host. Briefly, a mucosal cell suspension is prepared from a biopsy of the gut epithelium and incubated with the substance or preparation to be tested for genotoxicity. The cells are then spread on a microscope slide in a layer of agar, lysed gently in alkali and subjected to electrophoresis. Any DNA damage

(strand breaks) induced by the test chemical causes DNA to "unwind" and spread out under the influence of the electric field to form a "comet tail" (visualised by staining with ethidium bromide). In general the length of the comet is proportional to the degree of DNA damage. The assay can be applied to *in vitro* and *in vivo* studies and cells isolated from human biopsies can be investigated. Clearly the relevance of such DNA damage to tumour initiation and promotion needs to be established. Ultimately, large scale human intervention trials on the influence of diet and/or probiotic treatment on the incidence of colonic cancer, and other types of cancer (e.g. breast cancer) will have to be performed. At this moment these types of studies have only been carried out on the influence of fibres on the colonic carcinogenesis.

Experimental data

During the discussions, the some experimental results were mentioned. Two of the most remarkable were:

1. A study of Aso and Akaza (Urology International (1992) 49; 125-129) in which the influence of oral feeding with *Lactobacillus casei* on the recurrence of superficial bladder cancer was investigated. In this study, oral feeding of the lactobacilli significantly reduced the recurrence of the tumour. However, because of its descriptive nature of this study, it is not clear what the molecular or cellular tumour suppressive mechanism is.
2. Daenen and co-workers (manuscript in preparation) found that rats with chronic leukaemia responded much better to chemotherapy when their gut microflora was modulated. In this study the facultative, anaerobic, Gram-negative rods (Enterobacteriaceae and Pseudomonadaceae) were removed from the flora. In the rats with the modulated flora, leukaemia developed less quickly and, because the response to the treatment was better, the mortality in this group was significantly reduced. The experiment, although no molecular mechanisms are known to date, clearly demonstrates that gut microflora plays an important role in carcinogenesis but also in its therapy.

Ammonia production

Although it was only mentioned briefly by Dr. Rowland, this aspect of host-gutflora interaction may become of more interest in the future because the recent developments in experimental and clinical hepatology have increased the survival rate of severely ill hepatological patients. As a result of this development the group of patients which are at risk of hepatic coma has also increased. If probiotic treatment can decrease the level of intestinal urease activity, the incidence of hepatic coma may be reduced considerably. Three possible mechanisms which may reduce the ammonia production of the gut microflora were proposed during the discussions: (i) substrate competition of the probiotic with the urease-producing inhabitants of the gut microflora (ii) probiotic production of antimicrobial substances which inhibit the growth of the urease producers in the gut and finally (iii) probiotic production of urea antagonists in order to reduce the intestinal urease activity.

BIOCHEMICAL FUNCTIONING OF THE GUT MICROFLORA

In contrast to the therapeutic effects, which were mentioned above, this section will deal with the biochemical functions of the gut microflora. With

this term the probiotic modulation of host-related characteristics is described. Until now there is one claim on which some scientific work has been performed i.e. the reduction of levels of serum cholesterol by means of probiotic microflora modulation. The potential clinical relevance of this category of probiotic treatment was stressed by Dr. van der Waaij who referred to the function of HDL in the transportation of endotoxin to the liver. Probiotic treatment may be applied in stimulating the clearance of endotoxin as long as liver functions are normal. The effect of probiotic supplementation is uncertain. Dr. Rusch mentioned some research that was performed at the Institute for Microecology in which the probiotic treatment of (mice) with *Escherichia coli* was without effects on the levels of serum cholesterol.

Even though the experimental data are inconsistent an attempt was made to suggest some mechanisms which may explain the phenomenon:

1. Utilisation of cholesterol by the probiotic. There is some *in vitro* evidence for this mechanism but because of the low efficiency of this process it is still not clear whether this phenomenon is not merely an artefact.
2. Formation of cholesterol inclusion bodies. During the discussions none of the participants was able to come up with references to studies concerning this subject.
3. Interruption of the enterohepatic cycle by elevation of the bile salt hydrolase activity in the gut microflora. This latter possibility cannot be regarded as beneficial because of the adverse effects of these enzymes on the membranes of the enterocytes in the gut epithelium.

Again, no decisive data on one or more of the effects mentioned above in humans is present today.

HUMORAL AND CELLULAR IMMUNOMODULATION OF THE HOST AT THE MUCOSAL AND AT THE SYSTEMIC LEVEL

Mucosal surfaces are continually exposed to antigenic material and the effectiveness of the ensuing mucosal immune response and the development of systemic non-responsiveness (tolerance) is of great importance in maintaining the health of the host. Probiotics may be useful in presenting extra antigenic information to the host thus enlarging its number of different T-memory clones. This extra immunological information may be of protective value when the host is faced with known, or related antigens, or when the host faces a large amount of known antigens. This principle of immunomodulation may become more important because of the growing number of immunocompromised patients and because of the growing number of pathogenic bacteria which are multiple resistant against antibiotics. Furthermore some of the functions related to the gut depend on the presence of non-pathogenic microflora which the immune system must learn to disregard.

Antigenic contact

Antigens present in the gut may come into contact with the Gut Associated Lymphoid Tissue (GALT) via 2 different pathways: (i) translocation over the enterocyte membrane and (ii) translocation over the membrane of M-cells which overlay the Peyer's Patches. In the first case, the antigen is most likely to be transported to the mesenteric lymph nodes by macrophages, while in

the second case the antigen may be presented locally to T- and B-cells by dendritic cells.

Macrophages.

The phagocytic, or non-specific immune system is comprised mainly of macrophages. The functioning of the phagocytic system can be assessed by measuring the amount of lysozymal enzymes released by macrophages or by measuring the amount of B- and T-cell stimulating factors like interleukins. Perdigon and co-workers (Infection and Immunity 53, 404-410, 1986) found that oral feeding of *Lactobacillus delbrueckii* spp. *bulgaricus* and/or *Streptococcus salivarius* spp. *thermophilus* (6 x 10^9 cells) resulted in a significant increase in the production of the lysozymal enzymes indicating an increased phagocytic activity. Bacteria were also administered via the intraperitoneal route but this route of administration would not be suitable for tests on human volunteers. The increased activity of the phagocytic system induced by lactobacilli administered orally was accompanied by a decreased translocation rate of enteric bacteria to lymphoid organs like spleen and liver (Perdigon: Journal of Food Protection 53, 404-410 (1990). However, these results were obtained in mice. In humans there are some experimental data (Rusch: Drug Research 44, 691-695,1994) but this author tested the activity of circulating mononuclear blood cells. Although no decisive experimental data were obtained in humans, the results of the studies on the non-specific phagocytic immune system may well account for the observation that probiotics stimulate a response to a wide range of non-related antigens.

Humoral immune system

Antigens originating from the gut which have passed the membrane of M-cells are phagocytised by macrophages. These cells synthesise and release interleukin-1ß and other cytokines. Interleukin-1ß is able to directly activate resting B-cells while it also triggers T-helper cells which subsequently start production of interleukin 2, which is essential for the proliferation of a resting B-cell to a mature plasma cell. Interleukin 6 has a function in the late stage of the B-cell proliferation. Once B-cells have developed their interleukin 6 receptor they differentiate to immunoglobulin producing plasma cells. Plasma cells in the GALT mainly produce secretory immunoglobulins, which are "sprayed" into the mucus. Antigens translocated to the spleen may induce a comparable response. However, in this case the antibodies produced are transferred to the circulation.

The cellular immune system

Besides B-cell regions there are T-cell regions in the Peyer's Patches. When a gutflora-associated antigen is translocated over the M-cell membrane, antigen presenting cells will induce T-helper cell generation resulting in both a humoral and a cellular immune response. Apart from the already mentioned induction of B-cell proliferation, T-helper cells also induce T-cytotoxic cells which, in co-operation with antigen presenting cells are responsible for the clearance of the antigen. Furthermore, T-helper cells will induce proliferation of T-memory clones. Immune complexes are formed early on during a primary contact of the (probiotic) antigen with the cellular immune system of the host These complexes stimulate T-helper cell generation necessary for the "switch mechanism" of immunoglobulin isotype from IgM to IgA antibodies in the GALT and for generation of B-memory. Subsequently, they generate the higher-affinity antibodies of the IgG isotype forming

immune complexes which neutralise and remove the antigen. The latter also activate the T-suppressor cell circuit which suppresses any further T-helper cell generation. Prolonged exposure of the host to a (probiotic) antigen may induce the T-suppressor clones to such a degree that the cascade of events leads to a decrease in both the humoral and the cellular immune reactivity but not in the phagocytic activity. This mechanism appears to be in concordance with experimental data in which a lowering of titres of circulating IgG against *Enterococcus faecalis* was observed after oral feeding of the strain to human volunteers (Jansen: Infection 21, 193-194, 1993).

In conclusion, it may be stated that probiotic immunomodulation will probably be one of the hardest problems to tackle because the system is antigenically complex (the gut-microflora) and is further complicated by the fact that individual hosts may have experienced profound differences in their immunological history, and therefore have large differences in the information stored in their T-memory clones.

SAFETY ASPECTS OF PROBIOTICS

The safety of a probiotic depends on several factors. The ones which were discussed most intensively during the 8th Old Herborn University Seminar were:

1. Ability to transfer genetic material.
2. Sensitivity to antibiotics.
3. Ability to produce hazardous substances.
4. Immune status of the host organism.
5. Non-infectious nature .

Regarding the first factor it was stated that the more competent a probiotic strain is to transfer genetic material (irrespective of whether the underlying mechanism is transduction, conjugation or transformation) the less it is suited for probiotic purposes. However, this problem may have a self-limiting nature because a large "plasmid-load" may have inhibitory effect on the multiplication rate of the cell. In other words: bacterial cells which harbour vast amounts of plasmids (and therefore have a high chance of carrying genetic information which may -when expressed- have a detrimental effect on the host) are less well suited to withstand the selective forces which maintain the ecosystem.

The second factor, although discussed separately, is merely an aspect of factor number one since resistance to antibiotics is often plasmid encoded. Although there are some reports of transfer of genetic material in the intestinal ecosystem, no convincing data concerning intestinal transfer of genetic information, which may be associated with pathogenity, is known presently. The third factor was not discussed intensively because it is obvious that probiotic strains should not produce any substance which may harm the host.

The fourth factor concerning the immunological handling of intestinal antigens by the host organism is more extensively dealt with in the previous paragraph. With regard to this issue it is sufficient to mention some of the most significant host-related factors which may determine the safety of the treatment: (i) the nutritional status of the host has a profound influence on the immunological defence capacity (ii) the immunological history of the host organism, in other words the antigenic information stored in the host's memory clones.

With respect to the fifth factor; probiotics need -of course- to be non-infective to both the normal and the immune impaired host.

Typical for the gaps in our knowledge of the host-gutflora interaction is the diversity in safety precautions which exists today. Some manufacturers of probiotics do perform their own research on the safety of their products (Rusch: Microecology and Therapy 10, 173-203, 1993) others do not. It is clear that much research on host-gutflora interaction needs to be done to reach a widely accepted set of safety regulations.