MICROBIAL THERAPY WITH *ENTEROCOCCUS FAECALIS* AND *ESCHERICHIA COLI*: EXPERIMENTAL AND CLINICAL DATA

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SUMMARY

Microbial therapy with microbial preparations and autovaccines is a therapeutic method based upon experimental and controlled clinical studies recognised by social and private health insurances in Germany.

The treatment of various diseases ranging from acute and chronic infections in the ENT-field, in the respiratory tract, in the gastrointestinal area, in the urogenital tract, up to allergies and other, originally developed empirically and hardly understood from the conventional point of view, in these days wins comprehension via latest immunological insight. The embryologically, morphologically and immunologically conceivable mucosal immune system leads to a determinable basis of the broad spectrum of indications and opens new perspectives in therapy. Immunmodulation was in the centre of the scientific interest of a group at Herborn-Dill, Germany (Medical Association for Microbial Therapy), conducting numerous experimental and controlled clinical studies taking into consideration most recently measurable immunological parameters. Altogether, eleven randomised, double-blind and placebo controlled clinical trials were performed including a total of 2,334 patients. Hypotheses concerning efficacy were confirmed in animal experiments and in *in vitro* lymphocyte cultures of human volunteers. Different immunological signals intervene effectively in the complex network of the mucosal immune system, the immune system, the nervous system, the metabolism and the hormone system. Thus, forty years of careful medical observations in therapy with microbial preparations and autovaccines become validated.

FUNDAMENTALS

Immunological aspects

The basis of immunomodulatory therapies is the immune system of which we now know that it possesses a very much more complex structure than was originally assumed (*Roitt* et al., 1989). The immune system provides not only "immunity", i.e. protection from infection, but also serves for the performance of numerous other tasks in conjunction with the microflora, mucosae, metabolism, nervous system and hormone system. From the phylogenetic viewpoint, the intestine is to be regarded as the cradle of the immune system. The skin and especially the mucosae in the digestive tract are the direct borders with our environment so that it is quite logical to see a special immunological significance in these areas. Only recently,

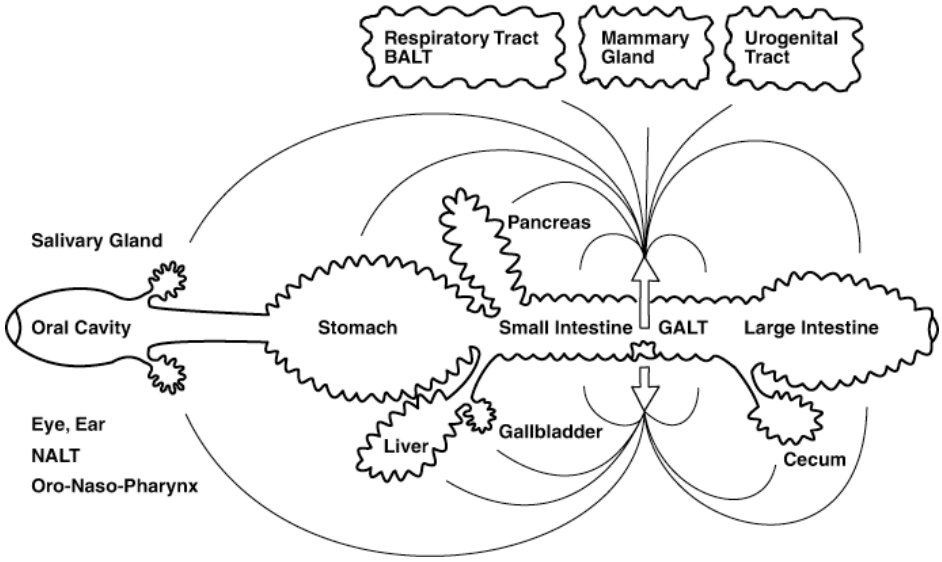

Figure 1: The mucosal immune system. Lymphocytes are sensitised at specific points of the mucosal immune system (e.g., in the lymphatic tissue associated with the intestine (GALT), move into the lymph capillaries and pass through the mesenteric lymph nodes, the thoracic duct and the blood stream (continuous arrows). These lymphocytes then migrate (interrupted arrows) to various mucosae of the gastrointestinal system and to mucosal tissues outside the intestinal tract (*Castro*, 1989).

it was learned, that about 85% of the immune system are to be attributed to the mucosae of the human organism. The morphological substrate of this relatively autonomous system are the organs developing from the entoderm in ontogenesis. It was first realised that there is an independent lymphatic tissue associated with the intestine (GALT). The latest findings have indicated that the mucosal immune system is common to all the mucosae, that the most diverse organs are linked with each other (*Gemsa* et al., 1991; *MacDonald* et al., 1990; *Ogra* et al., 1994; *Stern*, 1992). These concepts are shown in Figure 1. The boundary areas represented in the mucosal immune system cover an exceptionally large area: 400 m^2 small and large intestine, 80 m^2 lung (2.5 m^2 skin). A large part of these mucosae is normally colonised by microorganisms (*Fuller* et al., 1995; *van der Waaij* et al., 1990).

Microbiological aspects

Human beings are colonised by a fantastic variety and number of microorganisms. Microbe populations of different compositions in different densities colonise different habitats: in the digestive tract (mouth, nasal and pharyngeal tract, stomach, small intestine, large intestine), the vagina, while also the skin is populated by microbes. The small intestine and the large intestine alone are colonised by about 300 to 500 different species of microbes that can be divided into 17 families and 47 genera. From intensive studies on germfree animals, very much was learnt about the significance of the microflora, especially concerning the structure and the functions of the immune system. In germfree animals, 85% of the immune system, i.e., the mucosal immune system, is not developed at all. The number of granulocytes is substantially reduced and the function of these granulocytes is

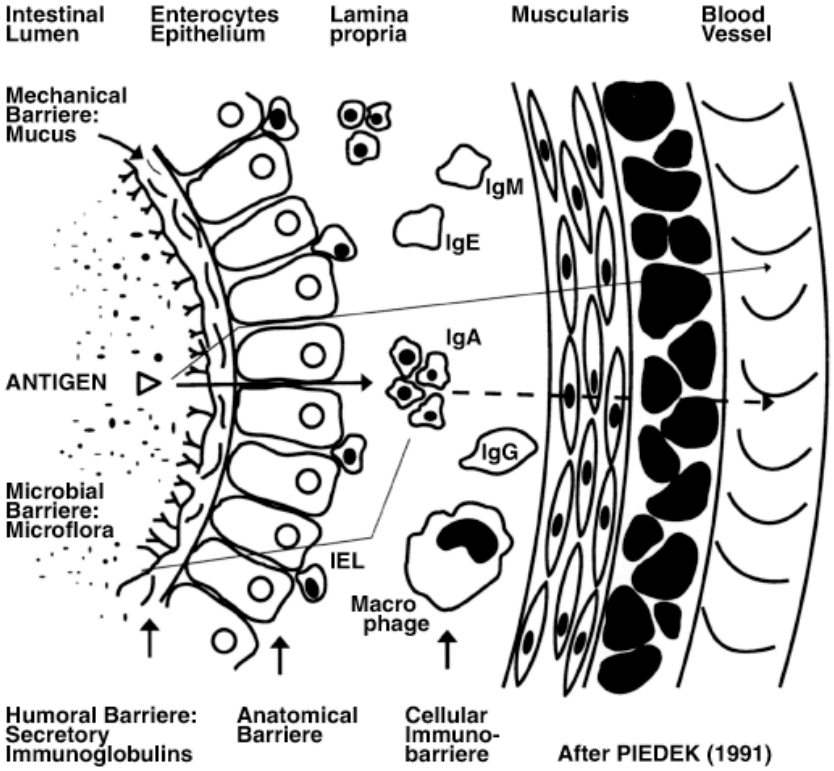

Figure 2: Graphical representation of the barrier function of the mucosal immune system on the example of the intestinal mucosa. IEL = intraepithelial lymphocytes (*van der Waaij* et al., 1995b).

limited. Relations between the microbes were studied by inoculation of germfree animals with microorganisms and it was experienced that the complex microbial populations stabilise in equilibria that resist colonisation by invading microbes from outside. This resistance is called colonisation resistance. Therefore the normal microflora is of decisive importance for the structure and the functions of the mucosal immune system. In addition, the normal microflora on its own provides a certain protection against pathogenic invaders. The microflora is thus an extremely important factor for health or disease. In the arrangement of the defence devices in most of the mucosae, the normal microflora represents one of the first defensive barriers (Figure 2) (*Fuller* et al., 1995; *van der Waaij* et al., 1990).

Symbiosis

Anton Heinrich de Bary defined the term "symbiosis" in 1879: "Symbiosis is the persistent and intimate living together of dissimilarly named organisms. Parasitism is the most popular and most exquisite phenomenon of symbiosis". Despite early misinterpretations and still existing misuse of the original meaning is accepted in modern biological sciences, as expressed during a Symposium of the Society for Experimental Biology in Cambridge by Starr in 1975: "'Symbiosis' is an eminently appropriate term. But an unfortunate second usage, in which the term 'symbiosis' has been limited to those organismic associations that are mutually beneficial, has crept in. It is high time to reverse the semantic deterioration. I have resolved, not only to use and to foster the use of the term

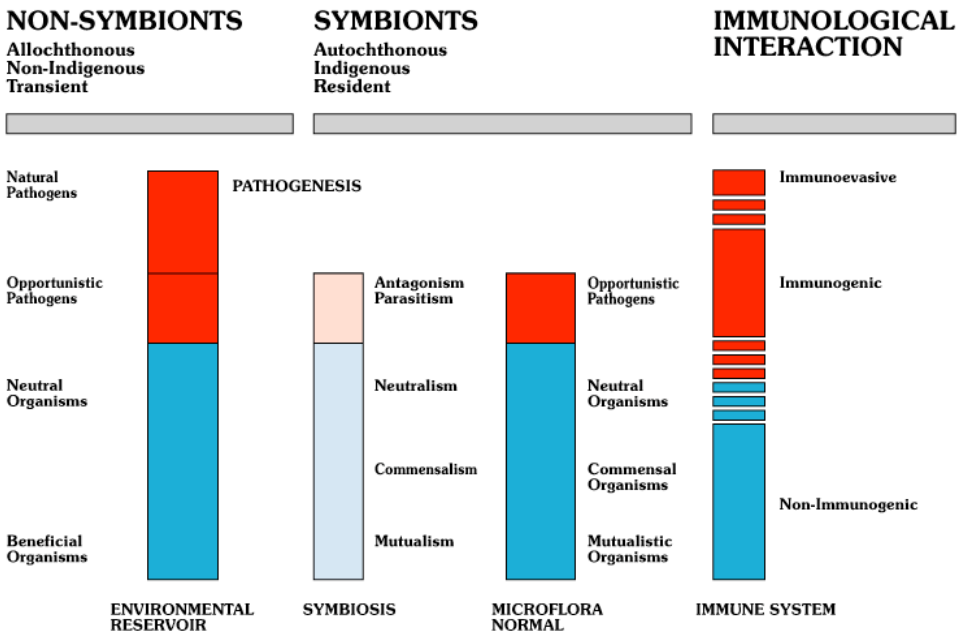

Figure 3: Microflora populations in the digestive tract (*Rusch*, 1989).

'symbiosis', but also to have it mean pretty much what de Bary intended." The definition of symbiosis covers a wide span of associations, including parasitism, neutralism, commensalism and mutualism. "The value of the concept resides in the widening of the concept of organism as structural unit to include heterogeneous systems as a "functional unit" or "functional field" (*Gregory*, 1951; *Rusch*, 1989).

This concept was transposed by an international study group to the consideration of microflora populations in the digestive tract (*Rusch*, 1989). The resulting classifications are represented in Figure 3. Completely in the sense of de Bary, man and microbes are understood as a symbiotic unity. The "normal" human microflora also contains opportunistic microorganisms with which humans live together in persistent and intimate partnership, microbes, normally under the control of neutral, commensal and mutualistic microorganisms and therefore cannot develop their pathogenic properties. Figure 4 is constructed on the concepts shown in Figure 3 and illustrates possible therapeutic consequences (*Rusch*, 1989).

MICROBIAL THERAPY

Definition

Microbial Therapy is defined as the oral and parenteral use of different live and/or killed microbes and/or their constituents for therapeutic purposes. The essential elements of Microbial Therapy are microbial preparations and autovaccines. About 35 microbial preparations are available in Germany (*Kolb* and *Maaß*, 1991). In spite of the tremendous variety of species of symbiotic microbes, essentially only four species are the basis for most preparations. *Enterococcus faecalis*, *Escherichia coli*,

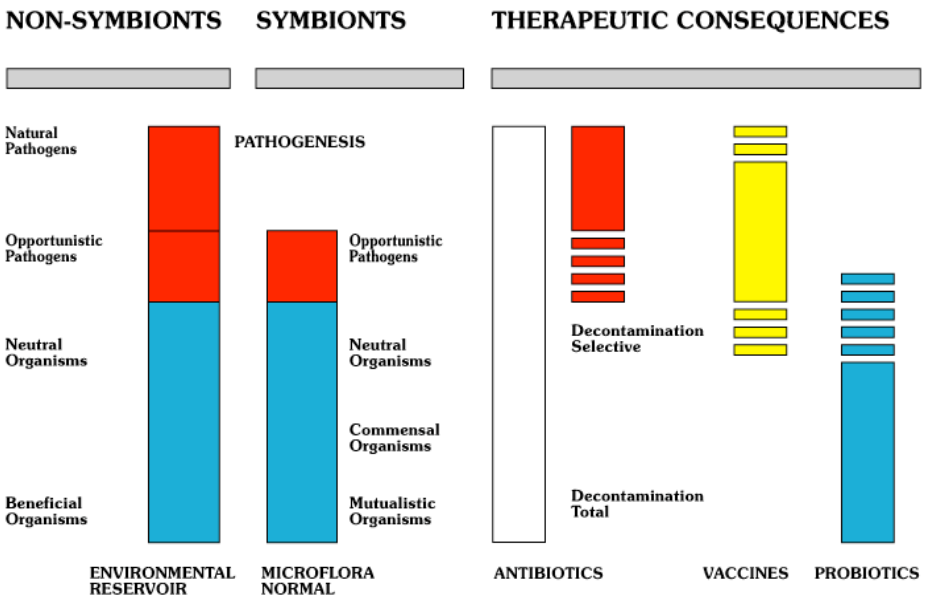

Figure 4: Therapeutic consequences in relation to the classification of symbionts and non-symbionts. (*Rusch*, 1989).

Bifidobacterium bifidum and lactobacilli. For non-symbionts, *Saccharomyces boulardii* are used.

In the context of therapy with microbial preparations, there exist terms such as "symbiosis treatment" (with several widely different interpretations), "therapy of dysbiosis", "microflora regulation", "microecological therapy" and similar connotations and terms reflecting obsolete views or which have little scientific background and are controversial. Microbial Therapy is to be clearly distinguished from these various therapy concepts (*Kolb* and *Maaß*, 1991).

Mode of action

Already in the seventies, the Medical Association for Microbial Therapy was confronted with the mode of action of microbial preparations and autovaccines. At the time, possible parameters for the determination of the efficacy were: flora modulation, modulation of metabolic activities of the host and immunomodulation. Because of major diagnostic problems and lacking clinical relevance, the flora modulation parameter was discarded. For similar reasons the metabolism parameter faded into the background.

Experimental and clinical studies

During the second half of the 1970's, the rapid advance in the knowledge of immunology led to a number of experimental studies in the USA to establish the foundations for later clinical tests. In the 1980's, several clinical trials were conducted on this basis. Two of these studies may serve as examples. On 106 patients with chronically recurrent disease of the upper respiratory tract, a randomised double-blind placebo study was conducted with the oral administration of an enterococcal preparation. Details of this study were published (*Kalinski*, 1986, 1987; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987). The clinical results of this study are illustrated in Figure 5. After a treatment of three months, a significant

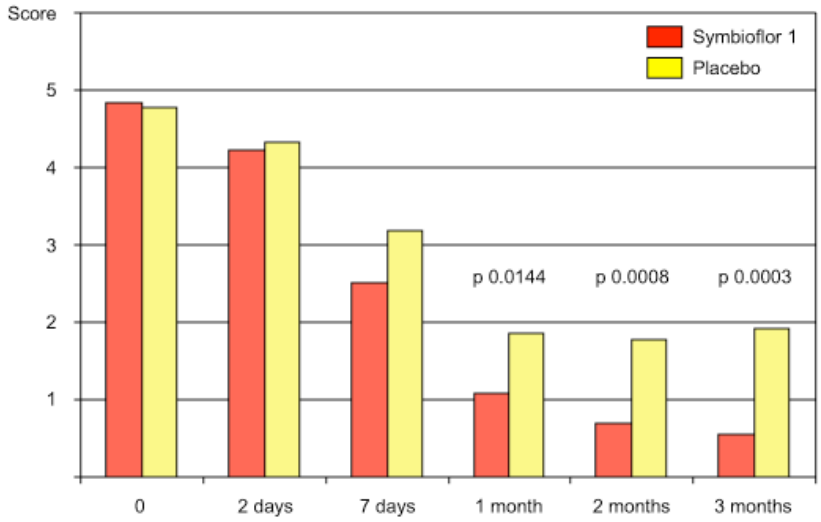

Figure 5: Clinical results (summarised in a score of clinical findings: the higher the score, the worse the findings) of a study with *Enterococcus faecalis* preparations (*Kalinski*, 1986, 1987; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987).

difference between the verum and the placebo group was established. From the clinical point of view, the outcome of the study was very satisfying. Regarding the possible influence on immunological parameters, the evidence of corresponding tests were less satisfactory. Although the determination of serum immunoglobulins showed significant differences between the beginning

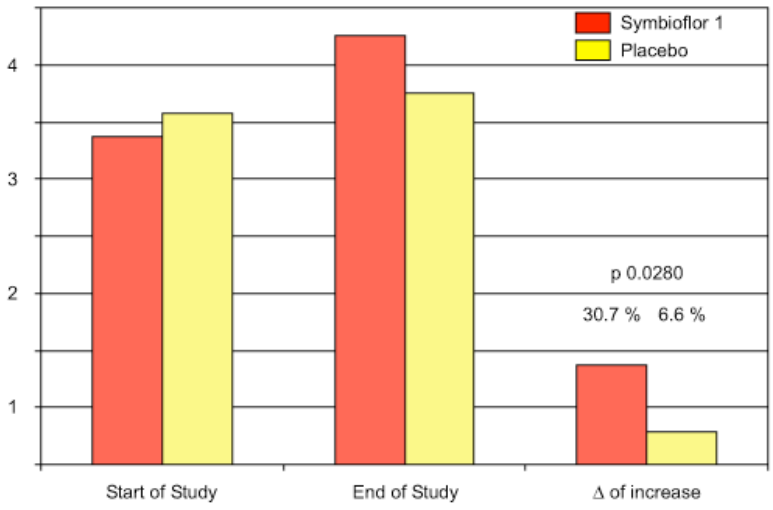

Figure 6: Evaluation of cellular immunity (Multitest Merieux) in the context of a clinical study with *E. faecalis* (see Figure 5). The increase of reactivity is significantly higher in the verum group than in the placebo group (*Kalinski*, 1986, 1987; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987).

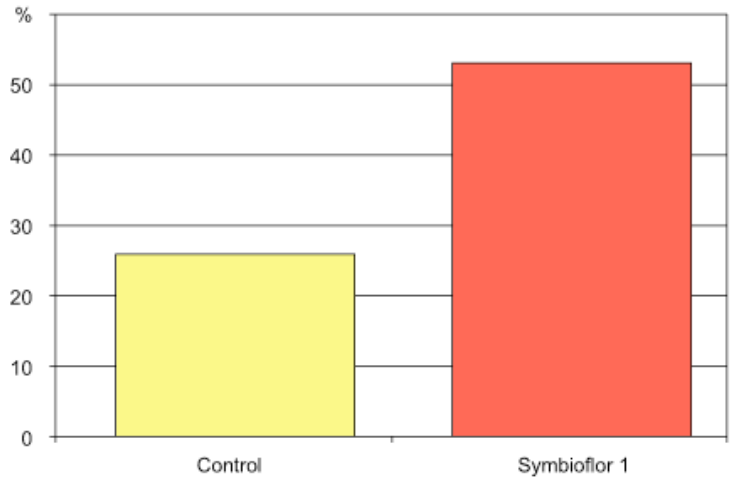

Figure 7: Determination of the granulocyte activity of C57Bl mice after three weeks of oral administration of an enterococcal preparation: Demonstration of the rate of intracellular killing of *Staphylococcus aureus*. The observed difference between the group of treated and untreated animals is significant (*Kalinski*, 1986, 1987; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987).

and end in the treated group in some instances, these changes are within the limits of the immunological norm. However, in cellular immunity it was possible to demonstrate a remarkable difference between the two groups (Figure 6). With the help of the Mérieux Multitest skin test it was found that the increment of the increase in cellular reactivity was significantly

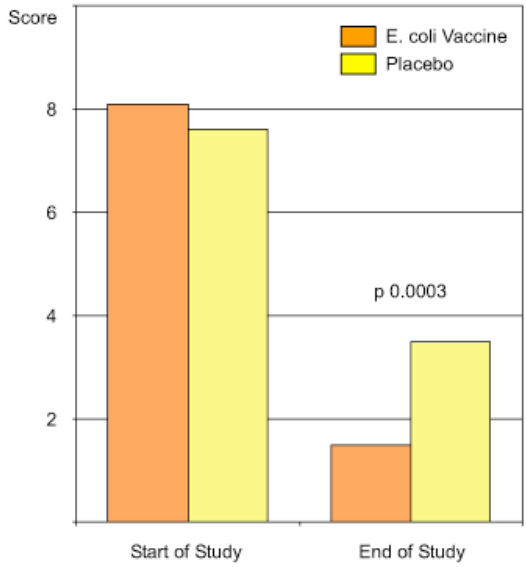

Figure 8: Clinical results (summary of clinical findings in a score, the higher the values, the worse the findings) of a study with *E. coli* vaccine for the treatment of chronic sinusitis (*Kalinski*, 1986, 1987; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987).

Table 1: Randomised double-blind placebo-controlled clinical trials with microbial preparations (statistical analysis according to FDA-criteria).

Enterococcus faecalis* - live, oral					**Publication**
01	Tonsillitis	n = 160	significant	1986	published
02	Upper respiratory tract infections	n = 106	significant	1987	published
03	Immune status	n = 42	significant	1991	in preparation
04	Bronchitis*****	n = 140	significant	1991	in preparation
05	Sinusitis*****	n = 140	significant	1991	in preparation
06	Tonsillitis*****	n = 200	open	1991	in evaluation
07	Tonsillitis*****	n = 200	open	1991	in evaluation
08	Acute non-specific enteritis	n = 774	open	1992	in evaluation
***E. coli* and *E. faecalis* - killed, oral** **					
09	Irritable colon	n = 299	significant	1989	published
E. coli* - live, oral **					
10	Irritable colon	n = 299	significant	1989	published
E. coli* - killed, parenteral* **					
11	Sinusitis	n = 114	significant	1991	published
11 (total number of studies)		*2,334 (total number of patients)*			

* Symbioflor 1.
** Pro-Symbioflor.
*** Symbioflor 2.
**** Symbioflor-Antigen.
***** Determination of the PBMC-activity under evaluation.
Publications: see *Kalinski* (1986, 1987), *Rosenkranz* and *Grundmann* (1994), *Rusch* et al. (1986), and *Rusch* (1987) for quotations.

higher under verum than with placebo. In the middle of the 1980's, the routine clinical immunological diagnostics were still very limited, so clear statements could only be obtained from studies on animals. An example of this is shown in Figure 7.

The second element of Microbial Therapy are autovaccines. As placebo-controlled studies cannot be conducted with autovaccines, microorganisms, as they are usually employed by the Herborn group for the production of autovaccines, were pooled and used as hetero-vaccines. These *E. coli*-vaccines were used in a randomised double-blind placebo study on 114 patients with chronic sinusitis. The results are shown in Figure 8. Clinically significant results were achieved but with the techniques of that time did not lead to relevant immunological evidence. With the same vaccines, studies on animals were also conducted with significant results (*Kalinski*, 1986, 1987; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987). It may seem paradoxical that an immunological signal from the colon (*Escherichia coli*!) is used in the treatment of an indication as sinusitis located far away from the colon - the mucosal immune system is the link. Details of this are given below.

This paragraph contains an overview of the so far conducted clinical tests of the Herborn study group. The overview

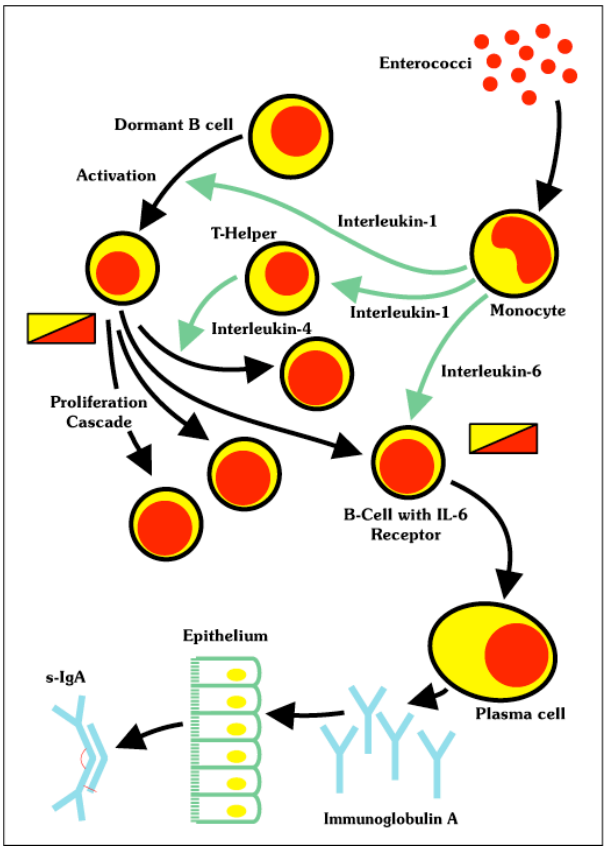

Figure 9: Schematic representation of a hypothesis on the efficacy of microbial preparations (*E. faecalis*). Enterococci stimulate monocytes/macrophages to produce interleukin-1ß and interleukin-6. This stimulates the activation and proliferation of B-cells with subsequent IgA and s-IgA production (*Gemsa* et al., 1991; *Kalinski*, 1986, 1987; *Kolb* and *Maaß*, 1991; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987).

is given in Table 1. Based on the experience of studies conducted in the second half of the 1980's, several further studies were performed at the beginning of the 1990's. In eleven randomised double-blind and placebo-controlled studies, 234 patients were involved. All the studies have been concluded: three of them are still in the evaluation phase. Eight of the studies yielded clinically significant results. The statistical analysis was carried out according to the criteria of the US Food and Drug Organisation. With the results of these studies, the criteria for the determination of efficacy have been observed. The following remarks concern the immunomodulatory aspects.

IMMUNOMODULATION

Hypothesis

In a series of preliminary trials with lymphocyte cultures of several volunteer groups, it was found that cytokine activities can be stimulated by microbial preparations. In more recent clinical trials, based on the results of these examinations, lymphocyte cultures of the

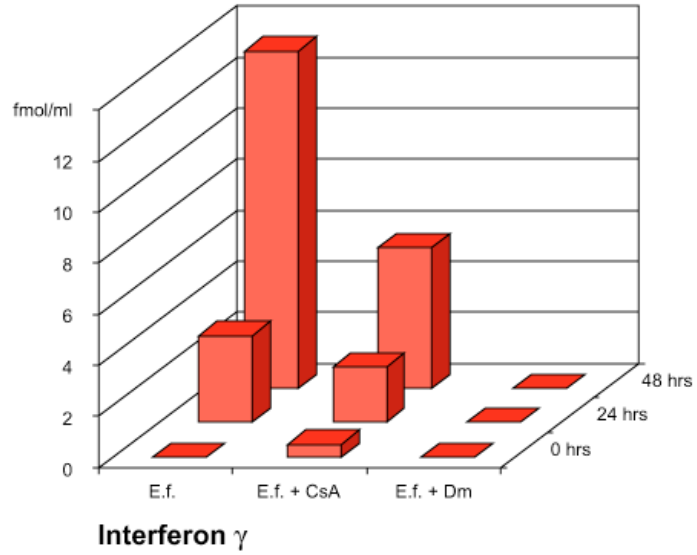

Figure 10: Stimulation of the release of gamma-interferon with *E. faecalis*. The release is inhibited by cyclosporin A (CsA) and dexamethasone (Dm) under simultaneous addition of *E. faecalis* (E.f.) to the incubation medium (*Rosenkranz* and *Grundmann*, 1994).

patients involved were also produced and the cytokine activities determined. The results are currently the subject of statistical analysis (see Table 1). The results of the preliminary trials led to the formulation of a hypothesis on the efficacy of microbial preparations.

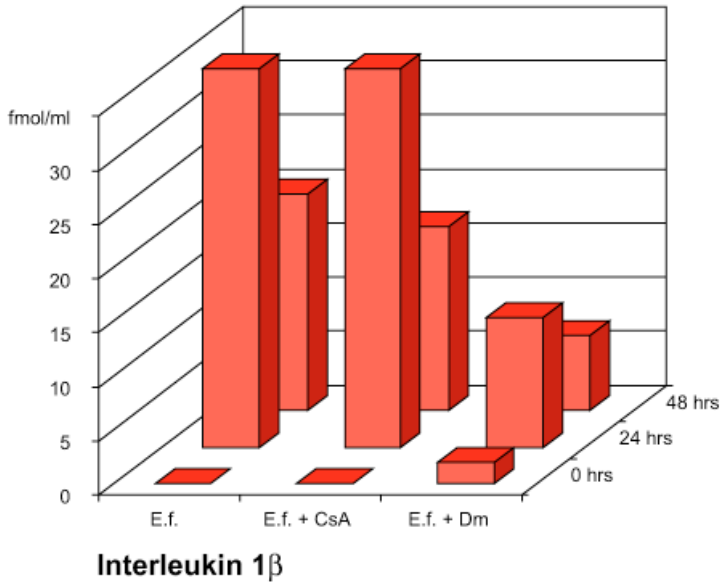

Figure 11: Release of interleukin-1ß due to stimulation with *E. faecalis* (E.f.). The release is inhibited by dexamethasone but not by cyclosporin A (*Rosenkranz* and *Grundmann*, 1994).

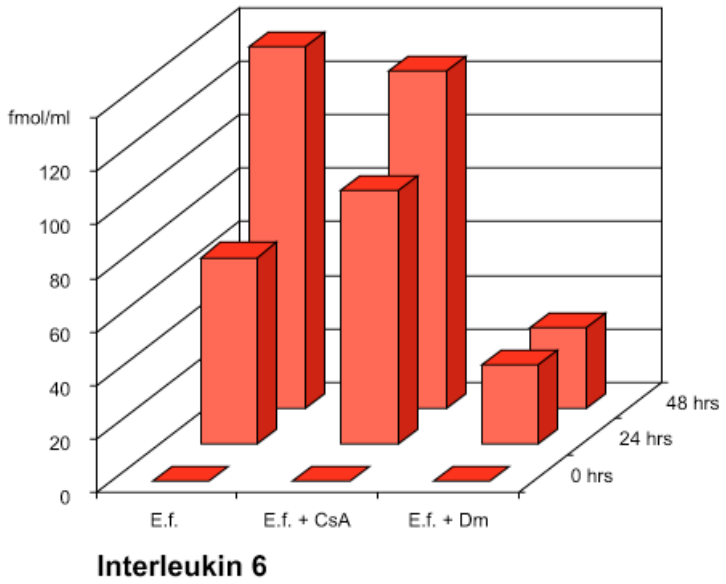

Figure 12: Release of interleukin-6 due to stimulation with *E. faecalis* (E.f.). The release is influenced by cyclosporin A (CsA) and inhibited by dexamethasone (Dm) (*Rosenkranz* and *Grundmann*, 1994).

Data

The hypothesis represented in Figure 9 was tested in two trial approaches. On the one hand, the effect of *Enterococcus faecalis* on the formation of cytokine was investigated in *in vitro* experiments with human peripheral, mononuclear blood cells; on the other, it was studied with mini-pigs whether *Enterococcus* leads to the stimulation of immunocompetent cells after oral administration and whether the mucosal system is stimulated. The *in vitro* tests on cell cultures with mononuclear peripheral blood cells of healthy volunteers prove the following effect of *Enterococcus faecalis*:

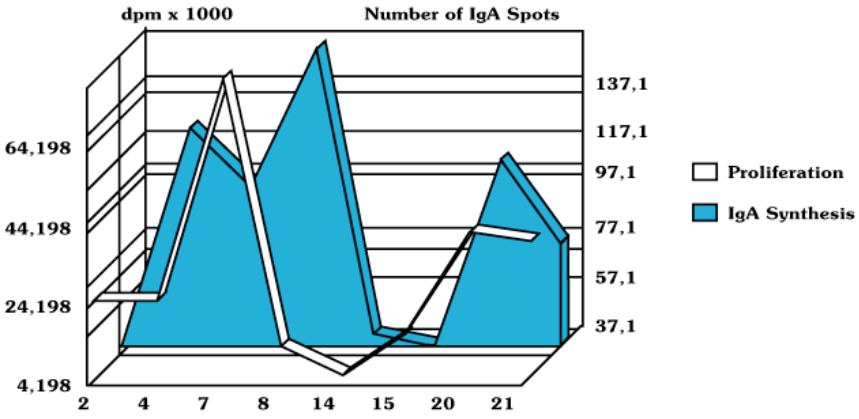

Figure 13: Comparison of the proliferation rate of immune cells in mini-pigs orally administered with *E. faecalis* and the IgA production in the Elispot-assay (*Ottendorfer*, 1994).

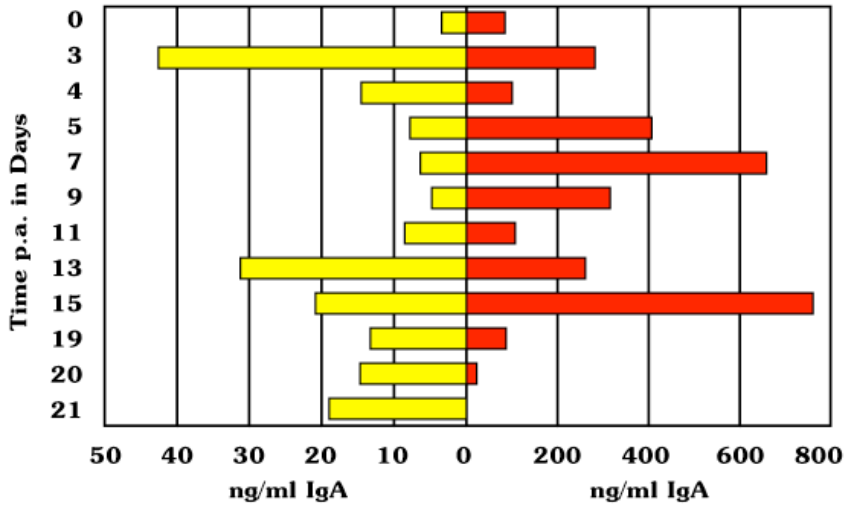

Figure 14: Concentration of total IgA in the saliva of mini-pigs during the administration of *E. faecalis*. The highest and lowest values are presented (*Ottendorfer*, 1994).

1. The release of interleukin-1ß and interleukin-6 is stimulated directly and dose-dependently. The release can be inhibited with dexamethasone; with cyclosporin A no inhibition is achieved.
2. Gamma interferon is liberated directly and dose-dependently. The release can be inhibited with dexamethasone and cyclosporin A.
3. The phytohaemagglutinin-induced release of gamma-interferon and interleukin-2 is inhibited in a dose-dependent manner.

Steps 1 and 2 of the hypothesis illustrated in Figure 9 are proved by the results of these tests. The results of these studies are represented in Figures 10, 11 and 12 (*Kalinski*, 1986, 1987; *Ottendorfer*, 1994; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987).

In tests with mini-pigs, it was demonstrated that the oral administration of *Enterococcus faecalis* activates dormant B-cells leading to a prolifera-tion in the sense of a cascade reaction and eventually to an increase in the IgA synthesis (Figure 13). The stimulation of the activities of the mucosal immune system could be proved by the increase in secretory IgA's in the saliva of the mini-pigs (Figure 14). Steps 3 and 4 of the hypothesis illustrated in Figure 9 have been confirmed by the results of these tests (*Kalinski*, 1986, 1987; *Ottendorfer*, 1994; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987).

Network

The complex mechanism of the mucosal immune system where the immune system, the nervous system, the hormone system and metabolism are interlinked is stimulated differently by different immunological signals of the microflora. Data from lymphocyte cultures of healthy volunteers can again serve as an example. In these tests live enterococci, live *E. coli*, a mixture of killed *Enterococcus faecalis* and *E. coli* (for oral administration) and killed *E. coli* (for parenteral administration) were studied. The live bacterial preparations were capable of promoting the production of interleukin-1ß; the liberation of gamma-interferon was not stimulated. On the other hand, the production of

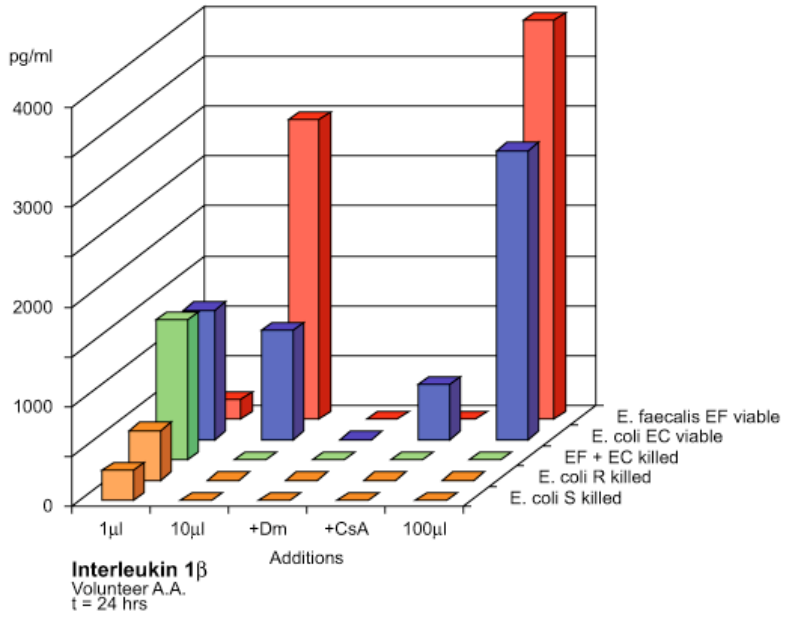

Figure 15: Influence of different bacterial preparations on the release of 1ß-interleukin in lymphocyte cultures of volunteers. Note the columns on the right (100 µl substance). The release is clearly promoted by live bacteria. The release is inhibited by the addition of dexamethasone (Dm) and cyclosporin A (CsA) (*Tarkkanen*, 1994).

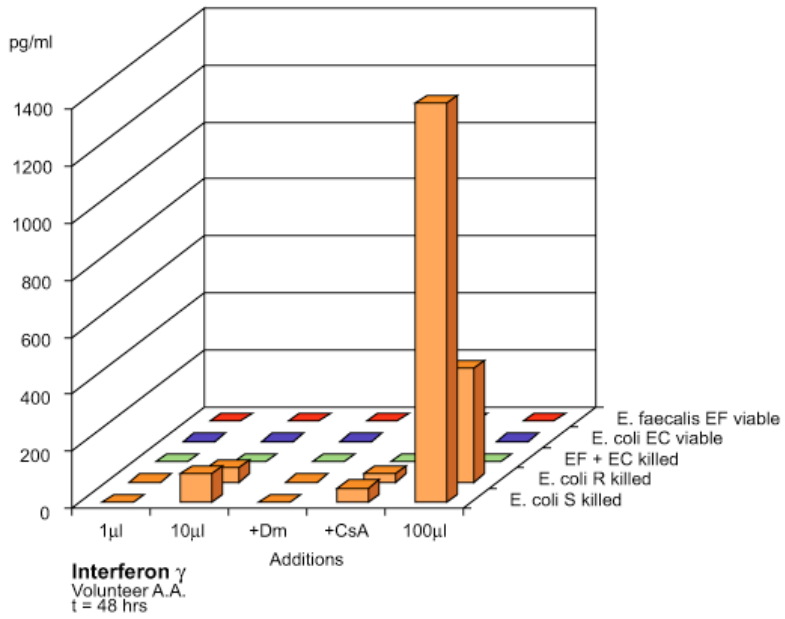

Figure 16: Liberation of gamma-interferon in lymphocyte cultures after stimulation with different bacterial preparations. The release of gamma-interferon is exclusively stimulated by killed *E. coli* and inhibited by the addition of dexamethasone (Dm) and cyxlosporin A (CsA) (*Tarkkanen*, 1994).

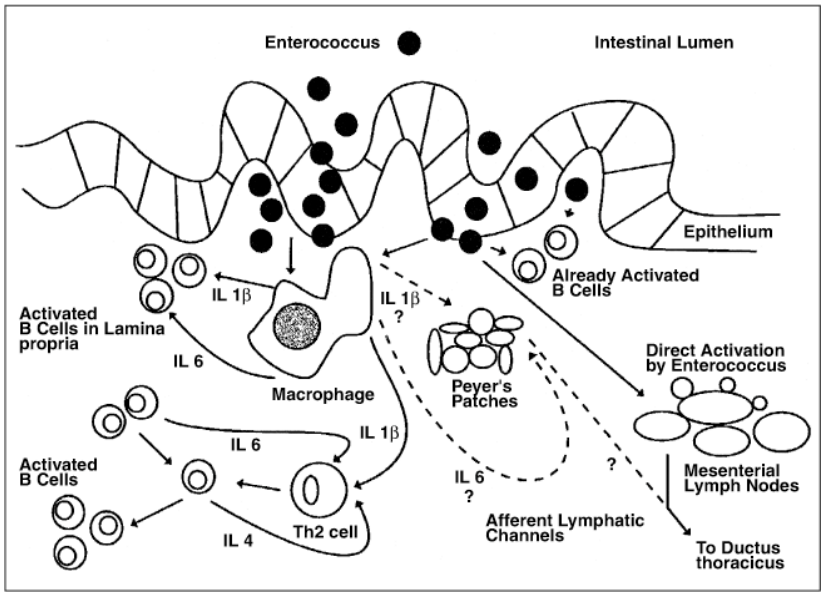

Figure 17: Schematic representation of the complex sequence in the stimulation of defence mechanisms of the mucosal immune system with *E. faecalis* demonstrated at the example of lymphatic tissue associated with the intestine (*Ottendorfer*, 1994).

gamma-interferon was stimulated by preparations of killed *E. coli* but not the release of interleukin-1ß. These data are set out in Figures 15 and 16. A further hypothesis, illustrated in Figure 17, is constructed on the basis of these data and the previously mentioned data illustrating the mode of action of microbial preparations based upon the example of the mucosal immune system associated with the intestine (*Jansen*, 1994; *Kalinski*, 1986, 1987; *Ottendorfer* 1994; *Panijel* and *Burkard*, 1993; *Rosenkranz* and *Grundmann*, 1994; *Rusch* et al., 1986; *Rusch*, 1987, 1989; *Schaffstein*, 1993; *Tarkkanen*, 1994).

Indications

Indications confirmed by controlled clinical trials are: immunomodulation (activation of endogenous resistance), colds, chronically recurrent infections of the respiratory tract, chronic sinusitis, inflammations in the mouth, nasal and pharyngeal cavity, middle ear, gastrointestinal disorders, irritable colon and urinary tract infections. Indications resulting from general experience are subject to controlled clinical trials: gastro-enteritis, enterocolitis, postantibiotic colitis, colitis ulcerosa, Crohn's disease, hepatopathies, cholecystopathies, skin diseases, allergic diseases and mycoses. All these indications concern areas of the human organism that are derived from the entoderm and cross-linked with the mucosal immune system. The wide spectrum of indications is explained by the mucosal immune system as a substrate (*Castro*, 1989; *Stern*, 1992). The inhibition of the phytohaem-agglutinin-stimulated clearance of gamma-interferon and interleukin-2 by *Enterococcus faecalis* in the *in vitro* lymphocyte cultures could become the pharmacological basis for further indications. The increased production of gamma-interferon and interleukin-2 under the action of the endotoxins of pathogenic Gram-negative bacteria could be reduced by *E. faecalis*. It is therefore feasible that *E. faecalis* could have some

importance in the treatment of hospitalism. This is indicated by the results of studies on lymphocyte cultures, animal experiments and human studies.

CONCLUSION

Microbial Therapy with microbial preparations and autovaccines is a therapeutic method based upon experimental and controlled clinical studies.

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