

## **RESULTS OF AN OPEN, NON-PLACEBO CONTROLLED PILOT STUDY INVESTIGATING THE IMMUNO-MODULATORY POTENTIAL OF AUTOVACCINE**

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### **SUMMARY**

Autovaccines, a fundamental concept within Microbiological Therapy, are prepared from autologous, human, non-pathogenic, „rough“ variants of *E. coli* derived from the stool flora of individuals according to a highly standardised procedure (SymbioVaccin, Germany). These autovaccines are mainly used to treat chronic inflammatory disorders associated with impaired immune reactions resistant to standard therapeutic treatments. Although immunomodulatory effects of outer membrane components or cell wall fragments of gram-negative bacteria on innate or adaptive immunity are widely accepted, mechanisms of actions of these autovaccines remained largely obscure, despite some recent publications about other autovaccine preparations of different origin. Therefore a pilot study was conducted with 78 outpatients from a physician in general practice. The patients suffered from variable disorders, ranging from recurrent respiratory infections to diffuse gastrointestinal complaints. To investigate whether the application of autovaccine affects non-specific and specific parameters of the immune system, the patients received their autologous bacteria parenterally in increasing doses. Before application and 4 to 6 weeks after application of autovaccine, blood samples of the patients were taken to investigate a range of immunological parameters such as acute phase proteins, serum antibodies and cytokines. The results revealed, that autovaccines were able to modulate significantly the release of three potent immunoregulatory cytokines but induces only slight changes in specific humoral immunity. From these results it may be concluded that the autovaccine mainly acts antigen non-specifically on the cytokine level rather than inducing a high level of specific antibodies against autovaccine. Further studies with more detailed kinetic measurements of cytokines will have to verify these results.

## INTRODUCTION

Autovaccines in the context of this paper are derived from human, non-pathogenic rough variants of *E. coli* isolated from the faecal flora of each patient. From the manufacturer of autovaccines (SymbioVaccin GmbH, Herborn, Germany), these bacteria were prepared according to a highly standardised procedure followed by heat-inactivation for 2h at 70°C (Zielinski et al., 1998). Autovaccines are used to treat chronic inflammatory disorders either associated with impaired resistance to infection or with hyperactivation of the immune system resistant to standard therapeutic strategies (Rusch, 1986). In fact, these heterogeneous indications underlying many sometimes unknown dysregulations of the immune system will require well-balanced immunoregulatory actions of autovaccines still unproved by relevant clinical observations up to now.

Although the application of autovaccines have a long traditional use going back to the beginning of the 20<sup>th</sup> century (Wright and Douglas, 1904), the underlying mechanisms of action are far from being clear. However, several recent reports described another form of autovaccine therapy inducing a specific immunisation, which has been shown for example by Zaluga (1998). This group examined immunological effects of an autovaccine preparation composed of *Propionibacterium acnes*, isolated from the skin of patients. These authors found a significant improvement in 47.6% of patients accompanied by functional changes in the immune system as for example a generation of specific antibodies against structural antigens of *P. acnes* (Zaluga, 1998). These observations were confirmed by others (Rubisz-Brzezinska et al., 1994) and agreed with reports demonstrating a therapeutic successful use of those

autovaccines in children with severe nasal sinusitis (Okrasinska-Cholewa, 1994) or purulent otitis media (Wilczynski et al., 1995). Therefore the present study sought to investigate the immunomodulating potential of autovaccine preparations of autologous origin to clarify, whether there will be similarities or differences in immunological effects compared to those other autovaccines described recently. Thus, to elucidate the influence of the autovaccine on the immune system, an outpatient (n=78) collective recruited by a physician in general practice was used for the following study to examine changes in immunological parameters. The patients received the autologous bacterial preparation derived from their own stool flora and immunological parameters were determined before application and 4 to 6 weeks after application of the autovaccines.

As this was only a preliminary pilot study no placebo control was included. Because it was not known, which immunological effects, if any, would be elicited by the autovaccine, a broad panel of investigations was performed. Patients' peripheral blood leukocytes (PBLs) were used to determine *ex vivo* cytokine release by specific enzyme immunoassays (ELISAs). Among the cytokine family, granulocyte-macrophage-colony stimulating factor (GM-CSF), interferon-gamma (IFN- $\gamma$ ), interleukin-1beta (IL-1 $\beta$ ), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumour-necrosis-factor alpha (TNF- $\alpha$ ) were examined. In addition, non-specific serum parameters characterising inflammatory processes such as neopterin, C-reactive protein, soluble interleukin-2 receptor (sIL-2R),  $\beta$ 2-microglobulin and interleukin-1 receptor antagonist (IL-1RA) were also deter-

**Table 1:** Demographic data of the participating patients

Number of patients	78
Patients available for two blood donations	60
Time span between sample donation	31 days (range 21-56) 25% percentile 28 days 75 % percentile 35 days
Female/male ratio	55/23 total 41/19 (second blood donation)
Age, median (range in parentheses)	36.5 (16-74) 25% percentile, 30 years 75% percentile, 44 years
Number of patients with previous history for autovaccine therapy	41/60 (68.9%)

mined. Finally, serum samples of patients tested before and after autovaccine therapy were used to detect a potential increase of specific antibodies of the

IgG, IgM or IgA isotype against antigens of the autovaccines, using an ELISA technique.

## MATERIALS AND METHODS

### Recruitment of patients

The study population comprised 78 outpatients from a primary care physician (P.N.) at Berlin-Charlottenburg and were recruited for the present study during a time span between end of 1995 and beginning of 1997. Patients' informed consent was obtained to draw two blood samples. The demographic data of the patients are shown in Table 1. Further details were recently published (Rusch et al., 2001).

### Test substances

The autovaccines used for application to the patients were prepared by the manufacturer from the stool flora of each patient. For the stimulation of PBLs from each individual donor, the corresponding autologous autovaccine preparation was used at 6 different concentrations ( $3 \times 10^2$  –  $3 \times 10^6$  bacteria/ml). A second control consisted of a polyclonal stimulus. This lectin mixture contained pokeweed mitogen (PWM), phytohaemagglutinin A (PHA) and concanavalin A each at a concentration of 1

ng/ml (all purchased from Seromed, Berlin, Germany). Lipopolysaccharide (1 ng/ml) derived from *E. coli* 055:B5 was obtained from Sigma/Aldrich Chemicals, Munich, Germany.

### Preparation of peripheral blood leukocytes from the patients

For the investigation of the immunological effects of autovaccines, peripheral blood leukocytes (PBLs) of the patients were prepared as described recently (Rusch et al., 2001). Briefly, blood was taken immediately before application of autovaccine and 4 to 6 weeks later. For each patient, only a limited number of blood could be drawn, usually 30 ml, of which all cytokine determinations and preparation of serum or plasma had to be performed. After separation of cells by density centrifugation over Ficoll-Hypaque (Pharmacia fine chemicals, Freiburg, Germany) and several washing steps; the cell number was adjusted to  $1 \times 10^6$  cells/ml with cell culture medium.

### ***Ex vivo* treatment of patients PBLs with the test substances**

To further investigate the role of the respective autologous bacteria used for the treatment of the patients, six different concentrations of each autovaccine were used for the additional *ex vivo* stimulation of the patients PBLs. The final concentration of autovaccine in the cell cultures were  $3 \times 10^2$ ,  $3 \times 10^3$ ,  $3 \times 10^4$ ,  $3 \times 10^5$ ,  $1.5 \times 10^6$  and  $3 \times 10^6$  bacteria/ml, together with  $1 \times 10^6$ /ml PBLs. A polyclonal stimulus was also used, to determine the level of immunological reactivity of the patients' PBLs under these experimental conditions. Negative controls included medium treated cell cultures and all experiments were performed in duplicate wherever possible, using 24-well cell culture plates (Nunc, Wiesbaden, Germany). The incubation period of the cultures ( $37^\circ\text{C}$ , 5%  $\text{CO}_2$ ) started with the addition of the PBLs from the patients. Cells were incubated over a period of 6h for the determination of TNF- $\alpha$ . IL-1 $\beta$ , IL-2, IL-4 and GM-CSF were measured after 24 h; IFN- $\gamma$ , IL-6 and IL-10 were measured after 48 h of incubation. To the indicated time points, cell culture supernatants were harvested by centrifugation (1200 rpm, 10 min.,  $4^\circ\text{C}$ ). After aliquotation, supernatants were stored at  $-80^\circ\text{C}$  (4 weeks at least) before the determination of all cytokines with specific sandwich ELISA tests.

### **Preparation of serum for the determination of specific antibodies and markers of the acute phase response**

Serum was prepared from whole blood after complete coagulation at room temperature for 3h according to *Rusch et al.* (2001). Aliquots were stored frozen at  $-25^\circ\text{C}$  until determination of acute-phase markers and specific antibodies.  $\beta$ 2-microglobulin and neopterin (IBL diagnostics, Hamburg,

Germany), IL-1RA (R&D Systems, Wiesbaden, Germany) and sIL-2R (Biosource, Ratingen, Germany) were all determined by specific ELISA tests. Because the sensitivity of the commercially available ELISA test for CRP was too low, CRP was determined with an ELISA developed by Affina Immunotechnik GmbH (sensitivity of  $< 0.03$   $\mu\text{g/ml}$  CRP).

### **ELISA procedures: Cytokines**

The details of the ELISA procedures were described recently by *Rusch et al.* (2001). All cytokines were determined with specific Sandwich ELISA-tests, using commercially available antisera. For coating of the plates the following antisera were used: Murine anti-human IL-1 $\beta$  ( $2\mu\text{g/ml}$ , Genzyme, Munich, Germany), rat anti-human IL-2 ( $2\mu\text{g/ml}$ , PharMingen, Hamburg, Germany), murine anti-human IL-4 ( $1\mu\text{g/ml}$ , PharMingen, Hamburg, Germany), murine anti-human IL-6 ( $1\mu\text{g/ml}$ , Boehringer Mannheim, Germany), rat anti-human IL-10 ( $1\mu\text{g/ml}$ , PharMingen, Hamburg, Germany), goat anti-human IFN- $\gamma$  (kindly supplied by Prof. Dr. Noll / MDC,  $5.2\mu\text{g/ml}$ ), murine anti-human-TNF- $\alpha$  ( $1\mu\text{g/ml}$ , PharMingen, Hamburg, Germany) and rat anti-human GM-CSF ( $1\mu\text{g/ml}$ , PharMingen Hamburg, Germany).

The appropriately diluted standards and samples, both diluted with complete cell culture medium as follows: the IL-2, IL-4, IL-10 and GM-CSF standards were purchased from PharMingen (Hamburg, Germany), whereas IL-6 and IL-1 $\beta$  were supplied by Boehringer Mannheim (Germany). The TNF- $\alpha$  standard was supplied by Calbiochem (Heidelberg, Germany) and the IFN- $\gamma$  standard was kindly supplied by Prof. Cheperanov (Moscow, GUS). After incubation for 1 h at RT and three to four washes (each with 200  $\mu\text{l}$  PBS/0.05% Tween 20) the second anti-

**Table 2:** Acute phase response markers in 39 serum/plasma samples available for testing before and after autovaccine application. The data are presented as median and range

Acute-phase protein	Before autovaccine application	4-6 weeks after autovaccine application	p-value
CRP (µg/ml)	0.55 (0.05-6.62)	0.41 (0.03-5.42)	0.042*
SIL-2R (pg/ml)	87.4 (28.5-1535.0)	79.2 (35.8-1737.2)	0.187*
β <sub>2</sub> -Microglobulin (mg/l)	0.84 (0.66-1.54)	0.85 (0.67-1.18)	0.807**
IL-1RA (pg/ml)	332.9 (177.7-726.6)	311.4 (127.1-1021.1)	0.572*
Neopterin (nM/L)	4.7 (2.6–10.4)	4.5 (2.8-9.2)	0.728*

\*p-value calculated with the Wilcoxon-Test, Median

\*\* p-value, calculated with the paired t-test, Mean

bodies were added as described recently, followed by the washing steps (Rusch et al., 2001). Thereafter, the substrate solution (Tetramethylbenzidine, TMB, Fluka via Sigma/Aldrich chemicals, Munich, Germany) was added (100 µl/well) and the plates were incubated at RT in the dark. The reaction was stopped with 50 µl 2N H<sub>2</sub>SO<sub>4</sub> (Merck, Darmstadt, Germany). The optical density was measured at 450 nm in a microplate reader (Titertek Multiscan®, Flow Laboratories, Meckenheim, Germany).

#### Acute phase proteins and determination of specific antibodies and total immunoglobulin

The determination of CRP in patients serum/plasma samples and the determination of specific antibodies was per-

formed as described recently (Rusch et al., 2001).

#### Statistics

The calculation of the significance of the data focused generally on an ANOVA principle including the comparison of the mean values ± standard deviation (S.D.) for cytokines for all 60 donors. Unpaired and paired t-test were used to compare cytokine values, acute phase markers and immunoglobulin differences between the different patients in the case of normal distribution of the data. Not normally distributed data were calculated with the Rank Sum test according to Wilcoxon and to Mann-Whitney. P-values between 0.001 and 0.05 were considered to be statistically significant.

## RESULTS

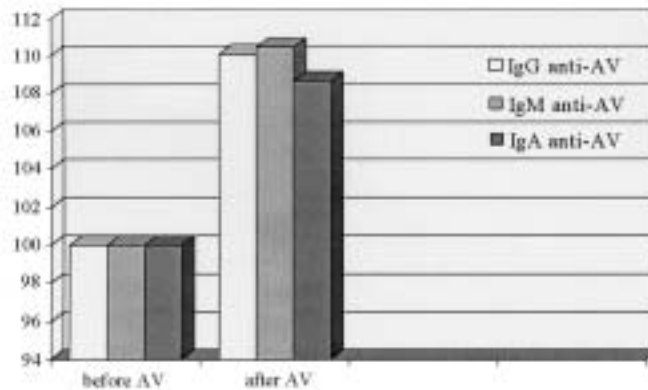
#### Acute phase proteins

At the end of the study, plasma or serum samples from 39 patients were available for measurement of non-specific serum markers of inflammation (Table 2). No statistically significant differences between values obtained before autovaccine application and 4 to 6 weeks after therapy with autovaccine were seen. However, the median value

for CRP was reduced about 23.5% compared to the concentration found before application of autovaccine. This reduction in CRP reached borderline significance (p= 0.042).

#### Specific antibodies and total immunoglobulin

The determination of total immunoglobulin demonstrated that an applica-



**Figure 1:** The percent values of autovaccine-specific immunoglobulins determined from 39 pairs of serum/plasma samples (taken the pretreatment values as 100%). Total antibody levels were measured by laser nephelometry. They did not change above pre-treatment values (data not shown). Autovaccine-specific immunoglobulins of the different isotypes were detected with an ELISA as described in materials and methods. Only at least 10% increase in specific immunoglobulins was seen.

tion of autovaccines during the observed time span of 4 to 6 weeks did not influence the total levels of the immunoglobulins IgG, IgM or IgA in plasma samples derived from the patients (the identical 39 pairs of samples as shown in Table 1, data not shown). There was no difference in antibody levels compared to pre-treatment. Only a very slight increase of specific antibodies against autovaccine of at least 10% was detected for the isotypes IgG, IgM and IgA. This is shown in Figure 1.

#### **Influence of autovaccine on *ex vivo* cytokine release by patients PBLs**

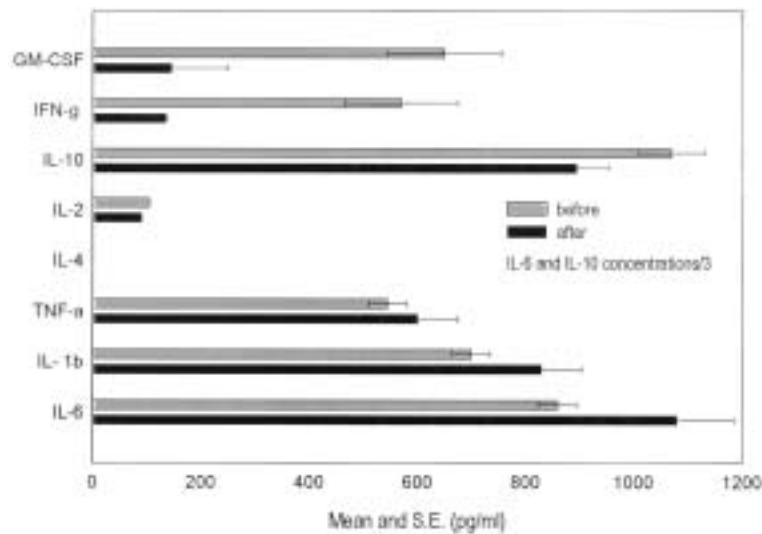
The comparative analysis of all cytokine mean values ( $\pm$ S.D.) for differences between the first examination (U1 = before autovaccine treatment) and the second time point (U2 = 4 to 6 weeks

after application of autovaccine) revealed the following results: Figure 2 summarises the cytokine values for 60 patients under therapy with autovaccine after additional *ex vivo* stimulation of their PBLs with the respective autologous bacteria. The *ex vivo* stimulation of patients' PBLs resulted in a significant decrease of the cytokines GM-CSF ( $p = 0.004$ ) and IFN- $\gamma$  ( $p = 0.007$ ). In contrast, the cytokine IL-1 $\beta$  was significantly increased at U2 ( $p = 0.04$ ). Although IL-6 showed a tendency to increase after application of autovaccine, this enhancement reached no statistical significance ( $p = 0.058$ ). All other cytokines measured remained unchanged under application of autovaccine, when the mean values of U1 and U2 were compared, whereby IL-4 remained below the detection level (see Figure 2).

## **DISCUSSION**

This open pilot study with 60 patients demonstrated for the first time, that autovaccines prepared from autolo-

gous bacteria according to a standardised procedure are able to modulate the human immune system preferentially in



**Figure 2:** The cytokine mean values  $\pm$  S.E. in pg/ml measured from cell culture supernatants from peripheral leukocyte cultures of 60 patients, before autovaccine application and 4 to 6 weeks after autovaccine application. The PBLs from each patient were treated *ex vivo* with the respective autovaccine and cytokines in the culture supernatants were determined as described in materials and methods. Statistically significant differences in cytokine production by patients' PBLs were observed with IFN- $\gamma$  ( $p < 0.007$ ) and GM-CSF ( $p < 0.004$ ) as both mediators were significantly diminished after application of autovaccines. In contrast, the cytokine IL-1 $\beta$  was found to be enhanced ( $p = 0.04$ ). Please note that the concentrations of IL-6 and IL-10 had to be divided by the factor 3 for reasons of better graphical illustration.

an antigen-non-specific way, affecting the key „communication“ molecules within in the immune response, the cytokines. Based on the literature concerning the immunological effects of other autovaccine preparations (Zaluga, 1998; Rubisz-Brzezinska et al., 1994; Okrasinska-Cholewa, 1994; Wilczynski et al., 1995), differences as well as similarities emerge between those autovaccines and the bacterial preparation tested in this study. Some of the bacterial preparations described elsewhere were for example obviously capable of inducing high levels of specific antibodies whereas the autovaccines examined herein failed to do so. On the other hand, thy cytokine profiles of patients PBLs were profoundly affected. The question is what causes these immunological differences between different preparations of autovaccines?

One explanation might come from the different origin of the bacterial strains used for the preparation of both types of autovaccines. Whereas for example Zaluga et al. (1998) used skin-derived autologous strains of *Propionibacteriaceae*, the autovaccines used in our trial were derived from the autologous faecal flora of the individual patients. The gastrointestinal flora nowadays is accepted as playing a major role not only in the pathogenesis of inflammatory gut diseases but also in the modulation of physiological reactions of the immune system (Sartor, 1997). Present knowledge on functions of the indigenous microflora of the gut includes profound effects on anatomical, physiological and immunological development of the host (Berg, 1996). Thus, it may not be a surprise that the individual faecal autologous bacteria of each

patient failed to induce a dramatic increase in specific antibodies above normal levels of pre-existing „natural“ antibodies.

However, even the small rise of Autovaccine-specific immunoglobulin isotypes opens the possibility for the interaction of different types of Fc-receptors with complexed gut-derived autologous *E. coli* antigens, which could initiate potent inflammatory pathways when not handled appropriately by the immune system. Generally receptors for the Fc domain of IgG (Fc $\gamma$ R) represent a crucial link between the humoral and cell mediated immune responses. The ligation of these receptors can trigger a variety of immune effector functions (Van der Winkel and Caperl, 1993; Daeron, 1997; Ravetch and Bolland, 2001). So far the structural diversity of the different classes of FcR and their variable capabilities to deliver activating (Fc $\gamma$ RI, Fc $\gamma$ RIIIa, Fc $\gamma$ RIIIa) as well as inhibitory signals (Fc $\gamma$ RIIb), as described by *Dijstelboem et al.* (2001), depends on the presence of an immunoreceptor-Tyrosine-based Activation Motif (ITAM) or an immunoreceptor Tyrosine-based Inhibitory Motif respectively. Therefore it is reasonable to assume that the control of cellular activities of both the innate and the adaptive immune system provides an efficient means by which FcR mediates immunoregulatory activities in controlling non-specific and even specific inflammation. This might have consequences for ongoing or memory T-cell and B-cell responses in terms of a down regulation of excessive activation. For example the inhibitory FcR $\gamma$ II was described to set thresholds for B-cell activation upon cross-linking with surface Ig, a mechanism whereby immune complexes can suppress the production of antibodies. In view of the inflammatory potential of non-cleared immune complexes this observation suggest for

a profound role of FcR mediated immunoregulatory processes and constitutes an ideal link for the autovaccine to control innate and adaptive immunity.

In view of the outstanding role of the gastrointestinal immune system and the autologous flora in maintaining a certain state of tolerance against harmless luminal and food antigens but inducing an active immune response against infectious agents the tight control of peripheral immune responses should not be underestimated. With regard to cellular activation this recently demonstrated clearly by the experimental work of *Duchman et al.* (1995), showing tolerance to the gastrointestinal microbial flora but an enhanced peripheral immune response once tolerance was broken and conditions of impaired mucosal barrier. The report of *Kimura et al.* (1997) agreed very well with these observations. A large body of evidence derived from a vast amount of experimental animal data lead to the suggestion, that the gastrointestinal flora may also participate in the generation of mucosal inflammation (*Elson et al.*, 1995; *Strober and Kelsall*, 1998), so that a tight control of immunoregulatory circuits (*Strober et al.*, 1997) controlling the immune responses in the gut towards potential harmful infectious or harmless dietary antigens are of utmost importance.

In view of the cytokine profiles measured with patients PBLs in this trial, IL-1 $\beta$ , IL-6 and IFN- $\gamma$  were reported to exert profound pro- as well as anti-inflammatory and immunoregulatory activities within the immune response (*Billau*, 1996; *Borish and Rosenwasser*, 1996; *Barton*, 1997; *Murphy et al.*, 2000). Notably, in view of the pattern of cytokines influenced by the autovaccine treatment of patients it seems noteworthy to mention that in our study classical „pro-inflammatory“ cytokines, normally readily released in a



co-ordinated manner upon contact with bacteria and immune cells, such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6, seem not to be upregulated simultaneously. It is generally accepted that in gram negative bacteria the Lipid A portion of LPS is the main component in stimulating these cytokines (Brandenburg et al., 1996). Our observation of immunological differences between the autovaccines described elsewhere in the literature and the autovaccine tested in this study, the latter stimulating rather the release of cytokines than inducing a specific antibody production, might be a hint for structural differences between those bacterial preparations and our autovaccine. Due to the amphiphilic nature of the LPS complex important structure-function relationships of endotoxines or the free Lipid A molecule exist (Schromm et al., 2000). Thus it may be conceivable that the *E. coli* strains used for the autovaccine preparation, representing particularly „rough“ variants, may possess structural modifications of the Lipid A-core-polysaccharide complex leading to distinct biological responses of human leukocytes. Two recent studies with the application of purified endotoxines of normal *E. coli* to

human volunteers showed only partial agreement with the cytokine profile measured with the autovaccines in our study (Zimmer et al., 1996; Lauw et al., 2000). Both reports described a marked downregulation of TNF- $\alpha$ , IL-2 and Interferon- $\gamma$  shortly after low-dose intravenous endotoxin administration to healthy human volunteers. On the other hand, a concomitant short rise in IL-4 and IL-10 was observed. These observations led the authors to suppose a down regulation of cell mediated immunity. These reports may support the idea of structural differences between highly purified *E. coli* LPS preparations and the autovaccine used herein. However, taking into account the broad time span for measurement of *ex vivo* cytokine release of patients' PBLs, which was due to the preliminary character of the present study and the limited amount of blood available, differences in cytokine profiles after more detailed kinetic studies cannot be excluded. Further clinical examinations are needed to confirm these preliminary results and most importantly to obtain more information on the immunomodulating role of this highly interesting bacterial preparation.

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## LITERATURE

- Barton, B.E.: Molecule of the month. IL-6: Insights into novel biological activities. Clin. Immunol. Immunopathol. 85, 16-20 (1997).
- Berg, R.D.: The indigenous gastrointestinal microflora. Trends Microbiol. 4, 430-435 (1996).
- Billau, A.: Interferon- $\gamma$ : Biology and role in pathogenesis. Adv. Immunol. 62, 61-130 (1996).
- Borish, L. and Rosenwasser, L.J.: Update on cytokines. J. Allergy Clin. Immunol. 97, 719-733 (1996).
- Brandenburg, K., Seydel, U, Schromm, A.B.,

- Loppnow, H., Koch, M.H.J., and Riet-schel, E.Th.: Conformation of lipid A the endotoxic center of bacterial lipopolysaccharide. *J. Endotoxin Res.* 3, 173-178 (1996).
- Daeron, M.: Fc receptor Biology. *Ann. Rev. Immunol.* 15, 203-234 (1997).
- Dijstelbloem, H.M., van de Winkel, J.G.J., and Kallenberg, C.G.M.: Inflammation in autoimmunity: Receptors for IgG revisited. *Trends Immunol.* 22, 510-516 (2001).
- Duchmann, R., Kaiser, I., Hermann, Mayet, W., Ewe, K., and Meyer zum Büschenfelde, K.-H.: Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). *Clin. Exp. Immunol.* 102, 448-455 (1995).
- Elson, C.O., Sartor, B.F., Tennyson, G.S., and Riddell, R.H.: Special reports and reviews. Experimental models of inflammatory bowel disease. *Gastroenterology* 109, 1344-1367 (1995).
- Kimura, K., McCartney, A.L., McConnell, M.A., and Tannock, G.: Analysis of fecal populations of Bifidobacteria and Lactobacilli and investigation of the immunological response of their human host to the predominant strains. *Appl. Env. Microbiol.* 63, 3394-3398 (1997).
- Lauw, F.N., Ten Hove, T., Dekkers, P.E.P., de Jong, E., van Deventer, S.J.H., and van der Poll, T.: Reduced Th1, but not Th2 cytokine production by lymphocytes after *in vivo* exposure of healthy subjects to endotoxin. *Infect. Immun.* 68 (3), 1014-1018 (2000).
- Murphy, J.E., Robert, C., and Kupper, T.S.: Interleukin-1 and cutaneous inflammation: A crucial link between innate and acquired immunity. *J. Invest. Dermatol.* 114, 602-608 (2000).
- Okrasinska-Cholewa, B.: Clinical evaluation of treating accessory nasal sinus diseases in children using autovaccine. *Med. Dosw. Mikrobiol.* 46 (1-2 Suppl.), 67-73 (1994).
- Ravetch, J.V. and Bolland, S.: IgG Fc Receptors. *Ann. Rev. Immunol.* 19, 275-290 (2001).
- Rubisz-Brzezinska, J., Wilk-Czyz, R., Brzezinska-Wcislo, L., and Rosenwasser, S.: Clinical evaluation of serious forms of acne treated with autovaccine. *Med. Dosw. Mikrobiol.* 46 (1-2 Suppl.), 35-42 (1994).
- Rusch, V.: Zur Bedeutung der Autovaccinen in der Medizin von heute. *Arzneimittelforsch./Drug Res.* 36 (II), 1290 (1986).
- Rusch, V., Ottendorfer, D., Zimmermann, K., Gebauer, F., Schrödl, W., Nowak, P., Skarabis, H., and Kunze, R.: Results of an open, non-placebo Controlled pilot study investigating the immunomodulatory potential of autovaccine. *Arzneimittelforsch./Drug Res.* 51 (II), 690-697 (2001).
- Sartor, R.B.: The influence of normal microbial flora on the development of chronic mucosal inflammation. *Res. Immunol.* 148, 567-576 (1997).
- Schromm, A.B., Brandenburg, K., Loppnow, H., Moran, A.P., Koch, M.H.J., Rietschel, E.Th., and Seydel, U.: Biological activities of lipopolysaccharides are determined by the shape of their lipid A portion. *Eur. J. Biochem.* 267, 2008-2013 (2000).
- Strober, W., Kelsall, B., Fuss, I., Marth, T., Ludviksson, B., Ehrhardt, R., and Naurath, M.: Reciprocal IFN- $\gamma$  and TGF- $\beta$  responses regulate the occurrence of mucosal inflammation. *Immunol. Today* 18, 61-64 (1997).
- Strober, W. and Kelsall: To be responsive or not to be responsive, that is the mucosal question. *Gastroenterology* 114, 214-217 (1998).
- van de Winkel, J.G.J., and Capel, P.J.A.: Human IgG Fc-receptor heterogeneity: molecular aspects and clinical implications. *Immunol. Today* 14, 215-221 (1993).
- Wilczynski, K., Kozminska, J., and Bilinski, A.: The testing of auto-vaccination of patients with chronic purulent otitis media. *Otolaryngol. Pol.* 49 (Suppl. 23), 183-185 (1995).
- Wright, A.E. and Douglas, S.R.: On the Action exerted upon the *Staphylococcus pyrogenes* by human body fluids and an elaboration of protective elements in the human organism in response to inoculation of a *Staphylococcus* vaccine. *Proc. Royal Soc. London* 74, 147 (1904).
- Zaluga, E.: Skin reactions to antigens of *Propionibacterium acnes* in patients with acne vulgaris treated with autovaccine. *Ann. Acad. Med. Stetin* 44, 65-85 (1998).
- Zielinski, T., Zimmermann, K., Ottendorfer, D., and Rusch, V.: Quality control and documentation of effectiveness of bacterial autovaccines with the use of flow cytometry. *Arzneimittelforsch.* 48, 502-511 (1998).

Zimmer, S., Pollard, V., Marshall, G.D., Garofalo, R.P., Traber, D., Prough, D., and Herndon, D.N.: The 1996 Moyer Award.

Effects of endotoxin on the Th1/Th2 response in humans. *J. Burn Care Rehabil.* 17, 491-496 (1996).