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# **Eliminating Hydrocortisone-induced Immune Suppression with *Pseudomonas aeruginosa* in vitro**

**by**

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## 1. Introduction

The investigations „Identification of Immune modulatory properties of *Pseudomonas aeruginosa*“ made it clear that here was a substance that intervened in the regulatory cycles of cytokine production of mononuclear blood cells.\* In its interaction with immune complexes, an increased immunocyte reaction was observed in the induction of cytokines in vitro. The results of these investigations have already been published in SANUM Post.<sup>1,2</sup>

Quite remarkable was the increase in production of the granulocyte monocyte colony stimulating factor (GM-CSF), a regulatory or hematopoietic cytokine. These results led to conclusions concerning the possibilities inherent in a deeper analysis of the immune modulatory potential of *Pseudomonas aeruginosa*.

As regards the use of *Pseudomonas aeruginosa*, there have been a number of clinical observations that give an indication of the product's immune modulatory effectiveness. *Pseudomonas aeruginosa* is evidently able to eliminate immunologically based therapy blockages. The goal of the investigations reported on here was to make these effective properties of *Pseudomonas aeruginosa* in vitro visible

\* Homeopathic preparation consisting of polysaccharides from *Pseudomonas aeruginosa* from a homeopathic-isopathic product line from Germany.

under defined experimental conditions, using the example of hydrocortisone induced immune suppression.

Hydrocortisone was chosen because it is a physiologically occurring immune suppressor. It is produced by the body itself and can induce therapy blockages. In diseases within the indication range of *Pseudomonas aeruginosa*, hydrocortisone probably plays a special role. The idea of investigating the effect of *Pseudomonas aeruginosa* on hydrocortisone induced cytokine suppression, with the users of the preparation in mind, therefore came quite naturally.

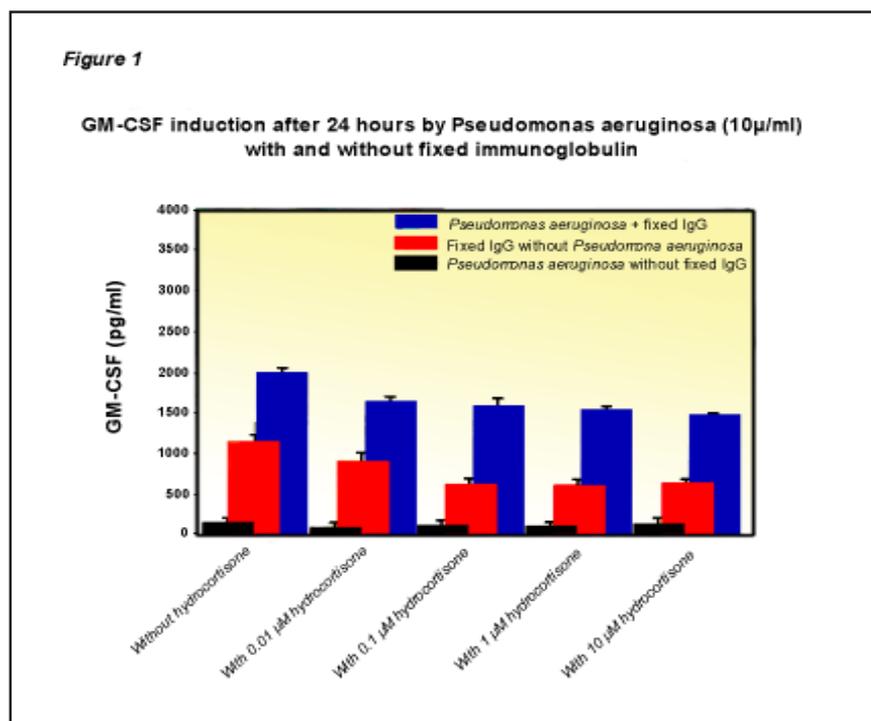
## 2. Results

We investigated experimentally whether *Pseudomonas aeruginosa*, in combination with fixed immunoglobulins (immune complexes), influenced the

regulatory or pro inflammatory cytokines GM-CSF and interleukin 1b in the presence of a substance which blocks immune activity (hydrocortisone).

To this end, peripheral mononuclear blood cells (PMBC) from the blood of healthy donors were isolated and incubated with human IgG. Cytokine production was stimulated through binding to the Fc receptors while saturating PMBC's absorptive binding capacity. Next, the dependence of the formation of the cytokines GM-CSF and IL-1b on increasing concentrations of hydrocortisone in the presence of increasing concentrations of *Pseudomonas aeruginosa* (and with regard to time) was investigated.

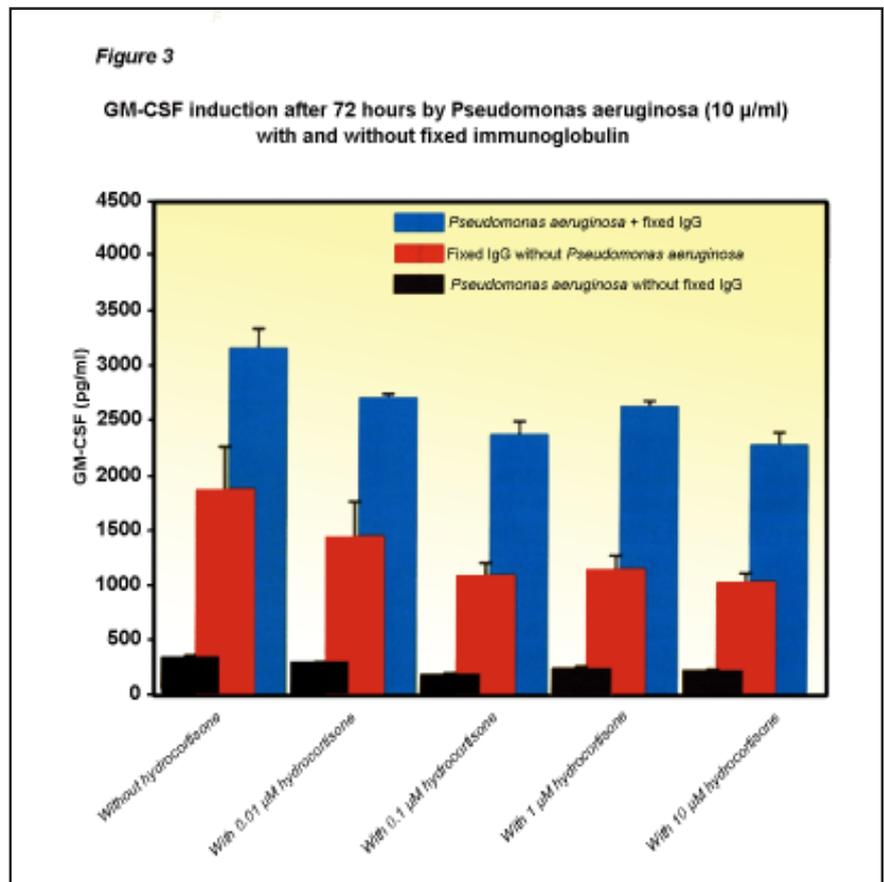
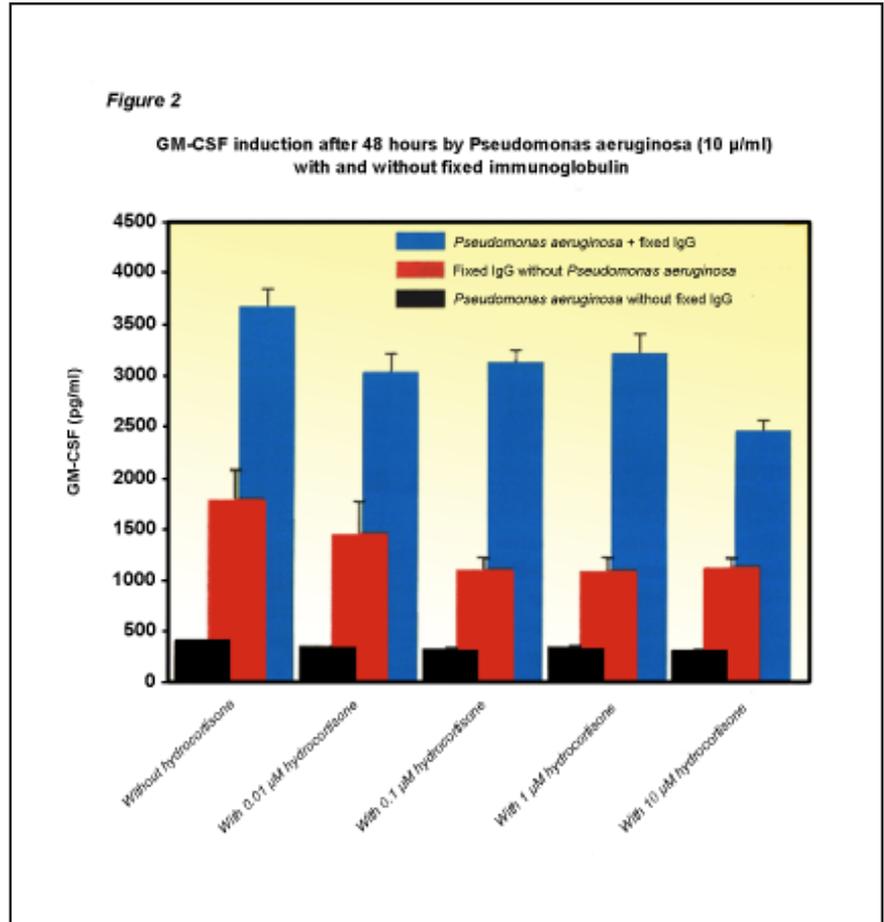
The hydrocortisone concentrations used (0.01 10 µM)



cover the human blood plasma concentration range of hydrocortisone, which (subject to a circadian rhythm) varies between 0.11 and 0.55.

The data from one donor representative for purposes of analysis are presented in detail in *Figs. 1 & 6*. The experiments were set up so as to be able to look at individual cases. On this level, relevant results have already been attained.

Based on *Figs. 1 & 3*, the current data are presented as examples. All cell culture preparations were done in parallel. In the culture preparation without hydrocortisone or immunoglobulin G, *Pseudomonas aeruginosa* itself generates a clearly demonstrable GM-CSF level (1st column in *Figs. 1 & 3*). Fixed immunoglobulin by itself generates a clearly higher cytokine signal (2nd column in *Figs. 1 & 3*). The combination of immunoglobulin G with *Pseudomonas aeruginosa* increases the cytokine signal considerably (3rd column in *Figs. 1 & 3*). In *Figs. 2 & 3*, in which GM-CSF was set for a later time, this effect becomes even clearer. These data serve as a reference system for the hydrocortisone experiments. An earlier research report detailed the superadditive effect of GM-CSF induction by *Pseudomonas aeruginosa* in combination with immune complexes.<sup>2</sup>



In the presence of hydrocortisone (Figs. 13), there is a more or less concentration dependent immune suppression of cytokine production. In combination with fixed immunoglobulins, *Pseudomonas aeruginosa* can, at all tested concentrations and at all times, reduce or eliminate hydrocortisone induced immune suppression.

The situation is structured similarly for interleukin 1b (Figs. 4 & 6). Here also one can note dosage effect relationships between *Pseudomonas aeruginosa* and hydrocortisone. In combination with fixed immunoglobulin G, *Pseudomonas aeruginosa* is once more able to eliminate hydrocortisone induced suppression. Timewise, the induction of the two cytokines does not differ significantly.

In order to be able to summarize the influence of the amount of *Pseudomonas aeruginosa* and the various donors, the values measured with and without *Pseudomonas aeruginosa* under fixed IgG were normed to the cytokine production measured with only fixed IgG without *Pseudomonas aeruginosa* or hydrocortisone (cytokine value = 100 %).

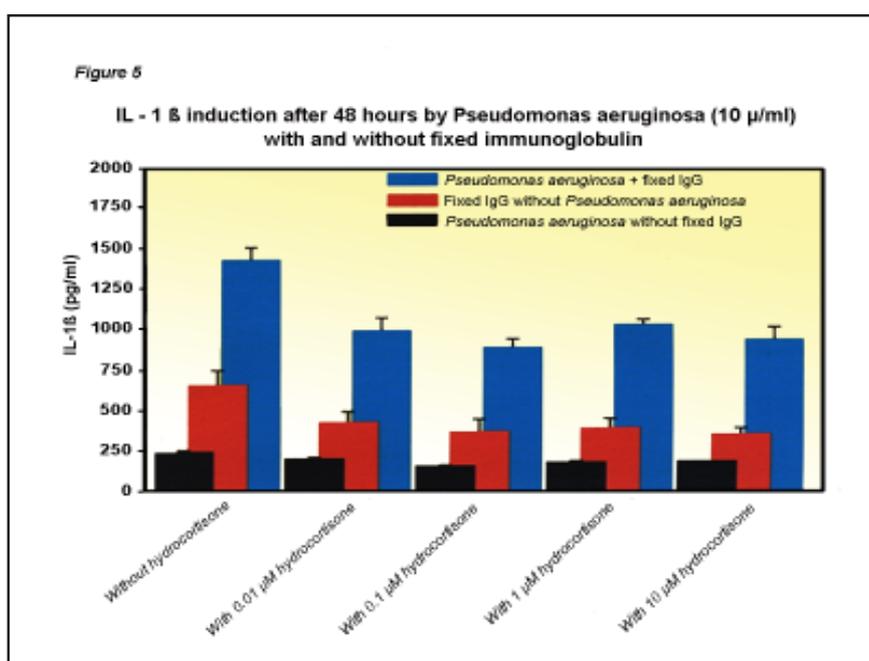
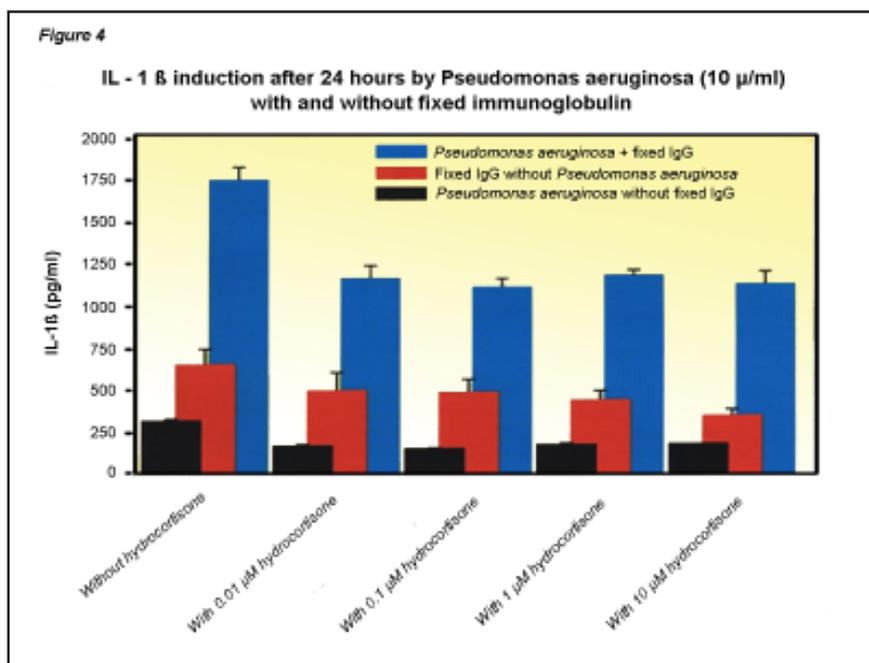
A 3D bar chart was chosen to clarify the relationships in the

reaction triangle hydrocortisone / *Pseudomonas aeruginosa* / induced cytokine signal. In Figs. 7 & 8, one can see that the four donors reacted in very nearly the same way. Increasing *Pseudomonas aeruginosa* concentration can more and more reduce or eliminate hydro-

cortisone induced suppression. The cells of the four donors react similarly at all three of the time points Chosen for cytokine determination.

### 3. Discussion

At the moment, there exists no commonly accepted model for



the molecular mechanisms which lead to compensation or elimination of hydrocortisone induced immune suppression. For the induction of cytokine signals, specific stimuli originating in extracellular space are transmitted via specific receptors to the interior of the cell, and there induce the release or production of cytokines.

Cytokines induced in the second manner can be switched off by hydrocortisone.<sup>3,4</sup> The cell interior has receptors for this molecule which, ultimately, take part in regulating protein synthesis.

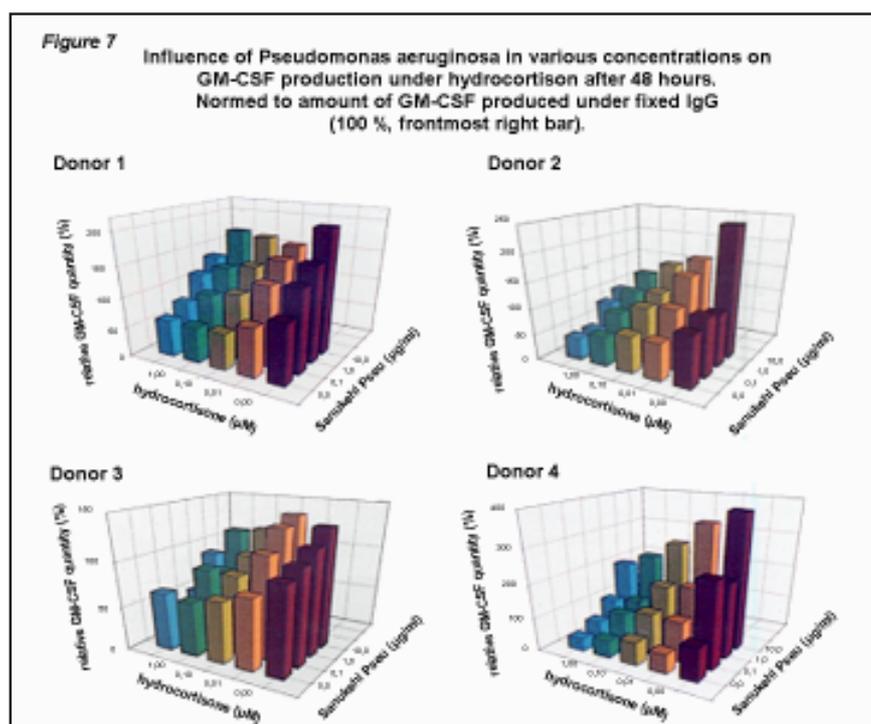
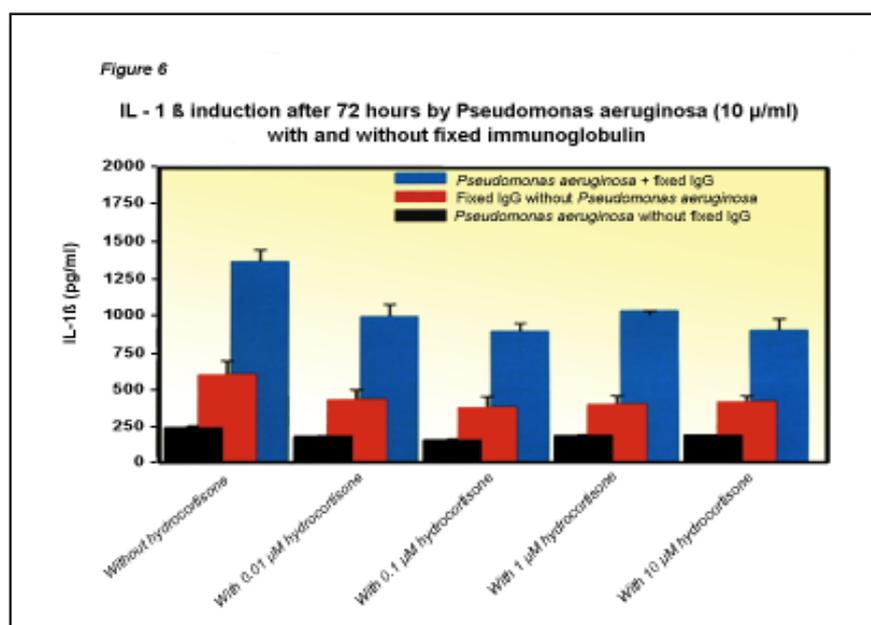
There are a number of additional receptors available for the induction of cytokines. These include, for example, the endotoxin receptor CD14 and the Fc receptors to which immunoglobulins or immune complexes bind. These also induce a reaction cascade within the cell,<sup>5,6</sup> which leads to cytokine production. This is in all likelihood the place to begin in seeking to understand the effect of *Pseudomonas aeruginosa* in combination with fixed immunoglobulins or immune complexes.

Other mechanisms are conceivable which could explain the results presented here. A cross linkage i.e. a simultaneous mutual interaction between a ligand or ligand pair (immune complex) and two receptors on the cell surface<sup>7,5</sup> can likewise

result in activation of the cell. Both *Pseudomonas aeruginosa* and immunoglobulin bound to bacterial antigen could effect the cross linkage via Fc at two receptor types on the cell surface. Another possibility not to be excluded is that different types of cells react with the immune complex or

*Pseudomonas aeruginosa*, and metabolic products from one type of cell activate another type of cell, which ultimately produces cytokine.

Furthermore, it is conceivable that the effect of *Pseudomonas aeruginosa* is based on activating a hitherto un-



discovered cytokine or chemokine which is involved in the reduction or elimination of hydrocortisone induced cytokine suppression.<sup>8,9,10,11</sup>

In the immunological technical literature, two molecular mechanisms for corticosteroids are discussed:

- On the genetic level, they inhibit in a complex with their receptors, by binding on the „key“ transformation factors of protein and thus also cytokine synthesis. The particular factors involved are AP 1 and NF KB.<sup>3,4,12</sup>
- As physiological opponents of MIF (macrophagemigration inhibitory factor: possesses immune activating properties), they modulate the reaction potential of macrophages.<sup>10,11</sup>

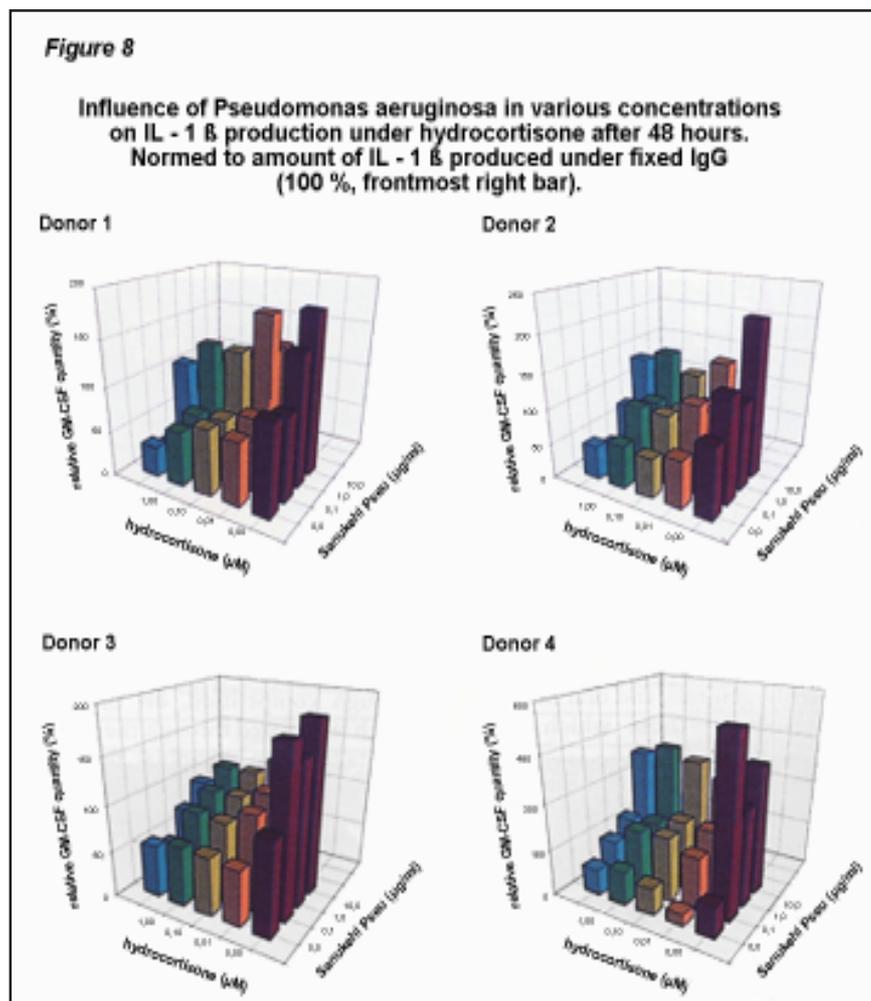
From the viewpoint of clinical immunology, the immune modulatory effect of *Pseudomonas aeruginosa* is of fundamental significance for the understanding of its effect on patients. The dependence on the immunopathological processes of various diseases permit adding new areas of indication for the product, at least theoretically at first. What we here have in mind is influencing neuroimmunological processes or else the breakup of immunosuppressive feedback systems set in motion by other substances or processes. This includes, for example, the immunosuppressive, cytostatic effect of Methotrexate or Cyclosporin-A but also

radiation induced immune suppression. Immune suppression observed in cases of long term physical or psychic stress might be a future area of indication for *Pseudomonas aeruginosa*.

Another possibility is that of influencing the immunological balance of the TH1/TH2 subpopulation, which regulates the immunological phenotyp (dominance of cellular or of humoral immunity). In the last 8 years, the analysis of the significance of the TH1/TH2 Subpopulation for the development of disease pictures has

developed into an independent research field of its own.<sup>13,14,15</sup> Hydrocortisone and other similarly structured immune suppressors can intervene in a fundamental way in the life cycle of cells.<sup>3</sup>

Apoptosis, programmed and regulated cell death, has been recognized in recent years as one of the most important processes in the regulation and maintenance of immune homeostasis. It can be assumed that *Pseudomonas aeruginosa* positively influences at least some populations of immuno-





competent cells, and protects them from hydrocortisone induced or accelerated apoptosis.

These experiments, or the results there from, show that *Pseudomonas aeruginosa* in combination with fixed immunoglobulins can minimize or eliminate immune suppression triggered by hydrocortisone.

The observations coming from the clinical application of *Pseudomonas aeruginosa* clearly demonstrate that, with this preparation, existing blockages in which various other attempts at naturopathic therapy have failed to improve the condition of the affected patients can be broken up.

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