Eliminating Hydrocortisone-induced Immune Suppression with Pseudomonas aeruginosa in vitro

by

Dr. R. Kunze and J. Hartmann (Ph. D., biology)
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.1,2

Quite remarkable was the increase in production of the granulocyte monocytecolony 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 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)

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

Figure 1
GM-CSF induction after 24 hours by Pseudomonas aeruginosa (10µ/ml) with and without fixed immunoglobulin

Semmelweis-Institut GmbH
Verlag für Naturheilkunde · 27318 Hoya · Germany
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 GMCSF induction by Pseudomonas aeruginosa in combination with immune complexes.²
In the presence of hydrocortisone (Figs. 1 & 3), 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 1β (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 hydrocortisone induced suppression.

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. 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, 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 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 chemo-
kine which is involved in the
reduction or elimination of
hydrocortisone induced cyto-
kine suppression.\textsuperscript{8,9,10,11}

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 11 and NF KB.\textsuperscript{3,4,12}

- As physiological opponents
  of MIF (macrophagemigration
  inhibitory factor: possesses immune
  activating properties), they
  modulate the reaction potential
  of macrophages.\textsuperscript{10,11}

From the viewpoint of clinical
immunology, the immune
modulatory effect of Pseu-
domonas 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 immuno-
suppressive feedback systems
set in motion by other
substances or processes.
This includes, for example,
the immunosuppressive, cyto-
static 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 develop-
ment of disease pictures has
developed into an independent
research field of its own.\textsuperscript{13,14,15}

Hydrocortisone and other
similarly structured immune
suppressors can intervene in
a fundamental way in the life
cycle of cells.\textsuperscript{3}

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-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure8.jpg}
\caption{Influence of Pseudomonas aeruginosa in various concentrations
on IL-1 \& production under hydrocortisone after 48 hours. Normalled to amount of IL-1 \& produced under fixed IgG (100 \%, frontost right top bar).}
\end{figure}
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.

Bibliography


First published in the English language in the EXPLORE! magazine (Vol.8/3 1997)

© Copyright by Semmelweis Institut GmbH
27318 Hoya, Germany
All Rights Reserved