

The antiinflammatory action mechanism of an antihomotoxic composita remedy

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[Summary]

Introduction

In order to use preparations in an effective, targeted manner with as few side effects as possible, attempts must be made, beyond the realms of empirical investigation, to reveal the underlying mechanism of action [5].

Traumeel, a combination homoeopathic preparation, has been used successfully for the treatment of trauma, inflammation and degenerative processes for more than 30 years (review in [11]). It has been shown in experimental investigations, primarily in ex-vivo studies in whole-blood cultures to which the individual components of the preparation had been added, that this homoeopathic antihomotoxic remedy stimulates the release of antiinflammatory cytokines, and particularly the inflammation-inhibiting “lead cytokine”, transforming growth factor beta (TGF- β), from leucocytes [6, 12, 13]. This gave rise to the theory that the anti-inflammatory action of certain antihomotoxic remedies could be based on an “immunological bystander reaction” [13]. In this process, depending on the very low concentrations of active substances in the preparation (plant and mineral extracts, D2-D8), a TGF- β -producing type of lymphocyte is evidently formed (Th3 cells) which regulates immunological tolerance. This type of cell was first described by the Weiner study group after the administration of minute quantities of autologous antigens (in the lowest microgram range per unit of body weight) in autoimmune diseases, and was christened “bystander suppression” [22, 23]. The term immunological bystander reaction was introduced into the biological and medical literature by Heine in 1997 [9].

The aim of this study is to check whether, in the early stage of rheumatoid arthritis (RA) (stage I and II) the antihomotoxic remedy Traumeel can stimulate the development of antiinflammatory Th3 cells and thus trigger an antiinflammatory immunological bystander reaction.

Only the early and side-effect-free treatment of the joint inflammation can prevent the incipient destruction of the joint cartilage or at least delay it for long periods. Conventional nonsteroidal antiinflammatories (NSAIDs) but also aggressive basic therapies such as methotrexate, gold, chloroquine and others often have only limited success, and in some cases have severe side effects [19].

Methods

Patients

10 volunteers with early-stage rheumatoid arthritis (7 men and 3 women between 39 and 60 years of age) were included in the study. 9 patients were in stage I, one case was classified as stage II. The main exclusion criteria were an acute episode of the disease and pregnancy. Overall, the laboratory parameters were uncharacteristic (C-reactive protein, erythrocyte sedimentation; only one case (patient DE) was positive for rheumatoid factor). In 3 cases the differential blood pictures showed mild lymphocytosis which returned to normal under Traumeel.

After a washout period of one week with the withdrawal of all medications, the patients were treated with Traumeel for 14 days (3×15 drops/day). The Th3 cells were determined before and after the 14-day period of treatment with Traumeel (Table 1).

Patients	Th3 before Traumeel treatment	Th3 after	Assessment
HETGFBK	0	0	No change
HETGFLK	0	0.16	Rise
HETGFPG	0.2	0.32	Rise
HETGFLB	2	3.2	Rise
HETGFGC	2	2.1	Rise
HETGFDS	0	1.5	Rise
HETGFDE	0	5.7	Sharp rise
HETGFGE	0.5	4.1	Sharp rise
HETGFPW	0.6	0.14	Reduction
HETGFDML	2.4	0	Reduction

Table 1: Change in the percentage proportion of Th3 cells in 10 patients with mild chronic rheumatoid arthritis (stage I and II) after 14 days' treatment with Traumeel. Data in % Th3 cells per million Th cells

Collecting Th3 cells

The Th3 cells were isolated from the blood before the start and after the end of the 14-day period of treatment. 30 ml of freshly collected heparinized blood had the same volume of buffer (Pharmacia, Freiburg) added to it and the lymphocytes were isolated by means of Ficoll-PAQUE (Pharmacia, Freiburg). The pellets were in each case resuspended in 50 ml buffer and washed twice. After removal of the supernatant, the material was again resuspended in the buffer and the cell count was determined in a Neubauer counting chamber. The Th3 cells were characterized using magnetic beads coated with a specific antibody to the membrane antigen CD4 which is typical of Th cells (CD4 Positive Isolation Kit; Dynal, Hamburg). The beads were first washed in 1 ml PBS/2% FCS and added to the lymphocyte pellet (1×10^7 beads to $0.5 \text{ ml} \times 10^7$ cells). After an incubation period of 20 min at about 8°C the test tubes were placed in a magnetic rack. The supernatant was removed after 3 minutes and the beads were washed 5 times in PBS/2% FCS.

After resuspension, DETACHABead (Dynal, Hamburg) was used to break down the DNA linker between the CD4 antibody and the magnetic bead and the beads were removed by being placed in a magnetic rack. The Th cell suspension was washed 3 times in a further reaction vessel, combined with the supernatants and the Th3 cell count was determined (see below).

In-vitro study

Part of the Th3 cell suspension from the baseline sample (before Traumeel therapy) was added to short (3 day) cultures with Traumeel (10 ml per sample) and the Th3 cell count was then determined. This was intended to check whether the constituents of Traumeel have a mitogenic action on Th3 cells.

Detection and determination of TGF- β -producing Th3 cells

Because of the very low Th3 cell count in the blood (see control values), the FACS technique is not suitable, since it requires much higher cell counts for cell determination. Very large ranges of variation are produced as a result [14]. An ELISpot for the qualitative determination of individual cells is not currently available for TGF- β -forming Th3 cells.

After drying of the Th cell suspension pipetted onto microscope slides, they were fixed for 10 min with isopropanol, then rinsed for 2 min with PBS.

Figure 1: Short culture of isolated Th3 cells. Immunological detection of TGF- β (brown, cell-bound reaction product) by the streptavidin-biotin method. Magnification 60 \times

The Th3 cells were detected immunologically via the visualization of TGF- β (a rabbit TGF- β 1 antibody [1:100] was used as primary antibody: Promega, Mannheim) using a monoclonal antibody and optical detection (streptavidin-biotin [20], DAKO-Chem Mate detection kit [DAKO-Chem, Hamburg]) in an automatic staining machine (the H₂O₂ which is formed converts TGF- β into its active form [Fig. 1]) [20, 21]). The DAKO detection kit works on the basis of the avidin-biotin-peroxidase complex technique. Use of this reaction to detect TGF- β was described in detail by Franchini et al. [8].

In brief, the microscope slides with the fixed Th3 cells were first incubated with TGF- β antibodies. After washing with PBS they were again incubated with biotinylated anti-rabbit immunoglobulins. After renewed washing in PBS the microscope slides were incubated with ABC for presentation of the reaction times, then washed in PBS, after which the peroxidase activity was detected using DAB (3,3-diaminobenzidine tetrahydrochloride). After washing in distilled water, the cell nuclei were counterstained with haematoxylin (Fig. 1). Controls were performed using the primary antibody with nonimmunogenic serum. The specimens were dehydrated by means of an increasing alcohol series and then covered. The positive cells were evaluated using an automatic measuring system (Ahrens ICM, Hamburg). 6 microscope slides (3 for the first and 3 for the second examination) were evaluated per patient.

Results

With the exception of one woman (patient KB) for whom no Th3 cells were detectable optically by immunofluorescence, all other patients showed changes in the Th3 cell count after 14 days' treatment. The values were between 0.16% and 5.7% (Table 1). With the controls, on the other hand, these were between 0.1% and less than 1%. In addition to a moderate increase for half the patients, two patients showed a very large increase and two more showed a decrease in the overall Th3 cell count (Table 1).

The patient group selected according to clinical criteria showed no conspicuous clusters of laboratory parameters which could be used to explain the behaviour of the total Th3 cell count. Only in one case (DE) was the patient positive for rheumatoid factor. This man is one of the two patients with a sharp rise in the Th3 cell count.

The in-vitro findings showed that the incubation of cultivated Th3 cells with Traumeel does not lead to any proliferation of Th3 cells. The changes in the Th3 cell counts in vivo are thus attributable to the concerted action of the constituents of Traumeel.

Discussion

The results show that the number of antiinflammatory Th3 cells can be increased by the antihomotoxic remedy. It must be borne in mind that Th3 cells are located mainly in the tissues and that, as the controls showed, only small numbers pass into the blood [17, 22].

The regulatory Th3 cells oversee immunological tolerance in the body by releasing the antiinflammatory cytokine TGF- β and IL-10 as necessary [10, 11, 13, 23]. In patient BK, the woman in whom no Th3 cell changes could be detected, the defence system may be blocked. With the other two patients who had a reduced Th3 cell count, latent tissue acidosis might be a possibility, as with BK. It has long been known that latent tissue acidosis practically always develops in the presence of a chronic disease (review in [7]). Neither biological nor conventional medical treatments have any effect where such a disease is present. This also gives rise to a blockade or loss of immune system function which could also be the reason for the non-response of the Th3 cells in the three cases mentioned.

It is at present estimated that almost half the German population is affected by tissue acidosis [7]. The result is an individually variable blockade of immunological reaction processes and resistance to treatment. The causes are mainly stress syndromes such as toxic environmental influences, incorrect diet and prolonged stress. In this situation the latent acidosis must be tackled by means of stress management and a change in diet [7, 11].

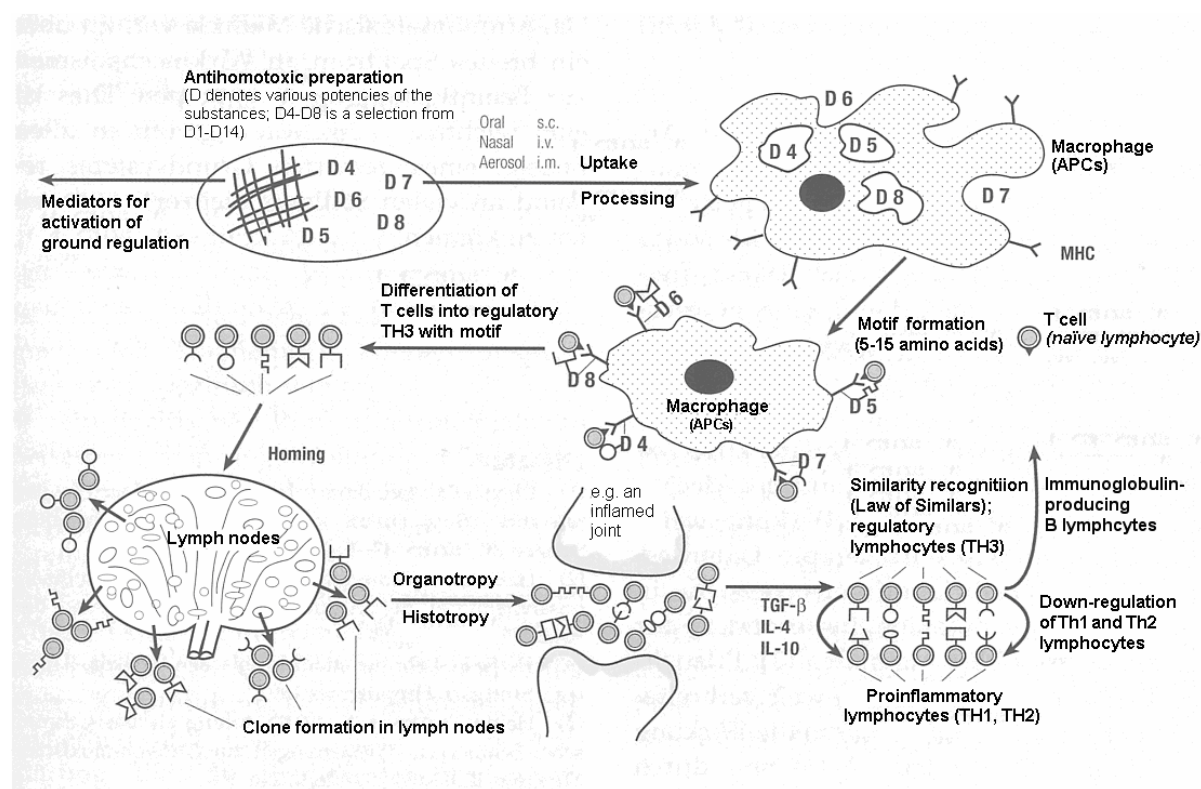


Figure 2: Immunological bystander reaction. Low to medium-potency combination homeopathic preparations generate regulatory lymphocytes of the Th3 cell type which, after contact with proinflammatory T lymphocytes (Th1 and Th2 cells; h = helper), release the antiinflammatory cytokines TGF- β (transforming growth factor beta) and interleukin (IL) -10 (from [9])

With unphysiological deviations in immunological tolerance, increased amounts of Th3 cells can be formed to restore tolerance via the forced stimulation of an immunological bystander reaction. This process is evidently also at the root of treatment with composita antihomotoxic remedies [9-13] (Fig. 2). To trigger an immunological bystander reaction, fresh (“naïve”), immunologically inexperienced Th cells must be loaded with certain short-chain (5-15 amino acids) constituents of an antigen (“motifs”) by antigen-presenting cells (APC) such as macrophages, monocytes, M cells (macrophages incorporated in the intestinal epithelium), dendritic cells and B lymphocytes [16, 17, 22, 23]. A motif is formed after the uptake of an antigen into an APC. The antigen is then lysosomally degraded apart from the antigenic motif. It is transported into the endoplasmic reticulum where it is bound to an MHCII molecule and brought to the cell surface with that molecule. At the same time, the APC forms a chemotactic cytokine for naïve T lymphocytes, i.e. ones which are still fresh and immunologically inexperienced [12]. Motifs are then (probably with a certain portion of the MHC molecule) removed from naïve Th cells which then are transformed into Th3 cells [23]. The “motified” Th3 cells then migrate immediately into the nearest lymph node where they proliferate into as many Th3 cell clones as Th3 cells were motified [9, 22, 23].

Chemokines from a focus of inflammation then attract the Th3 cells to the site of the inflammation, where they compare their motifs with the surface-bound antigens of the proinflammatory Th1 and Th2 cells. If there is any similarity (molecular biological similars, cross-reaction), the Th3 cells release TGF- β by means of which the proinflammatory cytokines TNF- α , IFN- γ , IL-1 and IL-2 can be down-regulated [11, 13, 23]. The fact that every organ evidently has its own chemokine spectrum protects the immunological bystander reaction typical for each organ [9, 23].

In rheumatoid arthritis (RA), Th1 cells synthesize large quantities of the cytokine osteoprotegerin ligand (OPGL) which, via the RANK receptor (receptor activator of the proinflammatory nuclear factor kappa B [NFkB]), activates osteoclasts and leads to bone degradation. This explains the periarticular and systemic osteoporosis often seen in RA. Since TGF- β inhibits the expression of OPGL, rheumatic “concomitant osteoporosis” is at the same time prevented by the activation of an immunological bystander reaction (review in [15]).

An immunological bystander reaction can be triggered only by very small quantities of substance ($\leq 1 \mu\text{g}$ per kg body weight), since with larger quantities the APC can fend off the antigen and with a larger influx of antigen ($\geq 1 \text{mg}$) the proinflammatory Th1 and Th2 cell populations are at the same time eliminated by anergy or deletion [23]. In addition, very small but varied quantities of antigen (as in antihomotoxic remedies) yield a large number of different Th3 cell clones, so that the molecular biological similarity is maintained [10].

Antihomotoxic medicine thus seems to intervene in the restoration of immunological tolerance essentially via cooperation and by supplying cytokines. These cellular messenger substances form an information network in the body which reacts to any change in homeostasis. Cytokines are effective in the smallest concentrations; for example, TGF- β is most active at 10^{-10} g/ml [1]. The small but very varied substance concentrations in antihomotoxic remedies take effect together with the Arndt-Schulz Law, according to which small stimuli activate regulation mechanisms and high concentrations paralyse them [9, 11]. The advantage of triggering an immunological bystander reaction is thus obvious: when treating an organ-specific disease it is not necessary to know the specific antigen [23], and a circulating antigen does not need to be detectable [23].

The results indicate that an immunological bystander reaction is involved in the well-documented successful treatment of inflammation using composita antihomotoxic remedies. Further confirmation must be reserved for more extensive investigations.

References

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Comments on the article

In this article, Heine et al. have – as they did in the earlier article on CFS – shown themselves to be pioneering scientists in the field of intercellular tissue research.

The reader is amazed by the attention to detail and the complexity of the research needed to establish the ground rules, and by the preconditions needed to perform investigations of this kind. What is remarkable – and this repeatedly characterizes Heine's work – is the link with practical work which is usually missing from similar studies in conventional research. The article thus provides general practitioners with substantial arguments in favour of using combination homoeopathic or antihomotoxic preparations.

As Heine hints, the homoeopaths' similars at the level of the ground substance can be understood and detected. What is also very important is the reference to the existence – repeatedly confirmed in practice – of other background conditions, i.e. in this case the chronically sick person's acidosis. This makes Heine a scientist who does not look at the evaluation of a series of experiments just so that he can add the final results to his list of publications; he is also a researcher and doctor who thinks in practical terms and looks beyond the edge of the test tube.

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