Induction of the Immunological Bystander Reaction by Plant Extracts

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Abstract

This study of whole blood cultures from healthy subjects demonstrates for the first time that low potencies of plant extracts (such as those used in combination preparations of antihomotoxic medicine) stimulate lymphocytes to synthesize and secrete the cytokine TGF-ß (transforming growth factor ß). Endogenous anti-inflammatory and homeostatic processes revolve around TGF-ß. Our findings confirm recent reports that antihomotoxic therapy activates the immunological bystander reaction.

Introduction

The immunological "bystander reaction," in which regulatory lymphocytes actively help control inflammatory reactions by producing the messenger substance TGF-ß (transforming growth factor ß), has been discussed as one of the significant working mechanisms of antihomotoxic homeopathic combination preparations.[3] This reaction is fully in accordance with the process of regressive vicariation.

By now it is generally accepted that the primary effect of TGF-ß as a mediator is to inhibit other immune-system cells. Weiner et al., in particular, who have been laying the groundwork for this since 1994, were able to prove that peroral stimulation of intestinal T-lymphocytes by using antigens in a defined range of low dosages induced tolerance-like phenomena,[5,6,7] achieving very extensive inhibition of autoimmune reactions in experimental animals, for example. The therapeutic inhibition achieved in this way proved to be reversible by neutralizing TGF-ß, which was thus clearly characterized as the determining factor in this control mechanism.[3,5,6,7]

The goal of the current study was to test whether plant components of antihomotoxic preparations are also capable of stimulating T-cells to secrete the inhibiting cytokine TGF-ß.

Materials and Methods

Because of the ability of lymphocytes to recirculate (tissues → lymph system → bloodstream → tissues), blood samples yield both antigen-triggered and naive T-cells, which can then be cultured and exposed to the relevant stimulation.

Investigational Model: Whole Blood Culture

Culturing whole blood from healthy donors was the test system selected. Because it incubates leukocytes in their natural surroundings rather than in a medium that is "naked", i.e., low in serum, whole blood culture offers significant advantages over culturing isolated leukocytes and more closely approximates conditions *in vivo*. Because of the complex composition of whole blood, its use *in vitro* typically weakens the effect of medications, as also occurs *in vivo*. Thus, in whole blood cultures, test samples produce recognizable changes in cellular reactions only if their ingredients remain active in spite of the presence of all of the blood factors that tend to modify their effects. Transferability of results to actual circumstances in the body is clearly more reliable in whole blood culture than in the culture of isolated leukocytes.

All three plant samples were tested on the blood of three different donors. Test samples were added to the cultures in the form of a 2X potency. In each case the samples were incubated with the whole blood for a 24-hour period. The solvent used in preparing the plant extract served as the control in each instance.

Mediator Synthesis

After obtaining the part of the culture that remained viable upon conclusion of the 24-hour incubation period, the concentration of TGF-ß that had been synthesized was determined by means of a commercially available specialized immunoassay (TGF-ß-ELISA, Hölzel Co.) Changes induced by the sample were calculated as percentages of increase or inhibition in comparison to the control value.

Test Samples

The samples tested were selected at random from the repertory of plant extracts available to antihomotoxic therapy. The following plant extracts were used, each in a 2X potency: Atropa belladonna, Bellis perennis, and Conium maculatum.

Results

At a potency of 2X, all three plant extracts tested proved capable of stimulating lymphocytes to release TGF-ß. Incubating the three blood cultures with Bellis perennis and Conium maculatum produced very distinct activity in all cases, while the effects of Atropa belladonna, although clearly weaker, must still be categorized as legitimately stimulatory (see Table).

An important secondary finding was that the three plant preparations showed distinct differences with regard to their pharmacological effects (secretion of TGF-ß) on individual donors.

Discussion

The results of this study demonstrated for the first time that lower potencies of plant extracts, such as those used in antihomotoxic preparations, are capable of stimulating production of the inhibitory cytokine TGF-ß in whole blood cultures. This clearly suggests that these potentized plant ingredients directly stimulate a very specific subtype of regulatory lymphocytes (Th3 cells). It is somewhat misleading to describe these cells as Type 3 *helper* T-cells, since it is quite clear that they play a predominantly suppressive role. They are described as being present wherever the immune system reacts to endogenous structures. In the context of immune-system derailments of this sort, it has been possible to achieve very extensive inhibition of lymphocytes that respond to autoantigens by administering the autoantigen in question *perorally* in its pure form and, above all, at a very low dosage.[6,7]

Detailed analyses have shown that this inhibition is underlain by the activation of Th3 cells with antigen-specific responses. After migrating into the inflamed areas, these cells appear to considerably reduce the activity of lymphocytes involved in the autoimmune reaction. This is known as the "bystander reaction."

Activation of Th3 cells ordinarily takes place as follows: Proteins that are known antigens are first taken in by macrophages, broken into fragments of a specific length (chains of approximately 10 amino acids) and transported back to the surface of the cell (antigen processing). There these fragments are attached to specific membrane proteins (MHC molecules) and the resulting complexes are presented for the T-lymphocytes to recognize (antigen presentation). When their antigen-binding receptors are strong enough, the T-lymphocytes are activated and begin to "recirculate," moving through the body in search of corresponding structures (see Figure).

Specifically, wherever these lymphocytes recognize signs of inflammation, they migrate from the blood vessels into diseased tissue areas and undergo renewed activation. In the case of Th3 lymphocytes, the results of stimulation include TGF-ß synthesis and the subsequent transmission of inhibitory signals. Through local production of TGF-ß, Th3 cells can prevent other, pro-inflammatory lymphocytes (especially Th1 and Th2 cells) from continuing to support the actual inflammatory reaction. In this process, the Th3 cells need not necessarily recognize the same fragments as the Th1 or Th2 cells; it is apparently sufficient for the TGF-ß-producing cells to recognize similar antigen fragments from the tissue where the inflammatory reaction is taking place. This can be seen as a manifestation of the simile principle on the cellular level in the immune system.[6,7]

The effects observed here can certainly be explained as antigen-like stimulations. Alternatively, however, it is also conceivable that an antigen-unspecific mechanism exists, requiring different receptors than the T-cell receptor. This would mean that the Th3 cells and other T-lymphocyte subpopulations would display different activation routes. It has only recently become possible to attribute the mechanism mediated by TGF-ß specifically to the Th3 subpopulation. We have Weiner et al. to thank for devoting more attention to this endogenous system of inhibiting activated leukocytes and controlling chronic inflammatory processes.[1,2,7]

Studies performed by Weiner et al. show that the effective antigen preparations that enabled their team to suppress autoimmune reactions developed full efficacy only at low dosages (µg quantities). At least in part, the homeopathic plant remedies tested in this current

	TGF-ß [%]		
Plant extracts	Blood sample A	Blood sample B	Blood sample C
Atropa belladonna 2X	18	8	24
Bellis perennis 2X	69	250	173
Conium maculatum 2X	177	325	364

Table: Increase in TGF-ß synthesis in three different samples of whole blood after incubation with preparations of the antihomotoxic ingredients Atropa belladonna, Bellis perennis, and Conium maculatum (Control solvent = 100%).

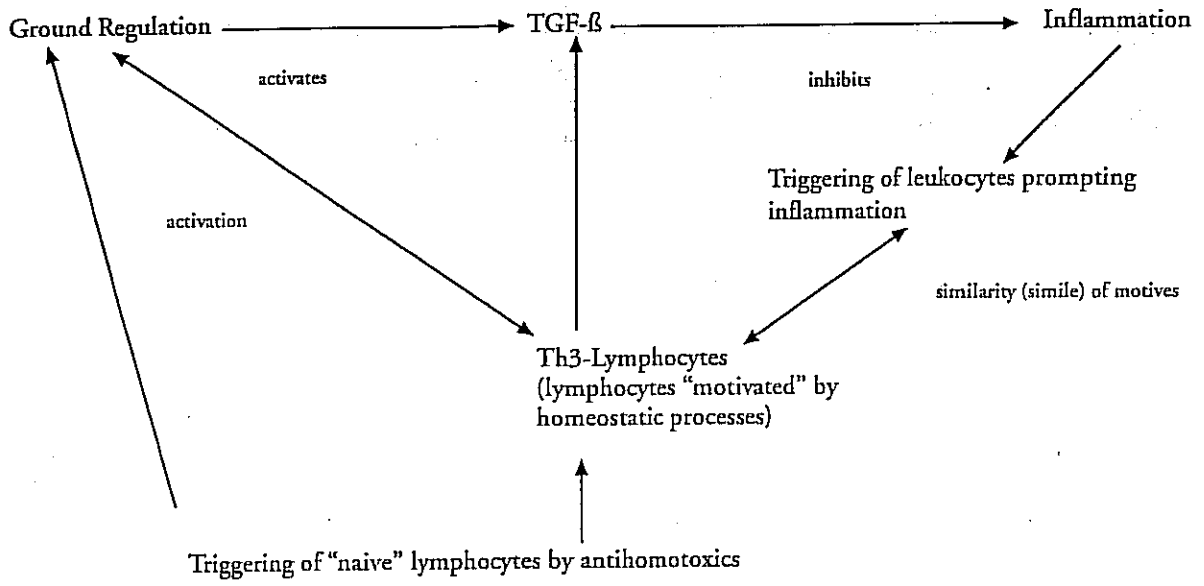

Figure: Immunological bystander reaction. Potentized low-dose antigens from homeopathic antihomotoxics lead to the formation of Th3 lymphocyte populations carrying fragments of potentized low-dose antigens (short amino acid chains) on their surfaces. Their similarity to membrane antigens of pro-inflammatory leukocytes (especially T4, Th1, and Th2 lymphocytes) leads, on contact, to the release of the inflammation-inhibiting cytokine TGF-ß (transforming growth factor ß) by Th3 lymphocytes. Additional components of homeopathic antihomotoxics promote homeostasis.

study have a very potent influence on TGF-ß synthesis by leukocytes in whole blood. The well-known positive clinical effects of these homeopathic substances on inflammatory processes permit the conclusion that stimulation of TGF-ß synthesis plays an essential part in their therapeutic effects.[3,7]

All three of the arbitrarily selected plant extracts stimulate TGF-ß production. Further studies will be necessary to demonstrate whether this effect is as common among single homeopathic remedies as these initial investigations suggest. If this tendency is confirmed, one of the principles behind the efficacy of the antihomotoxic therapy developed by Reckeweg will have been discovered.

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