

The
Instant Leukocyte Culture System – ILCS,
(US patent No. 6,410,334 B1)

Background

One of the most prominent features of the human immune system is its highly subject-specific behaviour. This is best documented by the highly variable pattern and amount of cytokines produced in response to experimental stimuli (when cells of different individuals are cultured *in vitro*).

Whenever changes in the activities of leukocytes are to be measured, this variability has to be taken into account thoroughly. Moreover, when testing the performance of leukocytes from volunteers or patients it has to be kept in mind that the procedure used to isolate these cells as well as their preparation for different tests (e.g. the determination of cytokine synthesis in isolated leukocyte cultures or of CD-markers in flow-cytometry) usually causes a loss of current activities or – on the other hand – even the expression of newly acquired ones.

Another major disadvantage of especially cultures of isolated leukocyte fractions or – much worse – sub-populations of white blood cells is that therein these cells are only able to set up parts of the multi-layered messenger network basically needed for the expression of a sufficient inter-cellular communication (as would be the case *in vivo*).

Even the smallest (and in most instances almost completely neglected) “cellular” elements of the blood, the platelets, provide significant additional signalling input into the network of messages. They not only secrete arachidonic acid metabolites, like prostaglandins and leukotrienes, but also lead to the expression of important activating receptors on leukocytes (e.g. CD11b on monocytes and granulocytes; Li et al, 2000).

Most important, the availability of biologically active cytokines in the cultures is strongly modified by the presence of enzymes released by neutrophil granulocytes (Bank and Ansorge, 2001).

Last, but not least, also red blood cells contribute to the development of an *in vivo* like condition by forming buffer surfaces for excess mediators (Olszyna et al., 2001).

These examples demonstrate clearly the importance of having all active elements of the blood present in experimental cultures. Only this way artificial findings can be avoided. This is particularly true in the preclinical and clinical evaluation of immunologically active drugs (immuno-suppressors, immuno-modulators, adjuvants, vaccines etc.).

Another critical issue in the experimental detection of cytokines is the choice of methods by which this is accomplished. Despite the wide spread use of mRNA based technologies to detect cytokines, it is noteworthy to mention that the amount of mRNA of a given cytokine does not necessarily reflect that of the secreted mediator (O'Hehir et al., 1996).

Not only because of all these drawbacks, data derived from "classical" test systems often suffer from a serious lack in reliability. In addition, the majority of these methods is characterised by a complex methodology. This gives rise to a poor reproducibility resulting from sometimes only slight differences in cell handling (not only from lab to lab but also from technician to technician).

Taking into account all of the drawbacks of the above mentioned so-called classical test systems, ILCS represents the most improved cell culture system available, providing an extremely versatile and valuable tool for clinical research.

Aims of ILCS

- Our on-site cell culture system ILCS was developed to eliminate most if not all of the above mentioned insecurities in the performance of leukocyte cell cultures. This is particularly important for multi-center trials.
- For ILCS is based upon a whole blood culture technique, its conditions resemble those found in vivo as close as possible in vitro.
- Furthermore, the overall test procedure was ultimately simplified so that virtually anybody allowed to draw blood is able to perform this highly standardised method and therefore obtain data of maximal reliability.

The advantages of ILCS

1. ILCS is performed as a "bed-side" test, i.e. at the site of blood-drawing.
2. ILCS prevents most of the artefacts known from the usual leukocyte culture techniques by eliminating manipulation as well as cell culture onset delay (caused by storing, shipping and preparing the blood over hours).
3. ILCS is a whole-blood culture system (culture conditions "like in the body").
4. The cells are cultured in a vessel reducing non-specific plastic adherence of the leukocytes substantially. This prevents unintended, artificial cellular activation and helps to avoid false positive or negative results.
5. The read-outs are endpoints of physiologically induced cellular activities (cytokines, enzymes, soluble receptors, receptor antagonists etc.). In addition, these are allowed to be released freely by the cells without the necessity of adding blocking reagents (as necessary in intra-cellular cytokine detection in flow-cytometry).
6. The hands-on time for the whole ILCS culture procedure is less than 15 min.
7. ILCS works outside specialised labs and with simple and inexpensive devices.
8. It can be performed by anybody allowed to draw blood.
9. Very small amounts of blood are needed: 3 ml suffice to perform 3 differently stimulated cultures (compared to 25-50 ml for isolated leukocyte cultures).
10. The supernatant of each single culture allows the determination of a whole series of cytokines or other activity markers of interest.
11. Despite providing data of much higher quality, ILCS is far less expensive than the traditional leukocyte culture techniques.
12. Only two steps are needed to perform ILCS: drawing blood and stopping the culture simply by inserting a plug. This ensures a maximum of standardisation and thus reproducibility.

ILCS OVERVIEW

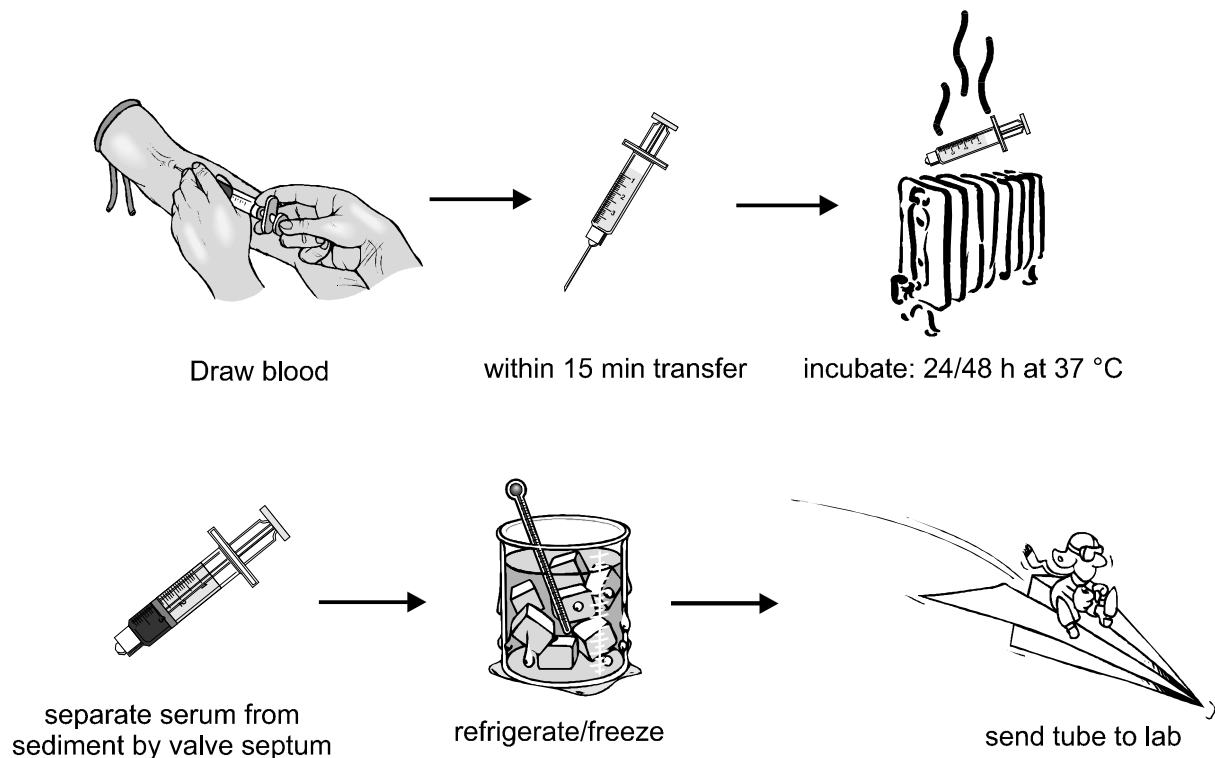
The main features

FACT	ADVANTAGE
Whole blood culture technique	closest to in vivo conditions
Tests leukocyte function, not CD markers	efficacy instead of morphology
Culture starts without any delay	no changes in cell activity
No cell activation by plastic adherence	no background activity without stimulus
Precise termination of cytokine synthesis	stop within seconds
Needs less than 15 mins of hands-on time	short term kinetics possible
Works with 1 or 2 ml of blood	increases patients´ compliance
Ultimately standardized	no lab to lab variations

System requirements

FACT	ADVANTAGE
No cell preparation or manipulation	no handling artefacts
No cell culture lab required	performable wherever current (or even a battery) is available
No need for cell culture experience	performance independent from experienced lab staff
Low cost system	fits into any budget

The Cartoon Flow-Chart
of the
Instant Leukocyte Culture System



Scientific Data

Data from 5 clinical trials demonstrate the outstanding value of the newly developed whole-blood on-site leukocyte culture system (ILCS). Many different mediators were chosen to test leukocyte functions, namely $\text{TNF}\alpha$, $\text{TGF}\beta$, G-CSF, $\text{IFN}\gamma$, IL-5, IL-6, IL-10, IL-12, Elastase and several chemokines (IL-8, Rantes, MCP-1). In addition we can determine the expression and activation of COX-1 and COX-2 in our ILCS tubes.

For some samples of test data see the figures on the following pages.

Fig. 1+2 show the data of 3 different healthy subjects taking the trial medication "FB" (mono-substance) 3 times (at 0; 5 and 10 hrs of the observation period). Notice the cumulation of drug effects seen in all 3 volunteers, although to different degrees and with slightly different schedules.

Fig. 1

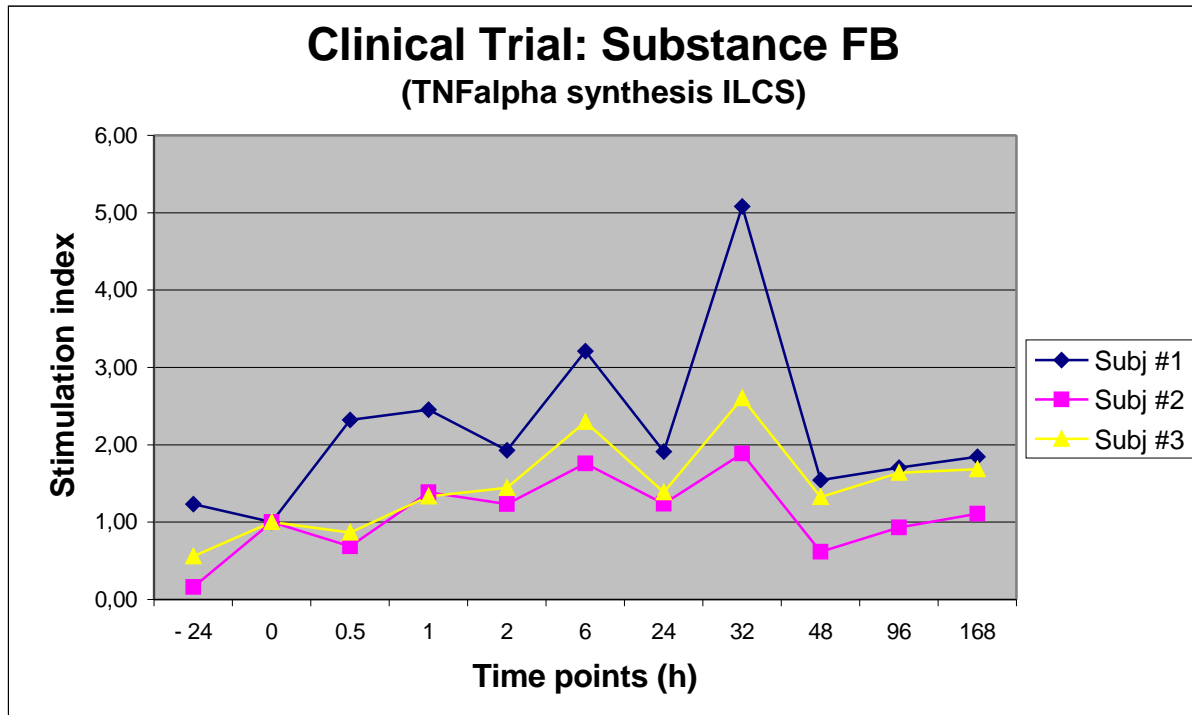


Fig. 2

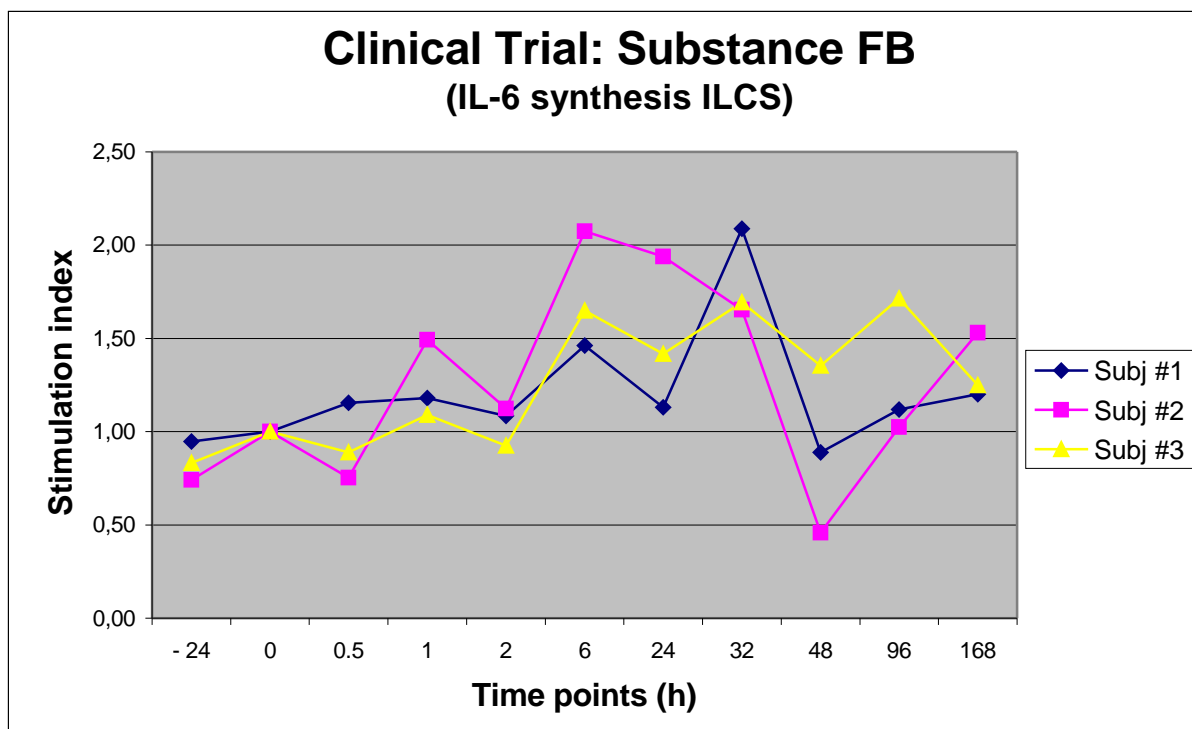


Fig. 3+4 depict results measured in a clinical trial using a complex homeopathic preparation. The drug was applied 3 times daily for 3 consecutive days, starting 1 hr before blood donation at day 0. Note the extremely low levels of IL-6 in the non-stimulated cultures (Fig. 3), nevertheless demonstrating the drug effects as precisely as the stimulated culture does (Fig. 4).

Fig. 3

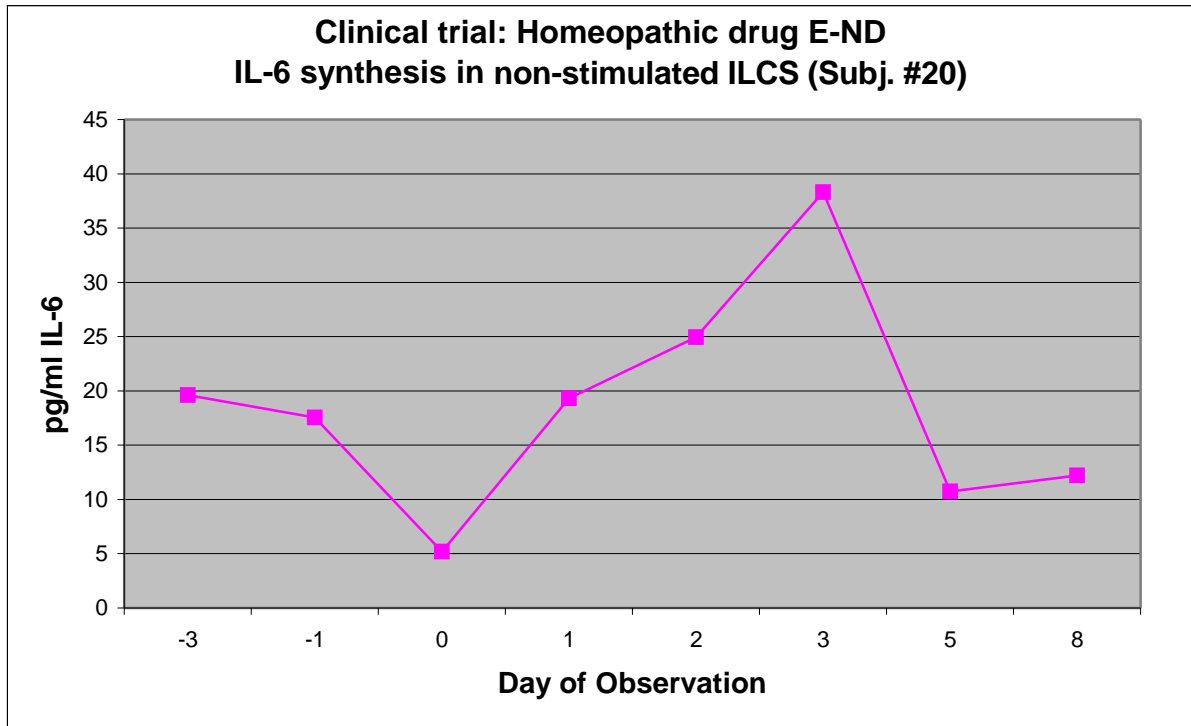
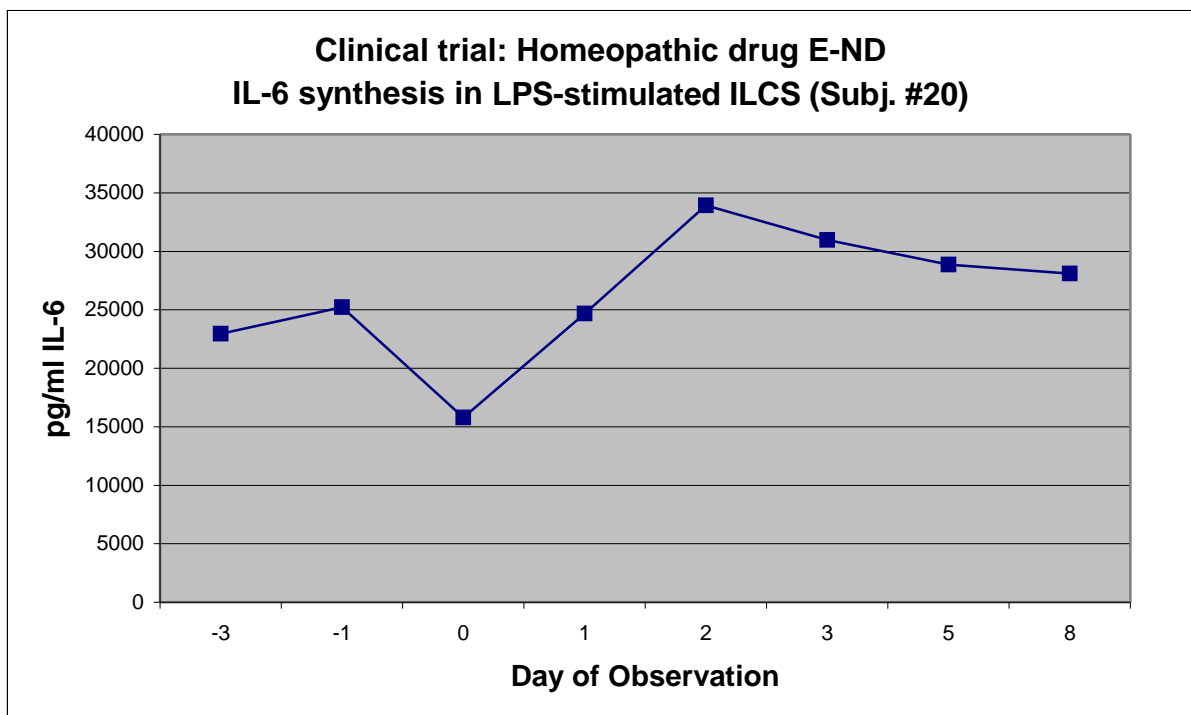


Fig. 4



Another effect frequently seen in clinical trials is the inter-individual difference regarding the reaction of immune systems of different volunteers or patients towards a drug. The example in Fig. 5 shows the response in three volunteers (A – C) towards an NSAID. Note the variability in time course and peak height.

Fig. 5

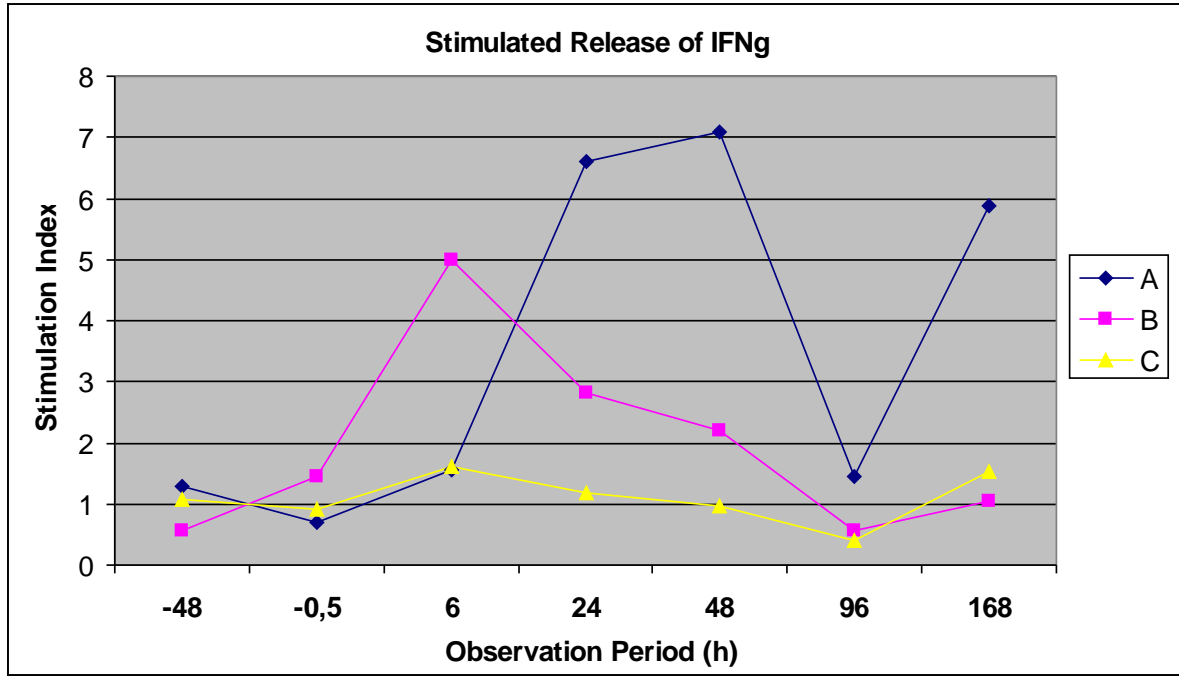
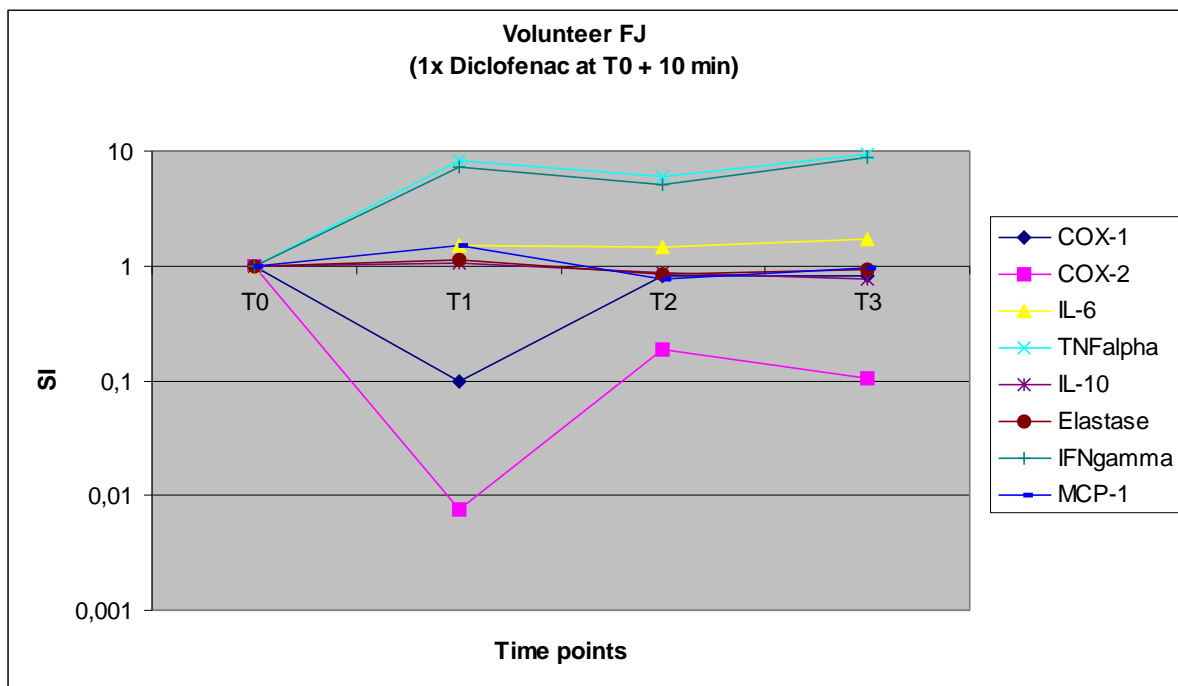


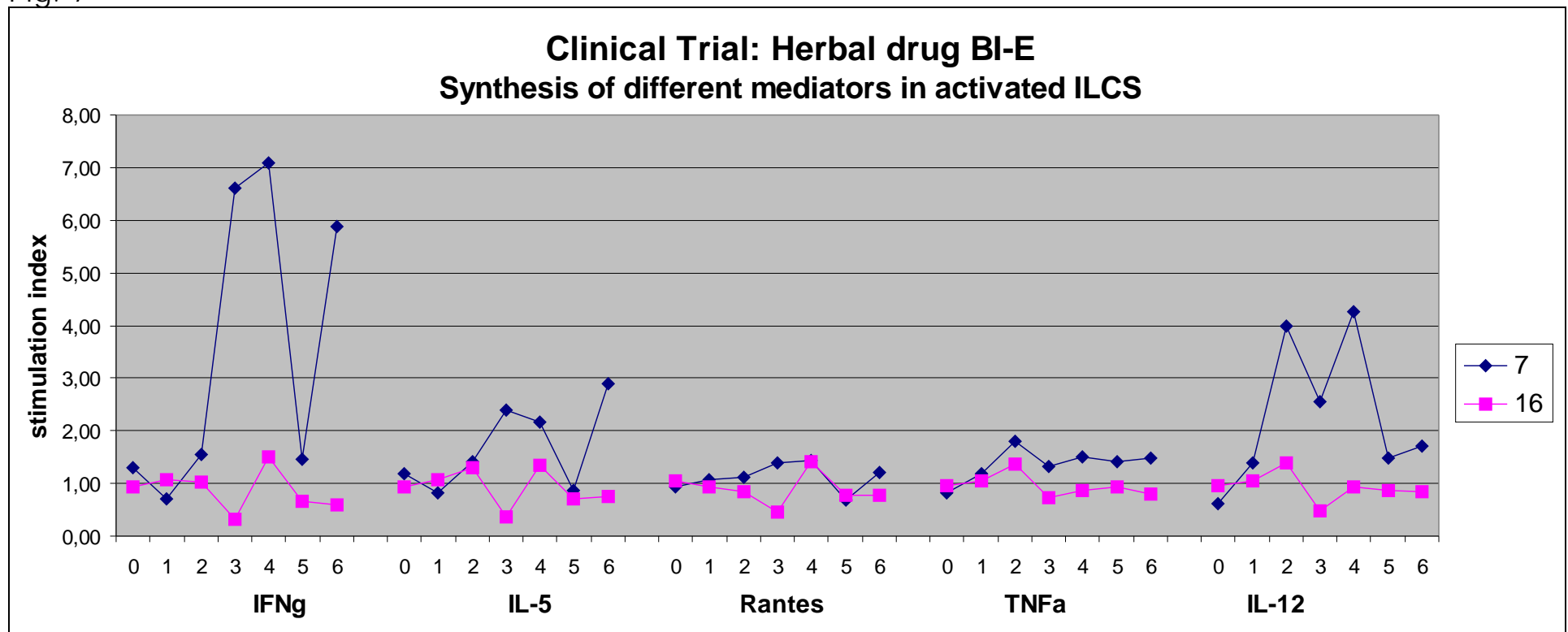
Fig. 6 shows an example from a trial with Diclofenac as control substance. The cytokines show little effects except for $TNF\alpha$ and $IFN\gamma$, both of which are clearly stimulated by suppressing COX-1 (90%) and COX-2 (99%).

Fig. 6

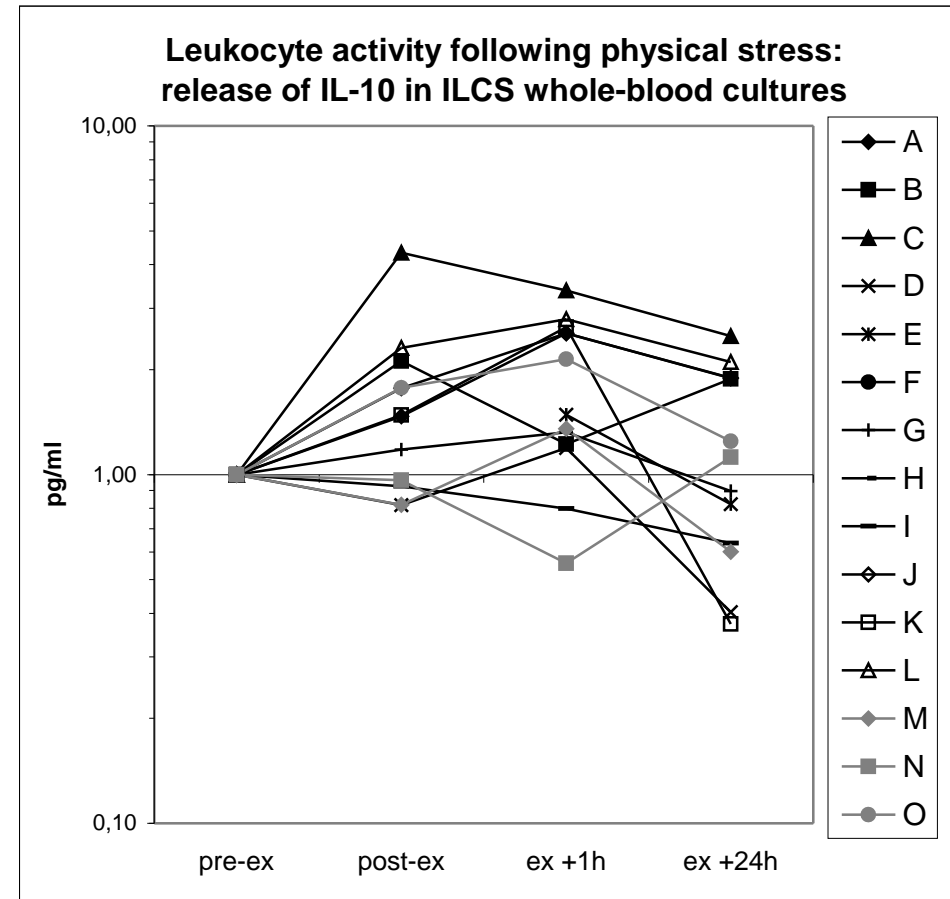
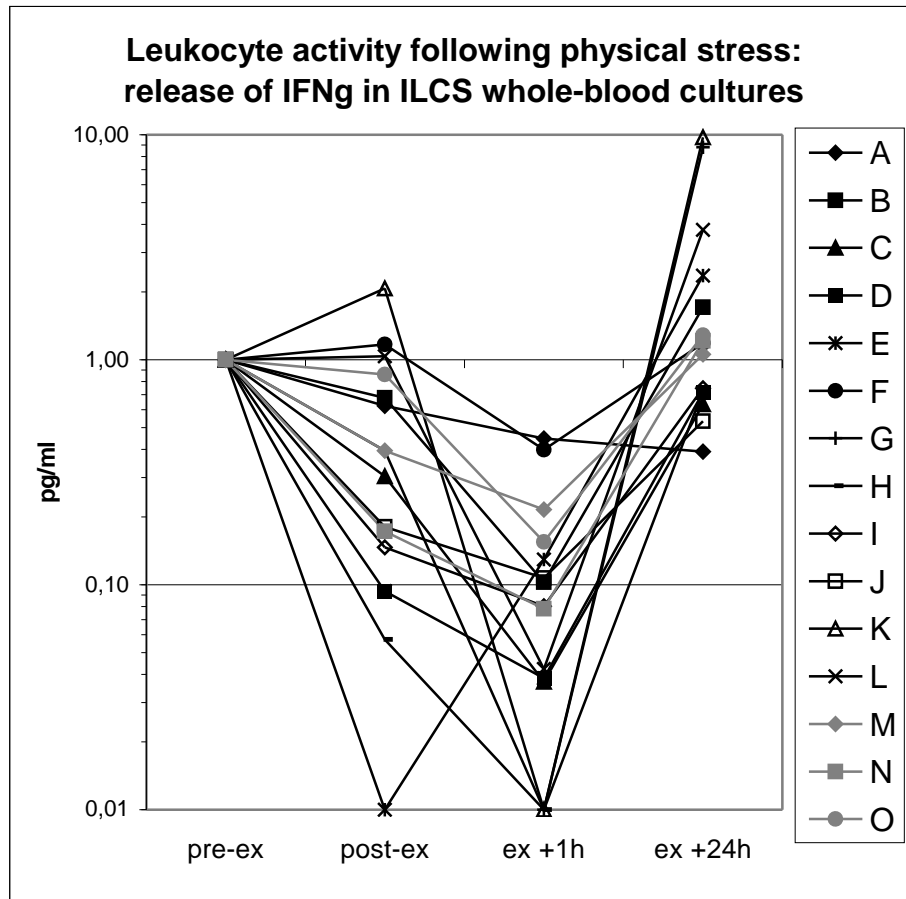


The highly active herbal preparation "BI-E" demonstrated its effects in a clinical trial looking at 5 different cytokines. Drug application was done 3x daily for 3 days, starting immediately before blood donation at day 2. The drug shows withdrawal (rebound) effects in T cells (IFN γ and IL-5). It is worthwhile to mention that the stimulation index was calculated to adjust control levels to 1.0 making it possible to compare highly different absolute concentrations between individual cytokines. For example: IFN γ with donor "7" (blue line) run up to about 25.000 pg/ml whereas IL-5 in this donor only reaches 50 pg/ml, which is at the lower end of the reliable dynamic range of the ELISA used to detect IL-5. Despite this, IL-5 mirrors precisely the effects the drug exerts on the T-lymphocytes as can be seen with IFN γ . This demonstrates best the sensitivity and reliability of ILCS, even if it faces borderline cell activities. Note also how differently both donors respond to the trial drug!

Fig. 7



In another clinical trial ILCS[®] was used to investigate under physiological conditions the immuno-depressive effects of physical exercise on the immune system of 15 healthy volunteers. Cytokine synthesis was measured immediately before and after as well as 1 h and 24 h post exercise. By use of ILCS[®] it was possible to clearly demonstrate a sharp decrease in IFN γ production that coincides with a rise in IL-10 secretion. Note the inter-individual differences in the time course, but also the extent of these responses towards physical stress.



References

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More Information / Ordering / Manufacturer

EDI (Experimental & Diagnostic Immunology) GmbH
Markwiesenstr. 55
D-72770 Reutlingen
GERMANY

T +49(0)7121-434103

F +49(0)7121-491074

E info@edigmbh.de

edigmbh@aol.com

H www.edigmbh.de