

# MISTLETOE TREATMENT INDUCES GM-CSF- AND IL-5 PRODUCTION BY PBMC AND INCREASES BLOOD GRANULOCYTE- AND EOSINOPHIL COUNTS: A PLACEBO CONTROLLED RANDOMIZED STUDY IN HEALTHY SUBJECTS

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## Abstract

**Objective:** Various immunological effects have been reported during application of mistletoe preparations. Because these data are heterogeneous, we performed a placebo controlled study to investigate (1) effects on peripheral granulocyte and eosinophil counts, (2) related cytokine levels and (3) whether effects are related to mistletoe lectin (ML).

**Methods:** 43 volunteers were randomized to receive the mistletoe plant extract Iscador Quercus spezial® (IQ), purified ML, IQ which was depleted from ML, or placebo subcutaneously twice per week for 8 weeks. Weekly, differential blood count and every four weeks spontaneous and IQ- and ML-induced cytokine production by peripheral blood mononuclear cells (PBMC) were analyzed.

**Results:** Leukocyte-, granulocyte-, and eosinophil counts were significantly higher during treatment in the IQ- and ML-groups than in the placebo group. Furthermore, a significant increase of antigen-induced production of GM-CSF, IL-5 and IFN $\gamma$  by PBMC was observed in the IQ- and ML-group but not in the groups receiving ML-depleted IQ or placebo. Severe side effects did not occur in any of the subjects. **Conclusions:** Treatment with IQ or ML stimulates the production of GM-CSF, IL-5 and IFN $\gamma$  by PBMC, and this is accompanied by an increase of eosinophil- and granulocyte-counts. These observations may, therefore, open rational therapeutic indications for mistletoe extracts.

**Key words:** mistletoe lectin, T-helper 2 response, eosinophils, granulocyte-macrophage-colony stimulating factor

## INTRODUCTION

Approximately 60% of patients with cancer use complementary therapies in Germany and two thirds of

them receive mistletoe preparations [1] which first have been suggested 1920 from Rudolf Steiner [2]. However, despite numerous studies have been performed since then, the efficacy of mistletoe extracts in anticancer therapy is still controversially discussed [3, 4]. Nevertheless, it became evident in the last 15-20 years that mistletoe extracts, which consist of different components such as mistletoe lectins (ML-1, -2 and -3), viscotoxins, oligo- and polysaccharides, exert immunomodulatory properties in vivo and in vitro affecting both, the innate as well as the specific immune system [5-10].

Subcutaneous mistletoe application is well tolerated [11-13], and severe side effects are very rare [14]. During exposure, dose-dependent local inflammatory reactions at the injection site, sub febrile temperatures and a mild to moderate eosinophilia in the differential blood count may occur [11-13]. Since interleukin (IL)-5 is one of the most potent factors inducing eosinophilia [15, 16] and is mainly produced by T-helper (TH) type 2 cells [17, 18], it was assumed that mistletoe extracts may also influence T-helper cell subtypes. In contrast, TH type 1 cells, which are involved in cytotoxic and delayed type hypersensitivity reactions, produce interferon-gamma (IFN $\gamma$ ) or tumor necrosis factor-beta (TNF $\beta$ ). During intravenous application of mistletoe extracts an increase of granulocyte count has been reported [19]. We therefore included in the analysis of immunological parameters the measurement of granulocyte-macrophage-colony stimulating factor (GM-CSF), being important for the release of granulocytes and eosinophils from bone marrow [20, 21]. In order to prove this hypothesis and to investigate, whether this effects can be attributed to ML alone, we conducted a randomized, placebo controlled study, exposing healthy individuals to IQ, pure ML, IQ which was depleted from ML, and placebo.

## SUBJECTS AND METHODS

Forty-three healthy subjects were recruited among students and physicians of the University Hospital Freiburg, Germany. Exclusion criteria were: (1) an acute or chronic illness (2) abnormalities in blood sed-

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imentation rate, differential blood count (eosinophils  $> 300/\mu\text{l}$ ), aminotransferases (alanine transferase [ALT] and  $\gamma$ -glutamyl transferase [gGT]) or protein electrophoresis, (3) pregnancy, (4) body mass index  $< 20$  or  $> 28\text{kg}/\text{m}^2$ , (5) smoking  $> 20$  cigarettes per day or alcohol intake  $> 20$  g per day (6) drug abuse and (7) prior application of mistletoe preparations. The study was approved by the local ethics committee. Informed consent was obtained from all subjects before onset of the study.

#### STUDY MEDICATION

Iscador preparations are aqueous, sterile whole-plant extracts of *Viscum album* L. (Iscador information for Health professionals, Verein für Krebsforschung, Arlesheim, Switzerland, 2003). Iscador Quercus spezial® (IQ), which is derived from mistletoes growing on oak trees, is standardized to its total lectin content. Depletion and isolation of ML from IQ was performed by affinity chromatography with immobilised  $\alpha_1$ -acid glycoprotein which binds to all three isoforms of ML.

The antiproliferative activities of the isolated ML, of the depleted preparation and the whole extract IQ were quantified on Molt4 cells [22]. The activities of the preparations corresponded exactly to the ML content measured by ELISA [23] (data not shown). One ampoule (1ml) of 5mg IQ, the highest concentration used in the study, contained 428 ng ML and 10.7  $\mu\text{g}$  viscotoxins. One ampoule (1 ml) of the isolated ML in equivalent concentration to IQ 5 mg had 401 ng ML, viscotoxins were not detectable. The ML-depleted IQ-preparation contained 22 ng ML/ml and 10  $\mu\text{g}$  viscotoxins/ml. Placebo was a physiologic saline solution. Numbered, identical ampoules of IQ, ML, ML-depleted IQ and placebo were provided by Weleda Company (Schwäbisch Gmünd, Germany) in identical boxes according to the randomization scheme and the injection schedule.

#### STUDY DESIGN

The study was prospective, randomized and double blinded. The allocation was concealed; the randomization list was compiled by an external center (Karl and Veronica Carstens-Foundation). During the 8-weeks study period, 1 ml of the respective preparation was injected subcutaneously twice per week at intervals of 3 and 4 days in increasing doses (week 1 = 0.01 and 0.1 mg, week 2 = 0.1 and 1 mg, week 3 and 4 = 1 mg, week 5 = 2.5 mg, week 6 = 2.5 and 5mg and week 7 and 8 = 5mg). The doses of IQ, ML and ML-depleted IQ were equivalent.

#### DIFFERENTIAL BLOOD COUNT AND EOSINOPHIL CATIONIC PROTEIN (ECP)

At baseline and at weekly intervals 24h after the injection differential blood count was determined. Biweekly ECP was measured with standard methods. Analyzes were performed concealed. All these parameters were determined in the quality controlled laboratory (good laboratory practice, GLP) of the University Hospital Freiburg.

#### CYTOKINE PRODUCTION

Heparinized blood was taken from the individuals before exposure and at week 4 and 8. Peripheral blood mononuclear cells were isolated within the first 24h after drawing the blood by Ficoll-Hypaque centrifugation.

For cytokine production,  $5 \times 10^5$  PBMC were cultured with IQ and pure ML in different concentrations found to give optimal results in previous experiments (IQ: 100 and 10  $\mu\text{g}/\text{ml}$ , pure ML: 10, 1, 0.1  $\mu\text{g}/\text{ml}$ ) for seven days at 37° C in a humidified atmosphere (5%  $\text{CO}_2$ ) in 24-well culture plates. Culture supernatants were collected at day seven and kept frozen at minus 20°C until quantitative cytokine determination. GM-CSF as well as the type 1 cytokine IFN $\gamma$  and the type 2 cytokine IL-5 were determined as recently described [24]. Measurements were performed in duplicates.

Cytokine release into the supernatants was considered positive above concentrations of 100 pg/ml for GM-CSF and IL-5, and 500 pg/ml for IFN $\gamma$ .

Antigen concentrations revealing the highest cytokine production were included into further statistical analysis. All analyses were performed blinded.

#### TOLERABILITY AND SAFETY PARAMETERS

Tolerability was estimated on a 4-point Likert scale (bad, moderate, good, excellent). Safety parameters (electrolytes, urea, creatinine, aminotransferases, creatin kinase, lactate dehydrogenase, glucose) were measured at baseline and after 2, 4 and 8 weeks.

#### STATISTICAL ANALYSIS

Treatment effects were estimated by fitting generalised linear models to the data. Essentially, we tested whether there is a shift in the mean response between any two treatment groups. For this, we assumed the course in time of each outcome parameter to be mixed of a piecewise linear part (week 0 to 4, week 4 to 8) and a quadratic term (starting at week 4). The serial correlation was assumed to be exponential within time. Baseline values and a four-level treatment effect were included in the model as linearly acting covariables, Outcome data was taken to the logarithm if necessary to obtain normality. The presented p-values were based on appropriate F-tests and were corrected for multiplicity by the Bonferroni-Holm-procedure for each outcome. All results were adjusted for baseline values to make the groups comparable and ensure that any group differences cannot be attributed to baseline differences.

#### RESULTS

##### BASELINE CHARACTERISTICS, SAFETY PARAMETERS AND TOLERABILITY

According to randomization, 10 subjects received IQ (age 26+6 years), 11 pure ML (age 27  $\pm$  5 years), 9 ML-depleted IQ (age 27  $\pm$  6 years), and 13 placebo (age 27  $\pm$  4 years). There were no significant differences in age, sex, body mass index, frequency of allergies and initial laboratory parameters (for leukocyte

counts  $p=0.09$ , for all other parameters  $p>0.1$ ). All 43 participants completed the study. None of the safety parameters was significantly influenced during the study (data not shown). Good or excellent tolerability was stated by 90% of the subjects receiving IQ, 91% receiving ML and 100% receiving the ML-depleted preparation or placebo. Moderate tolerability was reported from 10% and 9 % of the subjects in the IQ- and the ML-group respectively.

#### DIFFERENTIAL BLOOD COUNT AND ECP

As shown in Table 1 there was an initial increase in the general leukocyte counts in subjects receiving IQ or pure ML. Leukocytes were significantly higher in the IQ group and the ML group compared to placebo (versus IQ,  $p = 0.007$ , versus ML,  $p = 0.002$ ).

These differences could be attributed to an increase of granulocytes and eosinophils in the subjects exposed to IQ or pure ML (Table 1). The granulocyte counts increased in week 2 in the IQ- and the ML-group and were significantly different as compared to placebo (versus IQ,  $p = 0.006$ , versus ML,  $p = 0.0006$ ). However, in both groups the granulocyte counts decreased to baseline levels in week 8. Eosinophil counts increased week 4-6 during IQ and ML application and were also significantly higher than during application of placebo (versus IQ  $p=0.007$ , ver-

sus placebo,  $p=0.0002$ , Table 1). Accordingly, ECP increased in week 6 ( $p=0.001$  for IQ and  $p=0.002$  for ML versus placebo, Table 1). The lymphocyte counts remained constant.

Furthermore, in the IQ and ML-group total leukocyte counts ( $p = 0.01$  and  $p=0.006$ ), granulocyte counts ( $p = 0.04$  and  $p = 0.007$ ), eosinophil counts ( $p = 0.05$  and  $p = 0.003$ ) and ECP ( $p = 0.03$  and  $p = 0.04$ ) were significantly higher than in the group receiving ML-depleted IQ (Table 1). Between the group receiving ML-depleted IQ and placebo there was no significant difference (Table 1).

#### ANALYSIS OF GM-CSF

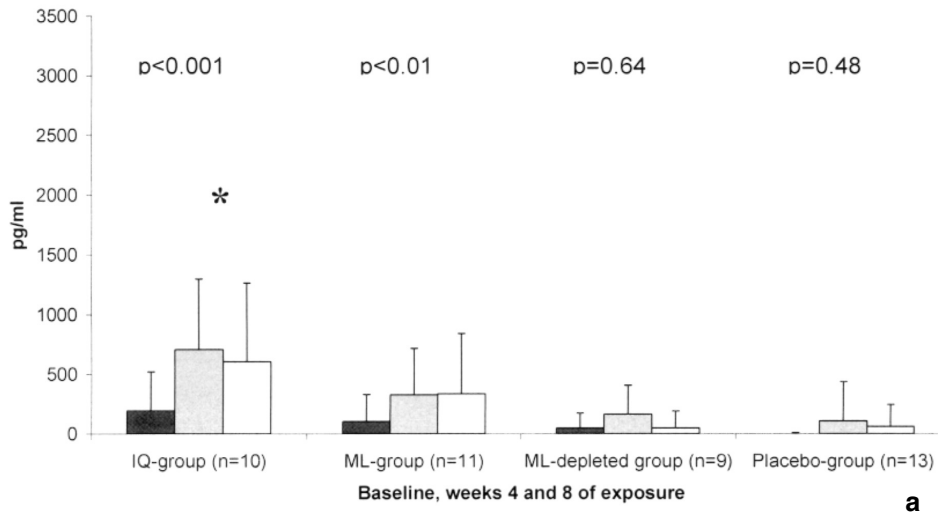
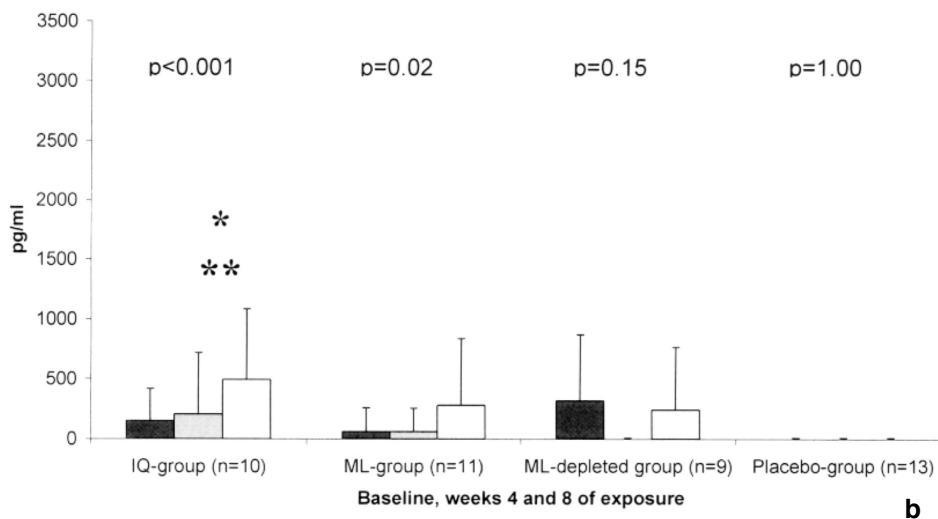
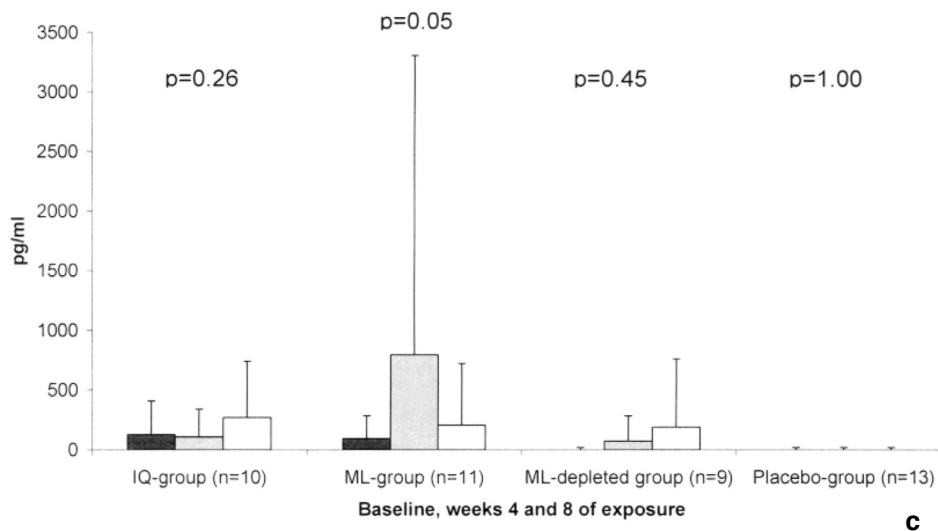
Only few subjects in all groups had a detectable GM-CSF production at baseline. Thus, there were no significant differences between groups at baseline. There was a significant increase of GM-CSF production by PBMC from subjects of the IQ- and ML-group after in vitro stimulation with IQ or ML (Fig. 1a, b). This increase was in the IQ group significantly different to the placebo- and ML-depleted IQ-group (IQ-induced GM-CSF-production: IQ- vs. placebo:  $p = 0.02$ , IQ- vs. IQ-ML:  $p = 0.02$ ; ML-induced GM-CSF-production: IQ- vs. placebo  $p = 0.04$ , IQ- vs. IQ-ML  $p = 0.07$ ). Between the ML-group and placebo or IQ-ML the difference did not reach statistical significance

Table 1. Differential blood counts and eosinophil cationic protein (means  $\pm$  standard deviation) in the course of 8 weeks treatment with IQ (n = 10), ML (n = 11), ML-depleted IQ (n = 9) and placebo (n = 13)

IQ = Iscador Quercus® (Weleda Company, Schwäbisch Gmünd, Germany), ML = mistletoe lectin derived from IQ, IQ-ML = IQ, depleted from ML, n.d. = not done

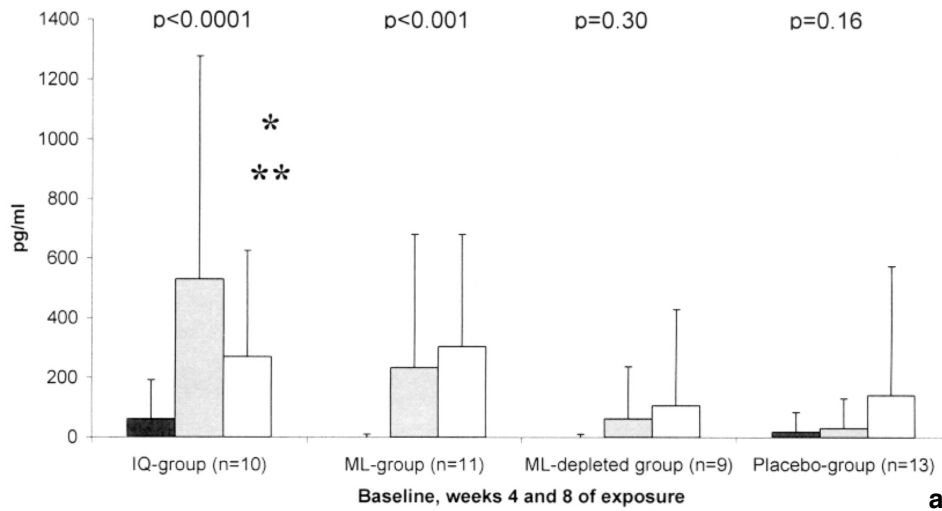
\*  $p<0.01$  versus placebo, \*\*  $p<0.001$  versus placebo \*\*\*  $p\leq 0.05$  versus ML-depleted IQ, \*\*\*\*  $p<0.01$  versus ML-depleted IQ

		Week				
		Baseline	2	4	6	8
<b>Leukocytes</b> (x 1000/ $\mu$ l)						
	IQ * ***	7.9 ( $\pm$ 2.1)	9.1 ( $\pm$ 2.0)	8.2 ( $\pm$ 1.8)	7.5 ( $\pm$ 1.4)	6.9 ( $\pm$ 1.4)
	ML * ****	7.1 ( $\pm$ 1.7)	9.1 ( $\pm$ 2.2)	7.8 ( $\pm$ 1.3)	8.3 ( $\pm$ 1.3)	6.8 ( $\pm$ 0.9)
	IQ -ML	7.7 ( $\pm$ 1.9)	6.6 ( $\pm$ 2.1)	6.7 ( $\pm$ 1.9)	7.4 ( $\pm$ 1.9)	6.6 ( $\pm$ 1.6)
	Placebo	6.2 ( $\pm$ 1.0)	6.6 ( $\pm$ 2.2)	6.3 ( $\pm$ 1.5)	6.2 ( $\pm$ 1.4)	6.2 ( $\pm$ 1.0)
<b>Granulocytes</b> (x 1000/ $\mu$ l)						
	IQ * ***	4.7 ( $\pm$ 1.7)	5.8 ( $\pm$ 2.0)	4.9 ( $\pm$ 1.3)	4.2 ( $\pm$ 1.1)	3.9 ( $\pm$ 0.9)
	ML** ****	4.4 ( $\pm$ 1.5)	5.7 ( $\pm$ 1.5)	4.8 ( $\pm$ 1.1)	5.3 ( $\pm$ 1.4)	4.0 ( $\pm$ 0.9)
	IQ -ML	4.9 ( $\pm$ 1.9)	4.0 ( $\pm$ 1.9)	4.0 ( $\pm$ 1.5)	4.6 ( $\pm$ 1.7)	4.0 ( $\pm$ 1.3)
	Placebo	3.6 ( $\pm$ 0.8)	4.0 ( $\pm$ 1.9)	3.6 ( $\pm$ 1.2)	3.5 ( $\pm$ 1.0)	3.5 ( $\pm$ 0.9)
<b>Lymphocytes</b> (x 1000/ $\mu$ l)						
	IQ	2.4 ( $\pm$ 0.6)	2.4 ( $\pm$ 0.5)	2.4 ( $\pm$ 0.9)	2.4 ( $\pm$ 0.6)	2.2 ( $\pm$ 0.6)
	ML	2.1 ( $\pm$ 0.5)	2.1 ( $\pm$ 0.7)	2.1 ( $\pm$ 0.4)	2.2 ( $\pm$ 0.3)	2.0 ( $\pm$ 0.5)
	IQ -ML	2.1 ( $\pm$ 0.7)	1.9 ( $\pm$ 0.4)	2.0 ( $\pm$ 0.5)	2.1 ( $\pm$ 0.6)	2.0 ( $\pm$ 0.6)
	Placebo	2.0 ( $\pm$ 0.5)	2.0 ( $\pm$ 0.3)	2.1 ( $\pm$ 0.5)	2.1 ( $\pm$ 0.4)	2.1 ( $\pm$ 0.5)
<b>Eosinophils</b> (x 1000/ $\mu$ l)						
	IQ * ***	0.2 ( $\pm$ 0.2)	0.1 ( $\pm$ 0.1)	0.3 ( $\pm$ 0.1)	0.3 ( $\pm$ 0.2)	0.2 ( $\pm$ 0.1)
	ML** ****	0.1 ( $\pm$ 0.1)	0.1 ( $\pm$ 0.1)	0.2 ( $\pm$ 0.1)	0.3 ( $\pm$ 0.1)	0.3 ( $\pm$ 0.1)
	IQ -ML	0.1 ( $\pm$ 0.0)	0.1 ( $\pm$ 0.0)	0.1 ( $\pm$ 0.0)	0.2 ( $\pm$ 0.1)	0.2 ( $\pm$ 0.1)
	Placebo	0.1 ( $\pm$ 0.1)	0.1 ( $\pm$ 0.1)	0.1 ( $\pm$ 0.1)	0.1 ( $\pm$ 0.1)	0.2 ( $\pm$ 0.1)
<b>Eosinophil cationic protein</b> ( $\mu$ g/l)						
	IQ * ***	13 ( $\pm$ 20)	n.d.	15 ( $\pm$ 11)	30 ( $\pm$ 20)	15 ( $\pm$ 10)
	ML * ***	10 ( $\pm$ 7)	n.d.	14 ( $\pm$ 9)	25 ( $\pm$ 20)	21 ( $\pm$ 13)
	IQ -ML	10 ( $\pm$ 4)	n.d.	11 ( $\pm$ 4)	15 ( $\pm$ 3)	14 ( $\pm$ 10)
	Placebo	12 ( $\pm$ 10)	n.d.	12 ( $\pm$ 10)	12 ( $\pm$ 8)	14 ( $\pm$ 10)

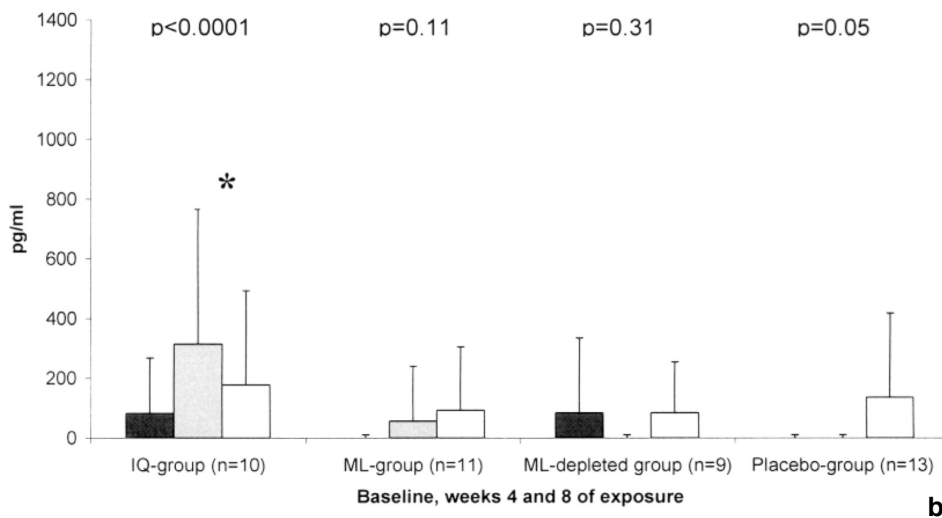
**IQ induced GM-CSF production by PBMC****ML induced GM-CSF production by PBMC****spontaneous GM-CSF production by PBMC**

*Fig. 1.* IQ- induced (a), ML- induced (b), and spontaneous (c) GM-CSF-production by PBMC from healthy subjects exposed to IQ, pure ML, ML-depleted IQ and placebo (means and SD). ■ before exposure, ■ after 4 weeks, □ after 8 weeks. P-values in the figures indicate the intra-group differences; inter-group differences: \*  $p < 0.05$  versus placebo, \*\*  $p < 0.05$  versus IQ depleted of ML.

**IQ induced IL-5 production by PBMC**



**ML induced IL-5 production by PBMC**



**spontaneous IL-5 production by PBMC**

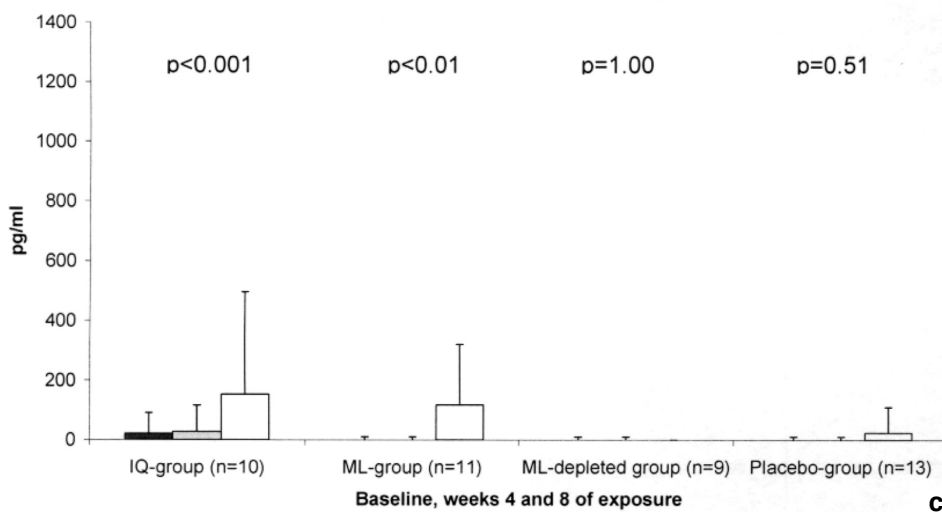
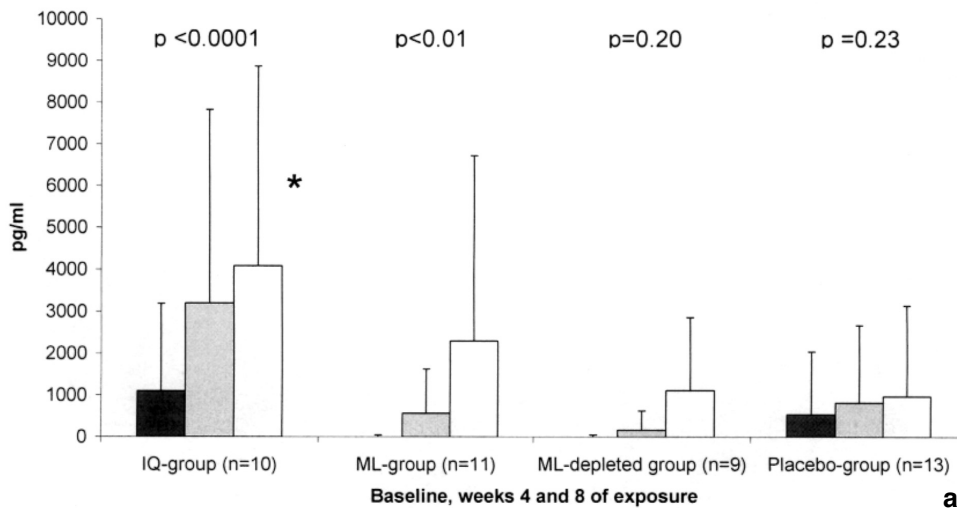


Fig. 2. IQ- induced (a), ML- induced (b), and spontaneous (c) IL-5-production by PBMC from healthy subjects exposed to IQ, pure ML, ML-depleted IQ and placebo (means and SD). ■ before exposure, ■ after 4 weeks, □ after 8 weeks. P-values in the figures indicate the intra-group differences; inter-group differences: \* p ≤ 0.05 versus placebo, \*\* p<0.05 versus IQ depleted of ML.

### IQ induced gIFN production by PBMC



### ML induced gIFN production by PBMC

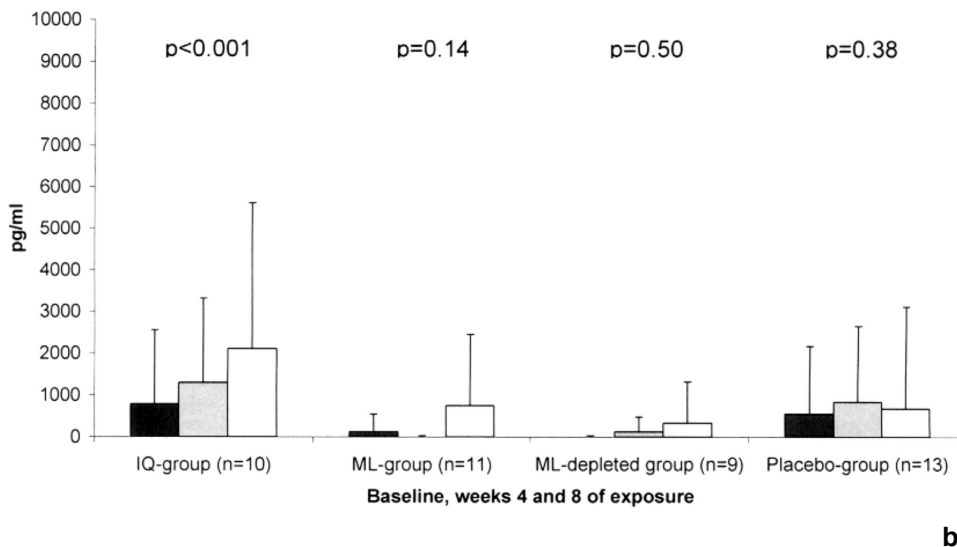


Fig. 3. IQ- induced (a) and ML induced (b) IFN $\gamma$ -production by PBMC from healthy subjects exposed to IQ, pure ML, ML-depleted IQ and placebo (means and SD). ■ before exposure, ■ after 4 weeks, □ after 8 weeks. P-values in the figures indicate the intra-group differences; inter-group differences: \*  $p < 0.05$  versus placebo.

( $p=0.2$  and  $0.3$  respectively). Furthermore, there were no differences between the groups receiving ML-depleted IQ and placebo.

The spontaneous GM-CSF release by PBMC slightly increased in the IQ- ( $p=0.3$ ) and the ML group ( $p=0.05$ ) (Fig. 1c). The differences between the groups were however not significant. The curves for the different concentrations of IQ and ML used for stimulation of PBMC were similar (data not shown).

#### ANALYSIS OF TYPE 1 AND TYPE 2 CYTOKINES

The effects of exposure to the different preparations on the IQ-, the ML-induced, as well as the spontaneous production of IL-5 and IFN $\gamma$  by PBMC are shown in Fig. 2 and 3.

There was a significant increase of the IQ-, the ML-induced- and the spontaneous IL-5 production

by PBMC especially in the IQ- and the ML-group (Fig 2a, b, c). Significant differences were partially found for the IQ- and ML-induced production of IL-5 between subjects of the IQ- and the placebo-group ( $p=0.05$  and  $p=0.03$  respectively) and of the IQ- and the ML-depleted IQ-group ( $p = 0.09$  and  $p = 0.02$  respectively) while the differences between the ML- and placebo- or ML-depleted IQ-group were not significant. The inter-group differences of the spontaneous production of IL-5 were not significant.

Analysis of the IQ- and ML- induced IFN $\gamma$  -production revealed an increase in the IQ- and ML-group (Fig 3a, b). Significant inter-group differences were only found between the IQ- and the placebo-group ( $p = 0.02$  and  $p = 0.08$ , respectively). The spontaneous IFN $\gamma$  -production was unchanged in the IQ and ML group (data not shown).

## DISCUSSION

In this study we showed, that subcutaneous injections of IQ and ML results in a significant enhancement of GM-CSF-production by PBMC in contrast to application of the ML-depleted IQ or placebo (intra-group analysis). Significant inter-group differences in GM-CSF-production by PBMC were, however, only found between the IQ- and the placebo- but not the ML- and the placebo-group. These findings may indicate that the GM-CSF releasing properties of pure ML are enhanced by other components present in the whole IQ-extract. Already in 1991, Schultze et al. showed that GM-CSF is increased in tumour patients receiving ML containing extracts, but this finding had never been taken up in the meantime [5].

Accordingly, granulocyte and eosinophil counts significantly increased in subjects exposed to IQ or ML, hereby confirming our previous assumption [12], that eosinophilia, which is quite frequently observed during mistletoe therapy, has to be related to ML. In contrast, lymphocyte counts were not affected. Similar observations were reported by Heiny et al [25] in patients with breast cancer during palliative chemotherapy being additively treated with an ML containing mistletoe extract. Interestingly, stimulated GM-CSF production is still increased in week 8, while granulocyte and eosinophil counts have already returned back to normal. As it is well known, that antibodies against ML occur after 4-8 weeks of treatment with ML containing mistletoe preparations [8], it can be supposed, that these antibodies block the immune response in vivo. Higher ML doses may have been required to maintain higher cell counts, and, indeed, it was shown that higher ML concentrations as used in this study may prolong the effect at least on eosinophil counts [12, 13]. In the serum samples, all T-cell-related cytokines were negative. It is however well known, that sera are not a suitable source for the determination of T-cell cytokines (24) due to their low concentrations. This speaks not against the postulated effects because it may take place locally or in the bone marrow.

One can, therefore, conclude that mistletoe extracts influence to some extent the TH1/TH2-balance as already postulated by other authors [9, 26]. Whether this effect can be related to the presumed efficacy in tumour diseases remains, however, still an open question. Nevertheless, an increase in TH1-activity has been postulated to be beneficial in tumour response, but there is also evidence that type 2 reactions and blood eosinophils may be associated with a better prognosis in different types of tumours [27, 28]. Indeed, IL-5 induced eosinophils are fully functional and have been demonstrated to kill antibody-coated schistosomula from *Schistosoma mansoni* and antibody-coated tumour cells [15]. While IL-5 is the most potent regulator of eosinophilia and appears to act on more mature progenitors causing them to proliferate and to differentiate into mature effector cells, GM-CSF regulates eosinophil development rather at a much earlier stage expanding the number of eosinophil-committed precursor cells [29]. Additionally, GM-CSF exerts most of its activities on progenitors of granulocytes and monocytes. It acts directly as a

growth factor for these cells, prolongs their life span and augments their functional capacity. The GM-CSF releasing properties of mistletoe extracts would explain the beneficial effect on granulocyte count and -function reported by several authors [7, 10, 25].

Furthermore, GM-CSF is an important immunopotentiating factor. By expanding the number of potent antigen-presenting cells (such as dendritic cells in the periphery) as well as augmenting the antigen presenting ability of mature macrophages, GM-CSF can greatly enhance the ability of the host to respond to antigens [30, 31]. Interestingly, preliminary studies indicate that – in vitro – mistletoe extracts lead to a pronounced maturation of dendritic cells [32].

The increase of GM-CSF production during exposure to ML containing mistletoe extracts seems for us, therefore, to be of particular relevance because it may open a new therapeutic field, namely conditions, in which the release of GM-CSF is desired. We are aware, that this hypothesis may be somewhat provocative and that the present study is hampered by the low number of individuals in each group; but nevertheless it seems to justify further controlled studies to see, for instance, whether in tumour patients under chemotherapy application of these extracts may protect against pronounced leukopenia with its consecutive complications, as it has been shown for the direct application of GM-CSF [30, 33, 34] – hereby taking advantage additionally of the beneficial influence of these extracts on further side effects of chemotherapy as well as on life quality outlined by several studies [for lit. rev. see ref. 4].

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