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Induction of anti-mistletoe lectin antibodies in relation to different mistletoe extracts

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Mistletoe extracts are frequently applied in adjuvant cancer treatment. The mistletoe lectins are especially suggested to mediate an antitumorous effect. During treatment with mistletoe lectin-rich extracts, anti-mistletoe lectin antibodies preferentially of the immunoglobulin G type are produced against mistletoe lectin (ML)-1. Interestingly, after application of mistletoe extracts containing natural micelles, anti-mistletoe lectin antibodies of the immunoglobulin G as well as of the immunoglobulin E type were induced in parallel, suggesting that the nature and preparation of the antigens within the extract modifies immune responses. Anti-mistletoe lectin antibodies were shown to neutralize the cytotoxic effect of mistletoe lectin on peripheral blood mononuclear cells *in vitro*. Thus, the mode of application of these extracts seems to be of importance with respect to the therapeutic effect.

Keywords: Mistletoe extracts, cytotoxicity, anti-mistletoe lectin antibodies, immune response.

Introduction

Mistletoe extracts are used for adjuvant cancer treatment, especially in Germany [1,2]. Different components have been isolated and characterized but one glycoprotein, the mistletoe lectin-I, has been reported to be responsible for the observed immunostimulatory properties [3]. However, there are reports demonstrating stimulatory effects on immunocompetent cells induced by other components such as oligo- and polysaccharides, small but unidentified peptides or vesicular substances [4–6]. In 1981, it has was shown by Franz *et al.* [7] that, in rabbits, anti-lectin antibodies were produced after mistletoe application. Stettin *et al.* [8] were able to demonstrate anti-mistletoe lectin-I antibodies in the sera of mistletoe-treated tumour patients. These studies were further extended to show whether this kind of humoral reactivity is dependent on the composition of the extract used.

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Detection of anti-mistletoe lectin-I antibodies

Sera from 23 tumour patients who were treated subcutaneously with an aqueous mistletoe extract (*Helixor* Mali) were studied with respect to anti-mistletoe lectin-I antibodies. Six patients were treated for up to 6 months (receiving 1–30 mg *Helixor* Mali), seven patients were treated for up to 2 years (dosage range 50–100 mg *Helixor* Mali) and 10 patients were treated for more than 2 years (dosage range 100–200 mg *Helixor* Mali). As demonstrated by enzyme linked immunosorbent assay (ELISA), anti-mistletoe lectin-I and anti-*Helixor* Mali antibodies of the immunoglobulin G type were produced in all these patients and the activity was correlated with the dosage of *Helixor* Mali and the length of the therapy (Fig. 1). These antibodies belonged mainly to the immunoglobulin G1, G2 and G4 subclasses, while immunoglobulin G3 anti-mistletoe lectin-I antibodies were present in only four out of the 23 patients. In 39% (n = 9), antibodies were also of the immunoglobulin A-type, while immunoglobulin M antibodies could not be found. Sera from untreated tumour patients and patients suffering from other disorders, including allergies, and healthy controls were negative [8].

From immunodiffusion studies, it became evident that the major antibody activity of the patient's sera was directed against mistletoe lectin-I. However, a further precipitation line could be detected when the sera were tested against the mistletoe extract *Helixor* Mali, suggesting that another antigen present in this mistletoe extract interacts with immunocompetent cells. It could be further shown by Western blotting that these anti-mistletoe lectin-I positive sera reacted with mistletoe lectin-I as well as another component (about 45 kD) present in the mistletoe extract *Helixor* Mali. However, following absorption studies these bands disappeared, indicating that the *Helixor* Mali-related bands were lectin-associated [8].

Further investigations were performed to obtain more information about antibody production in mistletoe-

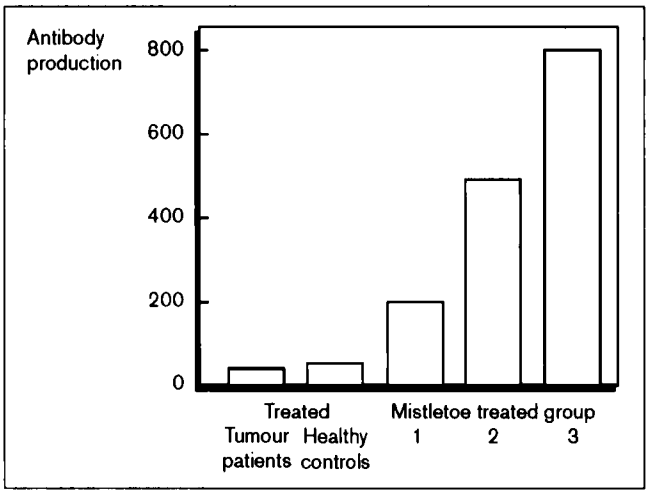

Figure 1. Anti-mistletoe lectin antibody production of the immunoglobulin G type in the sera from untreated tumour patients ($n = 20$), healthy controls ($n = 26$) and mistletoe extract (*Helixor* Mali)-treated tumour patients. Group 1, treated for up to 6 months ($n = 6$); group 2, treated for up to 2 years ($n = 7$); group 3, treated for more than 2 years ($n = 10$). Antibody units were measured as optical density.

treated individuals. From preliminary studies it became evident that patients treated with a mistletoe lectin-I-free preparation, containing only minute amounts of the other lectins, mistletoe lectin-II and -3, produced only low titres of anti-lectin antibodies. Patients, however, receiving mistletoe extracts containing natural micelles and a high amount of lectins for more than 2 years produced very high anti-lectin antibody titres of the immunoglobulin G type and especially of the immunoglobulin E type (about 60%). In these 25 patients, antibodies of the immunoglobulin A type were produced with similar frequency compared to the patients treated with *Helixor* Mali for more than 2 years (about 40% for both) [9]. These data strongly indicate that the mode of antigen preparation influences the type of immune response.

In vitro effects of anti-mistletoe lectin-I antibodies

Mistletoe lectin-I exerts cytotoxic effects on normal lymphocytes or phytohemagglutinine (PHA)-stimulated lymphocytes from healthy controls. Preincubation of mistletoe lectin-I with anti-mistletoe lectin-I antibodies abolished the inhibitory effect of mistletoe lectin-I on PHA-stimulated lymphocytes from healthy donors in a concentration range between 15 pg/ml and 8 ng/ml. At higher concentrations, the cytotoxic effect was only partially neutralized by the antibodies [8]. Similar observations were made when PHA-stimulated lymphocytes from healthy controls were incubated with a fraction of *Helixor* Mali derived by ultrafiltration, containing components with a molecular weight of more than 50 kD. The cytotoxic effect of this fraction was abolished by the addition of anti-mistletoe lectin-I positive sera [10].

Further studies on the lymphocyte proliferation of 25 *Helixor* Mali-treated tumour patients showed that only in the presence of anti-mistletoe lectin-I antibodies did the same fraction of *Helixor* Mali-containing components with a molecular weight of more than 50 kD induce mitogenesis in the lymphocyte cultures of four out of nine patients treated for up to 2 years (without co-stimulation) and in five patients who were treated for more than 2 years (only in the presence of PHA-co-stimulation). Another fraction derived from *Helixor* Mali containing components with a molecular weight of less than 10 kD, mainly consisting of viscotoxins, exerted a strong cytotoxic effect on patient lymphocytes which could not be influenced by the anti-mistletoe lectin-I antibodies. A stimulatory effect of this fraction could not be found. These data provide evidence that non-lectin components of *Helixor* Mali are able to induce lymphocyte proliferation.

Conclusions

The production of anti-mistletoe lectin-antibodies due to mistletoe therapy has been clearly demonstrated. This stimulatory response could be proven to be lectin-associated. However, another antigen has to be postulated to explain the finding that anti-mistletoe lectin-I-treated extracts were able to induce the lymphocyte proliferation.

Sponsorship

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