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Donor-dependent and dose-dependent variations in the induction of T lymphocyte locomotion in a three-dimensional collagen matrix system by a mistletoe preparation (Iscador)

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Controlled activation of non-specific and specific immune defence mechanisms can beneficially manipulate the host's ability to attack malignant cells. In this context, migration and tissue distribution of immunocompetent cells may be prerequisites for an efficient immune surveillance. The effect of various non-cytotoxic concentrations of the *Viscum album* L. (mistletoe) preparation Iscador QuFrF on the locomotory activity of immunomagnetically isolated human CD4+ and CD8+ T lymphocytes from healthy donors was investigated. Cellular migration was examined within a three-dimensional collagen matrix. Donor-dependent variations in baseline activities of spontaneously locomoting T cells were accompanied by individual response patterns of T cells from different donors in the presence of various concentrations of mistletoe preparation (0.25–2.5 μg/ml). Using the three-dimensional collagen matrix assay an induction of locomotory activity was detected in a highly reproducible fashion although the optimal concentration of mistletoe preparation and the time point of maximal response were individual for each donor. Our data suggest that the direct stimulation of T-cell migration by mistletoe components may modulate the system of immune surveillance and recognition in patients under mistletoe therapy.

Keywords: T lymphocyte, locomotion, collagen matrix, mistletoe preparation.

Introduction

Viscum album L. (mistletoe) extracts are widely used in complementary medicine as an adjuvant therapy in cancer treatment [1,2]. There are numerous data on its immunomodulatory and antitumorous activity [3–6]; however, the precise mode of action of mistletoe components are not yet sufficiently characterized.

Because of the importance of T lymphocyte migration in immune surveillance, we investigated whether the mistletoe extract Iscador QuFrF alters the migratory behaviour of human CD4+ and CD8+ T cells from normal donors in a three-dimensional collagen matrix. This collagen-based migration system was recently developed for the *in vitro* study of cell locomotion in a more physiological environment [7]. Migratory activity of T lymphocytes in the presence or absence of non-cytotoxic concentrations of Iscador QuFrF was quantitatively analysed by assessment of cellular displacement. Results from computer-assisted cell tracking and subsequent data analysis were presented but are, however, beyond the scope of this article [8].

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Materials and methods

Mistletoe extract

Iscador QuFrF, a clinically applied aqueous extract of *Viscum album* (European mistletoe) was provided by Verein für Krebsforschung (Arlesheim, Switzerland). It was produced from plants growing on oak (*Quercus*). The mistletoe preparation was provided as a dilution of 5 mg fresh plant extract/ml and contained about 750 ng lectins/ml [9].

Isolation of human CD4+ and CD8+ T lymphocytes

The cell separation was performed as previously described [7]. In brief, human peripheral mononuclear cells were isolated from heparinized blood of normal donors using density gradient centrifugation with Ficoll-Hypaque (ICN, Meckenheim, Germany). CD4+ and CD8+ T cells were positively selected (10 min, 4°C) using immunomagnetic beads (Dynabeads, Dynal, Hamburg, Germany). Subsequently, the cells were detached from the beads (45 min, room temperature) with polyclonal anti-F(ab') antibodies (Detachabead, Dynal, Hamburg, Germany). More than 98% of the cells were viable as assessed by Trypan Blue exclusion.

Preparation of three-dimensional collagen lattices

For cell migration studies, lymphocytes were mixed with a collagen solution consisting of 1.5 mg/ml type I bovine

dermal collagen (Vitrogen100, Cellon, Strassen, Luxemburg) in Earle's MEM (Flow), adjusted to pH 7.4. This cell suspension (2.5×10^5 cells in 100 µl) was transferred into sterile 96-well flat bottomed microtitre plates (Sarstedt, Newton, NC, USA). After polymerization, the collagen gels were overlaid with 100 µl of RPMI/10% FCS (Gibco BRL, Eggenstein-Leopoldshafen, Germany) or serum-free AIM-V medium (Gibco BRL) containing mistletoe extract at different concentrations.

Lymphocyte migration assay

Lymphocyte migration within the three-dimensional collagen matrix was microscopically recorded using time-lapse video microscopy, as previously described [7]. Cell migration was assessed for 60 min after 24 h, 48 h, 72 h and 96 h of culture in the collagen lattice. During the cultivation of lymphocytes in the collagen, no medium changes were performed.

Evaluation of migratory activity of T lymphocytes within three-dimensional collagen matrices

The percentage of motile cells was calculated from 80 cells randomly selected at the beginning of the analysis. Over a sample period of 60 min, locomoting cells were identified by their displacement from the individual starting point and the percentage of motile cells was calculated (80 cells ≡ 100%).

Statistical analysis

Statistical analysis to test for differences in displacement (Iscador QuFrF-exposed versus control cells) was performed using the paired two-tailed Student's t-test (eight and six independent experiments for CD4+ and CD8+ T lymphocytes, respectively).

Results

Spontaneous migratory activity of human peripheral CD4+ and CD8+ T lymphocytes in three-dimensional collagen lattices: baseline activity of control cells

Freshly isolated T lymphocytes incorporated into the collagen lattice displayed negligible migratory activity. During the first 60 min in the matrix, displacement never exceeded 5% (i.e. not more than four out of 80 cells were spontaneously motile). However, in the course of

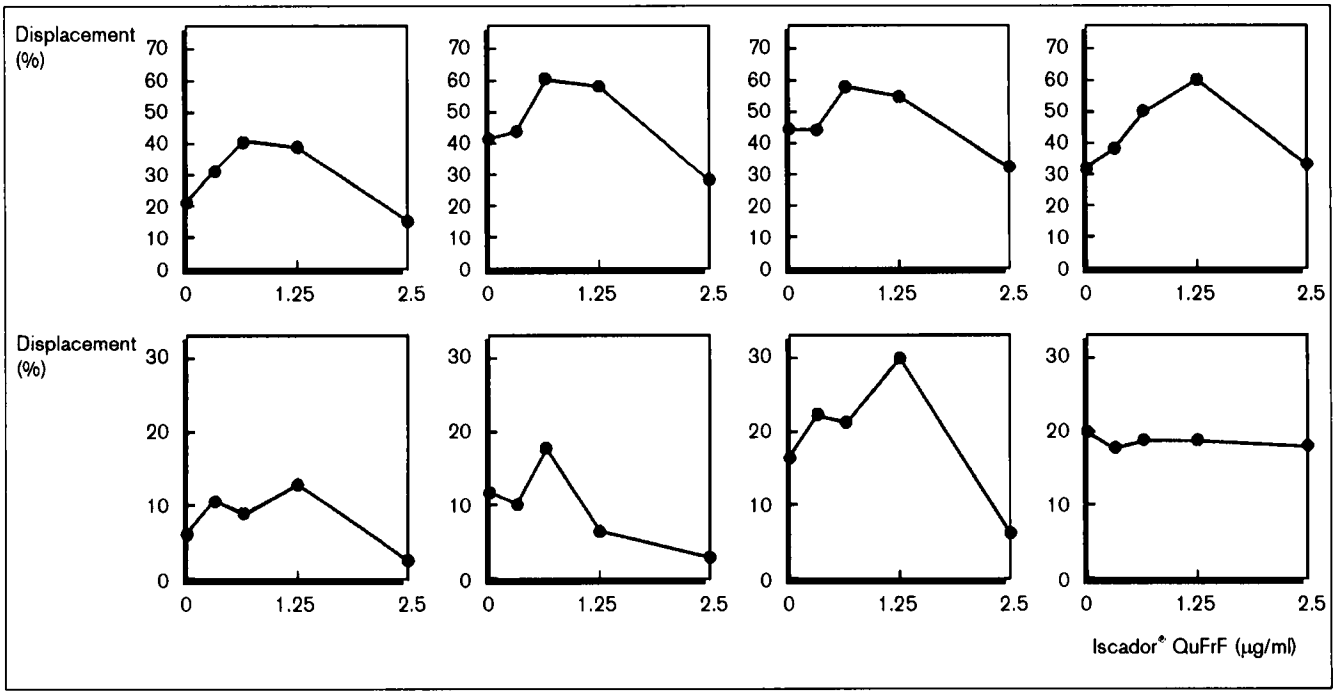

Figure 1. Migratory activity of CD4+ T lymphocytes from two different donors (top: C.M.; bottom: H.G. 1st in Table 1) over the course of 4 days in the presence of different concentrations of Iscador® QuFrF. Cellular migratory activity was expressed as displacement (% moving cells) during an observation period of 1 h at days 1, 2, 3 and 4 of culture in the collagen lattices. For each donor, a panel of concentrations of Iscador® QuFrF was tested (0.25, 0.5, 1.25 and 2.5 µg/ml).

cultivation in the collagen matrix, the migratory activity increased, reached a maximum and declined thereafter. Importantly, the kinetics of development of migratory activity exhibited a high degree of donor-dependent variation (Fig. 1 displays two representative examples). Furthermore, T lymphocytes isolated from the same donor at different time points displayed distinct kinetics of baseline activity (data not shown).

Locomotory activity of human peripheral CD4+ and CD8+ T lymphocytes in three-dimensional collagen matrices in the presence of mistletoe preparation: dose-dependent induction of T-cell migration by Iscador QuFrF

The migratory activity of both CD4+ and CD8+ T lymphocytes of different donors (Table 1) was analysed in the presence of various concentrations of Iscador QuFrF and the following tendencies were observed: (1) both CD4+ and CD8+ T lymphocytes displayed negligible migratory activity at high doses of Iscador QuFrF (25 μg/ml), indicating pronounced inhibitory, presumably cytotoxic, effects as compared to control conditions (data not shown); and (2) at low doses (≤2.5 μg/ml), the mistletoe preparation enhanced the locomotory activity of CD4+ as well as CD8+ T cells. Interestingly, the concentration of Iscador QuFrF which led to the most pronounced induction of locomotory activity, as well as the time point of maximal response, were individual for each donor (Table 1). In some cases, no stimulation of migratory activity was observed (CD4+ T cells, two out of eight donors; CD8+ T cells, one out of six donors; Table 1). In general, CD4+ T lymphocytes were more responsive to the migration-inducing potential of mistletoe extract than CD8+ T lymphocytes (CD4+ T cells, $P = 0.0078$, very significant increase; CD8+ T cells, $P = 0.0189$, marginally significant increase as assessed by the Student's t-test).

The locomotory activity of CD4+ T cells from two different donors in the presence of different concentrations of Iscador QuFrF are shown as representative examples underlining the donor-dependent variations in the observed response patterns (Fig. 1). Furthermore, Fig. 1 clearly demonstrates the dose-dependence in the induction of locomotory activity in T cells.

Table 1. Donor-dependent variations in the induction of locomotory activity in CD4+ and CD8+ T lymphocytes

Donor	Iscador® QuFrF (μg/ml)	Days of cultivation	Increase
CD4+ T cells			
W.R.	0.50	1	×1.97
C.M.	0.50	2	×1.44
C.K. (1st)			<1.2
K.S.	2.50	2	×1.92
H.G. (1st)	1.25	3	×1.85
H.G. (2nd)			<1.2
C.K. (2nd)	1.25	2	×3.00
U.W.	0.50	1	×2.86
CD8+ T cells			
W.R.	0.25	2	×1.61
C.M.	0.25	4	×1.23
H.G. (1st)	0.5	3	×1.39
H.G. (2nd)	1.25	2	×1.46
U.W.			<1.2
F.E.	1.25	2	×1.21

Migratory activity was assessed as displacement (% moving cells) for several donors over a 4-day observation period. The concentration of Iscador® QuFrF resulting in the most pronounced induction as well as the time point of maximal response are indicated for each individual donor. The increase factor was calculated by dividing the displacement of Iscador®-exposed T cells by the displacement of control T cells. Note that T cells isolated from the same donor at different time points displayed distinct response patterns. In two out of eight (CD4+ T cells: C.K. 1st and H.G. 2nd) and one out of six (CD8+ cells: U.W.) experiments, respectively, no induction of locomotory activity was detected (increase less than 1.2-fold).

Discussion

The recruitment of immunocompetent cells into target tissue is a critical prerequisite for immune surveillance. Imitating the encounter of T cells with mistletoe components within a three-dimensional tissue environment, potentially representing the situation of subcutaneous injection of mistletoe preparation, we approached the question whether mistletoe extract may contribute to immunomodulation by altering the locomotory activity of T cells. To this end, highly purified CD4+ and CD8+ T lymphocytes from normal donors were embedded into collagen lattices and exposed to the clinically applied mistletoe preparation Iscador QuFrF. Cell locomotion was monitored using time-lapse videomicroscopy [7] and quantitated by assessment of cellular displacement.

Monitoring T-cell migration over the course of 4 days, we detected that baseline activities of spontaneous T-cell locomotion drastically varied from donor to donor. It is conceivable that the distinct patterns of kinetics seen in the increase and decline of locomotory activity reflect the heterogeneity in CD4+ and CD8+ T cell subpopulations from different donors. Each of these subpopulations can be further subdivided according to functional properties, expression of various surface markers, and their ability to produce a different spectrum of cytokines [10,11]. We have previously published a study indicating that CD4+ and CD8+ T lymphocytes comprise further locomotory subpopulations related to the expression of different CD45 isoforms [12].

Analysis of displacement of T cells in the presence of various concentrations of Iscador QuFrF revealed a migration-inducing potential of this mistletoe extract. Both the dose of mistletoe extract that led to the most pronounced induction of locomotory activity and the time point of maximal induction were individual for each donor. In general, the induction of locomotion in CD4+ T lymphocytes was more pronounced than the induction in CD8+ T lymphocytes as assessed by statistical analysis. In three out of 14 experiments, no recruitment of T cells in the presence of Iscador QuFrF was detected. However, we cannot rule out that the optimal concentration of Iscador was not applied in these experiments.

Thus, the donor-dependent variations, with respect to both concentration of Iscador QuFrF that led to the most pronounced induction of locomotory activity in T cells and the time point of maximal response, may be attributed to individual donor compositions of T-cell populations and the cells' distinct responses to mistletoe components.

In conclusion, our data suggest that the examination of T-cell locomotion within physiological matrices in the presence of mistletoe extracts may provide a novel tool for unravelling the potential role of mistletoe components in the modulation of the locomotory behaviour of immune cells although pronounced donor-dependent variations in baseline activity were seen. Migratory-enhancing effects could be attributed to Iscador QuFrF in a dose-dependent manner.

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