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Interleukin-12 is associated with the in vivo anti-tumor effect of mistletoe extracts in B16 mouse melanoma

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Abstract

Viscum album (VA) preparations consist of aqueous extracts of different types of lectins of mistletoe. VA exert cytotoxic and immunomodulatory properties that may be relevant for the inhibition of tumor growth. We addressed the effects of VA preparation VA Qu FrF on growth of B16F1 melanoma implanted in mice and on proliferation and cytokine synthesis of splenocytes. In C57BL6 mice, inhibition of tumor growth by VA was associated with an enhancement of splenocyte proliferation and with an up-regulation of IL-12 secretion. In IL-12-deficient strain of mice the inhibition of melanoma growth by VA and the splenocyte proliferation were abrogated. Results from the present study strongly suggest a crucial role of IL-12 in the anti-tumor properties of VA extracts.

Keywords: Mistletoe; Melanoma; IL-12; Immunomodulation

1. Introduction

Viscum album (VA) extract preparations consist of aqueous preparation of European mistletoe plant. Biologically active components of VA extracts include Mistletoe lectins (ML) and viscotoxins. VA extracts also contain several other substances including amino acids, polysaccharides, and lipids. ML I, II, and, III belong to the ribosome-inactivating protein (RIP) family of type II, such as highly toxic ricin and abrin [1]. RIP of type II are composed of an *N*-glycosidase (A chain) and a galactoside-recognizing lectin (B-chain) connected by a disulfide bridge [2]. The treatment with VA extracts or with purified ML has been shown to be associated with tumor regression in several in vivo experimental model of tumoral implantation [3]. The mechanisms underlying the anti-tumoral activity of VA or ML are complex and involve apoptosis, angiogenesis and immunomodulation [4–12]. Similar to other members of RIP II family, ML exert cytotoxic activity towards several tumor cell lines from both human and rodent origins albeit to a lesser extent as compared to ricin [13]. To date, these findings have not supported the development of ML or ML-derived molecules for chemotherapy regimen in cancer patients [14,15]. More recently, several studies have clearly demonstrated that signaling by VA

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extracts and purified ML induce activation of transcription and secretion of pro-inflammatory cytokines such as IL-1, IL-6 and, TNFα in human PBMC, and endothelial cells [6,16]. Other studies have suggested that VA extracts may induce Th-1 polarization [17]. These promising results have lead several investigators to initiate uncontrolled clinical trials with VA-based regimen in cancer patients [14,15,18]. In the present study, we have investigated the mechanisms underlying the immunomodulatory effects of VA preparation on tumoral growth of B16F1 melanoma implanted in mice. We demonstrate that anti-tumoral effects of VA extracts are mediated by IL-12-dependent pathway.

2. Materials and methods

2.1. Mice and tumor cells

C57BL/6 mice were obtained from IFFA credo laboratory (L'Arbresle, France). B6.129-Il12b^{tmlJm} (IL-12$^{-/-}$) mice [19] with a targeted mutation of the IL-12 gene were originally purchased from the Jackson Laboratory (Bar Harbor, Maine, USA) and then bred in our own colony. Mice were maintained in a pathogen-free environment and used between 8 and 10 weeks of age. The experiments were conducted in accordance with the institutional guidelines and the recommendations for the care and use of laboratory animals put forward by the French Ministry of Agriculture. B16F1, a transplantable spontaneous murine melanoma of C57BL/6 origin was a kind gift of Pr E. Tartour (INSERM U255).

2.2. Viscum album extract

VA (Iscador Qu FrF) preparation was a gift from Weleda AG (Arlesheim, Switzerland). VA consists of aqueous extracts of mitletoe plants growing in oak tree. The preparation (1 ml ampules of 1 mg) contains a standardized concentration of ML and viscotoxins. The VA preparations were tested endotoxin-free, using the Ph.Eur.2.6.14. method E. The concentration of the mistletoe lectins and of the viscotoxins in the applied lot is of 200 ng/ml and 1.4 μg/ml, respectively.

2.3. Tumor model and in vivo treatment regimen

B16F1 melanoma cells (5×10^6 cells/100 μl in PBS) were subcutaneously implanted in the left flank as previously described [3]. Treatment of B16F1-bearing mice was initiated on the day of tumor implantation. The VA regimens consisted of a daily intra-peritoneal injection of various amounts of VA preparation diluted in PBS (T/VA mice). Untreated tumor-bearing mice received the vehicle alone (T/PBS mice). Some of the experiments included mice no tumor implantation and were left untreated (C/PBS). After 7 days of treatment, the subcutaneous tumors were removed and weighed.

2.4. Propidium iodide uptake assay for cell toxicity

Confluent B16F1 were incubated for 48 h with increasing concentrations of VA preparation. Cell death was determined by the uptake of propidium iodide (PI) (Sigma) followed by FACScan analysis as previously described [9].

2.5. Proliferation and cytokine production of splenocytes

Single cell suspension of splenocytes from control (PBS mice) and tumor-bearing mice under VA treatment (VA mice) was prepared as previously described [20]. Cells were then seeded in 96-well plates in triplicates (250×10^3 cells/well) in RPMI medium supplemented with 10% FCS and then incubated for 48 h in the presence or absence of polyclonal mitogen concanavalin A (conA) (1 μg/ml). In other experiments, splenocytes from untreated control mice were also incubated with various concentrations of VA preparation. After the incubation period, the supernatants were collected and were assayed for IL-4, IL-10, IFNγ and IL-12 by ELISA using matched Ab pairs (BD-Pharmingen, Le Pont de Claix, France). Tritiated thymidine (0.5 μCi/well) was then added to assess the proliferation of splenocytes. After an additional 24 h, the splenocyte proliferation was measured as previously described [20].

2.6. Statistical analysis

The statistical analysis was performed using a Statview® software using ANOVA and confirmed by a non-parametric Mann–Whitney test. Statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. VA inhibits the growth of tumor in C57BL6 mice bearing B16 melanoma

We first evaluated the in vivo anti-tumor properties of VA in B16F1 melanoma-bearing mice. In the present study, we selected B16F1 sub-line for the following reasons: its immunogenicity, its rapid growth associated with local involvement without any metastasis and because this subline does not induce any marked modification in the Th balance per se [21–23]. A great variability of the inhibition of tumor growth has been reported with VA extracts in both in vivo and in vitro experiments depending on the cell line investigated, the mouse strains, and the dose and type of VA extract used [3,24]. As illustrated in Fig. 1A, a short-course treatment with low doses of VA (Iscador QuFrF) (20 μg/mouse/day for 7 days) was associated with a