Pergamon

European Journal of Cancer Vol. 32A, No. 3, pp. 447–449, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

0959-8049(95)00541-2

Original Paper

Tumour Necrosis Factor (TNF) Soluble Receptors in Malignant Melanoma: Correlation with Soluble ICAM-1 Levels

J. Viac, C. Vincent, S. Palacio, D. Schmitt and A. Claudy

INSERM U346, Clinique Dermatologique, Hôpital Ed. Herriot, 69437 Lyon 03, France

It has been recently suggested that soluble tumour necrosis factor receptors (sTNF-Rs) may represent prognostic factors in cancer. In malignant melanoma, the intercellular adhesion molecule (ICAM-1) has been described as involved in progression of the disease and is upregulated by TNFα. We report in this study the serum concentrations of sTNF-R1 and sTNF-R2 in 32 patients with primary melanoma and in 21 patients with metastatic melanoma, in correlation with those of soluble ICAM-1 (sICAM-1). Significantly raised sTNF-R1 levels were detected only in patients with metastatic melanoma compared with normal controls ($P < 0.002$), whereas sTNF-R2 levels were increased both in primary and metastatic melanoma ($P < 0.001$). The ratio of type 2 to type 1 proteins increased in malignant melanoma compared with the controls but remained constant with the progression of the disease. A correlation between sTNF-Rs and sICAM-1 concentrations in patients' sera was observed in metastatic melanoma. The combined adverse effects of these soluble proteins on normal immune effector functions may contribute to tumour progression.

Key words: malignant melanoma, soluble TNF-R1, soluble TNF-R2, soluble ICAM-1
Eur J Cancer, Vol. 32A, No. 3, pp. 447–449, 1996

INTRODUCTION

TUMOUR NECROSIS factors (TNF) are pleiotropic cytokines that play a major role in inflammation and exert effects on a wide range of target cells including cytolytic activity on tumour cells [1, 2]. The intracellular signals for the response to TNF are provided by two distinct types of cell surface receptors (TNF-Rs), to which TNF binds with high affinity [3]. Truncated forms of these receptors exist as soluble binding proteins which arise from proteolytic cleavage of the membrane bound TNF-Rs and inhibit TNF activity [4]. They have been designated sTNF-R1 (TNFRβ or TR55) and sTNF-R2 (TNFRα or TR75). They are present in human serums and in patients with cancer [5–7]. It has been recently suggested that they may represent prognostic factors in cancer because tumour cells produce and shed large amounts of cell surface proteins and thus escape destruction by TNF [8].

In malignant melanoma, other soluble receptors such as the intercellular adhesion molecule-1 (ICAM-1) seem to be involved in progression of the disease in many cases [9]. Elevated serum levels of ICAM-1 are detected in patients with malignant melanoma and are significantly associated with a reduction in disease-free survival [10–12]. Moreover, soluble ICAM-1 (sICAM-1) shed from melanoma cells is able to inhibit MHC-restricted specific T cell/melanoma interactions *in vitro* [13, 14]. TNFα is a cytokine produced by immune and tumour cells which can induce an upregulation of cell surface ICAM-1 on melanoma cells [15]. Moreover, correlations between increased TNF levels and soluble receptor levels have been reported, suggesting that stimuli producing TNF release also induce shedding of TNF-Rs [16]. The aim of our study was to measure the levels of sTNF-Rs in patients with malignant melanoma and to determine whether these levels correlated with those of sICAM-1 and were involved in disease progression.

Correspondence to J. Viac.
Received 28 Mar. 1995; accepted 4 Jul. 1995.

PATIENTS AND METHODS

Patients and controls

A series of 53 patients with malignant melanoma (27 women and 26 men) ranging in age from 28 to 77 years was included in this study. This group comprised 32 patients with a primary malignant and 21 patients with metastatic melanoma. Serum samples were aliquoted and stored frozen at −20°C.

Sera from age- and sex-matched healthy individuals were included as controls.

Determination of sTNF-R1, sTNF-R2 and sICAM-1

The sera were tested for quantitative determination of soluble receptors using specific enzyme-linked immunosorbent assay (ELISA).

ELISAs were performed in 96-well microtitre plates according to the manufacturer's instructions (R & D systems, Abingdon, U.K.) using diluted (1/20 or 1/40) serum samples (each sample in duplicate). Soluble receptor concentrations were calculated from standard curves generated by standard dilutions of known concentrations (sTNF-R1 and sTNF-R2 ranging from 7.8 to 500 pg/ml; sICAM-1 ranging from 1.56 to 50 ng/ml). The assays were standardised against purified soluble forms of recombinant TNF-R1, TNF-R2 or ICAM-1.

The results were analysed by unpaired Student's *t*-test and correlations evaluated with Spearman's coefficient.

RESULTS

Soluble TNF-Rs in patients' sera

The values of sTNF-R1 were statistically higher in patients with metastatic melanoma (mean: 1849 ± 1132 pg/ml; range 942–5200 pg/ml) compared with the controls (mean: 887 ± 219 pg/ml; range 620–1350 pg/ml) ($P < 0.002$). No significant differences between primary melanoma (mean: 1451 ± 384 pg/ml; range 862–2210 pg/ml) and controls or between primary or metastatic melanoma were noted (Figure 1a).

Soluble TNF-R2 were detected in larger amounts in all serum samples. Their levels were significantly increased in patients with primary melanoma (mean: 5109 ± 2759 pg/ml; range 1900–9710 pg/ml) or metastatic melanoma (mean: 6765 ± 3875 pg/ml; range 2030–12 250 pg/ml) compared with controls (mean: 1926 ± 481 pg/ml; range 1410–2720 pg/ml) ($P < 0.001$). However, no significant difference occurred between primary and malignant melanoma (Figure 1b). The ratio of type 2 to type 1 proteins increased in malignant melanoma (> 3.5) compared with the controls (2.08) but remained constant with the progression of the disease (3.53 in primary melanoma versus 3.67 in metastatic melanoma).

Relationship between sTNF-Rs and sICAM-1 levels in patients' sera

The values of sICAM-1 were statistically higher in patients with primary melanoma (mean: 342 ± 129 ng/ml, range 170–650 ng/ml) and metastatic melanoma (mean: 482 ± 314 ng/ml; range 156–1280 ng/ml) compared with the controls (mean: 178 ± 34 ng/ml; range 153–258 ng/ml) ($P < 0.001$). A significant difference also occured between primary and metastatic melanoma ($P < 0.03$). Soluble ICAM-1 levels correlated with those of sTNF-R1 ($r = 0.646$; $P = 0.002$) and sTNF-R2 ($r = 0.518$; $P = 0.016$) in patients with metastatic melanoma. For these patients, a correlation between sTNF-R1 and sTNF-R2 was also observed ($r = 0.78$; $P = 0.001$).

DISCUSSION

Our results showed that sTNF-Rs levels are increased in malignant melanoma, with type 2 protein levels predominating and significantly raised in primary and metastatic melanoma. Although it was not possible to show a direct relationship with the progression of the disease, it is noteworthy that sTNF-Rs and sICAM-1 were correlated in patients with metastatic melanoma.

Increased serum levels of soluble receptors for TNF have been reported in other malignancies with, in some cases, a predominance of type 2 over type 1 [6]. In patients with malignant melanoma, the tumour cells are not the only source of TNF-Rs since activated immune cells also release TNF-Rs. Recent data indicated that melanoma cell lines exhibit low to moderate TNF-R1 and low but detectable TNF-R2 [17], and that TNF-R1 are critical signaling receptors for TNF action on melanoma cells [18]. It is, therefore, possible that TNF-R1 levels reflect tumour cell load and that TNF-R2 are preferentially linked to chronic activation of the immune system. Indeed, the two TNF-Rs are involved in different functions after TNF binding. Type 1 receptors are mostly responsible for cytotoxic and antiviral activities, and mediate the induction of cellular genes such as *ICAM-1*, whereas TNF-R2 are mostly responsible for the stimulation of T cell

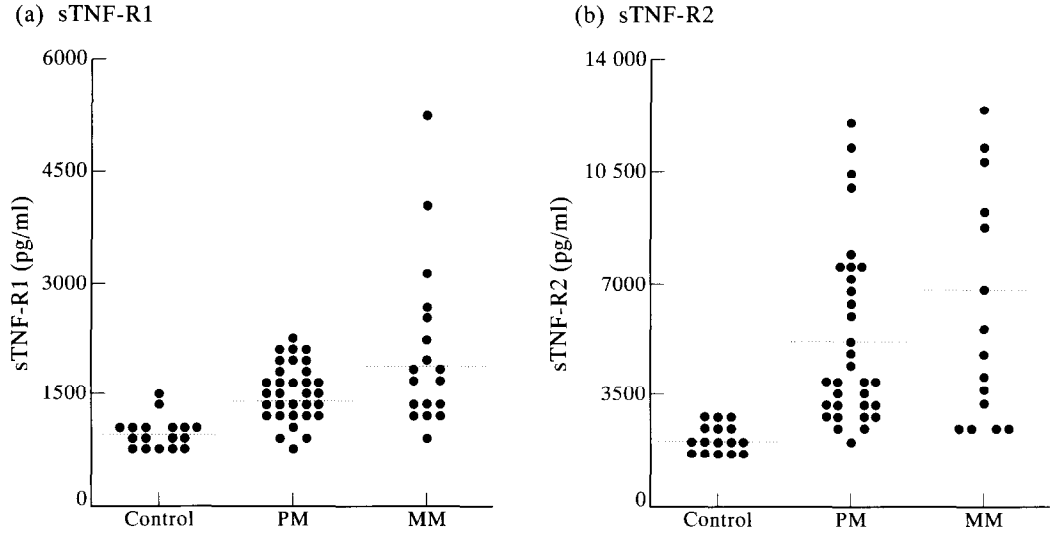

Figure 1. sTNF-Rs in the serum of healthy individuals and patients with primary melanoma (PM) and metastatic melanoma (MM). The data points represent single determinations. The horizontal lines indicate the mean values. (a) sTNF-R1; (b) sTNF-R2.

growth and mediate the induction of genes such as *IL2R* [19]. Moreover, the factors that are responsible for the *in vivo* release of sTNF-Rs are not clearly identified. TNF, IL-2 and interferon are physiological stimuli since administration of these cytokines to cancer patients is followed by increased sTNF-Rs [20, 21]. Among these cytokines, IL-2 and interferon are produced by activated T cells and TNFα by activated macrophages. As TNFα and IFNγ also upregulate ICAM-1, these cytokines may be involved in the host immune response to the tumour and may explain the increased sICAM-1 levels, as already reported [10–12] and the observed correlation between sTNF-Rs and sICAM-1 in metastatic melanoma.

The physiological role of the sTNF-Rs in tumour progression is not clear. High concentrations of sTNF-Rs can inhibit TNF activities and may thus represent a tumour escape mechanism from the destructive effects of TNFα [8]. If so, in metastatic melanoma this adverse effect combined with the ability of sICAM-1 to inhibit MHC-restricted specific T cell/melanoma interactions may dramatically contribute to tumour progression.

1. Tracey KJ, Vlassara H, Cerami A. Cachectin/tumor necrosis factor. *Lancet* 1989, **i**, 1122–1125.
2. Vilcek J, Lee TA. Tumor necrosis factor. *J Biol Chem* 1991, **266**, 7313–7316.
3. Tartaglia LA, Goeddel DV. Two TNF receptors. *Immunol Today* 1992, **13**, 151–153.
4. Nophar Y, Kemper O, Brakebusch C, *et al.* Soluble forms of tumor necrosis factor receptor (TNF-Rs). *EMBO J* 1990, **9**, 3269–3278.
5. Aderka D, Engelmann H, Hornick V, *et al.* Increased serum levels of soluble receptors for tumor necrosis factor in cancer patients. *Cancer Res* 1991, **51**, 5602–5607.
6. Digel WF, Porzsolt F, Schmidt M, Herrmann F, Lesslauer W, Brockhauss M. High levels of circulating soluble receptors for tumor necrosis factor in hairy cell leukemia and B-type chronic lymphocyte leukemia. *J Clin Invest* 1992, **89**, 1690–1693.
7. Denz H, Orth B, Weiss G, *et al.* Serum soluble tumor necrosis factor receptor 55 is increased in patients with haematological neoplasia and is associated with immune activation and weight loss. *Eur J Cancer* 1993, **29A**, 2232–2235.
8. Langkopf F, Atzpodien J. Soluble tumor necrosis factor receptors as prognostic factors in cancer patients. *Lancet* 1994, **344**, 57–58.
9. Hansen NL, Ralfkiaer E, Hou-Jensen K, *et al.* Expression of intercellular adhesion molecule-1 (ICAM-1) in benign naevi and malignant melanoma. *Acta Derm Venereol* 1991, 71, 48–51.
10. Harning R, Mainolfi E, Bystryn JC, Henn M, Merluzzi VJ, Rothlein R. Serum levels of circulating ICAM-1 in human melanoma. *Cancer Res* 1991, **51**, 5003–5005.
11. Altomonte M, Colizzi F, Esposito G, Maio M. Circulating intercellular adhesion molecule 1 as a marker of disease progression in cutaneous melanoma. *N Engl J Med* 1992, **327**, 959.
12. Viac J, Guéniche A, Faure M, Claudy A. Soluble intercellular adhesion molecule 1 and malignant melanoma. *Cancer Lett* 1993, 72, 191–194.
13. Becker CK, Termeer C, Schmidt RE, Bröcker EB. Soluble intercellular adhesion molecule-1 inhibits MHC-restricted specific T cell/tumor interaction. *J Immunol* 1993, **151**, 7224–7232.
14. Koyama S. Immunosuppressive effect of shedding intercellular adhesion molecule 1 antigen on cell-mediated cytotoxicity against tumor cells. *Jpn J Cancer Res* 1994, **85**, 131–134.
15. Temponi M, Romano G, D'Urso CM, Wang Z, Kekish U, Ferrone S. Profile of intercellular adhesion molecule-1 (ICAM-1) synthesized by human melanoma cell lines. *Semin Oncol* 1988, **15**, 595–607.
16. Lantz M, Malik S, Slevin ML, Olsson I. Infusion of tumor necrosis factor (TNF) causes an increase in circulating TNF-binding protein in humans. *Cytokine* 1990, **2**, 402–406.
17. Dekker SK, Vink J, Vermeer BJ, Bruijn JA, Mihm MC, Byers HR. Differential effects of interleukin 1α or tumor necrosis factor α on motility of human melanoma cell lines on fibronectin. *J Invest Dermatol* 1994, **102**, 898–905.
18. Smith DM, Tran HM, Soo VW, *et al.* Enhanced synthesis of tumor necrosis factor-inducible proteins, plasminogen activator inhibitor-2, manganese superoxide dismutase and protein 28/5.6, is selectively triggered by the 55 kDa tumor necrosis factor receptor in human melanoma cells. *J Biol Chem* 1994, **269**, 9898–9905.
19. Tartaglia LA, Goeddel DV, Reynolds C, *et al.* Stimulation of human T-cell proliferation by specific activation of the 75-kDa tumor necrosis factor receptor. *J Immunol* 1993, **151**, 4637–4641.
20. Miles DW, Aderka D, Engelmann H, Wallach D, Balkwill FR. Induction of soluble tumor necrosis factor receptors during treatment with interleukin-2. *Br J Cancer* 1992, **66**, 1195–1199.
21. Landmann R, Keilholz U, Scheibenbogen C, *et al.* Relationship between soluble tumor necrosis factor (TNF) receptors and TNF alpha during immunotherapy with interleukin-2 and or interferon alpha. *Cancer Immunol Immunother* 1994, **38**, 113–118.

Acknowledgements—This work was supported by the Association Vaincre le Mélanome and the Association de la Recherche sur le Cancer.