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The Vitronectin Receptor Alpha-V Beta-3, Contrary to ICAM-1, Is Not Modulated by Interferon-gamma and Tumour Necrosis Factor-alpha on Melanoma Cell Lines*

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A correlation was recently shown between expression of the vitronectin receptor (VnR) and the tumorigenic capacity of cultured human melanoma cell lines. On the other hand, modulation of VnR expression by interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) was observed on different non-melanoma cell lines.

We tested IFN-γ, TNF-α and interleukin-2 (IL-2), which are presumably released by infiltrating leukocytes in the melanoma lesional environment, on three melanoma cell lines. The VnR expression was assessed using FACS analysis and radioimmunolabelling.

The VnR did not show any modulation after treatment with any of the cytokines tested. By contrast, the expression of the intercellular adhesion molecule-1 (ICAM-1), tested as control, on five melanoma cell lines, was greatly enhanced by IFN-γ and TNF-α.

Thus, some host cytokines may preferentially induce melanoma cells to express ICAM-1 (which can increase host cytotoxic response against melanoma), other than the VnR (which instead might contribute to melanoma metastasis). ***Key words: metastasis; adhesion molecules; cytotoxicity.***

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A family of cell adhesion receptors termed integrins has been defined that favour a variety of cell-cell and cell-matrix interactions (1–8). The vitronectin receptor (VnR) is an integrin that mediates a wide spectrum of adhesive cellular interactions, including attachment to vitronectin, von Willebrandt factor, fibrinogen and thrombospondin (9,10). VnR is composed of an alpha subunit (alpha-v) non-covalently associated to a beta subunit (beta-3), although alternative forms of VnR were recently shown, composed of the same alpha-v subunit associated with either a beta-3b, or a beta-5, or a beta-s subunit. Alpha-v beta-3 is responsible for adhesive properties of both non-neoplastic cells such as cultured fibroblasts and human endothelial cells (11–13), and neoplastic cells such as lung cancer cells (14), breast cancer cells (14), glioblastoma multiforme cells (15), and melanoma cells (16).

Malignant melanoma is commonly initiated within the skin and is characterized by a proliferative and invasive growth at the primary site (17). This can ultimately lead to widespread metastatic disease, which is characterized by a multistep process that not only requires cell proliferation, but also depends on the ability of the tumour cells to migrate, and resist host immune functions (18, 19). It has been hypothesized that the malignant phenotype of melanoma and other human tumours is facilitated by tumour cell-matrix interactions. Support for this concept has come from studies indicating that tumour cell surface integrins may be involved in the malignant invasive phenotype of tumour cells (20). In fact, integrin alpha-v beta-3 is commonly found on melanoma cell lines (10) and in vertically invasive primary as well as metastatic melanoma lesions, while it is absent on normal melanocytes, naevi, and surgically treatable horizontal primary melanoma lesions (20).

Since VnR expression on endothelial cells in culture is susceptible to modulation by cytokines such as interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) (21), we investigated the possible modulation of the expression of alpha-v beta-3 in cultured human melanoma cells after treatment with cytokines, including TNF-α, IFN-γ and interleukin-2 (IL-2), which are presumably released by leukocytes infiltrating melanoma (22). As a control, the same cytokines were tested to investigate the expression on the same cell lines of the intercellular adhesion molecule-1 (ICAM-1), which was shown, although using different cell lines and antibodies, to be enhanced by IFN-γ and TNF-α (23). The results demonstrate that the expression of ICAM-1, but not of VnR, is enhanced by IFN-γ and TNF-α.

MATERIALS AND METHODS

Cell lines

Five melanoma cell lines were tested: FO-1, characterized for the lack of HLA class 1 antigen expression (24), Colo 38, not adherent on plastic, and the metastatic cell line MeWo, which was originally established from a lymph node metastasis (25); two further lines were provided by Prof. M. Maio (Aviano, Italy), namely, SK MEL 29 and SK MEL 33. All lines were cultured in RPMI medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, and antibiotics (1% penicillin-streptomycin and 1% gentamycin sulphate), as described previously (26). Tumour cells were cultured in 75 cm^2-tissue culture flasks (COSTAR, Cambridge, MA). On reaching confluence, adherent cells were passaged serially.

Antibodies

Four monoclonal antibodies (all of mouse origin) were used in this study: MAb TP 36.1 (alpha-v), MAb VF 27.263.15 and MAb LM 609 (10) (alpha-v/beta-3 heterodimer), and MAb CL 207.14 (27) which detects ICAM-1 (23). The MAbs were obtained by immunization of mice and then purified from ascitic fluid by sequential precipitation with caprillic acid and ammonium sulphate (28). Then a FITC-conjugated goat antiserum to mouse IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) was used in an

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indirect immunofluorescence assay. Alternatively, the secondary antibody was iodinated using the iodogen method (29) with ^{125}I; the immunoreactive fraction of the radiolabelled antibodies was at least 50%, as measured by the method of Lindmo et al. (30).

Cytokines

Each melanoma cell line was incubated for 72 h with the following: IFN-γ, TNF-α, a combination of IFN-γ and TNF-α (1000 U/ml IFN-γ and 1000 U/ml TNF-α), and IL-2. All such cytokines were obtained from Hoffmann-La Roche Inc., Nutley, NJ, and were diluted in RPMI at 37°C in a 5% CO_2 atmosphere. Human recombinant TNF-α was used at 10-100-1000 U/ml; human recombinant IFN-γ was used at 10-100-1000 U/ml; IL-2 was used at 10-100 U/ml. The cytokine treatment was always performed in complete RPMI.

Fluorocytometric analysis

Cells were detached with PBS/EDTA, washed once in growth medium, then twice in cold wash buffer (PBS/BSA 0.1%). Cells (2×10^5) in 50 µl volumes were incubated on ice for 30 min with 50 µl of primary antibody (neat supernatants, 1/100 dilution of ascites). After washing twice, 50 µl of the FITC-conjugated antiserum was added to the resuspended pellet for 30 min on ice. The cells were washed three times and analyzed by fluorocytometry on a FACScan analyzer (FACScan II, Becton Dickinson, Mountain View, CA).

Indirect radioimmunolabelling

After a cell suspension had been prepared as previously described, the iodinated antibody was used, adding approximately 4×10^5 cpm/well in 50 µl per assay. The assay consisted in 50 µl of cell suspension (containing 4×10^5 cells). The 96-wells-plates were then left in a cold room for 90 min and the cells were washed four times with cold PBS and dried under a hot lamp. The results were analyzed with a gamma-counter chamber.

Controls

The amounts of VnR and ICAM-1 expression were determined by comparison with a negative control (no primary antibody), both in FACS analysis and in radioimmunolabelling. In FACS analysis the control mean fluorescence intensity was set <1 fluorescence units.

RESULTS

Analysis of VnR expression

The VnR, whose expression was studied using MAb such as TP 36.1, VF 27.263.15 and LM 609 (the latter was then discarded), did not show any modulation ($p > 0.05$), either after treatment with TNF-α (Fig. 1), IFN-γ (Fig. 2) and a combination of these two cytokines (data not shown), or after incubation with IL-2 (Fig. 3). The number of observations for each cell line and each treatment was 48.

Analysis of ICAM-1 expression

On the other hand, ICAM-1 expression was increased ($p < 0.05$) after treatment with IFN-γ (Fig. 4) and/or TNF-α ($p < 0.05$; data not shown); by contrast, IL-2 did not affect the ICAM-1 expression ($p > 0.01$) (Fig. 5). The number of observations for each cell line and each treatment was 48.

Controls

When the primary antibody was omitted, negative results were obtained, both in FACS analysis and in radioimmunolabelling.

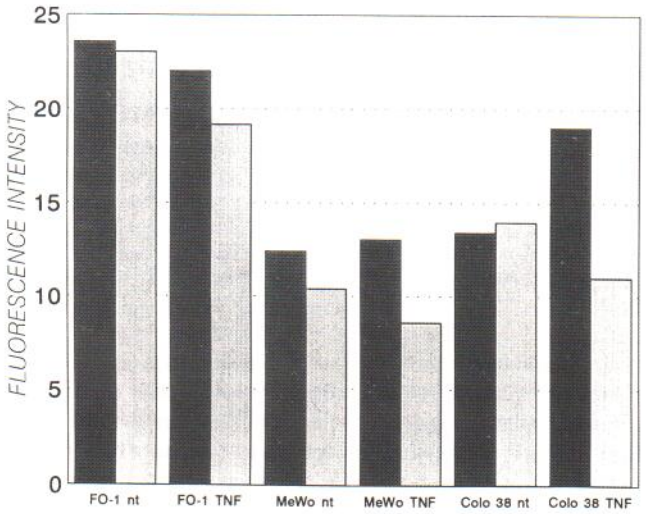

Fig. 1. Modulation of VnR expression in FACS analysis. Expression of VnR (TP 36.1, ■; VF 27.263.15 ▨) by different cultured melanoma cells (FO 1, MeWo and Colo 38) at basal conditions ("nt": not treated) and after treatment with TNF-alpha (1000 U/ml).

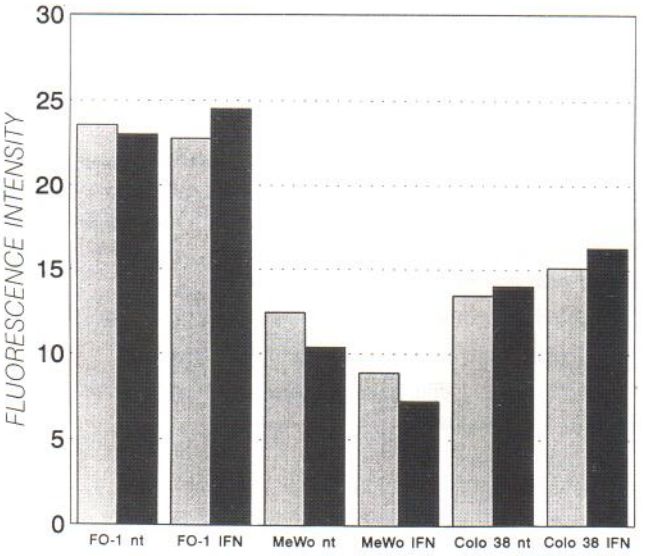

Fig. 2. Modulation of VnR expression in FACS analysis. Expression of VnR (TP 36.1, ▨; VF 27.263.15, ■) by different cultured melanoma cells (FO 1, MeWo and Colo 38) at basal conditions ("nt": not treated) and after treatment with IFN-gamma (100 U/ml). Similar results were obtained even at lower (10 U/ml) and higher (1000 U/ml) concentrations of IFN-gamma.

DISCUSSION

In the present study, we have shown that the cytokines TNF-α, IFN-γ and IL-2 are not able to trigger any modulation of alpha-v beta-3 on different melanoma cell lines. In fact the level of expression of alpha-v beta-3, as investigated by fluorocytometric and radiolabelling analyses, is not modified by any of the tested cytokines (Figs. 1,2,3); similarly, ICAM-1 expression on melanoma cells in culture is not affected after treatment with IL-2 (Fig. 5).

On the other hand, we confirm and extend the data showing the importance of ICAM-1 expression on melanoma cell lines (23) and its inducibility by IFN-γ (Fig. 4) and TNF-α (31).

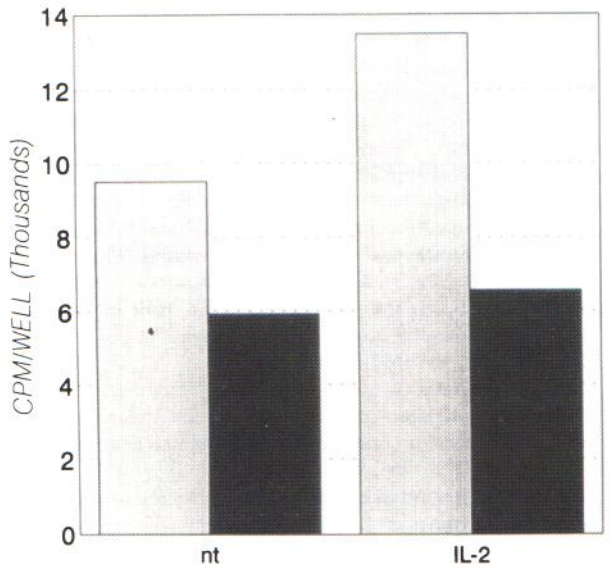

Fig. 3. Modulation of VnR expression in radiolabelling analysis. Comparison between VnR (TP 36.1, ▨; VF 27.263.15, ■) expression on a melanoma cell line (FO 1) at basal conditions ("nt") and after treatment with IL-2 (100 U/ml).

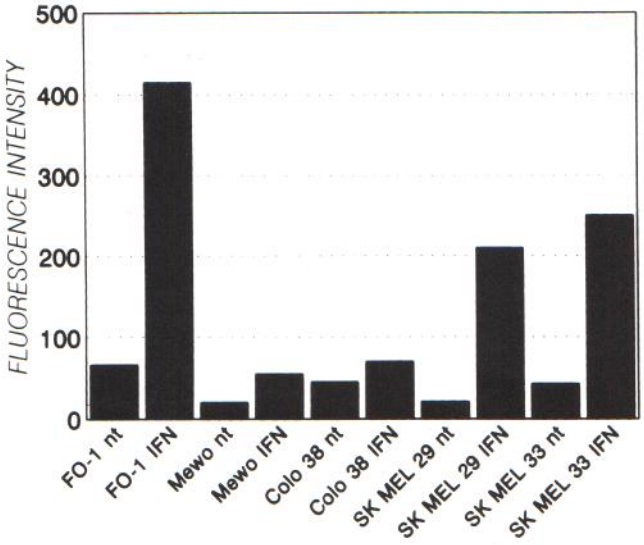

Fig. 4. Modulation of ICAM-1 expression in FACS analysis. Enhancement by cytokines of the expression of ICAM-1 (CL 207.14, ■) by different cultured melanoma cells (FO 1, MeWo, Colo 38, SK MEL 29 and SK MEL 33) incubated with IFN-gamma (1000 U/ml) in comparison with untreated cells ("nt") ($p < 0.05$).

The interaction of LFA-1 on leukocytes with ICAM-1 on tumoral cells appears to be a major functional step in many aspects of specific antigen recognition, cell migration into tissue, and immunologic cytotoxicity (32, 33). ICAM-1, which plays a major role in melanoma biology, is known to be actively induced by IFN-γ and TNF-α. Indeed, in the lesional melanoma environment, IFN-γ is released by cytotoxic T-lymphocytes (CTL) and lymphokine-activated killer (LAK) cells (34–36) beyond others, whereas TNF-α is released by macrophages (37). It is clear that additive and synergistic effects of macrophage- and T-lymphocyte-derived cytokines can greatly enhance ICAM-1 expression on melanoma cells,

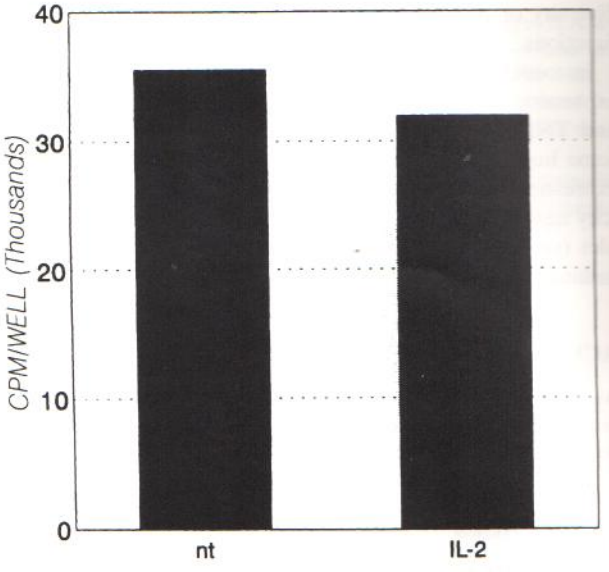

Fig. 5. Modulation of ICAM-1 expression in radiolabelling analysis. (Gamma counter analysis CL 207.14, ■). No significant variation of the expression on the melanoma cell line FO-1 was seen when IL-2 (100 U/ml) was added to the culture medium in comparison with untreated cells ("nt").

and this effect can be a major factor in initiating cytotoxic damage of target cells (32). Since ICAM-1/LFA-1-mediated adhesion appears to contribute to a large number of immune responses by mediating target cell/leukocyte binding, and in particular LAK cells and tumour-infiltrating CTL binding (22), we are tempted to hypothesize that this can be the way the host could try to block melanoma expansion. Support for this point of view comes from the evidence that cultured human melanomas (primary and metastatic) do not release IFN-γ and TNF-α (36), thus, as we show in the present study, not altering ICAM-1 expression; this may be explained as an attempt, for melanoma cells, to escape host defense.

Alternatively, however, ICAM-1 expression on melanoma cells may play a pro-metastasizing role, because the acquisition of ICAM-1 during the process of tumour progression was found to correlate with melanoma malignancy and the development of metastasis (38, 39). Furthermore, cytotoxicity mediated by natural killer and LAK cells could be abrogated either by purified soluble ICAM-1 or by melanoma cell culture supernatants containing shed ICAM-1 (40). In fact, high levels of circulating ICAM-1 were detected in the sera of patients afflicted with advanced stages of melanoma (41).

On the other hand, it is convenient for the host that the cytokines released by infiltrating macrophages (TNF-α) and CTL (IL-2, IFN-γ) do not affect VnR expression, which is a marker of a more malignant (i.e. metastatic) phenotype of melanoma (10, 16, 20); if not, host cells might contribute to the escape of the melanoma cells from immunosurveillance. Alternatively, these results might suggest that the interaction of melanoma cells with the extracellular matrix does not correlate with alpha-v beta-3 expression. The existence of alternative forms of VnR in melanoma cell lines (42), in which the alpha-v subunit is able to associate with another beta subunit which is not beta-3, might explain our findings. As a matter of fact, considerable heterogeneity exists regarding the association of alpha-v with different beta-subunits (42). This

diversity of structure might well be reflected in a diversity of functions.

In conclusion, the present study demonstrates that the expression of ICAM-1, but not of VnR, is enhanced by IFN-γ and TNF-α on melanoma cell lines, possibly suggesting that some host cytokines may be able to teleologically favour the expression of molecules (such as ICAM-1) mediating cytotoxicity against tumour cells, rather than the expression of molecules (such as VnR) mediating the metastasizing potential of melanoma.

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