Soluble Intercellular Adhesion Molecule 1 Is Released by Human Melanoma Cells and Is Associated with Tumor Growth in Nude Mice

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Abstract

We have studied the cytokine regulation of cell surface and soluble intercellular adhesion molecule 1 (ICAM-1) expression on the human melanoma cell line A375M. Unstimulated cells express ICAM-1 on their cell surface but do not secrete significant levels of soluble ICAM-1. Interleukin 1, interleukin 6, tumor necrosis factor, and γ-interferon all increased cell surface expression of ICAM-1. Tumor necrosis factor, interleukin 1, and γ-interferon also caused the release of soluble ICAM-1. The serum of melanoma patients has been reported to contain elevated levels of soluble ICAM-1; however, the source of this ICAM-1 is unclear. The serum from nude mice bearing s.c. human melanoma tumors was found to contain soluble human ICAM-1. ICAM-1 levels showed a positive correlation with tumor weight. The release of ICAM-1 from melanoma tumors, in response to host-derived cytokines, may have relevance to immune recognition of the tumor.

Introduction

Cell adhesion molecules which mediate homotypic and heterotypic cell interactions are thought to have several important roles in tumor progression. They can control tumor cell dissociation from the primary tumor and interactions with host cells during dissemination, adhesion and invasion of metastatic sites. ICAM-1 is originally shown to mediate homotypic lymphocyte aggregation and adhesion to cultured endothelial cells (1, 2). ICAM-1 has subsequently been demonstrated in a number of different cell lineages. Soluble forms of ICAM-1 have been described in normal human serum (3), and elevated levels have been shown in inflammatory diseases (4, 5). ICAM-1 can be found on cells of the melanocyte lineage, preferentially on cells undergoing malignant transformation, its expression correlated with the thickness of the lesion and was predominantly associated with metastases (6, 7). A recent report has described soluble ICAM-1 in the serum of patients with malignant melanoma (8), although the source of soluble ICAM-1, from either the host or the tumor, was unclear.

The nude mouse model is a powerful system with which to study human tumors in vivo. Human xenografts have been reported to maintain histological, molecular, and biochemical characteristics of the tumor of origin (reviewed in ref. 9). In this study, we have investigated the production of soluble ICAM-1 from cytokine-stimulated human melanoma cells in culture and have measured levels of soluble ICAM-1 in the serum of nude mice bearing tumors derived from the same cells.

Materials and Methods

Animals. NCr-nu/nu nude mice, 6 to 8 weeks old, were obtained from the National Cancer Institute Animal program, Frederick, MD. Mice were maintained throughout the experiments under specific-pathogen-free conditions and in accordance with institutional guidelines.

Tumor Lines. The human cell line A375M melanoma, HT29 colon carcinoma, and the SN12-K1 renal carcinoma were routinely cultured as described previously (10). Tumor cells were harvested by exposure to 0.25% trypsin-0.02% EDTA solution, washed twice, and resuspended in test medium. Tumor cells (2 x 10⁶) were seeded in 6-well culture plates in 2 ml of Dulbecco’s modified minimal essential medium containing 10% fetal calf serum. After 24 h, monolayers were washed and 2 ml 10% fetal calf serum-Dulbecco’s modified minimal essential medium with or without cytokines (IL-1, 500 units/ml; TNF, 500 units/ml; IFN-γ, 500 units/ml; IL-6, 200 units/ml) were added. After 72 h supernatants from treated and untreated wells were collected, centrifuged, and frozen at ~80°C. The cell monolayers were washed twice, subjected to two rounds of freeze/thawing, mechanically removed, sonicated to yield a cell lysate, and frozen at ~80°C. Tumor cells were also harvested from separate wells and processed for flow cytometric analysis.

Flow Cytometric Analysis. The expression of ICAM-1 was determined by indirect immunofluorescence using a FACScan analyzer (Becton-Dickinson). Briefly, after incubation with the appropriate primary antibody, cells were washed and incubated with an affinity-purified fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin antiserum (Tecno Genetics, Italy). Results were expressed as percentage of positive cells (after background subtraction) and mean channel fluorescence intensity.

Reagents and Antibodies. The following cytokines were a gift from the indicated sources: rIL-1 (specific activity, 3 x 10⁸ units/mg) from ScIov, Italy; rTNF (specific activity, 8.1 x 10⁸ units/mg) from Basf-Knoll, West Germany; rIFN-γ from NIBSC, United Kingdom, and rIL-6 (specific activity, 1 x 10⁶ units/mg) from British Biotechnology Products, United Kingdom. Antibodies specific for human ICAM-1 (BBIG-11 and BBIG-12) were obtained from British Biotechnology Products, and those to LFA-1 were from J. Ritz, Dana Faber Cancer Institute, Boston, MA.

ELISA. Microtiter ELISA plates (Nunc Immunoplates, Life Technologies, Paisley, Scotland) were coated with the capture antibody BBIG-11 specific for human ICAM-1. Antibody was diluted in 0.1 M bicarbonate buffer, pH 8.9, to a final concentration of 10 μg/ml and 50 μl were added to each well. Plates were incubated at 4°C overnight followed by washing twice with PBS-T. Blocking was achieved by adding 100 μl/well of PBS-T containing 1% casein and incubating at room temperature for 2 h. Following a further three wash cycles, the ICAM-1 standard and samples were added to the plate (50 μl/well) and left to incubate for 2 h at room temperature. After three washings with PBS-T the bound soluble ICAM-1 was detected using a human ICAM-1-specific biotin-labeled antibody BBIG-12. Antibodies were diluted in PBS-T to a final concentration of 5 μg/ml and 50 μl/well were added.
for 1 h at room temperature. The plates were washed another three times and 50 μl of a 1:1000 dilution of streptavidin horseradish peroxidase (Amersham International Amersham, United Kingdom) was added to each well. After incubation for 30 min and three final washes with PBS-T, the plates were developed using 50 μl/well of tetramethylbenzidine (Universal Biologicals, Kingston-upon-Thames, United Kingdom). The reaction was stopped after 30 min by the addition of 50 μl 1.0 M HCl/well and read using a Titertek MS-2 reader (ICN Flow, Rickmansworth, United Kingdom) at 450 nm. Soluble adhesins were quantified in arbitrary units/ml against an affinity-purified standard preparation of recombinant ICAM-1 expressed in CHO cells and solubilized by detergent extraction.

Tumor Growth. Tumor cell suspensions containing 2 × 10⁶ cells in 0.2 ml 0.9% NaCl were injected s.c. into the flank of nude mice. Tumor growth was followed by measuring tumor diameters with calipers and the tumor weight was calculated as length × width² × 0.2. Four weeks after injection, blood was obtained by intracardiac puncture. Serum was obtained by centrifugation, frozen, and maintained at −80°C before analysis.

Results

ICAM-1 Expression and Secretion by A375M Cells. As shown in Table 1, A375M cells express a basal level of ICAM-1, as measured by fluorescence-activated cell sorter analysis and by ELISA of ICAM-1 released from the cells by freeze-thawing (cell-associated). ICAM-1 expression was moderately increased by IL-1, IFNγ, or IL-6 stimulation but was significantly increased after treatment with TNF. Soluble ICAM-1 was not detectable in the supernatant of unstimulated cells, although cell-associated ICAM-1 was present. A375M cells stimulated with IL-1, IFNγ, and TNF released soluble ICAM-1 into the culture supernatant. IL-6 stimulation had no significant effect on soluble ICAM-1 release.

HT29 colon carcinoma cells could be stimulated by IFNγ to express high levels of cell-associated ICAM-1 but secreted only low levels of soluble ICAM-1. SN12-K1 renal carcinoma cells showed marginal increases in cell-associated ICAM-1 following IFNγ stimulation, and no soluble ICAM-1 production.

Serum ICAM-1 Levels in Nude Mice Bearing A375M Melanoma. Having found that A375M cells can secrete ICAM-1, we investigated ICAM-1 release into the serum of animals bearing a growing tumor (Fig. 1). Eight of the ten mice bearing the A375M s.c. tumor showed a significant level of circulating ICAM-1 in their serum (ranging from 6 to 31 units/ml); only two mice bearing tumor did not show detectable levels of ICAM-1 in serum. Six mice with no detectable tumor had no detectable ICAM-1 in their serum. Some correlation with the serum level of ICAM-1 and the tumor size was observed, the highest levels of ICAM-1 being found in the serum of mice with the largest tumors.

Sera collected from nude mice bearing SN12-K1 renal carcinoma (mean weight, 2.3 g), HT-29 colon carcinoma (mean weight, 2.8 g), or the murine melanoma B16 contained no soluble ICAM-1 (data not shown).

Discussion

Measurement of ICAM-1 expression has been proposed for the diagnosis and prognosis of malignant melanomas. We have shown that cultured human melanoma cells express ICAM-1 on their cell surface and have also shown that they can release a soluble form of ICAM-1. Some unstimulated melanoma cells express ICAM-1 constitutively, possibly due to autocrine stimulation by IL-1 (11), but can be stimulated by cytokines to increase expression. γ-Interferon treatment of the HT29 colon cells resulted in a massive up-regulation of cell surface/cell-associated ICAM-1, to levels greater than those seen on melanoma stimulated with TNF. Despite this, only 0.6 unit/ml of soluble ICAM-1 was detected in the cell supernatant, compared to 19.6 units/ml in the melanoma supernatants. This suggests that there is a specific mechanism of release from A375M cells.

A recent report has demonstrated elevated levels of soluble ICAM-1 in the blood of patients with melanoma, although the
source of the ICAM-1 was not identified. ICAM-1 could be produced either by the tumor or by the host cells in response to the tumor. We have shown that melanoma cells in culture can be stimulated by some inflammatory cytokines to release a soluble form of ICAM-1 and that nude mice carrying transplanted melanoma tumors have ICAM-1 in their blood.

Because our ELISA does not detect murine ICAM-1, the demonstration of soluble ICAM-1 in the serum of the nude mice bearing melanoma tumors confirms that the human tumor is the source of the ICAM-1. No ICAM-1 was detected in the serum of mice without tumors or those bearing the murine B16 melanoma. Nude mice bearing human HT29 colon carcinoma or SN12-K1 renal carcinoma showed no detectable ICAM-1 in their sera.

Locally produced cytokines may be important for expression and secretion of ICAM-1 from tumor cells. In the nude mice, endogenous murine IL-1 and TNF, which act across species, could stimulate the human tumors. Since murine IFN-γ is inactive on human cells it could not induce ICAM-1 expression (12). In the melanoma-bearing animals sufficient cytokine stimulation would seem to be available to cause the change from the nonsecreting phenotype, seen in cultured cells, to the ICAM-1 secreting phenotype observed in vivo.

ICAM-1 is a counter-receptor for leukocyte β2 integrins such as LFA-1, and plays an important role in the interaction of target cells with the immune system. Expression of ICAM-1 by tumor cells may therefore enhance immune recognition and be associated with a favorable prognosis (13). Down-regulation of ICAM-1 by Burkitt's lymphomas clones has been associated with their capacity to escape immune surveillance (14). It is, however, possible that ICAM-1 expression may help tumors escape the immune response. Interaction of the tumor with leukocytes could result in release of toxic cytokines or mediators. These may cause tissue damage and allow dissociation of the primary tumor, promoting tumor migration and extravasation. Circulating tumor cell/leukocyte aggregates could also be prone to arrest in capillary beds, thus enhancing metastasis.

The demonstration of soluble ICAM-1 release by tumor cells may provide additional escape mechanisms. Shedding of ICAM-1 by melanoma cells in response to cytokines could leave insufficient cell surface ICAM-1 to allow effective recognition by cytotoxic lymphocytes. It is also possible that high local concentrations of soluble ICAM-1 could act to block β2 integrin sites on the leukocytes and therefore block immune recognition. Evidence to support this has recently come from Becker et al. (15) who have shown that soluble ICAM-1 from melanoma cells can block natural killer or lymphokine-activated killer cell-mediated cytotoxicity. Whatever the functional role of cell surface or soluble ICAM-1 in tumor progression, the detection of soluble ICAM-1 in serum represents a potential prognostic marker for malignant melanoma. The diagnostic potential may, however, be limited, inasmuch as serum ICAM-1 can be elevated in a wide range of inflammatory diseases (5). In this respect, the nude mouse/human melanoma model system represents a powerful tool for further studies on the role of ICAM-1 in tumor progression.

References

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