Advances in Brief

Serum Levels of Circulating Intercellular Adhesion Molecule 1 in Human Malignant Melanoma

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Abstract

Current reports have suggested a role for intracellular adhesion molecule 1 (ICAM-1) in the progression of human malignant melanoma and other cancers. Stage I, II, and III patients with histologically diagnosed malignant melanoma had significantly increased serum levels of circulating ICAM-1 (cICAM-1) and a striking increase in the incidence of positive sera. In Stage II and III patients, the level of cICAM-1 was inversely correlated with survival. Patients with elevated levels of serum cICAM-1 (greater than 2 SD units above control mean) had a significantly shorter mean survival. We suggest that elevated levels of serum cICAM-1 may be of diagnostic and prognostic importance in patients with malignant cutaneous melanoma.

Introduction

Cell adhesion molecules have been observed to play important roles in specific steps of the metastatic process (reviewed in Ref. 1). ICAM-1 mediates homotypic lymphocyte aggregation (2) and adhesion of lymphocytes to vascular endothelium (3, 4). Reports from Johnson et al. (5), Natale et al. (6), and Kageshita et al. (7) suggest that increased expression of ICAM-1 on melanoma cells may positively correlate with a greater risk of metastasis. In contrast, evidence from Vanky et al. (8) suggests that the expression of ICAM-1 on tumor cells may facilitate recognition by autologous lymphocytes and thus reduce metastasis.

ICAM-1 is expressed on keratinocytes and blood vessels in benign inflammatory disorders such as dermatitis and allergic eczema, and this expression appears to correlate with the extent of disease (9). In addition, the expression of ICAM-1 appears to play a role in the pathogenesis of asthma (10) and rheumatoid arthritis (11). Like soluble forms of other membrane receptors such as the interleukin 2 receptor, we have recently identified a circulating form of ICAM-1.4 Here we show that serum levels of cICAM-1 are significantly elevated in patients with malignant melanoma and that serum cICAM-1 may be of diagnostic and prognostic importance.

Materials and Methods

Sera and Patients. Sera were obtained from patients with confirmed Stage I (n = 14), Stage II (n = 24), or Stage III (n = 18) malignant melanoma and from 22 normal healthy individuals of both sexes from 24 to 50 years of age. Stage I disease was defined as the presence of a primary lesion with no clinically observable metastatic disease at the time of serum collection; Stage II disease as metastasis to regional lymph nodes, local recurrence with or without regional lymph node involvement, or initial presentation of melanoma in a single lymph node group with no identifiable primary lesion; and Stage III disease as remote cutaneous, s.c., or visceral metastases. Disease-free survival was measured (in months) from surgical resection of regional disease to first evidence of recurrence. Overall survival in Stage III disease was measured (in months) from first evidence of distant metastasis to death. Sera from Stage II and III patients were taken at the time of diagnosis (before surgery).

ELISA for the Detection of Circulating ICAM-1. The preparation of mouse mab CL203.4 against domain 4 of ICAM-1 has been previously described (12, 13). CL203.4 was provided by Dr. S. Ferrone (New York Medical College, Valhalla, NY). Mouse mab R6.5 against domain 2 of ICAM-1 was produced and purified as previously described (13). Purified mab R6.5 was biotinylated as follows. Protein concentration was adjusted to 5 mg/ml, and the mab was dialyzed against 0.1 M sodium bicarbonate at pH 9.5. The mab was mixed with 3 mg/ml N-hydroxysuccinimidobiotin (Sigma, St. Louis, MO) and dissolved in dimethyl sulfoxide at a 1:10 solution for 4 h at room temperature. The mab was then extensively dialyzed against DPBS. Sodium azide (0.025%; Sigma) was added, and aliquots were frozen at -70°C. Soluble ICAM-1 was prepared as previously described (13), serially diluted in DPBS with 1.0% bovine serum albumin (Sigma), and then aliquoted and frozen at -70°C. mab CL203.4 (10 mg/ml in DPBS, 50 µl/well) was added to 96-well flat-bottomed enzyme immunoassay microtiter plates (Linbro) and then incubated at room temperature for 1 h. Wells were washed three times with DPBS and then blocked with 200 µl/well of 2.0% bovine serum albumin in DPBS for 1 h at 37°C. Wells were "flicked" empty, and a titration of soluble ICAM-1 standards (8-1024 ng/ml) and sera (diluted with 1.0% bovine serum albumin in DPBS) was added (50 µl/well) and incubated for 1 h at 37°C. All sera were assayed in duplicate. Wells were washed three times with DPBS, biotinylated R6.5 mab (2 µg/ml, 50 µl/well) was added, and the plates were incubated for 30 min at 37°C. The wells were then washed three times with DPBS, and 50 µl/well of a 1:4000 dilution of horseradish peroxidase streptavidin (Zymed, San Francisco, CA) were added and then incubated at 37°C for 30 min. Wells were washed three times with DPBS and once with substrate buffer (Zymed), and then 50 µl/well of 2,2'-azino-di(3-ethylbenzthiazolone sulfonic acid) (Zymed) in substrate buffer were added. The plates were read on a Dynatech MR600 Microtiter ELISA reader (Dynatech, Chantilly, VA) at 410 nm. Mean absorbance readings were calculated from the soluble ICAM-1 titrations, generating a standard curve from which cICAM-1 concentrations in sera or supernatants were determined using regression analysis. Sera positive for cICAM-1 were defined as having cICAM-1 concentrations greater than 2 SD units above the control mean, i.e., >258.2 ng/ml. This ELISA in kit form is also available commercially from Bender MedSystems, (Vienna, Austria).

Statistical Analysis. Statistical analysis was conducted using the Waller and REGWQ programs of SAS (SAS Institute, NC).
Results

Serum cICAM-1 Level in Patients with Malignant Melanoma.
A total of 56 patients with malignant melanoma and 22 normal controls were evaluated for the presence of cICAM-1 (Table 1). Stage I patients demonstrated a mean cICAM-1 of 406.2 ± 33.4 ng/ml, significantly \((P < 0.001)\) greater than the mean level observed in normal controls \((155.3 ± 11.0)\) or Stage II patients \((P < 0.01, 232.9 ± 18.7 \text{ ng/ml})\). Stage II patients also demonstrated a significant increase in mean serum level of cICAM-1 \((232.9 ± 18.7 \text{ ng/ml})\) when compared with controls \((155.3 ± 11.0, P < 0.01)\). Mean serum cICAM-1 for Stage II patients was 303.8 ± 30.4 ng/ml, significantly greater \((P < 0.01)\) than that seen in normal controls. Interestingly, the frequencies of positive sera were high in all patients groups (Stage I = 100%, Stage II = 25%, and Stage III = 72%) when compared with normal controls (0%; Table 1).

Serum cICAM-1 Level and Survival in Patients with Malignant Melanoma. The presence of cICAM-1 and its relationship to survival were examined in Stage II (24 of 24) and Stage III (13 of 18) patients (Table 2). cICAM-1-positive, Stage II patients had a significantly lower \((P < 0.01)\) disease-free survival \((25.8 ± 5.4 \text{ months})\) than cICAM-1-negative, Stage II patients \((38.8 ± 2.2 \text{ months}; \text{Table 2})\). Similarly, Stage III patients positive for cICAM-1 showed a significant reduction in overall survival \((33.6 ± 6.3 \text{ months}, P < 0.05)\) when compared with cICAM-1-negative, Stage III patients \((64.3 ± 13.1 \text{ months})\).

Discussion

ICAM-1 is an inducible cell surface adhesion molecule, and the ICAM-1/LFA-1 pathway plays a major role in a variety of cell-mediated immune responses \((14-17)\). Recently, this molecule has been suggested to play a role in the progression of metastasis in malignant melanoma \((5-8)\) and other cancers \((8)\). In addition, the expression of ICAM-1 is enhanced in inflammatory diseases such as rheumatoid arthritis \((11)\), asthma \((10)\), dermatitis and allergic eczema \((9)\), and uveitis \((18)\).

In the current study we show that serum levels of cICAM-1 are significantly elevated in patients with Stage I, II, and III malignant melanoma. Abnormally high serum levels of cICAM-1 were present in 100% of Stage I, 25% of Stage II, and 72% of Stage III patients but were not (0%) observed in 22 normal individuals.

The presence of cICAM-1 in the sera of patients with malignant melanoma may be of diagnostic importance because all patients \((100\%)\) with Stage I disease have increased levels of cICAM-1. Serum levels of cICAM-1 may also be of prognostic significance because Stage II melanoma patients with elevated levels of cICAM-1 have a significant reduction in mean survival when compared with Stage II patients with normal levels of cICAM-1. Overall survival was also reduced in Stage III patients with abnormally high levels of cICAM-1. No correlation between levels of serum cICAM-1 and \((a)\) size, depth, or location of primary tumor; \((b)\) extent or location of metastases; \((c)\) age or sex of patient; or \((d)\) recurrence of primary tumor was observed (data not shown). Although these findings are of statistical significance, they represent a limited number of patients. Additional studies designed \((a)\) to increase the number of evaluable Stage II and III patients; \((b)\) to observe the level of serum cICAM-1 during the progression of disease; and \((c)\) to observe the levels of cICAM-1 in sera from patients with other cancers are in progress.

The source of cICAM-1 in humans remains unclear. Elevated levels of cICAM-1 have been observed in lymphocyte adhesion deficiency patients and in rheumatoid arthritis patients. cICAM-1 can be detected in cell culture supernatants of normal lymphocytes and established melanoma and lymphoma cell lines. Elevated serum levels of cICAM-1 observed in patients with malignant melanoma may be indicative of an enhanced host cell-mediated immune response to a primary tumor or may represent ICAM-1 shed in situ by melanoma cells. Melanoma and other tumor cells are immunogenic in both humans and in mice and may elicit a cell-mediated immune response \((19, 20)\) and/or a humoral response \((21-23)\) in syngeneic hosts. The source of cICAM-1 in patients with malignant melanoma may be circulating leukocytes activated in response to the rapidly growing primary tumor or, alternatively, cICAM-1 may be shed from the primary tumor. In either case, serum levels of cICAM-1 may be both diagnostic and prognostic for cutaneous malignant melanoma.

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References


R. Rothlein, unpublished observations.


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