Differences in Cytokine Levels in Melanoma Patients with and without Redness (Brenner Sign)

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Abstract. *Background: In view of several studies highlighting an observation of an erythematous eruption in the vicinity of or distant from the lesion in melanoma patients (The Brenner sign), this study sought to assess whether this phenomenon might be related to the blood level of cytokines IL-6 and IL-8. Patients and Methods: Sera specimens obtained from 27 patients with melanoma, of which 15 had erythematous eruptions and 12 did not, were studied by immunohistochemistry for the expression of IL-6 and IL-8. Results: IL-6 was detected in all melanoma patients in both groups. The mean level of IL-6 in the redness group (2.41 pg/L) was significantly higher than in the group without redness (1.25 pg/L). IL-8 was detected in all 27 melanoma patients in the two groups. The serum level was less than 5 pg/L in only 1 patient (6.7%) in the redness group, and in 6 patients (50%) in the group without redness, a statistically significant difference. Conclusion: The Brenner sign appears to reflect a more advanced disease and herald a poor prognosis according to its correlation with the IL-8 and IL-6 blood level. However, in view of the biphasic effect of IL-8 level on tumor progression, and IL-6's ability to inhibit early stage melanoma, redness in melanoma patients could be a sign of a better prognosis of the melanoma.*

The incidence of melanoma has steadily increased over the past few decades. It rose nearly 200% in the United States among non-Hispanic whites from 7.5 cases per 100,000 in 1973 to 21.9 cases per 100,000 in 2002 (1). Among the factors accounting for the increased incidence is earlier and better diagnosis resulting from public health campaigns aimed at increasing awareness of the disease. New technologies and research on the pathogenesis of melanoma are enhancing the efforts to achieve earlier recognition.

An observation of malignant melanoma patients presenting a reddish appearance not explicable by any medical cause including photosensitivity, called the Brenner sign, is gaining documentation from a number of studies (2, 3). The present study was undertaken to further determine the usefulness of this parameter in the diagnosis and prognosis of melanoma by assessing the serum levels of Interleukin 6 (IL-6) and Interleukin 8 (IL-8) in melanoma patients with and without the redness.

Patients and Methods

Sera specimens were obtained from 27 patients with melanoma hospitalized in the Ella Institute for Treatment and Research of Melanoma, Sheba Medical Center, Tel Aviv. The study was approved by the Hospital Ethics Committee. There were two groups of patients: the redness group consisting of 15 patients with the Brenner sign, 13 men and 2 women, age range 37-71 years, with Breslow level range 0.68-5.1 mm; and the group without redness consisting of 12 patients, 8 men and 4 women, age range 21-64 years, with Breslow level range 0.48-1.55 mm. There was no predominance of any subtype of melanoma.

Levels of cytokines (IL-6, IL-8) were evaluated by ELISA kits (R& D Systems, Inc., 9 Minneapolis, MN, USA). In all these ELISAs intra-assay precision was 2.2-4 CV% and inter-assay precision was 6.5-9 CV% .

Results

IL-6 was detected in all melanoma patients in both groups. The mean level of IL-6 in the redness group (2.41 pg/L) was significantly higher than in the group without redness (1.25 pg/L). IL-8 was detected in all 27 melanoma patients in the two groups. However, the serum level was less than 5 pg/L in only 1 patient (6.7%) in the redness group, and in 6 patients (50%) in the group without redness, a statistically significant difference.

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Discussion

An erythematous rash accompanying malignant melanoma lesions, confined mainly to the face (4, 5), was first described by Brenner and Wolf (4) and Tamir and Brenner (5). In one study 8 of 14 melanoma patients had an erythematous rash adjacent to and in the general vicinity of the melanoma, confluent with the surrounding tissue, with no distinguishable borders, while no such rash was observed in the control group of patients with keratinocyte derived tumors such as squamous cell carcinoma or basal cell carcinoma.(2) In another study platelet growth factor (PDGF) expression in biopsy specimens from patients with primary melanoma was positive in 6 of 6 melanoma patients with redness and in 4 of 7 patients without the redness. There was no difference in the expression of vascular endothelial growth factor (VEGF) between the melanoma patients with and without redness (3). The explanation for the erythematous rash lies in the pathogenesis of melanoma.

The progression of melanoma and the escape from regulatory mechanisms leads to the acquisition of a metastatic invasive phenotype, enabled by complex changes such as excessive production of cytokines and autocrine growth factors, deregulation in the expression of cellular adhesion molecules, matrix-degrading enzymes, and angiogenic factors. The process of leukocyte migration from blood vessels to an inflammatory site requires chemotactic factors. One such chemotactic cytokine, IL-8, is produced by mononuclear cells, myeloid precursors, natural killer cells, neutrophils, eosinophils, mast cells, fibroblasts, endothelial cells, and tumor cells.(6) Chemokines play a crucial role in paracrine and autocrine control over tumor cell proliferation, angiogenesis, invasion and metastasis (7). Angiogenesis – a crucial part of a neoplastic process – occurs when the equilibrium between pro-angiogenic and anti-angiogenic equilibrium is changed. Among the factors that induce neovascularization are over-expression of pro-angiogenic factors, such as AKt, a signaling molecule that upregulates vascular endothelial growth factor (VEGF) (7), platelet-derived growth factor (PDGF), and IL-8 (9).

Melanoma cells express several chemokines, including IL-8, which is not expressed by normal melanocytes (10). Serum levels of IL-8 were found to be elevated in malignant melanoma patients compared to healthy controls (11), and several studies found that the expression of IL-8 in melanoma correlates positively with disease progression, the stage of the disease, and disease-free survival. (11,12) IL-8 exerts its effect in melanoma development and angiogenesis through the autocrine and paracrine effect it has on tumor and endothelial cells. Antibodies against IL-8 were found to inhibit melanoma transendothelial migration in a co-cultured assay by 30% (13). IL-8 is regarded as one of the potent angiogenic factors secreted by melanoma cells. Metastatic melanoma cells secrete higher levels of IL-8 than non-metastatic cells (4). Transfecting non-metastatic cells and IL-8 negative nude mice melanoma cells with the IL-8 gene makes them tumorogenic with metastatic potential (14). IL-8 was not expressed in radial growth phase melanoma while it was expressed in 50% of vertical growth phase melanoma and in all metastatic lesions of melanoma (15). Decrease in IL-8 serum levels after chemotherapy in stage 4 melanoma patients indicates response to the treatment (16). When melanoma cells were stimulated with recombinant IL-8, vascular endothelial growth factor (VEGF) secretion was increased more than 2-fold, leading to stimulating of angiogenesis, tumor growth and volume (17).

According to most studies in the literature, IL-8 expression correlates positively with melanoma progression and metastasis, but, there may be a balance in IL-8 secretion by melanoma cells at different stages of the disease. In a study measuring tumor growth and neutrophil-mediated cytotoxicity in early melanomas, it was found that non-tumorogenic and early melanoma benefited from the growth effect promoted by IL-8 when its level was intermediate. When the level exceeded a certain threshold it caused strong infiltration of neutrophils, eliciting a cytotoxic effect. Lower levels of IL-8 are less cytotoxic but the remaining neutrophils can induce mutagenicity. Depletion of neutrophils restored tumor growth in non-tumorigenic melanoma even at high IL-8 levels (17). IL-8 was detected in the serum of all melanoma patients in this study, but the level was higher in the redness group. Thus, at first glance it appears that the Brenner sign reflects a more advanced disease and heralds a poor prognosis. However, in view of the biphasic effect of IL-8 level on tumor progression, redness in melanoma patients, which correlates with a higher serum level of IL-8, could be a sign of a better prognosis of the melanoma.

IL-6 is a pleiotropic cytokine that induces the acute phase response, produces both pro- and anti-inflammatory effects, stimulates B- and T-lymphocytes, and regulates the growth, differentiation and death of several cell populations including neurons and melanocytes (18). IL-6 levels are increased in several diseases, including Alzheimer's disease, autoimmune diseases such as rheumatoid arthritis, myocardial infarction, inflammation, solid tumors, neurological cancers, B-cell malignancies, other malignancies and malignant melanoma (19). IL-6 serum level was found to be significantly higher in patients with melanoma than in healthy controls, and was related to angiogenesis and tumor growth and survival (20). Elevated level of IL-6 was linked to poor prognosis in patents with stage IV melanoma; was associated with larger tumor burden, resistance to chemo- and immunotherapy, and shorter median survival rate; and was predictive of overall survival (21-23). An experimental mouse model showed that lack of IL-6 leads to a decrease in both melanoma incidence

and size, suggesting that IL-6 promotes development and progression of spontaneous melanoma (24). Interestingly, pretreatment serum level of IL-6 was found to be significantly higher in patients with longer rather than shorter relapse-free survival (20).

IL-6 in malignant melanoma has been reported to be a bifunctional growth factor having a paracrine inhibitory effect in early stage melanoma, and an autocrine stimulatory effect in advanced stage melanoma cells (25). Addition of IL-6 *in vitro* to early stage melanoma cells caused inhibition of tumor cell proliferation, while advanced stage tumor cells were resistant to this inhibitory effect (26).

Melanoma cells, but not normal melanocytes, contain histamine (27), histidine decarboxylase and histamine catabolic enzymes (28), and H1 and H2 receptors, accounting for the possible role of histamine in DNA synthesis, cell proliferation and chemotaxis (29). Histamine was found to have a concentration-dependent duel effect on proliferation of WM35 primary melanoma line. High concentration of histamine or administration of H1 agonist inhibited colony formation, while low concentration of histamine or H2 agonist increased such formation (30). Histamine has been shown to increase IL-6 expression through the H1 receptor-mediated mechanism in many cell types, including metastatic melanoma cells (31). On the other hand, IL-6 strongly arrested growth of WM35 cells, upregulated histamine production, and enhanced HI and inhibited H2 histamine receptors expression (30). A model for the mutual reaction between IL-6 and histamine in melanoma suggested that when histamine production and H1 receptor expression are elevated, and H2 receptor expression is decreased locally By IL-6, causing a strong growth inhibitory effect. Additional growth inhibitory effect is exerted by the increase in IL-6 by histamine acting on H1 receptors (31).

According to some of the above-mentioned studies, IL-6 has a dismal effect on melanoma; hence, the Brenner sign, which was found to correlate with IL-6, is part of that dismal prognosis. However, taking into consideration IL-6's ability to inhibit early stage melanoma, the erythema could be a sign of a better prognosis of the melanoma. Histamine, which contributes to one of the possible mechanisms exerting the erythema, combined with IL-6 may inhibit primary melanoma cell proliferation, making the erythema a good prognostic sign.

A constant effort is made to achieve earlier recognition and discovery of melanoma, including new technologies and insight to complement the clinical examination. We are continuing our studies further in order to clarify the exact role of redness in melanoma patients and it's relation to IL-6 and IL-8 as well as other cytokines levels, in the serum and melanoma specimens, in disease progression and escape from regulatory mechanisms.

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