Clinical Study

Intrathecal IgM response in disseminated cerebrospinal metastasis from malignant melanoma

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Key words: malignant melanoma, brain, CSF, TNFα, IL6, immunology

Summary

Neurological complications are a major cause of morbidity and mortality in patients with disseminated malignant melanoma. We have studied and correlated clinical and cerebrospinal fluid (CSF) findings in 20 patients with central nervous system metastases from malignant melanoma including 8 patients with metastatic meningeal melanomatosis (MMM) and 12 patients with solid cerebral metastases (SCM). The putative CSF tumor markers, fibronectin and β2-microglobulin, were elevated significantly in MMM but not in SCM patients. A prominent increase in the IgM index, which reflects intrathecal B-cell stimulation, and a rise of IgG index, interleukin-6, and tumor necrosis factor-α in MMM patients provide preliminary evidence for a local intrathecal immune response triggered by melanoma cell invasion of the subarachnoid space.

Introduction

Malignant melanoma is the third most common primary neoplasm in patients with central nervous system (CNS) metastasis [1] and CNS metastasis is an increasingly common cause of death in malignant melanoma patients. Solid cerebral metastases (SCM) may be treated by surgical excision and irradiation [2–4] but alternative approaches such as chemotherapy and immunomodulative therapy are probably required to manage diffuse leptomeningeal neoplasia (metastatic meningeal melanomatosis, MMM). We have previously reported abnormal cerebrospinal fluid (CSF) findings suggestive of an intrathecal inflammatory or immune response in carcinomatous neoplastic meningitis [5]. The preliminary observation of increased tumor necrosis factor-α (TNFα) levels in the CSF of malignant melanoma patients with leptomeningeal tumor spread [6] suggests that malignant melanoma might be specifically associated with a prominent host response within the CNS.

The present study investigates whether the analysis of humoral CSF parameters could further substantiate the hypothesis that invasion of the subarachnoid space by melanoma cells elicits a specific intrathecal immune response.

Patients and methods

Twenty consecutive patients presenting to our department with neurological complications of metastatic melanoma were studied. Primary lesions were superficial spreading melanoma (n = 6), lentigo maligna melanoma (n = 2), acral lentiginous melanoma (n = 2), and nodular melanoma (n = 5). No primary lesion could be identified in 5 patients, mostly (n = 4) because of prior surgical removal of supposedly benign lesions without proper histologic work-up. Twelve patients had solitary or multiple SCM, 8 suf-
ferred from MMM. All but 1 of the latter had also solitary or multiple solid cerebral metastases.

The diagnosis of MMM was based on positive CSF cytology and supported by neuroradiological and clinical findings. Four of 5 single SCM patients underwent elective tumor resection followed by cranial irradiation. The fifth as well as 2 of 7 patients with multiple SCM were given irradiation alone. Three of 7 patients received intrathecal chemotherapy using methotrexate, 120-125 mg cumulative dose, single injections of 15 mg. Individual therapeutic regimens were developed according to clinical progression and according to wishes and expectations of patients and relatives.

CSF obtained by lumbar puncture before surgery, radiotherapy, or chemotherapy was examined for lactate, glucose, cell count, cytology, albumin, IgG, IgM, fibronectin, β2-microglobulin, interleukin-6 (IL-6), soluble interleukin-2 receptor (sIL-2R), TNFα and oligoclonal immunoglobulin bands on isoelectric focusing of CSF proteins. CSF samples obtained from Ommaya reservoirs were not included. Control CSF (n=28) was obtained from patients with tension headaches and suspected but eventually disproven neurological disease. Albumin and IgG were quantified by nephelometry. IgM, fibronectin, β2-microglobulin, and TNFα were determined by enzyme-linked immunosorbent assay (ELISA) [7-10]. The following antigens and primary antibodies were used: mouse monoclonal anti-human IgM, goat polyclonal antihuman IgM, human IgM (Sigma, St. Louis, MO); mouse monoclonal anti-human fibronectin and rabbit anti-human fibronectin (Calbiochem, La Jolla, CA), fibronectin (Sigma), rabbit anti-human β2-microglobulin and rabbit anti-human β2-microglobulin peroxidase conjugate (Dakopatts, Glostrup, Denmark), β2-microglobulin (Sigma), sIL-2R ELISA (Eurogenetics, Tessenderloo, Belgium), IL-6 and TNFα ELISA (Biotechnology, Oxford, UK). The specificity of the ELISA protocols was controlled by omission of single sandwich components. Sample optical densities, which had at least to double the blank optical density to be considered positive, were converted to actual concentrations by linear regression analysis. IgG, IgM, and cytokine index data were calculated as described previously [8-9]. Intrathecal immunoglobulin synthesis was estimated quantitatively according to Reiber and Felgenhauer [11]. Statistical analysis was performed by one of us (AS) using rank sum tests (Wilcoxon) and multiple linear correlation analysis.

Results

The mean survival from the initial diagnosis of cutaneous melanoma did not differ significantly between SCM patients (49 months) and the MMM group (28 months). The mean survival from the diagnosis of cerebral metastasis in SCM patients (11.2 months) was longer than from first positive CSF cytology in MMM patients (3.1 months). The three methotrexate-treated patients survived for 4, 6, and 9 months.

Findings of the initial lumbar punctures only were included in the statistical analysis. All parameters included in table 1 except for serum β2-microglobulin and CSF lactate were significantly higher in MMM than in SCM. SCM patients differed from controls in parameters of blood-brain barrier dysfunction like CSF albumin, IgG, and IgM, as well as IgG and IgM indices, the parameters of B-cell activation. The quantitative estimate of intrathecal IgG and IgM synthesis [11] was below 10% in the majority of both MMM and SCM patients. Oligoclonal immunoglobulin bands were detected in 2 of 7 MMM and in 1 of 7 SCM patients. TNFα was detected in CSF in 4 of 7 MMM patients (57±22 ng/1) but not in SCM or control CSF or in serum samples. Three MMM patients who received intrathecal methotrexate had positive CSF TNFα on their first spinal fluid examination but negative CSF TNFα on subsequent lumbar punctures.

CSF IL-6 was positive in 3 of 4 MMM patients (785±466 ng/1) but only 1 of 4 SCM patients (37 ng/1). Two of these 3 MMM patients did not have IL-6 detected in the CSF following chemotherapy. No follow-up CSF was available for IL-6 determination in the third patient who was not treated. One additional SCM patient had detectable levels of serum IL-6 (95 ng/1). The same above-