Histamine and Cytokine Therapy

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Interleukin-2 (IL-2) and interferon-α (IFN-α) are potent activators of natural killer (NK) cells and other anti-tumor effector cells, but the results obtained in clinical trials with these cytokines have proved disappointing in many forms of cancer. It may be that IL-2 and IFN-α are often not sufficiently effective because intratumoral monocytes/macrophages (MO) inhibit the cytokine-induced activation of cytotoxic effector lymphocytes such as NK-cells at the site of tumor growth. An essential part of this inhibitory signal is conveyed by MO-derived reactive oxygen species (ROS), which potently inhibit NK-cell-related functions, including the constitutive and cytokine-induced cytotoxicity against tumor cells. Histamine, a biogenic amine, inhibits ROS formation in MO; thereby, histamine synergizes with IL-2 and with IFN-α to induce killing of NK-cell-sensitive human tumor cells in vitro. Furthermore, treatment of tumor-bearing mice with histamine potentiates cytokine-induced killing of NK-cell-sensitive murine tumor cells in vivo. In ongoing clinical trials, histamine has been added to IL-2 or IFN-α in immunotherapy of human neoplastic disease. The results of two pilot trials in metastatic melanoma suggest that the addition of histamine to IL-2/IFN-α prolongs survival time and induces regression of tumors, such as liver melanoma, which are considered refractory to immunotherapy with IL-2 or IFN-α. In acute myelogenous leukemia (AML), histamine and IL-2 have been given in order to protect patients in remission against relapse of leukemic disease. The potential benefit of histamine therapy in melanoma and AML will be evaluated in randomized trials.

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Whether intra- and peritumoral monocytes/macrophages (MO) are friends or foes in the host defense against tumor cells is a matter of considerable controversy (1). MO are endowed with an effective anti-tumor machinery and exert, inter alia, antibody-dependent cellular cytotoxicity against tumor cells in vitro. Indeed, many immunotherapy regimens aim at enhancing the anti-tumor activity of MO. Such regimens include compounds which expand the population of MO and/or granulocytes and the concomitant use of antibodies which armor these cells with specificity against tumor cells (2). Also, MO are antigen-presenting cells and produce cytokines, including IL-1 and IL-10, which are important for the development of adaptive immune reactions against tumor antigens.

On the other hand, MO within and surrounding tumors may be responsible for the relative lack of reactivity of cytotoxic lymphocytes at the site of tumor growth. Indeed, several investigators have demonstrated that functions of intra- or peritumoral NK-cells and T lymphocytes are impaired as compared with NK/T-cells in peripheral blood or in adjacent, non-malignant tissues. Recently, a lot of attention has been focused on the ζ chain of the CD3 complex, which is part of a signal transduction pathway of importance for T- and NK-cell activation. The expression of CD3ζ is down-modulated on intratumoral NK- and T-cells in many forms of human and experimental cancer (3–7), and products of the oxidative metabolism of adjacent MO (such as hydrogen peroxide and other ROS) are considered to be pivotal mediators of the inhibitory signal (reviewed in Ref. (8)).

Only few studies have monitored anti-tumor properties of immunoenhancing cytokines in vivo in tumors with a variable content of MO. However, the supposition that MO-derived inhibition of cytotoxic lymphocytes may have implications for immunotherapy with NK- or T-cell activating cytokines is bolstered by results obtained in animal experiments in which murine B16 melanoma was transfected with a gene encoding for an MO-attracting protein (MCP). MCP-expressing melanoma clones inoculated into mice produced tumors with a high content of intratumoral MO; these MO-containing tumors were considerably less susceptible than MCP-negative control tumors to the anti-tumor efficacy of IL-2 in vivo (9).

The concept that intratumoral cytotoxic lymphocytes may be anergized by adjacent MO brings up the question

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of whether compounds which inhibit the formation of MO-derived ROS are useful in supplementing immunotherapy of neoplastic disease with IL-2/IFN-α. The focus of this review is on our studies of the NK-cell regulatory effects of histamine, an effective inhibitor of radical formation in MO and other phagocytes, and in particular on the histaminergic regulation of interactions between MO and NK-cells. Updated results are presented from two ongoing clinical trials in which patients with AML and metastatic melanoma receive histamine dihydrochloride together with IL-2 and/or IFN-α.

MO SUPPRESS NK-CELL-ACTIVATING PROPERTIES OF IL-2 AND IFN-α

In earlier studies, we aimed at monitoring anti-tumor properties of resting and cytokine-activated NK-cells in vitro in a physiological microenvironment. Thus, whereas other investigators frequently studied NK-cell functions in preparations of isolated NK-cells, we attempted to reconstitute the cellular elements of the immune system which are likely to be present at the site of tumor growth. It was found that peripheral blood MO effectively inhibit the anti-tumor activity of the resting population of NK-cells (10) as well as the NK-cell activating response to IL-2 or IFN-α (11, 12). Indeed, NK-cells could not be activated by IL-2 or IFN-α to kill tumor cells even at a relatively low density of MO. Similarly, other features of NK-cells such as IL-2-induced proliferation and transcription of cytokine genes were effectively down-modulated by MO. Once triggered, the inhibition was irreversible; eventually, a sizeable fraction of NK-cells underwent fragmentation of nuclear DNA and died as a result of apoptosis after cell-to-cell contact with MO (13).

The mechanism underlying the inhibition is as follows: MO produce ROS (including hydrogen peroxide, hypohalous acids and hydroxyl radicals). MO-derived, toxic oxygen species rapidly inhibit NK-cell functions including the killing activity against tumor cells, cell cycle proliferation, and cytokine gene transcription (13, 14). The conclusion that ROS are the main mediators of the NK-cell-suppressive signal is based on the findings that MO recovered from patients with constitutively defective ability to generate ROS (chronic granulomatous disease) do not inhibit NK-cell function, and that catalase, a scavenger of hydrogen peroxide, completely reverses the MO-induced inhibition (14).

NK-cells appeared to be more sensitive to the toxicity of MO-derived ROS than, e.g. T-cells. For example, NK-cells, but not T-cells, acquired apoptotic morphology and fragmented their nuclear DNA after short-term exposure to autologous MO, and approximately five-fold lower concentrations of exogenous hydrogen peroxide were required to induce apoptosis in NK-cells compared with in T-cells (13).

IN VITRO STUDIES OF HISTAMINE AND NK-CELL-ACTIVATING CYTOKINES

The finding that NK-cells are effectively suppressed by MO suggests that a compound which counteracts the MO-induced inhibition could improve the activation of NK-cells induced by cytokines such as IL-2 or IFN-α. An ideal compound should inhibit radical formation in MO without affecting NK-cell function per se; thereby, NK-cells will not be exposed to MO-derived radicals, and activation of NK-cells could also occur in tumor tissues with a high content of MO or other radical-producing phagocytes.

Histamine inhibits the generation of oxygen radicals in MO (12). By this mechanism, histamine synergizes with IL-2 and with IFN-α to kill a variety of NK-cell-sensitive tumor cells, including cultured tumor cells and freshly recovered leukemic blasts. This effect of histamine is strictly mediated by histamine receptors of the H2 subtype: it is mimicked by dimaprit, a specific H2R agonist, and completely blocked by H2R antagonists such as ranitidine and cimetidine (10–14).

HISTAMINE, CIMETIDINE AND TUMOR GROWTH

The interest in histamine as a potential antineoplastic agent originated in the late 1970s from studies in mice, in which passive induction of local anaphylactic reactions reduced the size of established, chemically induced fibrosarcomas. These antitumor responses were blocked by cyproheptadine, an antagonist at histamine and serotonin receptors (15). Subsequent studies revealed that systemic treatment with histamine tended to retard tumor growth and prolonged survival in mice carrying syngeneic fibrosarcomas (16). Similar protective effects of histamine treatment were reported in rats with chemically induced intestinal tumors (17), in mice carrying transplanted human colorectal cancer tumors (18), and in rats with an ascitic sarcoma (19). In the latter study, an ultra-low dose of histamine (< 0.02 μg/kg) protected 80% of rats from a lethal inoculum of sarcoma cells.

A source of controversy is that cimetidine, an antagonist of histamine H2-receptors, has been reported significantly to delay tumor growth and improve survival in experimental tumor models. One example is the study by Gifford et al. in which mice receiving cimetidine (100–200 mg/kg in drinking water) were partially protected against a lethal inoculum of EL-4 lymphoma cells (20). Another example is the study by Gorczynski et al., in which cimetidine (at 100 mg/kg) reversed the tumor growth enhancement induced by conditioned immunosuppression in mice (21).

On the other hand, others have demonstrated that, whereas cimetidine suppresses the formation of B16 melanoma metastases in mice at a high dose (100 mg/kg), cimetidine and other H2R antagonists instead aggravate melanoma metastasis at lower doses (25–50 mg/kg) (22).

Cimetidine also has been reported significantly to increase the incidence of chemically induced intestinal tumors in rats (23), and long-term exposure to cimetidine (19 mg/mg) reportedly increases the frequency of spontaneous lymphoid neoplasms and potentiates chemical carcinogenesis in mice (24).

The anti-tumor properties of cimetidine in rodents have prompted many clinical studies in which cimetidine has been given to patients with metastatic tumor disease, in particular metastatic melanoma and colorectal cancer. Although several anecdotal cases of tumor regression in cimetidine-treated patients are found in the literature, larger trials have hitherto not confirmed these observations. In metastatic melanoma, Mandanas and co-workers reported a median survival time of only 5.3 months in patients receiving single-agent therapy with cimetidine (25), whereas Creagan et al. reported a median survival time of 6 months for patients treated with cimetidine and IFN-α (26). In a randomized trial in metastatic colorectal cancer, a median survival time of 16.8 months was reported for cimetidine-treated patients as compared with 19.3 months for patients receiving placebo (27).

The only significant benefit of cimetidine in controlled trials of human neoplastic disease is reported in patients with gastric cancer, in whom administration of cimetidine significantly prolongs survival time. However, this effect of cimetidine is probably unrelated to H2-receptor antagonism since ranitidine, a potent H2R antagonist, recently was shown to be ineffective in a large randomized trial in patients with gastric cancer (28).

HISTAMINE AND CYTOKINES IN EXPERIMENTAL NEOPLASIA

IL-2 as well as IFN-α can induce regression of NK-cell-sensitive tumors in animal models of metastatic tumor disease (see, e.g., Ref. (29)). The finding that histamine synergizes with these NK-cell-activating cytokines in vitro prompted us to treat experimental animals with histamine and IL-2 or IFN-α with the aim of improving such anti-tumor responses in vivo.

It was found that a single dose of histamine, administered before an intravenous inoculation of B16 melanoma cells, reduced the number of pulmonary metastases by approximately 30–50% in several strains of mice. The antimetastatic effect of histamine was H2R-restricted: it was blocked by ranitidine and other H2R antagonists and mimicked by the H2R agonist dimaprit but not by a chemical control to dimaprit or the histamine H1R agonist 2-pyridylethylamide (22). Depletion of NK-cells in vivo strongly aggravated B16 metastasis and abrogated the anti-tumor effect of histamine. T-cells presumably do not play a major role in the responses to histamine in B16 melanoma, since the compound was active in adult nude mice which have high NK-cell counts in blood but lack functional T-cells (22). The synergy between histamine and IL-2 on B16 metastasis was observed after combined treatment before inoculation of tumor cells as well as in established tumor disease, i.e. treatment given 1–3 days after the inoculation of tumor cells (30).

Disintegration of radiolabeled YAC-1 lymphoma cells in lungs of mice within 1–2 h after i.v. tumor cell inoculation is considered to reflect the cytotoxic activity of NK-cells in vivo. Clearance of YAC-1 cells was significantly enhanced in animals pretreated with histamine, acting via H2R, and reduced by ranitidine and other H2R antagonists (such as cimetidine, tiotidine, and famotidine). The effect of histamine on lung clearance of YAC-1-cells was completely abrogated in animals depleted of NK cells in vivo (22, 31).

In further in vivo experiments, mice were treated with histamine and/or IL-2. It was found that the addition of histamine effectively potentiated the anti-metastatic effect produced by IL-2 or IFN-α in B16 melanoma, and that treatment with histamine and IL-2 as well as with histamine and IFN-α apparently synergistically augmented the NK-cell-mediated killing of YAC-1 lymphoma cells in vivo (22, 31).

More recently, it was found that histamine potentiates anti-tumor effects of IL-2 in a rat model for established prostatic adenocarcinoma. In these experiments, Copenhagen/Fisher rats were inoculated subcutaneously with a Dunning prostate adenocarcinoma. The tumor was allowed to grow without treatment for approximately 3 months, during which the tumor volume increased >100-fold. Thereafter, treatment with histamine and/or IL-2 was initiated and continued for 6 weeks. It was found that the growth of tumors was significantly delayed by IL-2, and this effect of IL-2 was significantly potentiated by histamine. In this model, histamine as a single agent produced only minor effects on tumor growth (Johansson et al., manuscript submitted for publication).

IMMUNOTHERAPY WITH HISTAMINE IN HUMAN CANCER

Metastatic malignant melanoma

The clinical trials of immunotherapy with IL-2 and/or IFN-α in metastatic melanoma have hitherto yielded disappointing results. For example, only approximately 20% of IL-2- or IFN-α-treated melanoma patients achieve objective responses and most investigators report a mean or median survival time of 10–12 months (see, e.g., Ref. (32)). Metastases in skin and lymph nodes seem to be more prone to respond to immunotherapy than, for example, intra-abdominal tumors. Liver melanoma is considered almost completely refractory to IL-2 and/or IFN-α.

The favorable effects of treating mice with B16 melanoma with combined histamine/IL-2 prompted us to add histamine in IL-2-based immunotherapy of human melanoma. In a first pilot study, 16 patients with

metastatic malignant melanoma received high-dose infusions of IL-2 (18 MIU/m^2 × day) together with daily subcutaneous (s.c.) injections of IFN-α; 3 MIU/m^2 × day) in 5-day cycles. Nine of these patients were given histamine (1 mg s.c.) twice daily during treatment with IL-2 and IFN-α.

In the seven patients who did not receive histamine, one partial response (referring to a $>50\%$ reduction of the total tumor diameter) was observed in a patient with skin and lymph node melanoma. In the eight histamine-treated patients evaluated for response, four partial responses (50%) of the overall tumor burden were observed. Two other patients showed regression at one site of metastasis but tumors remained unchanged at other sites ('mixed response').

Two of the histamine-treated patients showed complete resolution of extensive liver metastasis. Survival was significantly prolonged in patients receiving histamine/IL-2/IFN-α as compared with IL-2/IFN-α. One patient has been free of metastatic tumor disease for >48 months after the initiation of treatment with histamine/IL-2/IFN-α (33).

In a second study on metastatic melanoma, 11 patients received with IL-2 at a much lower dose (2.4 MIU/m^2 twice daily) with histamine and IFN-α at doses equal to those in the first trial. The treatment in the second study was given in an out-patient ward. To date, objective regression of tumor mass during treatment has been observed in 4 of the 11 patients (36%, 1 CR and 3 PR). Five patients are still alive, and the median survival time is currently 15 months (30). Results from the two trials with histamine and IL-2/IFN-α are summarized in the Table 1.

Table 1

Pilot studies of histamine/IL-2/IFN-α in human metastatic malignant melanoma

High-dose IL-2 + IFN-α ± histamine
Objective response
IL-2/IFN-α: 1/7 (1 PR)
Histamine + IL-2/IFN-α: 4/8 (4 PR)
Mixed response
IL-2/IFN-α: 0/7
Histamine + IL-2/IFN-α: 2/8
Local response
IL-2/IFN-α: 2/16 sites of metastases
Histamine + IL-2/IFN-α: 11/15
Survival time
IL-2/IFN-α: 6.8 months
Histamine + IL-2/IFN-α: 13.3 + months ($2p<0.03$)
Low-dose IL-2 + IFN-α + histamine
Objective response
4/11 (1 CR, 3 PR)
Survival time
15.0 + months

Acute myelogenous leukemia (AML)

Approximately 20–25% of AML patients will remain in life-long complete remission (CR) after the first bout of leukemic disease, and the median time to relapse (i.e. the time at which 50% of patients have relapsed) is approximately 12 months (34). After relapse, the patients may achieve a second CR, which is usually shorter than the first remission period; only 10–20% of AML patients will achieve a second remission which is longer than the foregoing ('remission inversion') (see, e.g., Ref. (35)).

A rationale for treating AML patients in remission with IL-2 is that AML blasts are frequently sensitive to the lytic activity of IL-2-activated NK-cells in vitro. Furthermore, AML patients whose peripheral blood lymphocytes can be activated by IL-2 to kill conventional NK-cell-sensitive target cells (K 562 erythroleukemic cells) or autologous AML blasts in vitro reportedly have a lower risk of relapse of leukemic disease, and AML patients with bone marrow blasts sensitive to IL-2-activated cytotoxic lymphocytes (recovered from the peripheral blood of healthy blood donors) have a higher probability of achieving CR and a longer duration of event-free survival than patients with insensitive blasts (for review, see Ref. (36)).

AML in remission is a prototype for minimal residual disease. Indeed, patients with a small clone of dangerous malignant cells which can be lysed by IL-2-activated lymphocytes would seem to be ideal candidates for immunotherapy with IL-2. Therefore, it is not surprising that several investigators have used IL-2 in order to maintain disease-free remission in AML. However, the results obtained in four recent trials in which AML patients in CR1 or in subsequent CR have received post-consolidation treatment with IL-2 suggest that monotherapy with IL-2 is of limited or no benefit for AML patients in remission (37, 38), (reviewed in Ref. (36)).

Histamine synergizes with IL-2 to induce NK-cell-mediated killing of freshly recovered AML blasts in vitro. The mechanism is similar to that described for NK-cell-mediated killing of NK-cell-sensitive tumor cell lines: histamine abrogates an MO-derived, inhibitory signal and thereby allows activation by IL-2 also in the presence of suppressive MO (39, 40). These data prompted us to add histamine dihydrochloride to a low-dose regimen of IL-2 (intended dose: 1 μg/kg s.c. bid) in AML patients in CR1 or subsequent CR (39). In this ongoing trial, AML patients in remission receive histamine in addition to IL-2 in repeated courses of 21 days.

Histamine has been administered by s.c. injection twice daily, given immediately after the IL-2 injection. The intended dose is 0.7 mg bid in patients of ≤ 40 years of age and 0.5 mg bid in patients >40 years of age. To minimize acute side effects, histamine is injected slowly at 0.1 mg/min. The patients are advised to rest for 20 min after the injection. With these precautions, histamine has

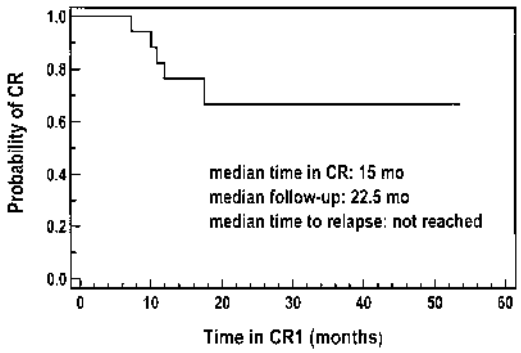

Fig. 1. Kaplan-Meier plot of CR duration in AML patients treated with histamine and IL-2 in first CR.

been well tolerated, and all patients but one have administered histamine and IL-2 at home without supervision throughout the study. It should be emphasized that careful instruction of patients before each treatment course is important. The rate of injection is particularly critical: at least three episodes of tachycardia, palpitations, severe headache, and intensive flush have occurred in patients who mistakenly injected histamine in < 1 min.

Twenty-nine patients with AML in first ($n = 18$) or subsequent ($n = 11$) CR ($CR > 1$) have been included in the study. The treatment has been continued until relapse or until a CR duration of 24 months, and most patients treated in $CR > 1$ have received low-dose chemotherapy (cytarabine and thioguanine) between the initial courses of histamine/IL-2. The median remission time achieved in patients treated with histamine/IL-2 in CR1 is currently 15 months (range 5–54) after a median follow-up of 22 months. The median time to relapse has not been reached. Five CR1 patients have relapsed after 7, 10, 11, 12, and 18 months (Fig. 1).

For patients in whom the treatment was started in CR2–4 ($n = 11$), the median time in CR is currently 15 months (range 7–51), and the median time to relapse 21 months after a median follow-up of 31 months. One patient who entered the protocol in CR4 relapsed after 13 months, two patients relapsed after 7 and 33 months in CR3, and four patients relapsed after 6, 8, 10 and 21 months in CR2. The four remaining patients (all in CR2) remain in CR at 15, 32, 35, and 51 months.

The duration of remission in patients treated with histamine/IL-2 has exceeded that of the foregoing remission in 8/11 patients (73%), and χ^2 analysis of Kaplan-Meier plots reveals that the patients treated in CR2–4 have achieved a significantly longer CR during histamine/IL-2 as compared with their own previous CR (median time to relapse in preceding CR: 12 months vs. 21 months in current CR, $p < 0.05$, Fig. 2). Further details on patient history and regimens are accounted for in two previous publications (36, 40).

CONCLUDING REMARKS

It is hypothesized that the inhibition of NK-cells by MO, and in particular the inhibition of the response to NK-cell-activating cytokines, may serve to explain why immunotherapy with IL-2 or IFN-α is frequently insufficiently effective in reducing tumor burden in human neoplastic disease: NK-cells cannot be induced to killing activity by IL-2 or IFN-α since the activity of cytotoxic lymphocytes is inhibited by MO at the site of malignant tumor growth. Histamine, which inhibits radical formation in MO, augments NK-cell function in mixtures of MO and NK-cells and allows compounds such as IFN-α or IL-2 optimally to activate NK-cells in vitro. Data obtained in tumor-bearing animals suggest that histaminergic mechanisms may be important in the regulation of NK-cell function in vivo and particularly that combined treatment with histamine and IL-2 or with histamine and IFN-α synergistically induces NK-cell-dependent destruction of tumor cells in vivo.

That histamine may be of benefit in supplementing immunotherapy with NK-cell-activating cytokines is supported by the findings that human neoplastic conditions characterized by pronounced hyperhistaminism in peripheral blood respond unusually favorably to IFN-α. Thus, elevated histamine levels in whole blood is a salient feature of chronic myelogenous leukemia, essential thrombocythemia, and polycythemia vera, and the therapeutic efficacy of IFN-α is much more striking in these diseases than in other hematopoietic malignant diseases or in most solid metastatic tumors.

Controlled clinical trials are needed to establish whether the addition of histamine is of benefit in immunotherapy with IL-2 or IFN-α. Recently, a nation-wide phase II trial in Swedish AML patients in first or subsequent CR has recently been started with the ultimate goal of initiating a controlled phase III trial in CR1 patients. In metastatic melanoma, a randomized trial including 200 patients with

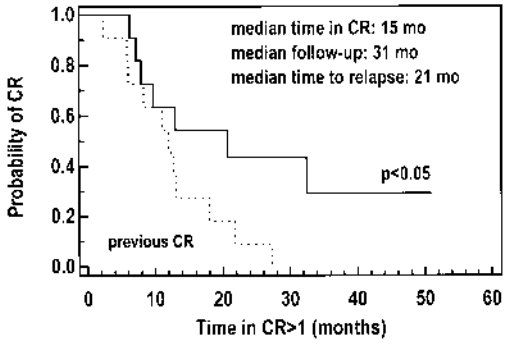

Fig. 2. Kaplan-Meier plot of CR duration in AML patients treated with histamine and IL-2 in second, third or fourth CR. Dotted line: CR in next foregoing remission. Solid line: CR during treatment with histamine/IL-2. Statistical evaluation using χ^2 analysis.

metastatic melanoma who receive histamine/IL-2 or monotherapy with IL-2 has recently been started.

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