Successful immunotherapy of murine melanoma metastases with 7-thia-8-oxoguanosine

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We have recently reported that a synthetic nucleoside, 7-thia-8-oxoguanosine (7T8OG) is a potent activator of a number of effectors which are involved in anti-tumor immune responses. 7T8OG was found to induce interferon (IFN) production, to activate asialo-GM$_1$ positive (AGM$_+$) killer cells, and to enhance specific antibody responses. In the present study, we investigated the effect of 7T8OG on growth of the murine pulmonary B16 melanoma and on formation of metastases. C57BL/6 mice were injected i.p. with 50–150 mg/kg 7T8OG before or after i.v. inoculation of B16 melanoma tumor cells, and 17–19 days after tumor inoculation, the number of metastases in the lungs were counted. 7T8OG given systemically in a single or a divided dose 24 h prior to the challenge of tumor cells reduced the number of lung tumor metastases by 89–99% which is highly significant as compared to untreated control (P < 0.001). Occasional extra pulmonary tumor growth in the thoracic cavity and neck lymph node was also completely inhibited. The reduction in the number of tumor nodules was dose dependent. A single dose of 150 mg/kg of 7T8OG was also effective in inhibiting the growth of 3–5 day old metastatic tumors. The cytotoxic activity of killer cells induced in vivo by 7T8OG was completely abolished by in vitro treatment of cells with anti-AGM$_1$ antibody plus complement. Administration of anti-AGM$_1$ antibody following the 7T8OG treatment completely abrogated the anti-tumor effect of 7T8OG, resulting in a massive increase in the number of tumor foci in the lungs. Administration of carrageenan or silica followed by injection of 7T8OG caused a significant increase (P < 0.01) in the number of pulmonary tumor nodules compared to treatment with 7T8OG only. These findings indicate that activated macrophages or perhaps their cytokine (tumor necrosis factor) also contribute to the host tumor defense by 7T8OG.

Introduction

Specific T cell mediated immune responses and nonspecific responses, involving activated effectors such as natural killer (NK) cells, lymphokine activated killer (LAK) cells and macrophages, have both been implicated in the defense against neoplasms. NK/LAK cells have been shown to be involved in the control of tumor metastases [9]. Direct evidence for a role of NK cells in providing resistance to metastatic tumors was demonstrated by the ability of adoptively transferred purified LGL to reduce tumor metastases [1]. It was postulated that the activation of NK cells with a concomitant enhancement of their activity by some immunomodulator(s) may provide a more efficient therapy for tumor

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control. Hana [7] demonstrated that selective activation of NK cells by periodate oxidized Corynebacterium parvum inhibited formation of tumor metastases. Augmentation of NK cell activity was also reported subsequent to activation of NK cells with a number of other natural and synthetic immunomodulators, including interleukin 2 (IL-2) and interferons [2, 5, 8, 10, 12, 13, 16, 19–21, 25]. LAK cells generated by incubation of splenocytes with IL-2 showed enhanced capacity to lyse B16 tumor cells in vitro, and when adoptively transferred to mice bearing established pulmonary melanoma metastases, they also reduced the B16 pulmonary tumor metastases [6, 14]. It was subsequently reported that repetitive systemic administration of large doses of IL-2 alone caused regression of established pulmonary metastases of tumors of different histologic origin [17]. High doses of recombinant IL-2 were also shown to induce the regression of some disseminated advanced tumors in humans [18].

Recently, we have described the diverse immunologic activities of a novel synthetic nucleoside, 7-thia-8-oxoguanosine [7T8OG] [11, 15, 22–24]. This drug, when injected in mice, activated asialo-GM1 positive (AGM1⁺) killer cells and induced interferon (IFN) production [11, 15, 22–24]. Since several of these induced effectors can exert effects on metastasis formation, we determined whether systemic administration of 7T8OG would prevent the formation or inhibit the growth of metastatic B16 melanoma.

Materials and methods

Animals

C57BL/6 mice were purchased from the Jackson Laboratory, Bar Harbor, MA. Mice were quarantined for a few days in our animal facilities before use and were 6 to 8 weeks old when used in the experiments.

Tumor

Murine B16 melanoma (B16-F10) syngeneic to C57BL/6 mice was obtained from the American Tissue Culture Collection and maintained according to the recommendations of ATCC. B16 cells were cultured and maintained in Eagle’s minimal essential medium as described previously [2]. Cells grown in monolayers were harvested by trypsinization, washed three times with Hanks’ balanced salt solution (HBSS), and resuspended in HBSS.

7T80G

This was synthesized according to the procedure described previously [23] and dissolved in 2% sodium bicarbonate. Doses of 50–150 mg/kg were administered i.p. in a single or divided dose. In the latter, the compound was dissolved in 1 ml sodium bicarbonate; 0.5 ml was given in one injection, and the remaining 0.5 ml was injected 2 h after the first injection.

Induction of pulmonary tumor nodules

Growth of the melanoma in the lungs was induced by injecting 10⁵ B16-F10 melanoma tumor cells into the tail veins of C57BL/6 mice. Visible pulmonary tumor nodules were established within 3 days after the injection [17]. Black nodules of B16 melanoma tumor were easily identified on the surface of excised lungs and counted at 4 × magnification. When foci were too numerous to count,