Sequential immunotherapy using interleukin-1 followed by interleukin-2 of ascitic MOPC104E-bearing mice

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Abstract

This study shows that intraperitoneal injection of interleukin-1 (IL-1), followed by interleukin-2 (IL-2), can effectively eradicate murine ascitic tumor cells. This antitumor effect of IL-1 and IL-2 was abolished when administration of IL-2 preceded that of IL-1. Solid tumors inoculated subcutaneously (s.c.) into the back of mice were also sensitive to this combined i.p. therapy, indicating a systemically-operating antitumor mechanism. Splenocytes from tumor-bearing mice treated with IL-1 followed by IL-2 showed a strong tumor-neutralizing activity. The population responsible proved to be Lyt2.2 (CD8)-positive cells.

Abbreviations: IL: interleukin; LAK: lymphokine activated killer; LU: lytic unit; MST: median survival time; SE: sonicated tumor extract.

Introduction

Many researchers have been investigating cancer immunotherapy using various types of cytokines in both experimental and clinical studies. Among the cytokines, IL-1 has not been fully examined concerning its immunocompetence in cancer therapy. Several reports indicate that IL-1 has a direct antiproliferative or tumoricidal effect against several human carcinoma cell lines [18] and that in vivo administration of IL-1 alone can eradicate murine cancer cells in some models [6, 9, 16, 17]. However, the in vivo antitumor effect of IL-1 can not be observed in general and moreover, severe toxicities make its clinical application difficult [4]. To increase therapeutic efficacy and to decrease side effects of IL-1, combination with other cytokines is required.

On the other hand, IL-2 immunotherapy of cancer has proven to be effective in a number of experimental animal models and clinical trials [1, 21, 22, 24]. IL-2 stimulates lymphocytes bearing IL-2 receptors and is capable of rendering them cytotoxic toward various targets in vitro [14]. Since IL-1 is reported to possess a strong immuno-stimulating competence toward immature T-cells, macrophages and natural killer cells [5, 19, 20], the combination of IL-1 and IL-2 is very attractive. However, reports examining the efficacy of this combination in tumor therapy are few. No clinical trial has been done as yet and in experimental models, only Ciolli et al. reported the antitumor effects of concurrent administration of IL-1 and IL-2 in tumor-bearing mice [2].

In this study, the possibility of sequential therapy with IL-1 followed by IL-2 was examined and we found that the effectiveness of this therapy is due to the mechanism that IL-1 induces antitumor lymphocyte precursors whose tumoricidal activities can be augmented by following IL-2 treatment.

Materials and methods

Mice and tumors. Inbred male BALB/c mice weighing 20–25 g were obtained from Japan SLC Co. Ltd.,
received s.c. inoculation with MOPC104E cells (5 x 10^5 cells) on day 0 and then were treated with 100 μg/kg of IL-1 i.p. once a day on days 8 and 9 and/or 3 x 10^5 JRU/kg of IL-2 i.p. three times a day (every 8 hours apart) on day 11 and 12. Saline was used for control mice. They were checked for survival daily till death. In another experiment, dose of IL-1 varied from 3.7 to 100 μg/kg to determine the minimal effective dose for the combined effect with IL-2. In addition, combined treatments consisting of IL-2 on days 8 and 9 and/or IL-1 on days 11 and 12 were also performed. The antitumor effects of therapies were evaluated in terms of survival and cure rate. Mice that had survived over 100 days were always in complete remission and rejected a rechallenge of MOPC104E cells, therefore they were regarded as being cured. In some experiments, mice received s.c. inoculation with MOPC104E cells (5 x 10^5 cells) on the back simultaneously with i.p. inoculation (1 x 10^5 cells) on day 0 (double grafted model) and then were treated with IL-1 and/or IL-2. This model was used to determine the efficacy of i.p. therapies against distant tumors. Tumor diameter was measured every two days for 25 days and expressed as the mean.

Culture of splenocytes. Spleens from normal mice, ascitic MOPC104E-tumor-bearing mice or IL-1-treated tumor-bearing mice were aseptically removed, minced and passed through a #100 stainless steel mesh. After erythrocytes were lysed for 5 min with 0.83% Tris-NH_4Cl, splenocytes were washed three times with Hanks balanced salt solution (HBSS) and suspended in an appropriate medium. Splenocytes were cultured for 8 or 11 days in a 24-well cell culture plate (Corning, NY) at 37°C in a humidified 5% CO_2 atmosphere. The culture medium consisted of RPMI1640 supplemented with 7% heat-inactivated human AB serum, 50 μM 2-mercaptoethanol, 20 mM HEPES, 100 μg/ml gentamicin, 0.2 μg/ml Fungizone (Gibco, UK), 120 JRU/ml IL-2 with or without sonicated tumor extract (SE, see below). Furthermore, 1 μg/ml Concanavalin A was added to the medium at culture initiation, and the culture medium was changed on days 5 and 8. Fresh IL-2 was added at this medium exchange. The cell concentration in culture was adjusted to 2.5 x 10^6 cells/ml on day 0, 7.5 x 10^6 cells/ml on day 5 and 6 x 10^5 cells/ml on day 8.

Sonicated tumor extract (SE). Tumor cells were purified from ascitic fluid of mice inoculated with MOPC104E by treatment with Tris-NH_4Cl and resuspended in RPMI1640 at a concentration of 2 x 10^7 cells/ml. After sonication for 90 s (20 kHz, 105W) with an Ultrasonic Disruptor (Tomy Seiko, Japan), the suspension was centrifuged for 90 min at 15,000 x g and the supernatant was passed through a 0.22 μm filter and stored at -80°C until use. After its protein concentration was measured, SE was used for splenocyte culture at a concentration of 50 μg/ml. We confirmed in our previous study that these cultured splenocytes contained both tumor-specific and non-specific killing activities[11].

In vitro 51Cr releasing assay. The killing activity of cultured splenocytes was assayed by a standard 4-h 51Cr releasing assay. Briefly, 1 x 10^6 MOPC104E cells were labeled with 100 μCi Na_2^{51}CrO_4 at 37°C for 60 min and washed with RPMI1640 supplemented with 5% fetal bovine serum (FBS) four times. Then, 5 x 10^5 target cells were mixed with graded numbers of effector cells and cultured in 0.2 ml medium (RPMI1640 supplemented with 10% FBS) per well in a 96-well round-bottomed microtest plate (Nunc, Denmark) at 37°C for 4 h in a humidified 5% CO_2 atmosphere. After the culture, the cell-free supernatant was harvested with Titertek Supernatant Harvesting Press (Flow lab, UK) and the radioactivity was measured by a gamma counter (Autowell Aloka, Japan). The percentage