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The Significance of Arginase I Administration on the Survival of Mice Bearing NS-1 Myeloma Cells

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Background. **Arginase I blood levels elevate in cancerous patients and correlate with cancer stages and poor prognosis. Since arginase is capable of enhancing cell growth, it is unclear whether its ominous effect on cancer progression is through the inhibition of immunity or through direct enhancement of cancer cell growth. We tried to clarify this question.**

Methods. **NS-1 mouse myeloma cells were inoculated intraperitoneally (i.p.) into mice. Purified mouse arginase I was injected daily either intravenously (i.v.) or i.p. for 6 d. A tumor-only control group received i.p. tumor cells without arginase. The survival rates of all mice were recorded.**

Results. **Survival rates were significantly lower in the i.v. group than in the i.p. group ($P=0.017$) or in the tumor-only control group ($P=0.034$). As spleen is readily exposed to i.v. arginase, its natural killer cells were studied and were found to have been significantly suppressed by arginase *in vitro* ($P<0.005$).**

Conclusion. **Our results indicate that the direct inhibition of the immune system by i.v. arginase is more significant in shortening the survival of tumor-bearing mice than localized (i.p.) arginase promotion of tumor cell growth. Thus, an elevation of arginase in a patient's blood is very harmful to the host immune system, e.g. splenic natural killer cells.**

Key Words: **NS-1 myeloma cell line; arginase-1; intravenous injection; intraperitoneal injection; natural killer cells, survival.**

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INTRODUCTION

Arginase originating from the liver (arginase I isozyme) is a rate-limiting enzyme in the liver urea cycle, which catalyzes arginine to ornithine and urea. Arginase has been found at increased levels in the serum and cancer tissue of cancer patients, such as gastric cancer [1, 2], colorectal cancer [3, 4], breast cancer [5], skin cancer [6], lung cancer [7, 8], prostate cancer [9], etc. The presence of these increased levels of arginase in cancer tissue may promote cell proliferation [10, 11] through the action of polyamines [11] formed from ornithine. The ornithine is generated from arginine by arginase.

As for immunology, arginase mediates a suppressive effect on T-cell proliferation and cytotoxic T-cell generation [12], as well as the suppression of natural killer (NK) cell function [13, 14]. It is conceivable that these suppressive effects on host immune defense allow tumor cells to grow prolifically.

The blood concentration of arginase I in cancer patients is positively related to the progression of cancer stages [1]. It is unclear whether the potential effect of arginase on tumor growth is through the suppressive effect of arginase on host immune defense, or through the direct contact of arginase with cancer cells to enhance tumor cell proliferation. To clarify this, we administered arginase either intravenously (i.v.) or intraperitoneally (i.p.) to BALB/c mice, which had been inoculated i.p. with tumor cells of the NS-1 myeloma cell line. The arginase administered i.v. reaches the immune system directly, such as the spleen, since the half-life of arginase I in human blood is as short as 1 h [15]. Administration i.p. of arginase enables direct contact between arginase and the tumor cells since the tumor cells were also introduced i.p.

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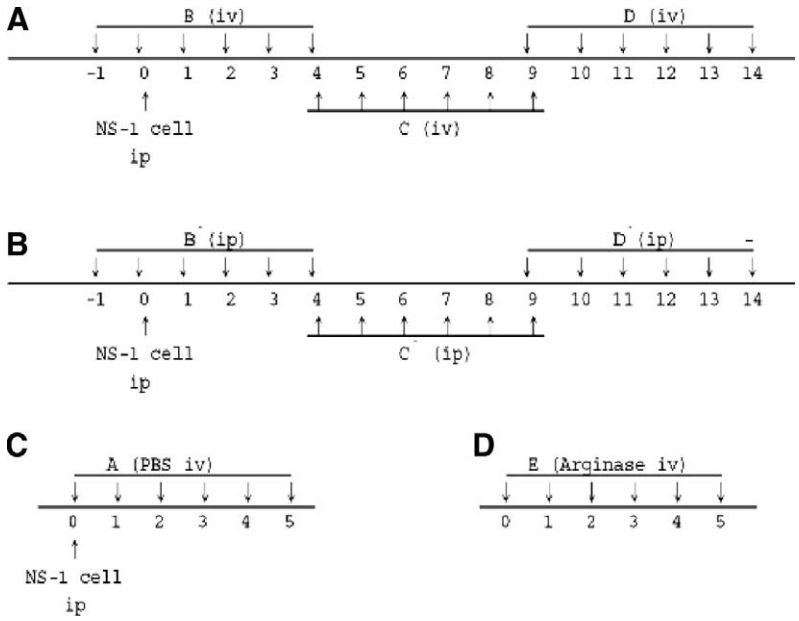

FIG. 1. Grouping of mice. Mice were divided into 8 groups (A, B, B′, C, C′, D, D′, and E) with 8 mice in each group. On d 0, NS-1 myeloma cells (3×10^6) were injected intraperitoneally (i.p.) into each mouse except for Group E. Sterile arginase (3 μg in 0.1 mL PBS) was injected intravenously (i.v.) into the mice of groups B, C, and D (A) and i.p. into those of groups B′, C′, and D′ (B). Arginase injections were conducted daily for 6 consecutive d starting from d −1 (group B and B′), d 4 (group C and C′), and d 9 (group D and D′) (A and B). (C) Group A served as a tumor-only control and received only PBS i.v. for 6 consecutive d starting from d 0. (D) Group E served as an arginase-only control and received arginase i.v. for 6 consecutive d starting from d 0 but was never inoculated with NS-1 cells.

If the arginase suppression on the murine immune system is significant to cancer progression, the survival rate of the mice in the i.v. group will be lower than that of the control group, which was injected i.p. with tumor cells alone. If the direct enhancement of tumor cell growth by arginase is significant to cancer progression, the survival rate of the mice in the arginase i.p. group will be lower than the control group. By comparing the rates of survival in these 2 groups, the relative importance of arginase action on cancer progression could be appropriated to either immune suppression or the enhancement of tumor cell proliferation by arginase. Heat-inactivated arginase was used as a control. Moreover, the effect of arginase on NK cells was evaluated for its mechanism.

MATERIALS AND METHODS

Experimental Design

BALB/c mice were divided into 8 groups (A, B, B′, C, C′, D, D′, and E) with 8 mice in each group (Fig. 1). On d 0, NS-1 myeloma cells (3×10^6 in 0.5 mL phosphate-buffered saline [PBS]) were injected i.p. into each mouse except for Group E. Sterile arginase (3 μg in 0.1 mL PBS) was injected i.v. into the mice of groups B, C, and D (Fig. 1A) and i.p. into those of groups B′, C′, and D′ (Fig. 1B). Arginase injections were conducted daily for 6 consecutive d starting from d −1 (group B and B′), d 4 (group C and C′), and d 9 (group D and D′) (Fig. 1A and B). The duration and time of the injection was arbitrarily determined. It was assumed that the later the injection, the bigger the tumor mass load. Group A (Fig. 1C) served as a tumor control with NS-1 cells inoculated i.p. and daily i.v. injections of PBS, instead of arginase, for 6 consecutive d starting from d 0. Group E (Fig. 1D) served as a negative control receiving i.v. arginase injections daily for 6 consecutive d starting from d 0 without tumor cell inoculation.

Arginase Preparation

Liver arginase was purified as previously described [16]. Briefly, liver homogenate from BALB/c mice was passed through a Sepharose 4B affinity column conjugated with an anti-arginase antibody [16, 17]. The active fractions were eluted with 0.1 M NaCl-NH_4OH, pH 11, and were concentrated and further purified by being passed through a Sephadex G-150 column (Pharmacia Fine Chemicals, Uppsala, Sweden). The largest single peak with the ability to degrade arginine was concentrated and dialyzed against PBS (0.01 M sodium phosphate, pH 7.4) [16]. The purified arginase was homogeneous as verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis [18]. The arginase activity was 243 U/mg protein by measurement of urea production as previously described [15, 17].

Animals

Specific pathogen-free BALB/c mice (male, 6–7 wk old, 20–22 g body weight) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The animals were kept in a controlled air-conditioned environment at 23 ± 2°C, 40–60% humidity, with a 12-h light/dark cycle. Standard mouse food and water were given *ad libitum*. This study was approved by the Institutional Animal Care and Use Committee, Chung Shan Medical University (Taichung, Taiwan).

Colorimetric MTT Assay for Cell Viability

To assay the cell viability, various numbers (1×10^2 to 1×10^5) of YAC-1 cells were cultured. The cell viability was assessed in microtiter plates as previously described [13], using a derivative of Mos-