学 位 論 文 の 要 旨

氏名 Kazunari Ishitobi

学 位 論 文 名	A Modulatory Effect of L-Arginine Supplementation on Anticancer Effects of Chemoimmunotherapy in Colon Cancer-Bearing Aged Mice
発 表 雑 誌 名 （巻，初頁～終頁，年）	International Immunopharmacology (in press)
著 者 名	Kazunari Ishitobi, Hitoshi Kotani, Yuichi Iida, Takahito Taniura, Yoshitomo Notsu, Yoshitsugu Tajima, Mamoru Harada

論 文 内 容 の 要 旨

INTRODUCTION

T cells play a crucial role in antitumor immunity; however, their functions are impaired with aging. Several mechanisms may account for impaired antitumor T cell responses with aging. Although the age-related increase of interleukin (IL)-6, a proinflammatory cytokine, dampens antitumor immune responses, another important mechanism is the increase of immunosuppressive cells, including myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), both of which increase in cancer-bearing aged hosts. MDSCs suppress antitumor immune responses via mechanisms involving arginase-I, inducible nitric oxide (NO) synthase, and indoleamine 2,3-dioxygenase (IDO). Although L-arginine and L-tryptophan are required for T cell proliferation/activation, arginase-I and inducible NO synthase metabolize L-arginine.

Recent studies have shown that some anticancer agents administered at low or moderate doses exhibit immune-modulating effects in cancer-bearing hosts. A combination of 5-fluorouracil (5-FU) and oxaliplatin (L-OHP) called FOLFOX has been clinically applied as adjuvant chemotherapy for colorectal cancer, as a first-line regimen. However, these drugs have immunomodulating effects; 5-FU can decrease MDSCs and L-OHP can induce immunogenic cancer cell death. In addition, cyclophosphamide (CP) can relieve Treg-mediated immunosuppression. We previously reported that immunogenic chemotherapy with 5-FU/L-OHP and CP in combination with anti-PD-1 antibody induced tumor regression in two

mouse colon cancer models. However, these preclinical models using young mice do not necessarily reflect the clinical situation, in which the majority of cancer patients are aged.

L-arginine is necessary during inflammation and wound healing. The tumor-bearing state may be regarded as reflecting chronic inflammation, and cancer-bearing aged hosts, as well as chemotherapy-induced leukopenia, require L-arginine to elicit antitumor T cell responses *in vivo*. Therefore, in this study, we compared the anticancer effects induced by 5-FU/L-OHP/CP between young and aged mice, and examined antitumor effects upon combination with anti-PD-1 antibody and/or L-arginine supplementation in colon cancer-bearing aged mice.

MATERIALS AND METHODS

Wild-type BALB/c and C57BL/6 female young (6–7 weeks old) and aged (60 weeks old) mice were used. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University (IZ2-74, IZ2-75, IZ3-97, IZ3-105, IZ3-116, and IZ3-127). CT26 and MC38 are colon carcinomas derived from BALB/c and C57BL/6 mice, respectively. Aged BALB/c mice were injected subcutaneously (s.c.) with 2–5 × 10^5 CT26 cells. In the MC38 model, aged C57BL/6 mice were injected s.c. with 5 × 10^5 MC38 cells. On days 12 and 20, the mice were injected intraperitoneally (i.p.) with 5-FU (50 mg/kg), L-OHP (6 mg/kg), and CP (50 mg/kg). On days 13 and 21, some mice were injected i.p. with anti-PD-1 mAb (200 μg/mouse). From day 12, mice were orally administered L-arginine (45 mg/mouse) at a volume of 200 μL. The tumor volume was calculated as follows: Tumor volume = (Length × Width^2) ÷ 2. In a liver metastasis model, BALB/c mice were injected with 2.0 × 10^4 LuM1 cells into the spleen in a volume of 50 μl. In a cytotoxicity assay, the spleen cells were cultured with the indicated tumor peptide (10 μg/mL) and IL-2 (20 U/mL) for 4 days. H-$2L^d$-binding AH1 peptide (SPSYVYHQF) and H-$2K^b$-binding p15E peptide (KSPWFTTL) were used as tumor antigenic peptides for CT26 and MC38, respectively. Tumor-infiltrating immune cells were stained with the indicated antibodies, and analysis was performed using the FACSCalibur. Protein extraction from tumor tissues were used for assays of arginase activity and immunoblot. Arginase activity was measured by the Arginase Activity Assay Kit (AK89). Plasma L-arginine and L-tryptophan levels were measured by liquid chromatography–mass spectrometry. Data were analyzed using Student's *t*-test or the Mann–Whitney *U* test for two groups, and by analysis of variance (ANOVA) followed by Tukey's post hoc test for more than two groups. Survival data were obtained using the Kaplan–Meier method and evaluated using the log-rank test. $P < 0.05$ was taken to indicate statistical significance.

RESULTS AND DISCUSSION

Therapy with 5-FU/L-OHP and CP significantly suppressed the *in vivo* growth of CT26 and MC38 colon carcinomas in young mice, whereas this effect was attenuated in aged mice. L-arginine monotherapy showed no effect in aged mice. However, additional therapy with anti-PD-1 antibody and L-arginine supplementation boosted the effect of chemoimmunotherapy in aged mice, and some mice were cured in aged mice. Tumor-specific cytotoxic T lymphocytes (CTLs) were generated from these cured mice. Importantly, tumor-specific CTLs were generated from mice with non-progressing tumor, but not from those with progressing tumor. In a liver metastasis model, the therapy with 5-FU/L-OHP and L-arginine was less effective in aged mice compared to young mice. Plasma L-arginine levels were lower in aged than young mice, and chemotherapy tended to decrease the plasma L-arginine levels in aged mice. When compared young and aged mice bearing CT26 or MC38, CT26-bearing aged mice decreased the arginase activity, the arginase-I expression, and the proportion of monocytic MDSCs in tumor tissues, whereas contrasting results were observed in MC38-bearing aged mice. In addition, the induction of tumor-specific (AH1 peptide tetramer^{+}) CTLs was impaired at lower doses of L-arginine *in vitro*, and the infiltration of CTLs into CT26 tissues after chemoimmunotherapy was promoted by L-arginine administration *in vivo*.

In terms of the mechanism by which L-arginine administration modulated antitumor effects in tumor-bearing mice, Cao *et al.* reported that L-arginine administration can decrease MDSC in tumor sites of 4T1 mammary carcinoma-bearing mice. In addition, Geiger *et al.* reported that L-arginine can modulate T cell metabolism from glycolysis to oxidative phosphorylation and enhance their survival *in vivo*. However, these experiments were done using young mice. In this study, we showed that the induction of tumor-specific CTLs and their cell numbers were dependent on L-arginine doses *in vitro*, and that chemoimmunotherapy with L-arginine, but not chemoimmunotherapy alone, significantly increased the proportion of CTLs in tumors. Based on these results, L-arginine administration could promote the induction/proliferation of tumor-specific CTLs and their infiltration into tumor sites.

CONCLUSION

In conclusion, we demonstrated that immunogenic chemotherapy was less effective in colon cancer-bearing aged mice, whereas additional therapy with anti-PD-1 antibody and L-arginine enhanced the therapeutic effect, even in aged hosts. In addition, our results suggest that L-arginine supplementation can modulate the therapeutic efficacy of chemoimmunotherapy via its effect on tumor-specific CTLs.