学 位 論 文 の 要 旨

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学　位　論　文　名　　Age-Associated Impairment of Antitumor Immunity in Carcinoma-Bearing Mice and Restoration by Oral Administration of *Lentinula Edodes* Mycelia Extract

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論 文 内 容 の 要 旨

INTRODUCTION

Age-associated impairment of T cell immunity should be considered at the setting of immunotherapy. Although several reasons have been proposed to explain immune dysfunction in the aged, the precise mechanisms have not been fully elucidated. Alternatively, the cancer-bearing state is accompanied by inflammation. Particularly, interleukin (IL)-6 and tumor necrosis factor (TNF)-α are the main mediators of cancer-associated inflammation. These cytokines are also increased in the aged. IL-6 inhibits the type 1 helper (Th1) response and induction of cytotoxic T lymphocytes (CTLs). Additionally, inflammation and IL-6 increase myeloid-derived suppressor cells (MDSCs), and MDSCs suppress the Th1 response.

We recently reported that oral ingestion of *Lentinus edodes* mycelia (L.E.M.) extract mitigates immunosuppression and restores melanoma-reactive $CD8^+$ T cells in melanoma-bearing mice. Additionally, oral ingestion of L.E.M. extract can suppress an increase in the serum level of IL-6 in mice that were inoculated with colon carcinoma into the cecum, suggesting that this reagent has the potential to attenuate inflammation with either cancer and/or aging. In this study, we tried to elucidate the mechanisms of impaired antitumor immunity in aged hosts by comparing CT26 colon carcinoma-bearing young and aged mice and determined whether oral administration of L.E.M. extract could mitigate impaired antitumor immunity in aged hosts.

MATERIALS AND METHODS

BALB/c and BALB/c nu/nu female mice (H-2^d: 6–7 weeks old (W)) were used as young mice. Retired BALB/c mice (45 or 60 W) were used as aged mice. Experiments were performed according to the ethical guidelines for animal experiments of the Shimane University Faculty of Medicine (IZ26-5) and for animal experiments of the Kobayashi Pharmaceutical Co., Ltd. CT26 is a colon carcinoma cell line derived from BALB/c mice. Dried powdered extract of L.E.M. was prepared with hot water before germination and after culturing mycelia in a medium composed of bagasse and rice bran. L.E.M. extract was diluted in PBS and was orally administered using gavage. For immunization, doxorubicin (DTX)-treated CT26 cells (1×10^6) were injected to the flank of mice. After 2 weeks, spleen cells were harvested and cultured with an H-$2L^d$-binding tumor peptide in the presence of IL-2 (20 U/mL) for 4 days. Thereafter, its cytotoxicity was measured using a 5-h ^{51}Cr-release assay. To assess the subsets of the spleen, the cell suspension was stained with mAbs and analyzed using flow cytometry. Serum levels of IL-6 and TNF-α were determined using the ELISA Development Kit. To neutralize either or both IL-6 and TNF-α *in vivo*, anti-mouse IL-6 antibody and/or anti-mouse TNF-α antibody were injected i.p. twice, one day before and simultaneously with vaccination of cancer cells. To compare protective antitumor immunity, mice were vaccinated s.c. in the left flank with 1×10^6 DTX-treated CT26 cells. Two weeks later, these mice were inoculated s.c. in the right flank with 5×10^5 viable CT26 cells. The L.E.M. extract was administered orally 1 week prior to and 1 week after vaccination for a total of 2 weeks. Data were evaluated using unpaired two-tailed Student's *t*-test and ANOVA with Bartlett's test. A P value of < 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

First, we compared the growth of CT26 in young (7 W) and aged (45 or 60 W) BALB/c mice. The tumor size was larger in aged mice compared with that in young mice. We next measured the serum levels of IL-6 and TNF-α in young (7 W) and aged (60 W) mice that were naïve or inoculated s.c. with 5×10^5 CT26 cells 10 days prior. The results showed that the serum levels of IL-6 and TNF-α were significantly elevated in aged mice compared with those of young mice and that the tumor-bearing state showed the tendency to increase IL-6. Such difference in tumor growth was not observed between young (7 W) and aged (45 W) nude mice,

whereas the level of IL-6, but not TNF-α, was elevated in the serum of aged nude mice. We compared the vaccine efficacy of inactivated cancer cells in the *in vivo* priming of tumor-specific CTLs between young and aged mice. Although tumor-specific CTLs were induced successfully from the spleen cells of vaccinated young mice, the vaccine efficacy was weak in aged mice. In addition, neutralization of TNF-α, but not IL-6, at the timing of cancer vaccine suppressed the induction of CT26-specific CTLs from aged mice. The percentages of $CD45^+$ immune cells were higher in aged mice than in young mice. However, there was no difference between young and aged mice in the percentage of MDSCs among $CD45^+$ cells in tumor sites.

We next determined whether oral administration of L.E.M. extract could ameliorate the impaired antitumor immunity in aged mice. CT26 growth in aged mice was significantly suppressed by oral administration of L.E.M.. In addition, oral administration of L.E.M. extract increased the CT26-specific cytotoxicity of DTX-vaccinated aged mice.

Since we observed that the growth of CT26 carcinoma was accelerated in aged mice, we compared the tumor growth between young and aged nude mice. However, no difference was observed. These results suggest that the difference in tumor growth between young and aged mice was ascribed mainly to the impaired antitumor T cell immunity in aged mice in this model.

What types of cells produce IL-6 and TNF-α in aged mice? The spleen cells from aged mice produced higher levels of IL-6 upon LPS stimulation than those from young mice. Regarding IL-6, LPS might trigger IL-6 production by macrophages and B cells in the spleen via TLR4-mediated signaling. We suppose that these cells in aged mice increase the potential to produce IL-6 as a result of an age-associated inflammatory environment. Of note is that serum IL-6, but not TNF-α, was elevated in aged nude mice. This finding indicates that IL-6 was increased in a T cell-independent manner in aged mice.

CONCLUSION

We demonstrated that the age-associated increase in IL-6/TNF-α and early infiltration of MDSCs into tumor sites, at least partially, contribute to impaired T cell immunity in aged mice, and that oral administration of L.E.M. extract beforehand can retard tumor growth, probably via attenuating aging-associated inflammation.