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Interleukin-17A is involved in enhancement of tumor progression in murine intestine

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ABSTRACT

Interleukin (IL)-17A is a cytokine involved in neutrophilic inflammation but the role of IL-17A in anti-tumor immunity is controversial because both pro- and anti-tumor activities of IL-17A have been reported. We hypothesized that constitutive expression of IL-17A in intestinal environment modifies tumor growth. To address the issue, mice were inoculated into subserosa of cecum (i.c.) with murine EL4 lymphoma expressing a model tumor antigen, and tumor growth was monitored. IL-17A-producing cells were detected both in tumor mass and in normal intestinal tissue of i.c. tumor-bearing wild type mice. Tumor size in the wild-type mice was significantly higher than that in the cecum of IL-17A gene-knockout mice. Furthermore, anti-IL-17A monoclonal antibody treatment of wild-type mice resulted in decreased tumor size in the cecum. Model tumor-antigen-specific interferon-γ production was not modified in draining mesenteric lymph node cells in the absence or after neutralization of IL-17A. All the results suggest that constitutive expression of IL-17A in intestine enhances tumor growth, and anti-IL-17A antibody treatment is a candidate of a new anti-tumor immunotherapy against intestinal tumors.

Introduction

Interleukin (IL)-17A was originally identified as a $CD4^+$ T cell-derived proinflammatory cytokine which stimulates differentiation and migration of neutrophils through induction of granulocyte-colony stimulating factor and chemokines such as CXCL1 and CXCL8 (Fossiez et al. 1996; Iwakura et al. 2006). IL-17A also induces expression of anti-microbial peptides such as β-defensins (Kao et al. 2004; Liang et al. 2006). IL-17A is involved in protective immunity against various infections (Matsuzaki and Umemura 2007) and pathogenesis of autoimmune diseases (Hirota et al. 2007; Langrish et al. 2005).

The host immune system plays an important role in control of tumor progression. Although cytotoxic $CD8^+$ T cells are considered as an important effector which directly kills tumor cells, $CD4^+$ T cells also participate in anti-tumor immunity through induction of $CD8^+$ T cells, Fas-mediated cytotoxicity, or sustain tumor regression upon oncogene inactivation (Corthay et al. 2005; Hahn et al. 1995; Huang et al. 1998; Rakhra et al. 2010). In contrast, $CD4^+Foxp3^+$ regulatory T cells have been reported to suppress anti-tumor immune response (Nishikawa and Sakaguchi 2010).

The role of IL-17A in anti-tumor immunity is controversial (Murugaiyan and Saha 2009; Zou and Restifo 2010). It has been reported that IL-23, a cytokine indispensable in establishment of $CD4^+$ T (Th17) cell response, is linked to malignancy of colon, ovarian, head and neck, breast, and stomach cancers in human (Langowski et al. 2006). Correlation between the presence of IL-17A-producing T cells and poor survival of patients was also reported on various tumors including gastric cancer and hepatocellular carcinoma (Zhang et al. 2008; Zhang et al. 2009). In murine experimental systems, IL-23- or IL-17A-expressing tumors showed increased growth when subcutaneously (s.c.) inoculated (Numasaki et al. 2005, 2003; Tartour et al. 1999; Wu et al. 2009).

Abbreviations: *Apc*, *adenomatous polyposis coili*; i.c., inoculation into subserosa of cecum; IFN, interferon; IL, interleukin; KO, gene knock-out; mAb, monoclonal antibody; MLN, mesenteric lymph node; r, recombinant; s.c., subcutaneous.

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Involvement of IL-17A in spontaneous tumor progression was also reported in *adenomatous polyposis coili* (*Apc*)-mutant $Apc^{Min/+}$ mice (Chae et al. 2010; Wu et al. 2009). In contrast, several reports have demonstrated that IL-17A-expressing tumors (Benechetrit et al. 2002; Hirahara et al. 2001) or introduction of IL-23 into tumor-bearing environment (Kaiga et al. 2007; Yuan et al. 2006) resulted in reduced tumor growth and enhancement of protective immunity against tumors. IL-17A-mediated anti-tumor protection was also reported in intravenously tumor-inoculated mice (Kryczek et al. 2009a; Martin-Orozco et al. 2009). Furthermore, IL-17A gene-knockout (KO) or IL-17 receptor KO mice showed increased tumor growth after s.c. tumor inoculation (He et al. 2010; Wang et al. 2009). The observations are consistent with a report on human ovarian cancer where IL-17A induction was correlated to $CD8^+$ T cell response and better survival (Kryczek et al. 2009b).

In the present report, influence of IL-17A on tumorigenesis was analyzed at the intestinal level where IL-17A is constitutively expressed (Ivanov et al. 2006). The results demonstrated that growth of tumor cells inoculated into murine cecum wall was enhanced by IL-17A, and antibody-mediated neutralization of IL-17A suppressed the tumor growth. Implication of the observation is discussed.

Materials and methods

Animals

Wild-type (WT) C57BL/6 mice were purchased from Japan SLC (Shizuoka, Japan). IL-17A KO mice (Nakae et al. 2002) were back-crossed to C57BL/6 mice more than eight times. The mice were maintained under conventional conditions. Experiments were conducted according to the Institutional Ethical Guidelines for Animal Experiments of the University of the Ryukyus under approval of the Animal Experiments Safety and Ethics Committee and the Living Modified Organism Experiments Safety Committee of the University of the Ryukyus.

Cell line

A C57BL/6 mouse-derived T cell lymphoma line EL4 was obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). EL4-85B cells were established by transfection of EL4 with plasmid pcDNA4 (Invitrogen, Carlsbad, CA) inserted with full length genomic Ag85B gene from *Mycobacterium bovis* BCG Tokyo strain obtained by PCR. Ag85B was selected as a model Ag because Ag85 contains a major mycobacterial antigenic determinant recognized by $CD4^+$ T cells of C57BL/6 mice (Yanagisawa et al. 1997). The transfected cells were selected using Zeocin (Invitrogen)-containing complete medium consisted of RPMI1640 medium (Wako, Osaka, Japan) supplemented with 10% FBS (Equitech Bio, Kerrville, TX), 100 U/ml of penicillin (Meiji, Yokohama, Japan) and 100 μg/ml of streptomycin (Meiji), then maintained in complete medium in the absence of Zeocin. In some experiments, 1×10^4 of the EL4-85B cells were cultured in 1 ml complete medium supplemented with or without 1 ng/ml murine recombinant (r) IL-17A (PeproTech, Rocky Hill, NJ) in 24 well plates, and cell growth was monitored daily by trypan blue exclusion method.

Reagents

Recombinant Ag85B was produced by yeast *Pichia pastoris* as secretory protein by using pPIC9K expression vector (Invitrogen) containing the full-length Ag85B gene cloned from the genomic DNA of the *M. bovis* BCG Tokyo strain.

S.c. tumor bearing model

A group of mice were s.c. inoculated in the left flank with 5×10^5 EL4-85B cells, with or without injection of 25 ng of rIL-17A at the tumor-bearing site everyday from day 7 to day 14 after the tumor inoculation. Tumor size was determined as (long diameter) × (short diameter) (mm^2) of the tumor mass.

Intestinal tumor bearing model

A group of mice were inoculated into subserosa of the cecum (i.c.) with 5×10^4 EL4-85B cells in 0.05 ml of PBS as previously reported (Harada et al. 1995). On day 14, mice were sacrificed and cecal weight was measured. The cecum was then longitudinally incised to expose lumen and size of the tumor was determined as (long diameter) × (short diameter) (mm^2) of the tumor mass. Mesenteric lymph nodes (MLNs) from the mice were suspended by passing through 30-mm stainless steel mesh. MLN cells (1×10^5 cells/well) were co-cultured with 5×10^5 30 Gy-irradiated syngeneic splenocytes as antigen-presenting cells in 200 μl of complete medium with or without 5 μg/ml Ag85B in 96 well plates. After 24 h, supernatants were collected and used to determine interferon (IFN)-γ concentration using an ELISA kit (R&D Systems, Minneapolis, MN).

Histological analysis

Paraffin-embedded sections of 10% formalin-fixed tissues were used for immunohistochemical analysis. For antigen retrieval, deparaffinized and rehydrated specimens were microwaved in a Retrieval kit (BD PharMingen, San Jose, CA). The slides were then incubated with the anti-IL-17A Ab (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight. The slides were subsequently incubated at room temperature with a biotinylated secondary Ab, horse radish peroxidase-conjugated streptavidin and then 3, 3′-diaminobenzidine, followed by counterstaining with hematoxylin.

Neutralization of IL-17A in vivo

WT mice were administrated intravenously with 0.2 mg of mouse-derived anti-mouse IL-17A monoclonal antibody (mAb) (MM17F3) (Uyttenhove and van Snick 2006) a day before i.c. inoculation of EL4-85B, and then twice a week. As the control, another group of mice were injected with PBS alone one day before and twice a week after i.c. inoculated with the EL4-85B.

Statistics

The statistical significance of data was evaluated using the Student's *t*-test. To compare survival rate, Kaplan–Meier method was used and evaluated using logrank test. All statistical analyses were carried out using STATCEL2 Software (OMS, Tokorozawa, Japan). A *P* value of <0.05 was considered to indicate a significant difference.

Results

Influence of IL-17A on growth of i.c. injected tumor growth

To analyze influence of IL-17A on tumor progression, we analyzed *in vitro* and *in vivo* growth of EL4-85B tumor cells in the presence of murine rIL-17A. We confirmed that the EL4-85B tumor cells did not produce IL-17A (data not shown). The proliferation of EL4-85B tumor cells cultured with rIL-17A was not different from that without rIL-17A (Fig. 1A), indicating that IL-17A does not affect directly on the growth of the tumor cells. In contrast, when the EL4-85B tumor cells were s.c. inoculated and rIL-17A was injected