Abstracts of the 2008th Maruyama Memorial Research Fund Prize Memorial Lecture (2)

Development of Antitumor Immunity by Oral Vaccination with Tumor Antigen and Cholera Toxin

Ayako Wakabayashi, Yohko Nakagawa, Masumi Shimizu and Hidemi Takahashi

Department of Microbiology and Immunology, Nippon Medical School

Introduction

Many malignant tumors originate from various epithelial tissues, such as the skin, and mucosal sites. Thus, a cancer vaccine must activate the mucosal and dermal immune systems, as well as the systemic immune system, with a suitable antigen, adjuvant, and route of administration. Mucosal immunization using cholera toxin (CT) adjuvant might be used to control mucosal tumors because CT primes both mucosal and systemic immunity. Induction of mucosal cytotoxic T lymphocytes (CTLs), which specifically recognize tumor-derived peptide antigens presented by the corresponding class I major histocompatibility complex (MHC) molecules, seems to be an important mechanism for eliminating tumor cells. Uptake of tumor antigens and their presentation via class I MHC molecules by dendritic cells (DCs) are essential for inducing antigen-specific CTLs. In the present study, we attempted to induce ovalbumin (OVA)-specific CTLs at mucosal compartments by oral vaccination with OVA and adjuvant CT and observed suppression of the growth of previously established OVA-expressing tumors. We also analyzed mucosal DCs that might be a key component for the induction of antigen-specific CTLs at mucosal sites.

Methods

Female C57BL/6 (H-2^b) mice were orally given 100 mg OVA plus 10 μ g of adjuvant CT or CT-B subunit. For systemic immunization, mice were given intraperitoneal or subcutaneous injections of OVA plus CT. Next, E.G7-OVA cells, OVA gene-transfected EL4 thymoma cells, were implanted into the gastric or dermal tissue of syngeneic C57BL/6 mice. Three days after implantation, when the tumor mass became visible, OVA plus CT was orally or systemically administered to tumor-bearing mice, and 7 days later booster doses of the same materials were given. Intraepithelial cells (IELs), spleen cells, and tumor-infiltrated lymphocytes (TILs) obtained from the mice were stained with both phycoerythrin-labeled H-2K^b/OVA tetramer-SIINFEKL and fluoroscein isothiocynate (FITC)-labeled anti-mouse T-cell receptor β , CD8 α , or CD8 β and analyzed with FACScan. For cytotoxicity assay, freshly isolated IELs, spleen cells, or TILs were measured with a standard ⁵¹Cr-release assay. Intraepithelial DCs and splenic DCs obtained from the mice were stained with both FITC-labeled anti-mouse CD11c and phycoerythrin-labeled anti-mouse 33D1 or DEC-205 and analyzed with FACScan.

Results

1. Suppression of Previously Established Tumors Growing through Mucosal CTLs Induced by Oral Administration of OVA with CT

The effects of oral administration of OVA plus CT adjuvant on the induction of antigen-specific mucosal CTLs and on tumor regression were investigated. OVA-specific T-cell receptors expressing CD8 $\alpha\beta$ T lymphocytes and OVA-specific CTL function were detected in freshly isolated IELs rather than in spleen cells 5 to 7 days after oral administration of OVA and CT (**Fig. 1**). When OVA and CT were orally administered to mice bearing E.G7-OVA in either gastric tissue or the dermis, tumor growth was significantly suppressed when compared with

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Fig. 1 Analysis of H-2K^b/OVA tetramer-positive cells (A) and OVA-specific cytotoxicities (B) in IELs and SCs after primary immunization of OVA plus CT (reproduced from J. Immunol. 180: 4000-4010, 2008).



Fig. 2 Intradermal tumor growth was significantly suppressed visually (A) and in tumor volumes (B) by oral but not systemic (C) vaccination of OVA plus CT (reproduced from J. Immunol. 180: 4000-4010, 2008).

that of mice treated orally with phosphate-buffered saline, OVA, CT alone, or OVA plus CT-B; however, subcutaneous or intraperitoneal injection of OVA plus CT did not produce any marked suppression (Fig. 2). Moreover, infiltration of such OVA-specific CD8⁺ CTLs was observed in the suppressed tumor tissues (Fig. 3). These results indicate that the growth of established tumors can be suppressed by tumor-specific CD8 $\alpha\beta$ CTLs induced by orally administered tumor antigen plus a suitable mucosal adjuvant.

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Fig. 3 Detection of H-2K^b/OVA tetramer-positive cells (A) and OVA-specific cytotoxicities (B) in TILs from suppressed tumor by the orally vaccination (reproduced from J. Immunol. 180: 4000-4010, 2008).

2. Increased DEC-205-expressing DCs in Mucosal Sites after Oral Administration of OVA plus CT and Its Involvement in Mucosal CTL Induction

To understand the precise mechanisms of mucosal CTL induction, we have examined the types of DCs involved. Recent studies indicate that DEC-205⁺CD11c⁺ DCs are important for class I MHC molecule-associated cross-presentation of an antigen, whereas 33D1⁺CD11c⁺ DCs are involved in presentation via MHC class II molecules. Although both DEC-205⁺ DCs and 33D1⁺ DCs were found at similar rates in the spleen, 33D1⁺ but not DEC-205⁺ DCs were predominantly observed in the surface mucosal areas of C57BL/6 mice. On the basis of these findings, we analyzed the expression of DEC-205 and 33D1 molecules on both intraepithelial and splenic DCs various days after the oral administration of OVA plus CT. Consequently, the number of DEC-205⁺ DCs was increased only in the intraepithelial compartment but not in the spleen 7 days after oral administration. In contrast, the number of 33D1⁺ DCs was decreased at 2 days and then increased in both the intraepithelial compartment and the spleen at 7 days. These results suggest that the mucosal DEC-205⁺ DCs may more efficiently take up antigens than do splenic DCs and will predominantly prime CD8⁺ CTLs at the mucosal compartment via oral administration of an antigen plus CT adjuvant.

Conclusion

In the present study, we have shown that oral vaccination with a tumor antigen plus CT adjuvant can efficiently prime mucosal antigen-specific CTLs via activation of mucosal DCs and suppress previously established tumors growing in the mucosal or dermal compartment. We are now studying mechanisms of mucosal DEC-205⁺ DC activation and CTL induction by oral vaccination to develop a powerful strategy for the treatment and control of tumors.