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CD4+ T helper responses in squamous cell carcinoma of the head and neck

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Summary Anti-tumor immunity plays an important role in the development of and protection from malignancy. However, there is a lack of information regarding induction of CD4+ T helper responses in patients with squamous cell carcinoma (SCCHN). To explore anti-tumor immune responses against SCCHN, a permanent cell line, Gun-1 was established from a squamous cell carcinoma of the hypopharynx. In addition to its characterization, we performed mixed lymphocyte-tumor cell cultures (MLTC) using peripheral blood lymphocytes and autologous tumor cells. Furthermore, T cell responses to wild type (wt) p53-derived peptides were assessed. Gun-1 cells overexpressed p53 and were negative for HLA-A2 expression. No tumor-specific or wt p53-specific CD8+ CTL lines could be established from peripheral blood mononuclear cells (PBMCs) of this patient. Autologous tumor-specific HLA-DR-restricted CD4+ T helper clone was obtained by limiting dilutions using bulk populations from MLTC. This clone produced IFN-γ but not IL-5 in response to autologous tumor cells. In addition, CD4+ T cells were generated from the patient's PBMCs which responded to two HLA-DP5-restricted wt p53-derived peptides. Our results suggest that the immune cells specific for autologous tumor

Abbreviations: SCCHN, squamous cell carcinoma of the head and neck; MLTC, mixed lymphocyte-tumor cell cultures; wt, wild type; PBMCs, peripheral blood mononuclear cells; CTLs, cytotoxic T lymphocytes; PBLs, peripheral blood lymphocytes; TILs, tumor-infiltrating lymphocytes; TGF, transforming growth factor; IL, interleukin; PGE-2, prostaglandin E-2; MHC, major histocompatibility complex; MMC, mitomycin C; HLA, human leukocyte antigens; CM, complete medium; FBS, fetal bovine serum; EBV-B, Epstein-Barr virus-transformed B; mAb, monoclonal antibody; IVS, in vitro stimulation; IFN, interferon; PMA, phorbol 12-myristate 13-acetate; DCs, dendritic cells; Th1, T helper type 1; Th2, T helper type 2; Th0, T helper type 0; TAAs, tumor-associated antigens.

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as well as wt p53-derived epitopes are present in the peripheral circulation of this cancer patient. However, helper-type CD4+ T lymphocytes represent the predominant anti-tumor response.

Introduction

It has been suggested that anti-tumor immunity plays an important role in the development of and protection from malignancy. T cells serve as effector cells responsible for specific long-lasting immunity against the tumor. CD8+ cytotoxic T lymphocytes (CTLs) can recognize and kill tumor cells expressing peptides that are presented by MHC class I molecules, while CD4+ T helper cells are activated by recognition of peptides presented by MHC class II molecules. To date, functions and interactions of these effector cells have been a focus of attention, and this contributed to a better understanding of the mechanisms necessary for the development of an effective immunotherapy. It has been determined that while CTLs play a major role in tumor eradication, the participation of CD4+ T helper cells is also needed for the development of optimal anti-tumor responses. Increasingly, evidence has accumulated indicating that CD4+ T cells have a pivotal role in generating, and maintaining anti-tumor immune responses through their interactions with CTLs, B cells, macrophages, and NK cells.[1,2]

In general, patients with cancer, including squamous cell carcinoma of the head and neck (SCCHN), are known to be immunologically compromised.[3,4] Considerable evidence for the presence of functional defects of peripheral blood lymphocytes (PBLs) and tumor-infiltrating lymphocytes (TILs) in patients with SCCHN has been accumulated.[5] It is also apparent that SCCHN not only actively corrupt the host anti-tumor response via various well-recognized mechanisms but also manage to effectively escape from the host immune system. For example, the production of immunosuppressive factors such as transforming growth factor-β (TGF-β), interleukin (IL)-10, or prostaglandin E-2 (PGE-2) and low levels or lack of expression of tumor antigens, major histocompatibility complex (MHC) molecules, or costimulatory molecules are some of the mechanisms facilitating tumor escape.[6] Therefore, it is not surprising that strong activation signals are necessary to induce and detect autologous tumor-specific T-cell responses in PBLs and TILs of patients with SCCHN.

In SCCHN, similar to other solid tumors, we and other groups have demonstrated that autologous tumor-specific CD8+ CTL can be generated from PBL or TIL.[7–9] On the other hand, tumor-specific CD4+ T cells restricted by MHC class II molecules have been studied in patients with lymphoma, sarcoma, colon cancer, and breast cancer;[10,11] however, there is little information regarding induction of tumor-specific CD4+ T helper cells in patients with SCCHN. In this study, we have established a permanent cell line from a squamous cell carcinoma of the hypopharynx. In addition to characterization of this cell line, we performed mixed lymphocyte-tumor cell (MLTC) cultures by using mitomycin C (MMC)-treated tumor cells and autologous peripheral blood lymphocytes. Furthermore, we examined T-cell responses to wild type (wt) p53-derived peptides in PBMCs obtained from the patient. We report here the analysis of CD4+ T helper responses in a patient with SCCHN.

Materials and methods

Patient

An 80-year-old man who was human leukocyte antigens (HLA)-A2+, A24+, B13+, B40+, Cw3+, Cw8+, DR9+, DR12+, DQ3+, DP2+, DP5+ presented in December 2004 with a squamous cell carcinoma of the hypopharynx in stage IVA (T3N2bM0) (Fig. 1A). The patient received systemic chemotherapy consisting of cisplatin and 5-fluorouracil as neoadjuvant chemotherapy, and then surgery was performed. Subsequently, the patient was treated with radiotherapy (60Gy). He has had no evidence of disease since then.

Cell lines

Tumor cell line Gun-1 was derived from the primary tumor obtained at surgery. These cells grew in a complete medium (CM) consisting of RPMI-1640 (Sigma–Aldrich, St. Louis, MO) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin (all reagents were from Invitrogen, Grand Island, NY). The cultures were maintained at 37 °C in 5% CO2 in air. The T2-A24 cell line, HLA-A*2402-transfected T2 cell line[12] and H0301 cell line, HLA-DPB1*0501 homozygous Epstein-Barr virus-transformed B lymphocytes (EBV-B cells) were kindly provided by Dr. Kuzushima (Aichi Cancer Center, Nagoya, Japan) and Dr. Nishimura (Kumamoto University, Kumamoto, Japan),

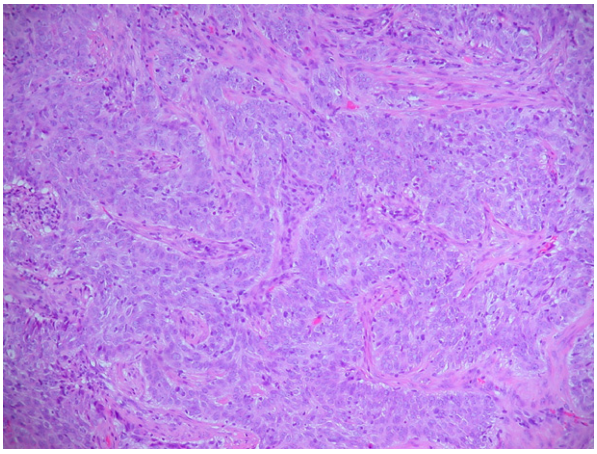

Figure 1A Histological sections of the original tumor obtained from biopsy. H&E (original magnification, ×100).