Th1 type CD4^+ T cells may be a potent effector against poorly immunogenic syngeneic tumors

Anti-tumor activity of Th1 type CD4^+ T cells

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Abstract

We examined the possibility that Th1 type CD4^+ T cells may be an effector against three kinds of syngeneic tumors such as highly immunogenic B16 melanoma (B16) and two poorly immunogenic lines of MCA fibrosarcoma (MCA) and 3LL carcinoma (3LL). In a proliferation assay, the Th1 type CD4^+ T cell clone (MH2) recognized the purified protein derivatives (PPD) derived from _Mycobacterium tuberculosis_. In a tumor-neutralizing assay, MH2 showed anti-tumor activity against both B16 and MCA. In a model of pulmonary metastasis, MH2 also showed anti-tumor activity against both B16 and 3LL. In an assay of cytolysis, MH2 showed a moderate level of tumor necrosis factor-dependent cytolytic activity only against MCA. In a cytostasis assay, MH2 showed a high level of interferon-γ-dependent cytostatic activity against the three tumors in the presence of macrophages. The anti-tumor activity of MH2 against B16 and 3LL was suggested to be, at least in part, attributable to the augmented natural killer activity. Taken together, these findings suggest that we may potentially be able to utilize Th1 type CD4^+ T cells as an effector for immunotherapy against poorly immunogenic tumors.

Abbreviations: PPD: purified protein derivatives; SC: spleen cells; MHC: major histocompatibility complex; IFN: interferon; TNF: tumor necrosis factor; s.c.: subcutaneous(ly); i.v.: intravenous(ly), CTLs: cytotoxic T lymphocytes; MMC: mitomycin C; IL: interleukin; LAK: lymphokine-activated killer; NK: natural killer; mAb: monoclonal antibody

Introduction

In several kinds of effector cells against various types of cancer, tumor-specific T cells have been regarded as the main effector [1, 2]. Indeed, tumor-specific CTLs^4 have been established [3, 4] and many laboratories have reported that such T cells could be an effector for adoptive immunotherapy against cancer [5–8]. However, this protocol appears to be ineffective against poorly immunogenic tumors because of the difficulty in inducing tumor-specific T cells. In addition, spontaneously-developing tumors are thought to be capable of escaping immunological surveillance and they might thus be poorly immunogenic, if at all [9]. From a clinical point of view, we therefore suppose that it is therapeutically meaningful to search for an effector that would be suitable for adoptive immunotherapy against poorly immunogenic tumors.

Recently, it was revealed that CD4^+ T cells could be divided into two groups, namely, the Th1 subset that produces IL-2 and IFN-γ and the Th2 subset that produces IL-4, IL-5 and IL-10 [10]. Considering their cytokine profiles, Th1 type CD4^+ T cells appear to be more useful for immunotherapy against cancer. In
fact, several researchers, including us, have reported that Th1 type CD4+ T cells can be an effector against syngeneic tumor [11, 12]. On the other hand, it has also been reported that PPD preferentially induces in vitro the appearance of Th1 type CD4+ T cells [13, 14]. In addition, PPD-reactive CD4+ T cells are easy to prepare and several researchers have already reported an immunotherapy model utilizing either these T cells or PPD [14–16]. These lines of evidence thus suggest the efficacy of PPD-induced CD4+ T cells as an effector for adoptive immunotherapy against cancers.

In this study, we examined the possibility that Th1 type CD4+ T cells, which were established by utilizing PPD, could be an effector against three kinds of syngeneic tumors. We also demonstrated that such T cells could show a remarkable degree of anti-tumor activity even against poorly immunogenic tumors. The implications of these findings are also discussed.

Materials and Methods

Mice

Eight- to ten-week-old female C57BL/6 (H-2b), BALB/c (H-2d) and C3H/He (H-2k) mice were purchased from Japan SLC (Shizuoka, Japan). All mice were bred in specific pathogen-free conditions.

Tumors

B16 is a highly immunogenic melanoma cell line. MCA and 3LL are a methylochortren-induced fibrosarcoma and a carcinoma, respectively. All tumor cell lines were of C57BL/6 origin. P815 is a mastocytoma of DBA/2 origin and is used as an NK-resistant and LAK-sensitive target cell line. All tumors were maintained in vitro.

Cell preparation

To prepare the SC, the spleens were removed aseptically and teased into suspension in a complete culture medium. RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (HyClone, Logan, UT), 5 × 10^−5 M 2-mercaptopethanol, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 30 μg/ml gentamycin (Schering Corporation, Kenilworth, NJ), and 0.2% sodium bicarbonate was used as the complete culture medium.

A PPD-recognizing CD4+ T cell clone (MH2)

We established PPD-recognizing T cell clones from the lymph node cells of C57BL/6 mice, as previously reported (submitted for publication). Briefly, 7 days after immunization with 100 μg PPD and complete Freund’s adjuvant, the regional lymph nodes were harvested and cultured with 5 μg/ml PPD in the presence of 3000 rad-irradiated syngeneic SC. Thereafter, the viable cells were collected and cultured with 5 μg/ml PPD, irradiated syngeneic SC and 5% Con A super-natant every 7 days. After the establishment of a cell line, two clones were isolated through the limiting dilution technique which showed the same characteristics. Then, one of them (designated as MH2) was used in this study.

The proliferative assay of PPD-recognizing CD4+ T cells

A proliferative assay was performed with standard [3H]dThd incorporation and scintillation counting. Briefly, 7 days after the last stimulation, MH2 cells were collected by Lymphocyte M (Cedarlane, Ontario, Canada). Two hundred thousand viable MH2 cells were seeded in each well of a flat-bottom 96-well culture plate (Corning 25860, Corning, NY) in a volume of 0.2 ml with 2 × 10^5 irradiated (3000 rad) SC for 72 h. The cells were then harvested and the incorporation of [3H]dThd was calculated with the Beta Plate system (Pharmacia LKB Biotechnology, Uppsala, Sweden). To analyze the proliferation of MH2 cells against PPD of Mycobacterium tuberculosis Aoyama B (BCG Inc., Tokyo, Japan), PPD was added to each well at 5 μg/ml. To analyze the proliferation of MH2 cells against syngeneic tumor cell lines, 1 × 10^4 MMC-treated tumor cells and 2 × 10^5 irradiated SC were then added to each well. The MMC-treated tumor cells were prepared by a co-culture with 100 μg/ml MMC (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) for 60 min.

Tumor-neutralizing (Winn's) assay

The anti-tumor activity of MH2 was assayed by the modified Winn’s technique [17]. Briefly, the tumor cells (2 × 10^5) were injected s.c. into the right abdomen either with or without MH2 cells (2 × 10^5) at a volume of 0.2 ml. After tumor inoculation, the tumor growth was inspected every 3 or 4 days.