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Original Article

A tritherapy combination of inactivated allogeneic leukocytes infusion and cell vaccine with cyclophosphamide in a sequential regimen enhances antitumor immunity

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Abstract

Background: Tumor-induced immunosuppression can impede tumor-specific immune responses and limit the effects of cancer immunotherapy. The aim of this study was to investigate the possible effects of sequential chemoimmunotherapeutic strategies to enhance antitumor immune responses.
Methods: Using the E7-expressing tumor TC-1 as the tumor model, the treatment groups were divided into the following groups: (1) inactivated allogeneic leukocyte infusion (ALI), (2) ALI + MMC-inactivated TC-1 cell vaccine, and (3) ALI + MMC-inactivated TC-1 cell vaccine + cyclophosphamide (CTX).
Results: In our study, we demonstrated that treatment with immune-modulating doses of CTX results in a beneficial tumor microenvironment with the suppression of Tregs. ALI has a limited therapeutic effect, as does the MMC-inactivated TC-1 cell vaccine. Our results showed that CTX preconditioning and persistent ALI treatment along with the MMC-inactivated TC-1 cell vaccine resulted in significant inhibition of tumor growth and extended survival.
Conclusion: Our study illustrated the effects of immune-modulating doses of a sequential chemoimmunotherapeutic strategy targeting the tumor and its microenvironment. The results suggest potential clinical effects for the immunotherapy of HPV-associated malignancies.

Keywords: Alloantigen; Cyclophosphamide; Immunotherapy; Regulatory T cells; Tumor microenvironment

1. Introduction

Human papillomavirus (HPV) accounts for approximately 5.3% of cancers throughout the world and consists of cervical cancer and subsets of genital and head and neck cancer.[1,2] It is estimated that 50 million women carry HPV, and approximately 500,000 women develop cancer yearly,[3] posing a threat to human health worldwide. Thus, there is a significant need to develop better strategies to treat HPV-induced lesions.

Tumor-induced immunosuppression plays a critical role in preventing tumor-specific immune responses and decreasing the effect of cancer immunotherapy.[4,5] There is growing acknowledgment that there may be significant advantages to eliciting immune responses against a broad spectrum of antigens expressed by cancer cells rather than targeting a single antigen.[6,7]

Allogeneic transplantation (including hematopoietic stem cell transplantation) has been used as a successful therapeutic method for tumor treatment.[8] It is well recognized that

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alloantigens can induce powerful cellular and humoral immune responses and play an important role in graft vs. host (GVH) or host vs. graft (HVG) response. However, both GVH and HVG biology have many antitumor mechanisms in common, such as antigen presenting cell (APC) activation,[9] fas- and perforin-based cytolysis[10] and IFN-γ-secreting type 1 T cells.[11] Therefore, it is possible to use these characteristics as therapeutic methods.

Over the past 20 years, the efforts to create antitumor immune responses have been based on the sophisticated mechanisms of T cell activation and antigen presentation. However, in an allogeneic transplantation model, the phenomenon in which the powerful host T cell immune reaction to allogeneic major histocompatibility complex (MHC) molecules has drawn little attention. A recent study illustrated the application potential for the allogeneic response to induce weak self-restricted T cell responses to tumor Ags.[12] This study raised the prospect of a novel allogeneic immunotherapy for patients with malignant tumors.

The tumor microenvironment plays an important role in impeding the clinical responses.[13] Novel strategies should be based on targeting the tumor and its microenvironment. It is widely believed that immune-modulating doses of chemotherapeutic drugs induce a powerful response of the immune cells in the tumor microenvironment and exert an antitumor reaction.[14−16] Therefore, the combination of effective chemotherapeutic agents with immunotherapy may elicit the optimal clinical response in cancer patients.

In the present study, to explore the antitumor effect, we combined cyclophosphamide (CTX), MHC-unmatched allogeneic leukocytes and inactivated TC-1 tumor cells in a mouse subcutaneous TC-1 tumor model. The purpose was to establish the theoretical and experimental basis for a novel sequential biological treatment for HPV-induced lesions. We hope that this will be a promising strategy for clinical study as a cure of HPV-related cancer in the near future.

2. Methods

2.1. Mice and cell lines

Inbred female C57BL/6 (B6, H-2) mice (8−10 weeks) were purchased from the Experimental Animal Institute of Peking Union Medical College. All animals were maintained under specific pathogen-free conditions, and all procedures were performed according to approved protocols and in accordance with recommendations for the proper care of laboratory animals.

TC-1 tumor cells derived from primary epithelial cells of C57BL/6 mice co-transformed with HPV-16 E6, E7 and c-Ha-ras oncogenes were provided by Dr. T.C. Wu from Johns Hopkins University. The following standard experimental mouse tumor cell lines were used in vitro and in vivo: B16-F10 (H-2b) melanoma and YAC-1 lymphoma.

TC-1, B16-F10 and YAC-1 cells were cultured in RPMI 1640 (Gibco-BRL, Carlsbad, CA) supplemented with 10% fetal calf serum containing 10% fetal bovine serum (HyClone, Logan, UT) in the presence of 200 μg/mL of Geneticin (G418) at 37 °C with 5% CO_2.

2.2. Cell vaccine preparation

Murine splenocytes were separated from freshly spleens of BALB/c mice. For inactivating splenocytes, 25 μg/mL mitomycin C (MMC) were taken into the cell suspension in a concentration of 1×10^7 cells/mL. Meanwhile, 100 μg/mL mitomycin C (MMC) were taken into the TC-1 cell suspension in a concentration of 1×10^6 cells/mL. And the cells were incubated for 60 min. At last the cell were washed with phosphate-buffered saline (PBS) and resuspended.

2.3. Reagents

CTX (Cat. C0768; Sigma−Aldrich, Milwaukee, WI) was dissolved at 10 mg/mL in deionized water.

2.4. Therapeutic tumor experiment protocol

On Day 0, mice were injected subcutaneously (s.c.) on the right flank with 1×10^5 TC-1 tumor cells. On Day 10, mice bearing tumors approximately 60 mm^3 were arbitrarily assigned to six groups as follows: the PBS control group; TC-1 cell vaccine group; ALI groups (4×10^7, 2×10^7, 1×10^7); CTX group; ALI and TC-1 cell vaccine group; and the CTX, ALI and TC-1 cell vaccine group. CTX was given intraperitoneally (i.p.) at a dose of 50 mg/kg on Day 10 after tumor challenge. On Day 11, the MMC-inactivated allogeneic leukocytes were administered intratumorally (i.t.) and repeated twice every 3 days. On Day 12, the 2×10^6 MMC-inactivated cancer cells were injected subcutaneously (s.c.) and then boosted twice every 3 days. Tumors were monitored every 3 days, and the survival of mice was recorded. Tumor dimensions were measured with calipers, and the values were inserted into the following formula: tumor volume (mm^3) = 0.52 × (length × $width^2$). The number of deaths was assessed at each interval. All measurements were obtained in a strictly blinded fashion.

2.5. Flow cytometry

TC-1 cancer cells were incubated for 30 min with an optimal concentration of FITC-conjugated anti-mouse MHC Class I (Cat. 11-5999; eBioscience, San Diego, CA) on ice and then washed twice with cold phosphate buffered saline (PBS).

For the samples obtained from fresh TC-1 carcinoma masses, separation of the tumor-infiltrating mononuclear cells was carried out by differential gradient centrifugation, and the tumor-infiltrating mononuclear cells were found at the interface of 75% and 100% Ficoll-Hypaque. For samples obtained from spleens, erythrocytes were removed by suspending the cells in lysis buffer and then rinsing the cells twice with RPMI 1640. Cells were incubated for 30 min with an optimal concentration of antibodies on ice and then washed twice with cold phosphate buffered saline (PBS). The following

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