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Preservation and redirection of HPV16E7-specific T cell receptors for immunotherapy of cervical cancer

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Abstract

Human papilloma virus (HPV) type 16 infections of the genital tract are associated with the development of cervical cancer (CxCa) in women. HPV16-derived oncoproteins E6 and E7 are expressed constitutively in these lesions and might therefore be attractive candidates for T-cell-mediated adoptive immunotherapy. However, the low precursor frequency of HPV16E7-specific T cells in patients and healthy donors hampers routine isolation of these cells for adoptive transfer. To overcome this problem, we have isolated T cell receptor (TCR) genes from four different HPV16E7-specific healthy donor and patient-derived human cytotoxic T lymphocyte (CTL) clones. We examined whether genetic engineering of peripheral blood-derived $CD8^+$ T cells in order to express $HPV16E7_{11–20}$-specific TCRs is feasible for adoptive transfer purposes. Reporter cells (Jurkat/MA) carrying a transgenic TCR were shown to bind relevant but not irrelevant tetramers. Moreover, these TCR-transgenic Jurkat/MA cells showed reactivity towards relevant target cells, indicating proper functional activity of the TCRs isolated from already available T cell clones. We next introduced an $HPV16E7_{11–20}$-specific TCR into blood-derived, $CD8^+$ recipient T cells. Transgenic CTL clones stained positive for tetramers presenting the relevant $HPV16E7_{11–20}$ epitope and biological activity of the TCR in transduced CTL was confirmed by lytic activity and by interferon (IFN)-γ secretion upon antigen-specific stimulation. Importantly, we show recognition of the endogenously processed and HLA-A2 presented $HPV16E7_{11–20}$ CTL epitope by A9-TCR-transgenic T cells. Collectively, our data indicate that HPV16E7 TCR gene transfer is feasible as an alternative strategy to generate human HPV16E7-specific T cells for the treatment of patients suffering from cervical cancer and other HPV16-induced malignancies.

Keywords: Immunotherapy; Cervical carcinoma; Retroviral transduction; TCR transfer

Introduction

Cervical cancer (CxCa) is the second leading cause of cancer-related death among women worldwide. Infection with the sexually transmitted human papilloma virus (HPV) is proposed to be associated with cervical cancer [1] because HPV DNA is detected in more than 99% of all tumors of the uterine cervix [2]. Vaccination strategies against HPV are currently based on the induction of HPV-specific, virus-neutralizing antibodies that reduce viral load and infection, thereby preventing the development of premalignant lesions and cervical cancer. The immunogens used for prophylactic vaccines consist of the late proteins, L1 and L2, which are structural elements of the virus. From the work of Koutsky et al. [3], it is clear that these prophylactic vaccines can indeed prevent the appearance of premalignant lesions. While prophylactic vaccines are useful in preventing HPV infection, they are useless in the treatment of existing, HPV-induced premalignant lesions and cervical cancer. The increased incidence and progression of HPV infections in immunosuppressed individuals suggest that T-cell-mediated

Abbreviations: CxCa, cervical cancer; CTL, cytotoxic T lymphocyte; GFP, green fluorescence protein; HPV, Human papilloma virus; IFN, Interferon; IRES, internal ribosome entry site; NGFR, nerve growth factor receptor; TCR, T cell receptor.

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immune responses may be important for the control and eradication of HPV-associated cervical neoplasia [4].

Therapeutic vaccines have been designed to prime antigen-specific T cell responses directed against virus-infected cells. The late proteins can be excluded from therapeutic vaccines because they are not expressed in CxCa or its precursor lesions. The early HPV proteins E6 and E7 are responsible for malignant transformation of HPV-infected cells. The constitutive expression of these proteins is necessary for the maintenance of a transformed phenotype and is therefore considered an ideal target for T-cell-mediated immunotherapy [5]. Therapeutic vaccines consisting of E6 and/or E7 have been tested in patients and have proven to be safe and effective against benign warts, however have had limited therapeutic effect so far in cases of cervical cancer [5]. Tumor-infiltrating lymphocytes directed against HPV16E7 have been found in cervical cancers; therefore, these cells might be attractive for adoptive T cell transfer [6,7].

Recent clinical studies have shown regression of human melanoma upon adoptive transfer of tumor-specific cytotoxic T lymphocyte (CTL) [8,9]. In the case of melanoma, the frequencies of melanoma antigen-specific CTL are usually high within tumor-infiltrating lymphocytes and also in peripheral blood. However, in the case of cervical carcinoma HPV16E7-specific CTL are relatively rare, hampering the isolation of tumor-specific CTL for T-cell-mediated adoptive transfer [10,11]. To circumvent this problem, we have used T cell receptor (TCR) gene transfer, which allows the generation of high numbers of HPV-specific CTL. Previous reports have indicated that TCR gene transfer can result in the successful redirection of antitumor reactivity [12–14], and that the avidity and peptide fine specificity are preserved following TCR gene transfer into human CTL [15,16]. Additionally, Kessels et al. [17] showed antigen-specific expansion and antitumor reactivity of TCR-transduced CTL in vivo in a murine tumor model.

In this study, we describe TCR gene transfer of a number of different TCRs, specific for the HPV16E7$_{11–20}$ epitope, isolated from different CTL clones. TCR-transduced T cells showed MHC restriction and specific reactivity against the endogenously processed and HLA-A2 presented HPV16E7$_{11–20}$ epitope. Our data indicate that TCR gene transfer might be an alternative strategy to generate HPV16E7-specific, CxCa-reactive T cells.

Materials and methods

Cell lines and CTL culture

The HPV16-positive, HLA-A2-negative CxCa cell line SiHa (American Type Culture Collection, ATCC, Manassas, VA) and the HPV16-positive, HLA-A2-positive CxCa cell line SiHa-A2 (SiHa transfected with HLA-A2.1, kindly provided by Dr. S. Man, University of Wales College of Medicine, Cardiff, UK) were cultured in keratinocyte serum-free medium (Life technologies, Paisley, UK) supplemented with 5% (v/v) fetal calf serum (FCS; Perbio, Helsingborg, Sweden), 20–30 μg/ml bovine pituitary extract (Life technologies), 0.1–0.2 ng/ml epidermal growth factor (Life technologies), and antibiotics (100 IE/ml penicillin and 100 μg/ml streptomycin, Life technologies). Jurkat/MA is a modified Jurkat cell line devoid of endogenous TCRβ, engineered to express both CD8α and an NFAT–luciferase construct [18]. These cells were cultured in Iscove's modified Dulbecco's medium (IMDM; BioWhittaker, Verviers, Belgium) supplemented with 8% FCS and antibiotics. CTL were cultured in Yssel et al.'s [19] medium supplemented with 1% human serum (HS; ICN Biomedicals, Aurora, OH) and antibiotics. The EBV transformed B cell line JY, which is homozygous for HLA-A2, was cultured in IMDM supplemented with 8% FCS and antibiotics. To obtain CD8$^+$ T cells, healthy donor-derived PBMC were isolated from an HLA-A2-positive buffy coat by density gradient centrifugation using Lymphoprep (Nycomed, Oslo, Norway). Subsequently, isolation of resting CD8β$^+$ CTL precursors from total PBMC was performed by positive selection on an automated magnetic sorting device (AutoMACS; Miltenyi Biotec, Bergisch Galdbach, Germany), using anti-CD8β mAb (clone 2ST8.5H7, Immunotech, Marseille, France) and microbead-conjugated anti-mouse IgG antibodies (Miltenyi Biotec), according to the manufacturer's protocol. The HPV16E7$_{11–20}$-specific CTL clones A9, JJR3.1, and JJR7.3 were obtained as has been described previously [10]. The HPV16E7$_{11–20}$-specific CTL clone C6 has been described previously [11]. Once a week, CTL were stimulated with an irradiated feeder mixture as described previously [10,19,20] and were maintained at 37°C in humidified air containing 5% CO_2.

RT-PCR and sequence analysis of TCR repertoire

Total RNA was isolated from 5 × 10^6 CTL using RNAzol (Campro Scientific, Veenendaal, The Netherlands) according to the manufacturer's instructions. Copy DNA was synthesized from 2 to 5 μg of RNA using oligo(dT) primers and reverse transcriptase (Life Technologies) according to the manufacturer's instructions. PCR was performed using 12 mixtures of four to five primers (a kind gift from Dr. T. Schumacher, The Netherlands Cancer Institute, Amsterdam, The Netherlands) complementary to the variable TCRα chain or the TCRβ chain in combination with the downstream constant α or β primer, respectively [21,22]. PCR was performed in the presence of 2 mM $MgCl_2$, 15 μM of each primer, 200 μM dNTPs, and 2.5 U of *Taq* polymerase (Roche, Almere, The Netherlands). When a band of the expected size was obtained, the PCR was repeated using each of the variable primers separately together with the constant TCR primer. PCR products were purified using a PCR purification kit (Qiagen, Leusden, The