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Human papilloma virus–specific T cells can be generated from naïve T cells for use as an immunotherapeutic strategy for immunocompromised patients

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Abstract

Human papilloma virus (HPV) is a known cause of cervical cancer, squamous cell carcinoma and laryngeal cancer. Although treatments exist for HPV-associated malignancies, patients unresponsive to these therapies have a poor prognosis. Recent findings from vaccine studies suggest that T-cell immunity is essential for disease control. Because Epstein-Barr Virus (EBV)-specific T cells have been highly successful in treating or preventing EBV-associated tumors, we hypothesized that the development of a manufacturing platform for HPV-specific T cells from healthy donors could be used in a third-party setting to treat patients with high-risk/relapsed HPV-associated cancers. Most protocols for generating virus-specific T cells require prior exposure of the donor to the targeted virus and, because the seroprevalence of high-risk HPV types varies greatly by age and ethnicity, manufacturing of donor-derived HPV-specific T cells has proven challenging. We, therefore, made systematic changes to our current Good Manufacturing Practice (GMP)-compliant protocols to improve antigen presentation, priming and expansion for the manufacture of high-efficacy HPV-specific T cells. Like others, we found that current methodologies fail to expand HPV-specific T cells from most healthy donors. By optimizing dendritic cell maturation and function with lipopolysaccharide (LPS) and interferon (IFN)γ, adding interleukin (IL)-21 during priming and depleting memory T cells, we achieved reliable expansion of T cells specific for oncoproteins E6 and E7 to clinically relevant amounts (mean, 578-fold expansion; n = 10), which were polyfunctional based on cytokine multiplex analysis. In the third-party setting, such HPV-specific T-cell products might serve as a potent salvage therapy for patients with HPV-associated diseases.

Key Words: *cervical cancer, human papilloma virus, immunotherapy, T cell*

Introduction

Human papilloma virus (HPV) is the most common sexually transmitted infection in the United States, and is a recognized oncovirus. More than 70% of cervical carcinomas are associated with HPV, and the virus is also frequently seen in penile, vulvar, anal and oropharyngeal cancers [1,2]. Several serotypes of the HPV are designated as high risk for oncogenesis. Among these, HPV types 16 and 18 are the most common and most associated with different malignancies: high-risk HPV infections are responsible for approximately 5% of all human cancers (7.7% in developing countries, 2.2% in developed countries [2]) and are responsible for 7.5% of female cancer-related deaths worldwide each year [3].

The risks for HPV-associated infections and malignancies are particularly pronounced in immune compromised patients. Organ transplant recipients have twice the risk of developing cancer, and these malignancies are often more aggressive because of the prolonged immunosuppressive regimens required to prevent rejection [4]. Clearance of HPV is slow in patients with deficiencies in T cells or natural killer (NK)

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cells and severe HPV infection can occur in many forms of primary immunodeficiency [5]. In immunosuppressed populations, such as hematopoietic stem cell transplant (HSCT) recipients and human immunodeficiency virus (HIV)-infected patients, the incidence of HPV infection and associated malignancies is increasing, even as treatments for these patients continue to increase their life expectancy [6]. In a recent study of more than 28 000 allogeneic HSCT recipients, the risk of developing squamous cell carcinoma (SCC) was five times higher in patients with a history of chronic graft-versus-host disease (cGVHD) as compared with the general population [6]. The risk of an immunosuppressed patient developing SCC is 64 to 250 times higher than for an immunocompetent patient, and SCC in immunocompromised patients is more likely to be HPV-related [6,7]. In patients with HIV, a low CD4+ T-cell count is associated with multiple HPV infections, poor viral clearance and cervical dysplasia [8].

HPV vaccines are successful in preventing infections and the malignancies associated with the virus, but they have no impact on the risk of malignancies in patients with pre-existing chronic HPV infections and no therapeutic efficacy against malignancy [9–11]. Evidence from other opportunistic viral infections show that adoptive transfer of virus-specific T cells facilitates immune reconstitution and disease recovery [12]. T-cell immune therapies for these patients, notably in HSCT recipients, result in successful prevention and treatment of infections with viruses such as adenovirus, Epstein-Barr Virus (EBV) and cytomegalovirus (CMV) as well as the post-transplantation lymphoproliferative disease associated with EBV [13–17]. In contrast to monoclonal antibodies, T cells can expand *in vivo* and persist long-term after infusion [18].

Although T-cell therapies are promising, generating a product for individual patients is costly and time-consuming, which limits its utility [19]. These limitations can be overcome by the use of third-party banks to deliver an "off the shelf," partially HLA-matched T-cell therapy. The seroprevalence of HPV is not universal, differing widely by age and ethnicity. This presents a challenge when manufacturing donor-derived HPV-specific T cells because current protocols for *ex vivo* expansion depend on pre-existing donor immunity to the targeted virus. Ramos *et al*. successfully generated cytotoxic T lymphocytes (CTLs) from patients with HPV-associated cervical and oropharyngeal cancers, but generating them from healthy donors was unsuccessful [20].

Here, we present a modified, currently Good Manufacturing Practice (GMP)–compliant protocol for the manufacture of HPV-specific T cells with improved antigen presentation, priming and T-cell expansion. We used our previous success in expanding T cells from naïve cell sources [21,22] as a template for expanding HPV T cells regardless of donor source. These HPV-specific T cells represent a novel therapeutic option for patients with HPV-associated diseases [19].

Materials and methods

Patients/subjects

Blood was obtained after informed consent from healthy donors at Children's National Medical Center (Washington, DC), or from buffy coats provided by the National Institutes of Health (Bethesda, MD).

HPV16-E6 and HPV16-E7 peptide library

Overlapping libraries of 15-mer peptides encompassing HPV16-E6 (Protein ID P03126) and HPV16-E7 (P03129) were purchased from JPT Peptide Technologies. Peptides were reconstituted in dimethyl sulfoxide (DMSO; Sigma-Aldrich) at a working concentration of 200 ng/μL.

Monocyte isolation and dendritic cell generation

Peripheral blood mononuclear cells (PBMCs) were isolated using lymphocyte gradient centrifugation (Lymphocyte Separation Medium; MP Biomedicals). Monocytes were isolated from PBMCs by CD14 selection using MACS Beads (Miltenyi Biotec) and cultured in 24-well plates in dendritic cell (DC) medium (CellGenix medium [CellGenix GmbH] and 1% alanyl-glutamine [GlutaMAX; Gibco Life Technologies]), with 800 U/mL granulocyte/macrophage-colony stimulating factor (GM-CSF; R&D Systems) and 1200 U/mL interleukin (IL)-4 (R&D Systems). DCs were fed with GM-CSF and IL-4 on day 3. On day 5, DCs were pulsed with 1 μL peptide mixture (E6 and E7 mix) and matured by adding 1 mL of DC media containing 10 ng/mL Lipopolysaccharides from *Escherichia coli* (LPS; Sigma-Aldrich), 100 U/mL Recombinant Human Interferon-γ (IFN-γ; R&D Systems), 100 ng/mL IL-6 (R&D Systems), 10 ng/mL IL-1β (R&D Systems), 10 ng/mL tumor necrosis factor (TNF)α (R&D Systems), 1 μg/mL prostaglandin E2 (R&D Systems), 800 U/mL GM-CSF and 1200 U/mL IL-4.

HPV16 E6/E7-specific T-cell generation

After maturation, DCs were harvested and resuspended in CTL medium: 45% Click's medium (Irvine Scientific), 45% RPMI-1640 (HyClone), 10% human AB serum (Gemini BioProducts) and 1% GlutaMAX. CD14 negative PBMCs were thawed and underwent CD45RO depletion using MACS Beads (Miltenyi