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Identification of a novel HLA-A*24:02-restricted adenovirus serotype 11-specific CD8+ T-cell epitope for adoptive immunotherapy

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ABSTRACT

Subgroup B adenovirus serotype 11 (Ad11) occasionally causes fatal infections in immunocompromised patients. The present study describes a novel Ad11 epitope presented by HLA-A*24:02 that could be used for adoptive immunotherapy. Ten synthetic Ad11 hexon protein-derived nonamer peptides that bound to HLA-A*24:02 were selected by a computer algorithm and MHC stabilization assay. Stimulation of peripheral blood mononuclear cells from HLA-A*24:02+ donors with each of these synthetic peptides induced peptide-specific CD8+ T-cells for three peptides. Testing the reactivity of these peptide-specific CD8+ T-cells against various target cells confirmed that peptide TYFNLGNKF is naturally processed in Ad11-infected cells and is presented by HLA-A*24:02. Emergence of TYFNLGNKF-specific CD8+ T-cells coincided with the clearance of adenoviruses in a patient with Ad11 disease. Importantly, TYFNLGNKF-specific CD8+ T-cells were suggested to be not serotype cross-reactive. The novel HLA-A*24:02-restricted Ad11 epitope could be used for anti-Ad11 adoptive immunotherapy and to monitor immunity to Ad11 using MHC tetramers.

1. Introduction

Adenoviruses (AdV) cause lethal infections in immunocompromised hosts such as hematopoietic stem cell transplantation (HSCT) and chemotherapy recipients (Chakrabarti et al., 2002; Leen et al., 2006a; Yokose et al., 2009). Although antiviral agents, such as ribavirin and cidofovir, have been used for the treatment of AdV infection, their efficacy is limited by weak intrinsic activity against viruses and by toxicity (Ison, 2006; Lindemans et al., 2010). Furthermore, reconstitution of AdV-specific T-cells is required for the control of AdV infection (Feuchtinger et al., 2005; Heemskerk et al., 2005). These observations have led to the development of adoptive T-cell therapy for the management of AdV infection (Feuchtinger et al., 2006; Leen et al., 2006b).

There are several different approaches to generate virus-specific T-cells for adoptive therapy. In previous reports, peripheral blood mononuclear cells (PBMCs) were stimulated with the lysate of AdV-infected cells or with adenoviral vector-transduced cells to generate AdV-specific T-cells (Feuchtinger et al., 2004; Leen et al., 2004a). However, the clinical use of these strategies is complicated by the concerns associated with transferring live viral particles to patients who are immunocompromised. Another method to generate virus-specific T-cells is to stimulate PBMCs with immunogenic peptides derived from viral proteins. This method is advantageous in that synthetic peptides can be readily produced under good manufacturing practice conditions. Furthermore, the feasibility of this approach has been documented in clinical studies in which cytomegalovirus (CMV)-pp65 peptide-specific cytotoxic

Abbreviations: AdV, adenoviruses; Ad11, adenovirus serotype 11; BIMAS, Bioinformatics and Molecular Analysis Section; CM, culture medium; CMV, cytomegalovirus; CTLs, cytotoxic T-cells; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; E:T, effector to target; FBS, fetal bovine serum; HC, hemorrhagic cystitis; HSCT, hematopoietic stem cell transplantation; IFN-γ, interferon-γ; IL-2, interleukin-2; K562/A*24:02, K562 cells transduced with HLA-A*24:02; LCLs, Epstein-Barr virus-transformed B-lymphoblastoid cell lines; LYA, LYANSAHAL; mAb, monoclonal antibody; MFI, mean fluorescence intensity; PBMCs, peripheral blood mononuclear cells; TCRs, T cell receptors; TYF, TYFNLGNKF; VYS, VYSGSIPYL.

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T-cells (CTLs) were given therapeutically or prophylactically to HSCT recipients (Meij et al., 2012; Micklethwaite et al., 2007).

The wide clinical application of AdV-specific T-cells generated by stimulating PBMCs with immunogenic peptides requires knowledge of T-cell epitopes restricted by prevalent MHC molecules. Previous studies that have identified T-cell epitopes of AdV have focused exclusively on the subgroup C AdV (Leen et al., 2004b, 2008; Tang et al., 2006; Zandvliet et al., 2010). Subgroup B AdV serotype 11 (Ad11) is a major pathogen for hemorrhagic cystitis (HC) (Akiyama et al., 2001; Miyamura et al., 1989; Mori et al., 2012) and occasionally causes disseminated infection with fatal outcomes in immunocompromised patients (Taniguchi et al., 2012). However, T-cell epitopes of Ad11 have not been identified. Therefore, the goal of the following study was to identify a novel T-cell epitope of Ad11 presented by HLA-A*24:02, which is one of the most common HLA class I molecule in many ethnic groups (60% in Japanese population, 20% in Caucasians, and 12% in Africans) (Gomi et al., 1999).

2. Materials and methods

2.1. Donor and patient specimens

PBMCs and serum from HLA-A*24:02+ healthy volunteer donors and a patient with Ad11-associated HC were obtained after informed consent. In addition, urine was collected from a patient with Ad11-associated HC. Measurement of AdV DNA in the patients' serum and urine was performed by real-time polymerase chain reaction, as previously described (Funahashi et al., 2010).

2.2. Cell lines

Epstein-Barr virus (EBV)-transformed B-lymphoblastoid cell lines (LCLs) were generated by infection of PBMCs from healthy donors with concentrated EBV-containing supernatants of cultured B95-8 cells (Leen et al., 2004b). T2-A24 cells, the transporter associated with antigen processing-deficient B and T hybrid cell line T2 transfected with the HLA-A*24:02 gene, were kindly supplied by Dr. Y. Akatsuka (Aichi Cancer Center Research Institute, Nagoya, Japan). K562 cells were transduced with retroviruses that encode CD80 and CD86 and were selected to >90% purity by cell sorting for expression of these co-stimulatory ligands. CD80 and $CD86^+$ K562 were then transduced with retroviruses that encode a full-length HLA-A*24:02 (Phoenix-Ampho System; Orbigen) and sorted twice to obtain cells of >95% purity that expressed HLA-A*24:02 (named K562/A*24:02). Cell lines were cultured in RPMI-1640 medium (Sigma, St. Louis, MO) containing 10% fetal bovine serum (FBS). For preparation of peptide-pulsed LCLs, K562, and K562/A*24:02, the cells were washed once, resuspended in RPMI-1640 medium, and pulsed with the corresponding synthetic peptide at 5 μg/ml at room temperature for 2 h. The cells were then washed once and used in stimulation assays.

2.3. Infection of K562 and K562/A*24:02 with AdV

Ad11 isolated from patients was used in the experiments. K562/A*24:02 were infected with Ad11 at a multiplicity of infection of 100 and used for enzyme-linked immunosorbent assay (ELISA) 72 h after infection. Infection was confirmed by documentation of AdV hexon antigen expression by flow cytometry.

2.4. Peptides

A computer-based program (Bioinformatics and Molecular Analysis Section (BIMAS), HLA peptide binding predictions; http://www-bimas.cit.nih.gov/molbio/hla_bind/) was used to identify potential HLA-A24-binding peptides within the Ad11 hexon protein. The nonamer peptides with a score exceeding 100 were selected and synthesized. HLA-A24-binding peptide, QYDPVAALF, derived from the human CMV-pp65 protein (Kuzushima et al., 2001), and TYFSLNNKF, derived from the human AdV serotype 5 hexon protein (Leen et al., 2004b), were also synthesized. All peptides were synthesized by Medical & Biological Laboratories (Nagoya, Japan).

2.5. MHC stabilization assay

All candidate peptides were tested for their capacity to bind to HLA-A24 molecules on the surface of T2-A24 cells as described previously (Kuzushima et al., 2001). Briefly, T2-A24 cells (3×10^5 cells) were incubated with 200 μL RPMI-1640 medium containing 0.1% FBS, 5×10^{-5} M β-mercaptoethanol (Sigma), 3 μg/ml human β2-microglobulin (Sigma), and each of the peptides at a concentration of 10 μM at 37 °C for 16 h. Following the incubation, surface HLA-A24 molecules were stained with the anti HLA-A23/A24 monoclonal antibody (mAb) and anti-mouse FITC-labeled antibodies. Expression was measured by flow cytometry, and mean fluorescence intensity (MFI) was recorded. Percent MFI increase was calculated as follows: percent MFI increase = (MFI with the given peptide − MFI without peptide)/(MFI without peptide) × 100.

2.6. Generation and expansion of peptide-specific $CD8^+$ T-cells

PBMCs obtained from healthy volunteers were placed at a concentration of 2×10^6 cells per tube in a 14 ml polypropylene tube with 1 ml of RPMI-1640 medium with 10% human serum [referred to as culture medium (CM)] and directly stimulated with peptides at a concentration of 1 μg/ml. At day 3, CM was added to a final volume of 2 ml and supplemented with 25 IU/ml recombinant human interleukin-2 (IL-2) (R&D Systems, Minneapolis, MN). Cells were transferred to a 24-well plate at day 7, re-stimulated with peptides every 7 days, cultured until day 21 or 28, and tested by interferon-γ (IFN-γ) secretion assay for the presence of peptide-specific $CD8^+$ T-cells. To expand peptide-specific $CD8^+$ T-cells, $CD8^+$ cells producing IFN-γ in the presence of peptides were isolated using IFN-γ secretion assay, followed by expansion in the presence of OKT3 mAb (Janssen Pharmaceutical), IL-2, and feeder cells, as described previously (Sugimoto et al., 2009).

2.7. Antibodies and flow cytometric analysis

All antibodies were purchased from BD Biosciences (San Jose, CA) unless otherwise noted. The following anti-human antibodies for staining of cell surface markers and intracellular molecules were used: IFN-γ-FITC, CD3-PE-Cy5.5 (Invitrogen, Carlsbad, CA), CD8-PE, -APC, or -PerCP-Cy5.5, and HLA-class I-PE (eBioscience, San Diego, CA). PE-conjugated HLA class I tetramers folded with AdV peptides were used to stain virus-specific T cell receptors (TCRs) (Medical & Biological Laboratories). In addition, mouse anti HLA-A23/A24 (One Lambda, Canoga Park, CA) and mouse anti-AdV hexon protein (Abcam, Cambridge, UK) antibodies were used in combination with anti-mouse IgG/IgM-FITC.

The IFN-γ secretion assay was performed using the IFN-γ Secretion Assay – Cell Enrichment and Detection Kit (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions. Briefly, cells were re-stimulated for 4 h at 37 °C in the presence or absence of peptides (1 μg/ml). Cells were incubated with IFN-γ catch reagent and cultured for 45 min at 37 °C to allow for IFN-γ secretion, followed by staining with anti-IFN-γ, CD3, and CD8 antibodies. Intracellular cytokine staining assay for IFN-γ was performed as previously described with some modifications (Terakura et al., 2012). In brief, cells were re-stimulated with peptide-pulsed