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Research paper

HLA class I mono-specific APCs and target cells: A method to standardise in vitro CD8+ T cell expansion and functional assays

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Abstract

The introduction of in vitro T cell expansion and assay methods that are robust and easy to use would be welcome in cancer vaccine and infectious disease research. By coating HLA class I −ve B cells with recombinant HLA class I peptide complexes, we are able to produce antigen presenting cells and target cells expressing a single defined antigen in the context of costimulatory and adhesion molecules. HLA class I mono-specific cells promoted the in vitro expansion of CMV epitope specific CD8+ T cells from 0.03% to 30.6% in 2 weeks, which was comparable to using peptide-loaded dendritic cells. The HLA class I mono-specific cells were also shown to promote in vitro antigen specific T cell function in assays based on measuring cytokine production and cytolytic activity. HLA class I mono-specific cells are simple to prepare, can be used with any recombinant HLA class I allele/peptide combination and should provide a useful system for in vitro T cell expansion and functional analysis.

Keywords: Antigen presenting cell; T cell; Assay; In vitro; Immunotherapy

1. Introduction

The effective monitoring of antigen specific T cell numbers and activity is central to the assessment of vaccination and other immunotherapy strategies in oncology and viral infections (Nagorsen et al., 2004). The introduction of recombinant HLA class I tetramers has greatly facilitated the rapid and accurate enumeration of epitope specific CD8+ T cells (Altman et al., 1996). However the assays that measure the functional abilities of T cells via cytokine production and release, granzyme B production or in vitro lytic activity are frequently difficult to perform, of limited reproducibility and subject to considerable background 'noise' (Keilholz et al., 2002). A further difficulty is that the epitope specific T cells within PBMC populations are often present at low frequencies, necessitating in vitro expansion prior to analysis.

In CD8+ T cell expansion and functional assays, T cells interact with antigen presenting cells (APC) or target cells via the T cell receptor-HLA class I interface. The most

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widely used APCs or target cells are autologous B cells, autologous dendritic cells or partially HLA matched lymphoblastoid cell lines such as T2 cells (Scheibenbogen et al., 2000). However the use of these cells introduces a number of variables including, the ratio of T cells to APCs, the numbers of antigenic HLA class I/peptide complexes per cell and the presence of other HLA class I and II peptide complexes. These factors, combined with the lack of standardisation of any preceding in vitro expansion step, can lead to poor assay reproducibility. An approach that improves the specificity of assays, increases reproducibility and avoids the need for separate target cell lines for different HLA types would be a significant step forward.

We have previously described the use of a two step biotin–streptavidin anti-CD20 antibody targeting system to deliver recombinant HLA class I complexes to the surface of B cells. These complexes convert targeted B cells into highly effective APCs capable of producing significant expansion of peptide specific CD8+ T cells (Stebbing et al., 2004). In addition, coating B cells with recombinant HLA class I complexes converts them to effective targets for cytolytic T cells of the appropriate antigen specificity (Savage et al., 2002). By using Daudi B cells which do not express HLA class I, this system can be adapted to produce a homogenous population of B cells coated with a defined amount of a single HLA class I/peptide complex for use as APCs or functional assay targets. In this report we have investigated the use of these cells, termed HLA class I mono-specific cells, for in vitro T cell expansion and functional assays.

2. Materials and methods

2.1. Preparation of peripheral blood mononuclear cells (PBMCs)

PBMCs were purified from the heparinized blood of healthy volunteer donors by Ficoll–Hypaque density gradient centrifugation. HLA class I genotyping was performed using PCR sequence-specific primers (Olerup SSP; Genovision, Alpha Helix). CMV status was determined by ELISA for CMV specific IgG antibodies.

2.2. Preparation of HLA class I monomers

In-house recombinant biotinylated HLA class I monomers were constructed as described previously (Whitelegg et al., 2005) using the HLA-A*0201 binding immunogenic CMV peptide NLVPMVATV (pp65 protein 495–503), the HLA-B*0702 binding immunogenic CMV peptide TPRVTGGGA (pp65 protein 417–425) or an irrelevant HLA-A*0201 binding peptide TLWVDPYEV (BTG protein 103–111). Additional proprietary biotinylated HLA-A*0201 class I monomers incorporating the influenza peptide GILGFVFTL (MP protein 58–66), were purchased from ProImmune Ltd Oxford UK. The HLA class I monomers are referred to by the first three letters of the peptide.

2.3. Coating of Daudi cells with HLA class I monomers

HLA class I negative Daudi cells (1×10^6/ml) were incubated with recombinant B9E9 single-chain Fv-streptavidin (ScFvSA) fusion protein (10 μg/ml) diluted in phosphate-buffered saline (PBS) for 1 h at 4 °C (Schultz et al., 2000). After washing in PBS, cells were incubated with biotinylated HLA class I monomers (0.5 μg/ml in PBS) for 30 min at room temperature. Coating with HLA class I monomer was verified by staining with FITC-conjugated anti HLA class I antibody W6/32 (Serotec) and analysis by flow cytometry.

2.4. In vitro expansion of CMV-specific CD8+ T cells using Daudi cells coated with HLA class I monomers

Enrichment of CD8+ cells from PBMCs was performed using anti CD8 antibody-coated magnetic beads (Miltenyi Biotec) according to manufacturer's instruction. Daudi cells (0.2×10^6/ml) coated with HLA class I monomers were irradiated (100 Gy) and used to stimulate purified CD8+ cells (1×10^6/ml) in the presence of irradiated (30 Gy) autologous CD8 depleted PBMCs (1×10^6/ml) added back as non-proliferating feeder cells in RPMI media with 10% FCS and IL-7 (R&D Systems) at 10 ng/ml. IL-2 (R&D Systems) at 20 U/ml was added from day 4. The cultured cells were re-stimulated every 7 days.

2.5. In vitro expansion of CMV-specific CD8+ T cells using peptide loaded dendritic cells

To prepare monocyte-derived dendritic cells, PBMCs were cultured at 4×10^6/ml in RPMI media with 10% FCS in 6 well tissue culture dishes. Non-adherent cells were aspirated after 2–4 h. Adherent cells were cultured in 100 ng/ml IL-4 (R&D Systems) and 250 ng/ml GM-CSF (R&D Systems) and fresh media with cytokines was added every second day. After 6 days, dendritic cells were matured by overnight culture with 10 ng/ml TNF-α (R&D Systems) and 15 μg/ml poly (I:C) (Sigma-Aldrich). Maturation was confirmed by analysis of the CD80, CD83, CD86, CD11c and HLA-DR cell surface phenotype using fluorescent-labelled antibodies (BD Biosciences) and flow cytometry. Mature dendritic