Immunology Letters 119 (2008) 62–70

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/

Antigenic properties of HCMV peptides displayed by filamentous bacteriophages *vs.* synthetic peptides

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ARTICLE INFO

Article history:
Received 23 January 2008
Received in revised form 10 April 2008
Accepted 22 April 2008
Available online 19 May 2008

Keywords:
Synthetic carrier
HCMV epitopes
Biotechnology tools
Signal transduction
TCR

ABSTRACT

Several efforts have been invested in the identification of CTL and Th epitopes, as well as in the characterization of their immunodominance and MHC restriction, for the generation of a peptide-based HCMV vaccine. Small synthetic peptides are, however, poor antigens and carrier proteins are important for improving the efficacy of synthetic peptide vaccines. Recombinant bacteriophages appear as promising tools in the design of subunit vaccines.

To investigate the antigenicity of peptides carried by recombinant bacteriophages we displayed different HCMV MHCII restricted peptides on the capsid of filamentous bacteriophage (fd) and found that hybrid bacteriophages are processed by human APC and activate HCMV-specific CD4 T-cells. Furthermore we constructed a reporter T-cell hybridoma expressing a chimeric TCR comprising murine αβ constant regions and human variable regions specific for the HLA-A2 restricted immunodominant NLV peptide of HCMV. Using the filamentous bacteriophage as an epitope carrier, we detected a more robust and long lasting response of the reporter T-cell hybridoma compared to peptide stimulation. Our results show a general enhancement of T-cell responses when antigenic peptides are carried by phages.

1. Introduction

The system of antigen delivery by filamentous bacteriophage *fd* is a development of "phage display technology" based on phage virions displaying peptides encoded by oligonucleotide sequences that have been cloned into a gene coding for one of the viral coat proteins. The bacteriophage is a non-pathogenic and non-replicating vehicle in eukaryotic cells that we and others proposed to use as *in vivo* carrier of immunogenic peptides [1,2]. A peculiar characteristic of bacteriophages is their ability to display multiple copies of exogenous peptides on the capsid surface [3]. The viral assembly allows the incorporation into the capsid structure of 10–30% recombinant pVIII (the major coat protein) during hybrid phage synthesis, displaying one (single hybrid phage) or two (double hybrid phage) different epitopes. For this reason the molar concentration of specific peptides displayed by the carrier has to be established by N-terminal sequencing.

Filamentous bacteriophages have been shown to enter both the MHCI and MHCII pathway of antigen presentation and to prime antigen-specific T-cells. Of note, endocytosed phage particles have been shown to localize in phagosomes and endoplasmic reticulum (ER) suggesting that phages could represent the means by which exogenous antigen can reach the MHC class I pathway [4,5]. However, the underlying mechanisms are as yet largely unknown. In a previous report [6] the response of human CD4 T-cells against the phage-displayed peptide was determined by examining the reactivity of specific T-cell lines and clones obtained by *in vitro* priming of human PBMC with an HIV p66 peptide. We observed that not all p66 specific T-cells selected with the synthetic peptide were able to react with the hybrid phage because different TCRs have different abilities to recognize phage-derived peptide/MHC complexes as compared with their ability to recognize synthetic peptide/MHC complexes [6]. Furthermore we have previously demonstrated that the display of a single cytotoxic peptide on the virion surface is

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doi:10.1016/j.imlet.2008.04.004

sufficient to induce priming and to sustain long-term MHC class I restricted cytotoxic T lymphocyte responses in C57BL/6 mice immunized with recombinant bacteriophages [7]. In support of the efficacy of this polyepitopic carrier to generate a CTLs response, Sartorious et al. [8] have recently demonstrated that *fd* virions displaying tumor associated Ag-derived peptide elicit potent and specific CTL responses *in vitro* and *in vivo*.

Human cytomegalovirus (HCMV) is well controlled by immunocompetent individuals but causes disease in immunocompromised subjects such as transplant recipients or AIDS patients [9,10]. Cell-mediated immunity represents an essential host factor in the recovery from CMV disease and in the control of persistent infection. CTL are the principal effectors of cell-mediated immune response against CMV as demonstrated in HLA A*0201/Kb transgenic mice [11] and play a fundamental role during adoptive cellular immunotherapy [12–14], CD4 lymphocytes are equally relevant players, favoring long-term persistence of reinfused CTL or reconstitution of the endogenous repertoire [15,16]. Thus an antigen delivery system such as recombinant bacteriophages, capable to enter both MHCI and MHCII pathway of antigen processing and presentation may represent a powerful tool in designing a vaccine against HCMV.

To assess the potential of recombinant bacteriophages as HCMV epitope carriers we investigated the ability of bacteriophages to be processed by antigen presenting cells (APC) and to activate specific T-cell responses *in vitro*. In this study we focused on HCMV derived peptides showing different features of immunodominance and codominance that can be relevant in a diagnostic and clinical perspective. Specifically, we expressed on the phage capsid surface a CTL peptide or two different Th peptides of the immunodominant pp65 protein of HCMV, recognized by CD8 and by CD4 T-cells, respectively [14,17–19]. Here we demonstrate the ability of the *fd* antigen delivery system to activate HCMV-specific human CD4 T-cell lines. Moreover, we developed murine T-cell hybridomas transfected with a human TCR specific for an immunodominant HCMV peptide to test the antigenicity of the recombinant vaccine carrier for CD8 lymphocytes. Using this approach, we found that in the reporter T-cell hybridomas the NLV peptide displayed by *fd* bacteriophage results in a stronger and long lasting TCR signalling compared to soluble NLV peptide.

2. Materials and methods

2.1. Cell lines and plasmids

The TCR-negative murine T hybridoma 58ζ [20] and the OT-1 cell line were kindly provided by E. Palmer (Lab. of Transplantation Immunology and Nephrology, Dept. Research, University Hospital-Basel, Basel, CH). The 58 ζhCD8 line expressing human CD8 was a gift of HC. Chang (Dana-Farber Cancer Institute, Boston, US). The human lymphoblastoid cell line T2 (ATCC number CRL-1992) and splenocytes isolated from HLA-A2 transgenic mice, a gift from G. Bensi (Novartis Vaccines, Siena, Italy), were used to present the NLV peptide.

Plasmids included the pGα and pGβ cassette vectors [20] as well as the pBabe [21] and pcDNA3 (Invitrogen, Leek, The Netherlands) mammalian expression vectors. The pEGFP/NFAT-1D construct encoding GFP-fused NFAT was previously described [22]. The pGem-Teasy construct containing cDNAs encoding NLV-specific TCR α and β chains isolated from clone LSRA14 (Vα18S2Jα49 Vβ13S1Jβ1s2) and clone PBL3.1 (Vα15S1Jα44 Vβ20S1Jβ2S3) [23] were provided by B. Malissen and A. Kissenpfennig (Centre d'Immunologie, INSERM-CNRS, Marseille, France).

2.2. Construction and purification of hybrid bacteriophages

Single display bacteriophages were constructed by ligating a *Sac*II-*Sty*I oligonucleotide insert encoding peptide into fdAMPLAY88 phage genome [2]. Briefly, the following oligonucleotide sequences 5′-CCGCGGAGGGTAACCTGGTTCCGATGGTAGCTACCGTTGACGATCCCGCCAAGGG-3′, 5′-CCGCGGAGGGTGAATTCTTCTGGGACGCTAACGACATCTACCGTATCTTCGACGATCCCGCCAAGG-3′, and 5′-CCGCGGAGGGTCCGCTGAAAATGAACCTGATCCCGTCCATCAACGTTCACCACTACGACGATCCCGCCAAGG-3′encoding, respectively, for peptide NLV (pp65: aa 495–503, NLVPMVATV) [24] peptide128 (pp65: aa 512–524, EFFWDANDIYRIF) [17,18] and peptide 30 (pp65: aa 117–131, PLKMLNIPSINVHHY) [18,25] were used. The hybrid bacteriophage virions were purified and subjected to N-terminal amino acid sequencing analysis as described elsewhere [26]. The density of peptide displayed in a hybrid virion was calculated by N-terminal Edman sequencing. The construction of fdOVA has been described elsewhere [7].

2.3. Generation of CMV-specific human CD4 T-cell lines

CD4 T-cell lines were generated as previously described in detail [17,27,28]. Briefly, PBMC were stimulated with CMVpp65 peptides pep30 and pep128 in 24 well plates. IL2 was added on day 4. Cultures were split according to growth and restimulated with autologous, irradiated, peptide-pulsed PBMC after 4 weeks. IL2 was added on day 2. The cultures were split and restimulated every 3 weeks.

Specific proliferation was measured by thymidine incorporation on 3×10^4 T-cells cultured with 1×10^5 autologous irradiated PBMC and shown as Kcpm (cpm $\times 10^3$) [27]. The plates were pulsed after two days (0.018MBq ^{3}H-thymidine, Amersham, Aylesbury, UK) and harvested the next day. The filters were counted (Matrix-9600, Canberra-Packard, Meriden, CT). Specific response of CD4 T-cell lines was also tested by measuring IFNγ production in the 24 h supernatants by using a commercial ELISA kit (Mabtech, Stockholm, Sweden), following the manufacturer's instructions. Results are shown as OD_{405} after 2 h incubation.

2.4. Construction of plasmids encoding chimeric NLV-specific TCRs

The entire variable regions of two NLV-specific human TCRs were obtained by PCR amplification of a fragment of the cloned cDNAs encoding TCR α and β chains isolated from clone LSRA14 (Vα18S2Jα49 Vβ13S1Jβ1s2) and clone PBL3.1 (Vα15S1Jα44 Vβ20S1Jβ2S3).

The sense primers for Vα15 (5′-CGAATTCATGAAGACATTTG-3′) and Vα18 (5′-CGAATTCATGGAGAAGAATC-3′) included an *EcoRI* restriction site. The antisense primers for Vα (5′-AGGGTCAGGGTTCTGAATATT-3′) were designed on human constant α chain and included an *SspI* restriction site. The sense primers (5′-CGAATTCATGATGCTCTGCT-3′) for Vβ20 and (5′-CGAATTCATGAGCATCGCC-3′) for Vβ13 included an *EcoRI* restriction site. The antisense primers for Vβ (5′-CACCTTGTTCAGGTCCTC-3′) were designed on human constant β chain and included an *EcoO109* restriction site. The Vβ20S1 cDNA contains an internal *EcoO109* restriction (GGGGCCT) site which was modified by site-specific mutagenesis to GGCGCGT prior to performing the amplification.

The chimeric TCR chains, formed by murine constant regions and human variable regions (Vα15-Vβ20 and Vα18-Vβ13), corresponding, respectively, to the PBL3.1 and LSRA14 T-cell clones were generated by cloning the Vα and Vβ PCR products into the pGα and pGβ cassette vectors upstream of the murine constant regions, respectively. Chimeric α and β chains were subsequently