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Research paper

Identification of T cell epitopes by the use of rapidly generated mRNA fragments

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Abstract

Although the number of defined T cell epitopes of clinically relevant antigens is constantly increasing, there is still an enormous need to identify further peptides, processed from new antigens or presented by rare HLA molecules, respectively. Here we introduce a novel two-step approach for the rapid identification of T cell epitopes. It was established in the CMV infection model.

From the peripheral blood of healthy donors sharing HLA-A1 according to HLA serotyping we isolated $CD8^+$ T lymphocytes and generated dendritic cells (DCs). DCs were electroporated with CMV pp65 mRNA and tested for recognition by autologous $CD8^+$ T lymphocytes in IFN-γ ELISPOT assays. In all 10 CMV-seropositive donors, CMV pp65-specific $CD8^+$ T cells were readily detectable ex vivo. In 7 of them the response was at least in part restricted by HLA-A1.1 as verified in IFN-γ ELISPOT assays with pp65 mRNA-electroporated K562 cells stably transfected with HLA-A*0101 (K562/A*0101). In a subsequent step various 3′-deleted pp65 RNA fragments were rapidly generated by in vitro transcription of plasmid DNA-templates linearized with restriction enzymes at different sites within the pp65-coding sequence. Polyadenylated mRNA fragments were then electroporated into K562/A*0101 cells and tested for recognition by ex vivo $CD8^+$ T cells in IFN-γ ELISPOT assays. We thereby identified a 76 bp-long sequence as target of the HLA-A*0101-associated pp65-specific T cell response. From this region, 10 peptides predicted by current algorithms were synthesized and tested for recognition. Peptide pp65 364–373 (previously identified by a reverse immunology approach by [Hebart, H., Daginik, S., Stevanovic, S., Grigoleit, U., Dobler, A., Baur, M., Rauser, G., Sinzger, C., Jahn, G., Loeffler, J., Kanz, L., Rammensee, H. G., Einsele, H., 2002. Sensitive detection of human cytomegalovirus peptide-specific cytotoxic T-lymphocyte responses by interferon-gamma-enzyme-linked immunospot assay and flow cytometry in healthy individuals and in patients after allogeneic stem cell transplantation, Blood 99, 3830.]) was positively tested and found to be the dominant target epitope of the HLA-A1-restricted anti pp65 T cell response in all donors.

Abbreviations: APC, antigen-presenting cells; CTL, cytolytic T lymphocytes; DC, dendritic cells; CMV, cytomegalovirus; ELISPOT, enzyme-linked immunospot; IFN, interferon; PBMC, peripheral blood mononuclear cells; SD, standard deviation.

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We conclude that (i) the use of HLA-transfected K562 cells allows to dissect antigen-specific T cell responses to partial responses associated with defined HLA class I alleles and (ii) transfection of in vitro transcribed RNA fragments allows to identify immunogenic regions of a given antigen. The latter technique bypasses the need of prior cloning and sequencing of cDNA fragments, reduces the number of synthetic peptides to be tested and thus saves both costs and time.

Keywords: HLA-transfected K562 cells; mRNA fragments; ELISPOT assay; T cells; CMV

1. Introduction

The identification of T cell epitopes generally involves the determination of restricting HLA alleles followed by the identification of the respective immunogenic peptide. In this study we introduce a new approach to facilitate the identification of T cell epitopes of known antigens using (i) HLA-transfected K562 cells and (ii) mRNA fragments generated in vitro from antigen-coding cDNA.

We recently introduced HLA-transfected K562 cells as an alternative antigen-presenting cell line with ideal properties for the peptide-independent enumeration of T cell responses associated with HLA-A2.1 and -B7.2. The background reactivity against K562 cells stably transfected with HLA-A*0201 and B*0702 was generally low and the HLA-transfected K562 cells efficiently expressed whole antigens and presented antigenic peptides after electroporation with clonal mRNA (Britten et al., 2004).

A regularly applied intermediate step on the way to identify new T cell defined peptide epitopes is the identification of a short gene region of the targeted antigen and the subsequent synthetization of putative HLA-binding peptides from this region. This can be achieved by cloning ans transfecting truncated cDNA fragments of the respective antigen-coding full length cDNA (e.g. Schneider et al., 1998). Here we introduce a new and fast approach to obtain 3′-deleted gene fragments of any given antigen by using mRNA fragments instead of plasmids as antigen format. The antigen-coding circular plasmid was therfore linearized with restriction enzymes cutting within the coding sequence of the transgene. RNA fragments of different length were then generated by in vitro transcription of the differently digested plasmid templates. The resulting RNA fragments were then polyadenylated and electroporated into K562/HLA cells.

This two-step approach was established in the CMV infection model. $CD8^+$ T lymphocytes were isolated from healthy donors all carrying the frequently found HLA-A*0101 allele. The $CD8^+$ lymphocyte populations were tested in in IFN-γ ELISPOT assays for recognition of autologous DCs that were electroporated with pp65 mRNA. The detected ex vivo T cell responses against pp65 mRNA were then further dissected into a partial response associated with HLA-A1.1 by the subsequent use of mRNA-electroporated K562 cells stably transfected with HLA-A*0101. In order to attribute the detected HLA-A1.1-restricted ex vivo T cell response to a small and defined region of the pp65-antigen, K562/A*0101 cells were electroporated with different 3′-deleted pp65 mRNA fragments and applied as APCs in IFN-γ ELISPOT assays. In all donors tested we could further attribute the complete HLA A1.1-restricted anti-pp65 T cell response to peptide pp65 364–373 that can be found within this region and had been previously identified with different techniques (Hebart et al., 2002). This approach can be transferred to other known antigens that are recognized by T lymphocytes and will facilitate the identification of new T cell epitopes.

2. Materials and methods

2.1. Donors

Donors HD B 1–11 were healthy volunteers all HLA-A1-positive according to standard HLA serotyping procedures. All donors were CMV-seropositive with the exception of HD B 11 (Table 1). From all