Research paper

Use of replication restricted recombinant vesicular stomatitis virus vectors for detection of antigen-specific T cells

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ABSTRACT

Detection of antigen-specific T cells at the single-cell level by ELISpot or flow cytometry techniques employing intracellular cytokine staining (ICS) is now an indispensable tool in many areas of immunology. When precisely mapped, optimal MHC-binding peptide epitopes are unknown, these assays use antigen in a variety of forms, including recombinant proteins, overlapping peptide sets representing one or more target protein sequences, microbial lysates, lysates of microbially-infected cells, or gene delivery vectors such as DNA expression plasmids or recombinant vaccinia or adenoviruses expressing a target protein of interest. Here we introduce replication-restricted, recombinant vesicular stomatitis virus (VSV) vectors as a safe, easy to produce, simple to use, and highly effective vector for genetic antigen delivery for the detection of human antigen-specific helper and cytotoxic T cells. To demonstrate the broad applicability of this approach, we have used these vectors to detect human T cell responses to the immunodominant pp65 antigen of human cytomegalovirus, individual segments of the yellow fever virus polyprotein, and to various influenza proteins.

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1. Introduction

The development and application of techniques such as MHC tetramer staining, ELISpot, and intracellular cytokine staining have transformed the study of T cell immune responses to microbes, tumors, auto-antigens, and vaccines. These assays – which permit detection of antigen-specific cells at the single cell level – do not require difficult to reproduce *in vitro* expansion protocols, and are widely regarded as the new "gold standards" for the characterization of T cell responses. When optimal peptide epitopes and their MHC restriction elements are mapped, MHC tetramers provide the most rapid method for detection of antigen-specific T cells

and give direct access to physical phenotypes, but they do not detect function. In contrast, the ELISpot and ICS assays detect a specific function – the ability to produce one or more cytokines upon short-term stimulation with antigen – and they are considerably more flexible with respect to the form and range of antigens that can be used in the assays. Neither the ELISpot nor the ICS assay requires mapping of MHC restriction elements or optimal MHC-binding peptide epitopes, and both assays are often performed with "complex" antigens that might contain multiple distinct epitopes that are recognized by the T cell population of interest.

Although a wide range of antigens may be used for ELISpot or ICS assays, each has one or more significant limitations. Antigens delivered in the form of recombinant proteins, microbial lysates, and lysates of infected cells are largely restricted to exogenous antigen-processing pathways, and are therefore effective for stimulation of CD4 T cells but ill-suited to efficiently stimulate CD8 T cells. Plasmid DNA has been used to deliver

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antigens for T cell assays (Tscharke et al., 2005), but since primary cells have low transfection efficiencies, its use requires highly transfectable cultured cell lines with an additional requirement for co-transfection with expression plasmids for one or more MHC alleles. Recombinant vaccinia viruses have been extensively used to deliver antigens for detection of antigen specific CD4 and CD8 T cells (Larsson et al., 1999; Xu et al., 2003), but their production requires lengthy protocols involving homologous recombination and multiple rounds of plaque purification. Furthermore, pre-existing immunity prevents their use in vaccinia-exposed individuals, including those who have received experimental vaccinia-based vaccines. Finally, pools of overlapping peptides representing entire proteins or even small viruses are now commonly used to stimulate both CD4 and CD8 T cells, but it is not economically feasible to routinely purchase all of the peptides that are required to contain all possible epitopes for large viruses, such as those in the pox and herpesviridae families, or for broad coverage for highly variable viruses, such as HIV or HCV. Under defined and common circumstances, each of these forms of antigen has significant theoretical and/or practical limitations, and new options for antigen delivery are needed.

Our search for a suitable antigen-delivery system began with the following criteria. (1) The antigen-delivery system had to be capable of stimulating both CD4 and CD8 T cells. (2) It had to do so using only fresh, *ex vivo* cell populations such as PBMC, without recourse to cultured stimulator cells, such as B lymphoblastoid cell lines (B-LCL). In practice, this narrowed the search to viral vectors. (3) Once the search was narrowed to recombinant viral vectors, they had to be easy to produce from plasmid DNA, without recourse to systems that require homologous recombination (effectively ruling out poxviruses). (4) The viral vector had to have reasonably broad tropism, both at the cellular and species level (it would be desirable if we could use the same constructs in mice, non-human primates, and humans). (5) There had to be little-to-no pre-existing immunity to vector epitopes; this made us leery of adenoviral vectors, because although many of them express negligible amounts of vector antigens upon infection, it is possible that the input adeno structural proteins could stimulate cells, especially CD4 T cells. (6) The vector should have the capacity to accommodate reasonably large insert sizes (up to 4 kb). (7) The vector had to give high levels of antigen expression early after infection. (8) The vector should have relatively low cytopathicity. (9) The vector should be inherently safe, at least at the BSL2 level. One virus that seems to meet all of these criteria is vesicular stomatitis virus (VSV).

Vesicular stomatitis virus is a member of the Rhabdoviridae family. It has an extremely compact, nonsegmented negative strand RNA genome with five non-overlapping genes coding for viral proteins. Reverse genetics systems for efficient production of recombinant VSV from plasmid DNA were developed in the mid-1990s by the laboratories of Rose and Wertz (Whelan et al., 1995; Lawson et al., 1995). These techniques were originally designed to genetically manipulate the VSV genome for RNA virus assembly and replication studies. Further development of these methods soon made it possible to construct recombinant VSV encoding a foreign protein of interest in place of the viral glycoprotein (VSV-G) in the VSV genome (VSV-ΔG). VSV-ΔG vectors are safe for routine laboratory use,

because even though the recombinant virions are coated with VSV-G, they lack VSV-G in the recombinant genome and are thus capable of only one round of replication. Additionally, VSV-ΔG has a relatively large insert capacity (≥4 kb). For these reasons, we have used VSV-ΔG as a vector to introduce antigen to T cells. We generated a panel of VSV-ΔG constructs encoding various viral antigens and have developed a method to employ these constructs for antigen delivery in T cell ICS and ELISpot assays. Our results demonstrate that VSV-ΔG vectors represent an alternative and efficient antigen source for detecting antigen-specific T cells.

2. Materials and methods

2.1. ELISpot

The IFN-γ ELISPOT assay was performed as previously described (Larsson et al., 1999; Murali-Krishna et al., 1998). 2×10^5 to 5×10^5 human PBMC from a CMV-seropositive donor were infected with a recombinant VSV expressing the pp65 gene of human cytomegalovirus (VSV-ΔG.CMVpp65). PBMC from the same donor were also infected with a recombinant VSV encoding the nucleoprotein gene from lymphocytic choriomeningitis virus (VSV-ΔG.LCMV-NP). Since LCMV infection is uncommon in human populations, VSV-ΔG.LCMV-NP infection was not expected to stimulate cytokine production, and this vector was used as a negative control virus for our studies in human subjects.

2.2. Plasmid construction

Plasmid pVSV-ΔG is a Bluescript-based plasmid that encodes the anti-genome RNA of VSV. In this plasmid, the coding region for VSV-G has been removed and replaced with a polylinker (Takada et al., 1997). Genes of interest were inserted into the polylinker region of pVSV-ΔG using *Kpn I*, *Sph I*, and *Asc I* restriction enzyme sites. For some constructs, a gene of interest was inserted into pVSV-ΔG using a ligation-independent cloning (LIC) method (Aslanidis and de Jong, 1990). Briefly, the polylinker region of pVSV-ΔG was replaced with a LIC sequence that upon linearization with *Sma I* and subsequent treatment with T4 DNA polymerase and dATPs yields specific overhangs. In the absence of ligase, these overhangs anneal with complimentary sequences flanking genes of interest that have been amplified with LIC primers and treated with T4 DNA polymerase and dTTPs. The DNA primers used to amplify genes of interest from plasmid DNA or cDNA are listed in Table 1.

2.3. Recovery of recombinant VSV-ΔG

Recombinant VSV-ΔG was produced as previously described (Takada et al., 1997; Robison and Whitt, 2000; Whitt, 2010). Briefly, baby hamster kidney cells (BHK-21; American Type Culture collection) were maintained in Dulbecco's modified Eagle's medium (DMEM)/5% fetal bovine serum (FBS) at 37 °C. BHK-21 cells on 6-well plates (~95% confluent) were infected with recombinant modified vaccinia virus Ankara (MVA) expressing T7 polymerase (MVA-T7) at a multiplicity of infection (MOI) of approximately 1. Following a 90 min incubation, plasmids encoding VSV-N, P, L, and G proteins and pVSV-ΔG containing a gene of interest (VSV-ΔG) were transfected at a ratio of