Cytotoxic T cells generally recognize short 8-mer to 11-mer peptides in association with major histocompatibility complex (MHC) class I molecules on the surface of the antigen presenting cells (APCs). Usually, these peptides are produced in the cytosol of the APCs by proteolytic degradation of cytosolic proteins. The peptides are subsequently transported first to the endoplasmatic reticulum (ER), then to the cell surface and are bound to the MHC class I molecule. In the study of these events, transient expression from recombinant vaccinia viruses encoding short immunogenic peptides has been shown to lead to peptide presentation on the MHC class I molecules (2,6).

In this work, a novel retroviral vector for expression of peptides has been developed, which potentially could be used for intracellular expression of large random libraries of short peptides. Such retroviral vector-based cellular libraries can be prepared in the way that each cell only expresses a single peptide species derived from the library—the “one cell-one peptide” principle. Due to the replication cycle of the virus, expression of such a library using vaccinia viruses would have some limitations, because it will result in many different peptides being produced transiently in single cells. Vaccinia viruses infect most mammalian cells and replicate transiently in the cytoplasm where they inhibit host-protein synthesis and elicit a rapid cytopathic effect on the host cell (16).

A bicistronic retroviral vector encoding a single RNA transcript was constructed using standard recombinant techniques (Figure 1). The vector backbone is derived from the murine retrovirus Akv (13). Downstream from the packaging signal, the vector contains a polylinker enabling conventional cloning of peptide-encoding sequences or insertion by non-polymearse chain reaction (PCR)-mediated overlap extension (9). Following the polylinker, an internal ribosome entry site (IRES) derived from the encephalomyocarditis (EMC) virus is inserted (10). This IRES directs translation of a neo gene conferring G418 resistance to transduced cells. To ensure that the IRES cassette was functional in a retroviral vector context, the sequences encoding the peptide were exchanged with the phleomycin-resistance gene (pUT649; CAYLO, Toulouse, France). After transduction with more than $10^5$ NIH-3T3 cells of a diluted stock of this vector, 38 colonies resistant to phleomycin and 21 colonies resistant to G418 were isolated. All phleomycin-resistant colonies were G418 resistant, and all G418-resistant colonies were phleomycin resistant, indicating that both resistance genes were translated from the same vector’s RNA transcript.

As a test system for evaluating whether the vector could express such small peptides, we used a functional MHC-class I presentation assay. Two vectors encoding the peptides SIINFEKL and TPHPARIGL, respectively, were constructed. These peptides correspond to two previously described MHC-class-I-restricted T-cell epitopes derived from ovalbumin and Escherichia coli $\beta$-galactosidase, respectively (1.5). The peptides bind specifically to the mouse K$^b$ and L$^d$ MHC-class-I molecules, respectively. The vector harbors a methionine start codon in front of the peptide-coding regions and a stop codon immediately after. APCs of relevant MHC-I haplotypes were transduced using the Psi-2 packaging cells (14) with standard retroviral vector transduction techniques (Figure 1) (12). The APCs were selected for G418 resistance, and resistant colonies were pooled and assayed for peptide presentation on the relevant MHC-class-I molecules. Mixing the APCs with specific T-cell hybridomas causes
the T-cell hybridomas to start secretion of Interleukin 2 (IL-2). Production of IL-2 was determined in a standard cellular assay measuring $[^3]$H]thymidine incorporation in the IL-2-dependent HT-2 T-cell line (19). All cell culture work was performed according to standard procedures.

As shown in Figure 2, both peptides were: (i) translated in the cytosol from their respective vector RNAs, (ii) correctly processed, (iii) transported into the antigen presentation pathway and (iv) eventually presented on the relevant MHC-class-I molecules. Only a few hundred APCs were needed to obtain a detectable IL-2 production. Control cells, either untransduced or transduced with irrelevant peptides, did not activate the T-cell hybridomas (Figure 2). As controls, we have used 20 µM of synthetic SIINFEKL or TPHPARIGL peptides to obtain maximal pulsing of the APCs. This amount of synthetic peptide leads to the same level of IL-2 production from the T-cell hybridomas as that obtained by using the transduced APCs expressing the same peptides. Furthermore, we have found that addition of 20 µM of the relevant synthetic peptide to the LB27-4 SIINFEKL and 79.24.H8 TPHPARIGL-transduced cells during an antigen-presentation assay generally did not lead to further increase in the IL-2 production under our assay conditions (data not shown).

We have demonstrated that a small peptide, eight or nine amino acids long, can be stably produced in sufficient amounts for detection in a biological assay using a retroviral vector. The vector described here is a bicistronic, single-RNA-transcript vector enabling selection for positive vector transduction. By using a bicistronic single-promoter vector construct, selection for resistance (e.g., G418 resistance) will not result in promoter interference leading to shutdown of peptide expression during selection procedures (3,4).

This retroviral peptide expression system differs at some important points from the previously described vaccinia peptide expression system. The vaccinia virus does not integrate in the host genome but stays in the cytoplasm of the infected cells. Host cell protein production is shut down, and mainly viral proteins are produced. This means that vaccinia virus provides a high-transient production of a given peptide. In contrast, the retroviral vector is integrated before peptide production is initiated.

One exciting development of this work would be the application of retroviral vectors for use in generating intracellularly expressed random peptide libraries. Synthetic peptide libraries consisting of a population of different benchmarks.
peptides synthesized on beads following the “one bead-one peptide” concept have previously been described (11). Using such libraries, specific peptides interacting with various receptors can be identified by means of isolating and analyzing beads positive for receptor binding.

A one-cell-one peptide concept may, like the one bead one-peptide concept, be important for screening and selection of intracellular peptide libraries. The retroviral transduction system provides several clear advantages for that use compared to other intracellular expression systems. It has an efficient system for integration of the viral vector DNA into the host-cell genome that allows stable expression from the integrated retrovirus vectors. It has an efficient mechanism for the rapid identification of class-I MHC-restricted CTL epitopes. J. Immunol. 151:3971-3980.


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