

A modified cell surface marker gene for transgenic animal studies

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Received August 17, 1998; Revised and Accepted September 29, 1998

ABSTRACT

We developed a marker gene encoding a human cell surface molecule called CD8 for use in transgenic animal studies. The CD8 cDNA contains three mutations: one in the extracellular domain which prevents interaction with its ligand MHC class I and the other two in the cytoplasmic domain which inhibit its signalling function. The cDNA was linked to a fragment of the human growth hormone gene and in transgenic animal studies, expression was observed in the appropriate cell types using a CD2 enhancer. The advantage of the CD8 marker gene is that it is incapable of signalling via its only known signalling pathway and its expression can be monitored using monoclonal antibodies and microscopy or flow cytometry.

In order to characterize and study the function of regulatory elements for gene expression, DNA fragments with putative elements are often fused to a marker gene. Some of these marker genes are enzymes such as luciferase or β -galactosidase. These enzymes, being processive, allow for sensitive detection of low levels of gene expression. Other types of marker genes, such as green fluorescent protein (GFP) and some which encode cell surface proteins have also been used (1–3). Thus far, GFP has not been demonstrated to be useful in the study of hematopoietic cells. However, cell surface molecules, while not as sensitive as an enzyme, offer advantages for studying expression during hematopoietic or lymphoid differentiation. Expression on subsets of cells within mixed populations can be identified using flow cytometry with combinations of monoclonal antibodies conjugated to different fluorochromes. In addition, quantitative information is obtained, reflecting levels of expression on individual cells which is important in detecting position effect variegation. Living cells expressing the marker gene can be isolated for further analysis using FACS sorting or magnetic bead separation. Another advantage of monitoring expression of a human cell surface molecule on a murine background is that usually an antibody can be selected which will not bind to endogenous mouse cell surface proteins. A potential limitation, however, is that some human cell surface proteins can interact with murine signalling molecules or homologous murine ligands and therefore

may not be completely neutral in terms of effects on cellular processes such as activation and differentiation.

We have developed a marker gene encoding a modified cell surface protein that could be used for transgenic animal studies. The human CD8 glycoprotein is expressed on the cell surface of T cell subsets during lymphocyte differentiation and on subsets of NK cells and dendritic cells (4). Expression of the CD8 α gene in cells that do not normally express the protein leads to the formation of CD8 α/α homodimers. CD8 functions as an adhesion molecule binding to MHC class I (5), and as a signalling molecule through its association with the lymphoid specific tyrosine kinase p56^{lck} (6). We performed mutational analysis of the CD8 α chain to identify amino acids that were critical for interaction with p56^{lck} (7) and for interaction with MHC class I (8). We identified two cysteines in the cytoplasmic tail that were necessary for binding to p56^{lck}; cysteine to alanine substitutions at those two positions abolished detectable interaction with the kinase (7) and impaired signalling through CD8 upon crosslinking with antibody (9). A single point mutation of residue N99 in the CDR-3-like loop of the Ig-like domain severely impaired the ability of CD8 to interact with its ligand MHC class I (8).

To create the marker gene we incorporated these three mutated residues into the CD8 α chain cDNA so that the protein could not interact with MHC class I and could not signal through p56^{lck}. In addition, to increase the probability that a cDNA would be expressed in transgenic animals, we linked a DNA fragment containing the human growth hormone (HGH) gene (10) to the mutated CD8 α cDNA (Fig. 1). The presence of the HGH introns appears to increase transcription of linked cDNAs (11), and while the mechanism is not completely understood there is some evidence for effects on nucleosome alignment (12). The HGH fragment starts with a *Bam*HI site beginning at the second nucleotide in the first exon of the gene and contains the five exons, introns and polyA addition site.

To demonstrate that the new construct could be expressed, the CD8 α cDNA linked to the HGH region was subcloned into the pcDNA3 expression vector containing the CMV promoter. Purified plasmid was transfected into monkey kidney COS7 cells as previously described (8) and after 48 h, expression of CD8 on the cell surface was detected by the anti-CD8 α mAb OKT8. Similar expression of human CD8 was obtained with the CD8–HGH construct and another plasmid containing the wild-type CD8 α cDNA with an SV40 polyA addition site (data not shown).

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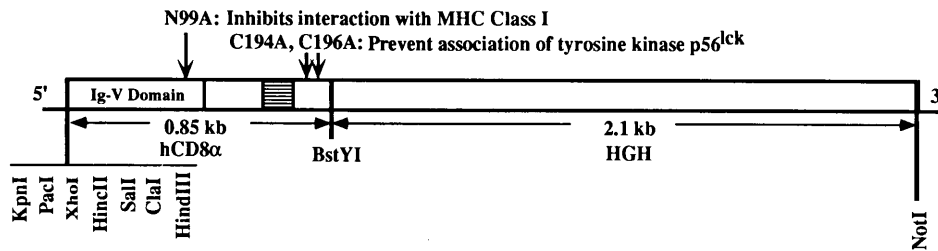


Figure 1. Schematic map of the CD8 α cDNA linked to the 2.1 kb HGH fragment in the vector pBluescript II SK. A *Bam*HI fragment starting within the first exon of the HGH gene and including the PolyA addition site (10) was subcloned into the Bluescript vector. A *Hind*III/*Bst*YI fragment containing the mutated CD8 cDNA without the 3' UT region was then subcloned into the vector. The unique restriction endonuclease sites flanking the region are shown. A *Pac* restriction endonuclease site was added at the 5'-end of the Bluescript polylinker to facilitate the cutting away of vector sequences from the 5' and 3'-ends with *Pac* and *Not*I, respectively, before pronuclear injection to generate transgenic mice. The mutated positions as well as potential sites for subcloning are indicated.

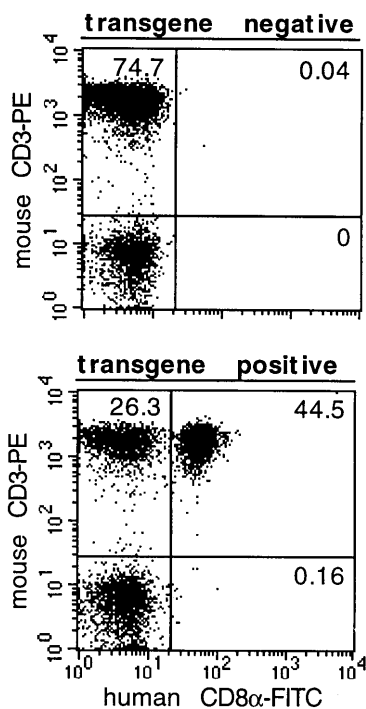


Figure 2. Expression of the CD8 α marker gene on lymphocytes of transgenic mice. Shown are flow cytometry profiles of peripheral blood lymphocytes stained with the phycoerythrin conjugated anti-murine CD3 mAb (clone 145-2C11, Pharmingen, San Diego, CA) and fluorescein conjugated anti-human CD8 α mAb (clone B9.11, Coulter-Immunotech, Miami, FL).

Given that the construct functioned in tissue culture cells, we went on to test its expression in transgenic animals. The CD8-HGH fragment was linked to a 2.1 kb *Kpn*I-*Asc*I fragment containing the CD8 β promoter and a 2 kb *Hind*III-*Bam*HI fragment from the 3' flanking region of the human CD2 gene containing an enhancer. The 2 kb CD2 fragment allowed position independent expression of a construct containing a CD2 minigene which included the CD2 promoter (13). We obtained expression of the transgene in two out of three founder lines and only a portion of the cells expressed the transgene (Fig. 2). The

expression pattern was typical for enhancer regions that are susceptible to position effects. Thus, the 2 kb CD2 fragment we used has enhancer activity but not LCR activity when paired with the human CD8 β promoter. The expression was observed in both major subpopulations of CD3⁺ T lymphocytes, the CD4⁺ and CD8⁺ cells, as expected for a CD2 enhancer (data not shown). Expression of the transgene in the appropriate cell populations indicates the marker gene is functional in transgenic mice. This new form of CD8 should be useful for gene regulation studies, particularly for genes expressed in subsets of lymphoid or hematopoietic cells.

ACKNOWLEDGEMENTS

We appreciate the excellent technical assistance of Ms Shanta Nag and Mr Mark R. Barr. This work was supported by an RO1AI35417 grant to P.K. and an NIH postdoctoral fellowship 1-F32-AI09700-01A1 to L.K.

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