Chapter 12

IN SITU MHC TETRAMER STAINING

In situ tetramers

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Abstract: With the onset of MHC tetramer technology came a wealth of new understanding of antigen specific CD8⁺ T cells. This chapter discusses the application of MHC tetramer technology to stain antigen specific T cells in tissue sections. In situ tetramer staining (IST) can be used to determine the localization, abundance, and phenotype of antigen specific T cells in their native environment. IST can be used to stain essentially any antigen specific T cell in any tissue for which MHC tetramers are available. In this chapter, an overview of the technique is described including advantages and disadvantages of using thick fresh sections vs. thin frozen sections, and using direct labeling vs. indirect labeling. A summary of experimental systems that have employed IST to gain understanding of antigen specific CD8⁺ T cells is provided, including some interesting biology that has been revealed from these studies. Finally, the prospects for using IST to evaluate cancer specific T cells in cancer patients undergoing vaccine therapy are discussed.

Key words: In situ tetramer staining (IST), MHC class I tetramers, CD8⁺ T cells, vibratome, confocal microscopy

1. INTRODUCTION

Antigen specific CD8⁺ T cells are required for clearance of several cancers and most viral infections and are therefore an important cell type to understand. The use of MHC-tetramers to stain antigen specific T cells has revolutionized the study of T cells and has led to increased understanding of how these important cells work (1). A few years ago, methodologies were developed to use MHC tetramers to stain antigen specific T cells in tissue sections (2-4). This technique is called in situ tetramer staining (IST). Where flow cytometric analysis of tetramer stained cells allows for the rapid
quantification of antigen specific cells, IST allows for the visualization of antigen specific T cells in their native environment, thus allowing the study of antigen specific T cells in 3-dimensional space and maintaining the relationship to surrounding cells within a tissue. Combining immunohistochemistry with IST allows for phenotypic characterization of antigen specific T cells and surrounding cells, including target cells, in tissues. In instances when tissue quantities are limited, such as tissue biopsies in which infiltrating cells are limited and not sufficient for flow cytometric analysis, IST is an attractive alternative. IST can be applied to essentially any tissue from any species to label any antigen specific T cell for which MHC-tetramer reagents are available. IST is a valuable tool to evaluate the localization and phenotype of cancer specific CTLs in tissues from cancer patients undergoing vaccine therapy.

2. **IN SITU TETRAMER STAINING (IST)**

IST involves applying MHC tetramers to fresh or frozen tissue sections (3, 5). IST with MHC class I tetramers is less effective or ineffective with fixed tissues (3, 5). IST appears to require mobility of the TCR on the surface of the T cells in order to gain avidity obtained by one MHC tetramer binding more than one TCR on the surface of the T cell. The use of peptide-loaded MHC class I molecules conjugated to dextran, referred to as MHC-multimers, has been described to stain antigen specific T cells in air dried acetone fixed tissue sections (6). Presumably MHC-multimers have enough range to bind multiple TCRs and obtain needed avidity regardless of TCR mobility.

Fresh tissue sections cut with a vibratome or scalpel offer several advantages over thin frozen sections for IST. First, the resultant tetramer and antibody stain is more crisp and has higher resolution. Second, thin sections are a single plane of one cell layer whereas thick sections contain many layers of cells. Because thick sections inherently contain more layers of cells, one can use a confocal microscope to examine antigen specific T cells deep into cellular layers and study interactions in 3-dimensional space. The third dimension is lost in thin sections. Nonetheless, frozen sections offer the great advantage over fresh tissue sections in that tissues can be collected, stored, and processed when convenient. Also, cutting tissue that is frozen is much easier than cutting fresh tissue.

IST can be performed in either a direct or indirect fashion. Direct labeling involves using tetramers coupled to a very bright fluorophore such as APC or by using MHC-dextran multimers (3, 6). Indirect labeling involves using tetramers coupled to a fluorophore such as FITC, where the