

Preface

Ever since A. Townsend, A. McMichael, and coworkers reported 26 years ago that T lymphocytes recognize short peptide fragments in association with major histocompatibility complex (MHC) encoded proteins (1), immunologists have been keen to understand how these fragments are produced, how they are transported to MHC molecules and how they assemble with them. It was not surprising that the new field of antigen processing attracted considerable interest, given that peptide presentation by MHC molecules is the key element in immunological self-non self discrimination, pathogen-specific immune responses, autoimmunity, and vaccine development. As a result, many aspects of cellular antigen processing are now understood in great detail, although some other issues (e.g., the nature of endogenous proteins giving rise to MHC class I ligands, or the cell biology of cross-presentation) remain to be clarified.

This volume aims to provide the reader with a comprehensive set of protocols for studying presentation of antigens produced in the standard processing pathways for MHC class I and class II molecules. In both cases, the book attempts to follow the chronology of intracellular events ending with recognition of peptide–MHC complexes at the cell surface by T lymphocytes. Surveying MHC class I antigen processing, we start by examining cytosolic proteases and the kinetics of peptide survival determined by them (Fig. 1). The next steps open to scrutiny are peptide transport into the endoplasmic reticulum and synthesis and loading of MHC class I molecules. Further protocols examine the fate of class I mol-

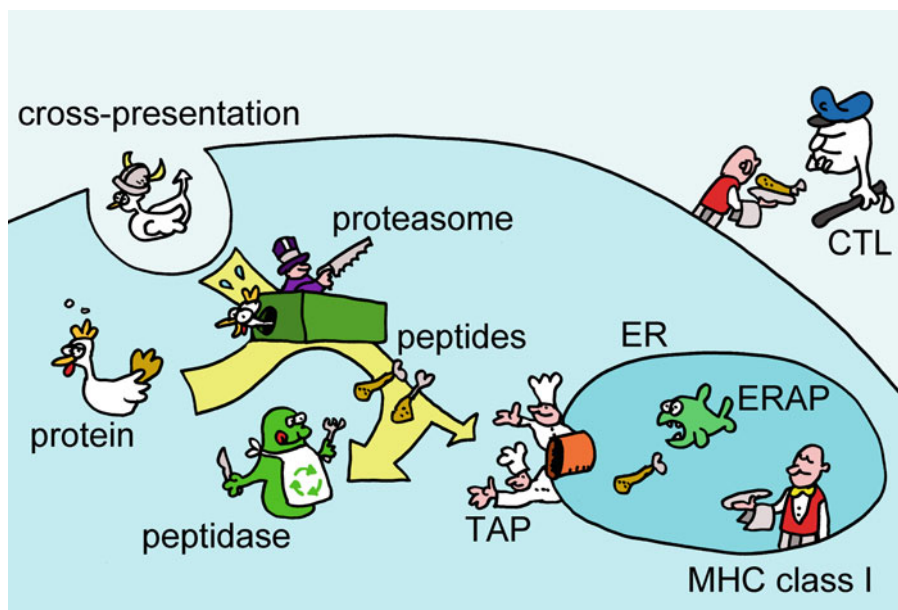


Fig. 1. The principal steps in processing of endogenous antigens for presentation by MHC class I molecules.

ecules beyond the endoplasmic reticulum including disposal upon ubiquitination and viral interference. A number of chapters are devoted to analysis of the final peptide–MHC complexes, using biochemical, immunological (T cell receptor-like antibodies), and immune-informatics approaches. Complementary chapters deal with CD8+ T cell assays and cloning, indispensable tools when studying MHC class I antigen processing.

A second set of chapters (Chapters 24–30) presents methods for studying antigen processing by dendritic cells, a cell population with a critical role in priming and orchestrating antigen-specific cellular immune responses. These chapters will enable the reader to purify and prepare dendritic cells, monitor their activation, transform them with lentiviruses, perform cross-presentation assays, and monitor intracellular routing of antigens in endocytic compartments. Another volume in the series (Dendritic cell protocols, Ed. S.H. Naik) provides a collection of additional protocols that will be of interest to the reader with a special interest in this section.

Section 3 (Chapters 31–40) deals with the principal steps in antigen processing for MHC class II molecules (Fig. 2). These include synthesis, assembly, and peptide loading of class II molecules. Intracellular transport of class II molecules is studied with emphasis on dendritic cells. Additional protocols address the role of macroautophagy, a mechanism with an important role in the loading of class II molecules with endogenous peptides, and endosomal proteases producing class II ligands. The section would not be complete without chapters dealing with class II ubiquitination, which controls MHC class II turnover and disposal, and with production of CD4+ T cell lines and clones. The final two protocols concern presentation of lipid antigens by nonclassical MHC molecules.

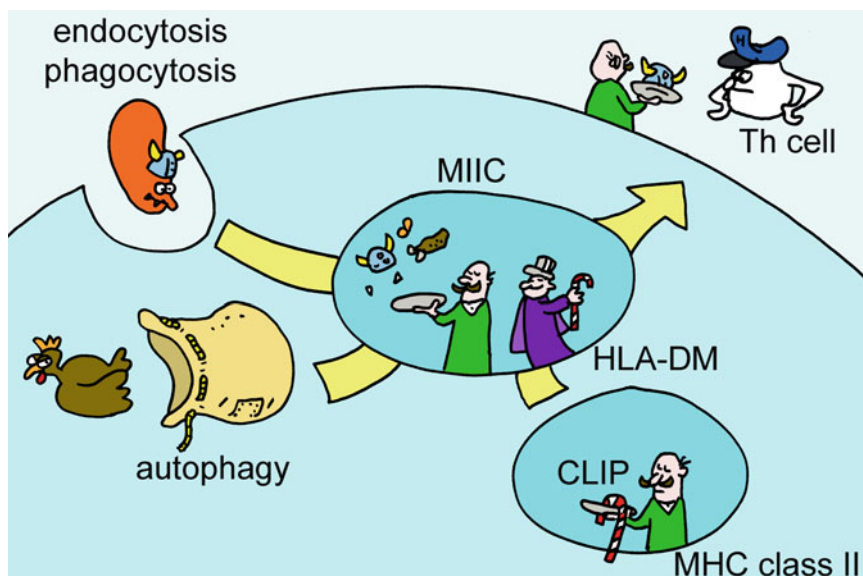


Fig. 2. The principal steps in processing of antigens for presentation by MHC class II molecules

This methods book is designed to be a bench-side companion for beginners and experts interested in studying antigen processing. In keeping with the spirit of the series, the protocols included hopefully will enable readers to confidently venture into the field of antigen processing. The editor wishes to express his gratefulness to all authors for their excellent contributions, to Christophe Marchi for help with editing, and to Eric Reits for his wonderful cartoons.

Paris, France

Peter van Endert

Reference

1. Townsend AR, Rothbard J, Gotch FM, Bahadur G, Wraith D, McMichael AJ (1986) The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 44:959–968

Antigen Processing

Methods and Protocols

van Endert, P. (Ed.)

2013, XVII, 589 p. 85 illus., 25 illus. in color., Hardcover

ISBN: 978-1-62703-217-9

A product of Humana Press