

MHC structure and function – antigen presentation. Part 2

Estrutura do MHC e função – apresentação de antígenos. Parte 2

Anna Carla Goldberg¹, Luiz Vicente Rizzo¹

ABSTRACT

The second part of this review deals with the molecules and processes involved in the processing and presentation of the antigenic fragments to the T-cell receptor. Though the nature of the antigens presented varies, the most significant class of antigens is proteins, processed within the cell to be then recognized in the form of peptides, a mechanism that confers an extraordinary degree of precision to this mode of immune response. The efficiency and accuracy of this system is also the result of the myriad of mechanisms involved in the processing of proteins and production of peptides, in addition to the capture and recycling of alternative sources aiming to generate further diversity in the presentation to T-cells.

Keywords: Major histocompatibility complex; Antigen presentation; HLA genes; Immune response

RESUMO

A segunda parte desta revisão trata das moléculas e processos envolvidos no processamento e apresentação dos fragmentos antigênicos ao receptor de célula-T. Apesar de variar a natureza do antígeno apresentado, a classe mais significativa é a das proteínas, as quais são processadas dentro da célula para enfim serem reconhecidas na forma de peptídeos, o que confere um grau extraordinário de precisão a essa forma de resposta imune. A eficiência e a precisão desse sistema se devem também à miríade de mecanismos envolvidos no processamento de proteínas e produção de peptídeos, além da captura e reciclagem de fontes alternativas de antígenos com o objetivo de gerar ainda maior diversidade na apresentação à célula-T.

Descritores: Complexo principal de histocompatibilidade; Apresentação do antígeno; Genes HLA; Resposta imune

INTRODUCTION

A central role for MHC in the immune response to protein antigens was suggested in the 1970 when

experimental evidence showed that T lymphocytes recognize portions of antigens (peptides) only when bound non-covalently to products of the HLA genes. Crystallographic analysis of the HLAI molecule carried out in 1987⁽¹⁾ disclosed how these peptides nest in the external part of the molecule, that is, in the peptide-binding groove.

PART 2 – PEPTIDE GENERATION: PROCESSING, MECHANISMS OF DIVERSITY, AND ALTERNATIVE SOURCES FOR PRESENTATION

Broadly, proteins are degraded inside cells, and peptides derived through this processing are coupled to HLA molecules and transported to the cell surface. HLAI and HLAII molecules transport peptides produced in different cellular compartments (proteasomes and endosomes, respectively), in which different proteolysis strategies employed generate the variety of peptides needed for an efficient presentation and recognition by T cell receptors. HLA plus peptide on one side bound to T-cell receptor on the other forms the trimolecular complex, which initiates and confers the necessary specificity to the effector immune response.⁽²⁾

Peptide presentation via HLAI (2)

The profile of peptides eluted from HLAI confirms the same molecule can present diversified sequences (approximately 10,000 distinct peptides per cell). Furthermore, the array of peptides presented by each HLA molecule varies according to the binding motif displayed by the respective grooves.^(3,4) The bewildering variety of peptides is mainly produced in the cytosol, within the proteasomes, multi-component complex

¹ Hospital Israelita Albert Einstein, São Paulo, SP, Brazil.

Corresponding author: Anna Carla Goldberg – Avenida Albert Einstein, 627/701, 2ss floor, building A – Morumbi – Zip code: 05661-900 – São Paulo, SP, Brazil – Phone: (55 11) 2151-1233
E-mail: goldberg@einstein.br

Received on: Mar 27, 2014 – Accepted on: June 27, 2014

DOI: 10.1590/S1679-45082015RB3123

structures capable of enzymatic proteolysis, and are therefore of endogenous origin.⁽²⁾ Peptide diversity is further increased by proteasome component substitution during the immune response that leads to production of novel peptide fragments.⁽⁵⁾

Proteins involved in the cellular metabolism are synthesized in the endoplasmic reticulum, and when unnecessary or defective (called DRiPs, for Defective Ribosomal Proteins) that is, truncated or altered due to misfolding or erroneous translation⁽⁶⁾ are coupled to ubiquitin units.⁽⁷⁻⁹⁾ The ubiquitin tail transfers the tagged proteins to proteasomes for processing inside the catalytic center to 9 amino acid-long fragments. Other components of the proteasome complex carry out binding and unfolding of ubiquitin-tagged proteins in preparation for their fragmentation.⁽¹⁰⁾ This major protein degradation pathway has been shown to be a fundamental and universal cell function, and its discoverers, A. Ciechanover, A. Hershko, and I. Rose, were awarded the 2004 Chemistry Nobel Prize.

Peptides surviving the proteasomal processing are translocated into the endoplasmic reticulum by the TAP (for Transporter associated with Antigen Processing) heterodimer.⁽¹¹⁾ The assembly of a HLAI molecule, its folding, and coupling of the peptide are complex processes which occur only with help of accessory proteins acting as chaperones.⁽¹¹⁾ The list includes calnexin that confers stability and ERp57 (a disulfide isomerase) that helps to bind beta-2 microglobulin. Calreticulin then replaces calnexin and together with ERp57 and tapasin will form a peptide-loading complex (PLC) in charge of loading and favoring the correct insertion of peptides unto the nascent HLAI molecule.^(12,13) Tapasin also serves as the bridge between the PLC and peptide-carrying TAP.⁽¹³⁾ Finally, endoplasmic reticulum-associated amino peptidases (ERAP1 and ERAP2) carry out the fine tuning trimming peptides to the 8 to 10 amino acid size ideal for coupling to HLAI.⁽¹⁴⁾ Finally, the stable trimmer made up of the α heavy chain, β 2- microglobulin, and peptide can be transported through the Golgi for glycosylation and arrival on the cell surface. This is the principal route for class I antigen presentation, and when devoid of peptides, HLAI exhibits a very short half-life and is rapidly degraded. In other words, empty HLA molecules are the exception.

This route of antigen processing, called cytosolic or endogenous, is excellently suited for viral peptide presentation when the cell is infected, as viruses employ the cellular machinery to produce their own proteins

and new virus particles. However, studies have shown that their presentation is even more efficient than that of other peptides (1.5 hour instead of 8 hours as usual). This rapid degradation is made possible because viral proteins are seen as DRiPs by the cell.^(2,15) The cytotoxic response (via CD8+ T lymphocytes) against viruses is an essential feature of defense by the infected organism, and is frequently targeted by the invading pathogen with immune evasion strategies. Examples are cytomegalovirus, herpes simplex virus, and human immunodeficiency virus, which synthesize proteins capable of interrupting the flow of peptides, blocking TAP and even synthesis of class I molecules.⁽¹⁶⁾

In addition to the processing described above, several others are operating, including some recently identified, which add to the variety of peptides generated and presented. A list of these extra features follows:

1. different speeds in transport of HLAI molecules to the cell surface, where HLA-B exhibits the best efficiency;⁽¹⁷⁾
2. presentation by HLA-E (non-classical) of peptides derived from classical HLAI leader sequences for recognition by CD94/NKG2A receptors on natural killer (NK) cells;⁽¹⁸⁾
3. presentation by HLA-G (non-classical) at the maternal-fetal interface, in which classical HLAI expression is very low, for recognition by KIR2DL4 receptors on NK cells;^(19,20)
4. presentation not of peptides, but of glycolipids by CD1 (a-d) molecules (belonging to the HLA family) for recognition by a subpopulation of $\gamma\delta$ T cells;^(21,22)
5. exchange of proteasome components, in the presence of interferon- γ secreted by activated T cells. Components of the 20S subunit are substituted to constitute a transient immunoproteasome, capable of producing more immunogenic peptides;^(23,24)
6. neo-peptide formation by the ligation of two smaller fragments during their passage through the proteasome. These peptides are immunogenic and considered to be an additional mechanism for tumor cell recognition by cytotoxic T lymphocytes;⁽²⁾
7. alternative peptide formation by unconventional proteases like thimet oligopeptidase or angiotensin-converting enzyme;^(25,26)
8. changes in the peptidome due to modified messenger RNA transcription patterns operated by micro-RNAs⁽²⁷⁾ or to immunogenic peptides produced from deliberately truncated messenger RNA;⁽²⁸⁾
9. peptide transfer between cells through the gap junctions;⁽²⁹⁾

10. cross-presentation of peptides from exogenous sources⁽³⁰⁾ or transported into the cell by Hsp70.⁽³¹⁾

Peptide presentation via HLAI

In spite of the great structural similarity and shared function of antigen presentation, HLAI and HLAII exhibit important differences in expression profile and source of peptides loaded, leading to a very different role in the adaptive immune response.

In HLAII molecules, the groove will also be usually filled with a peptide, but now resulting from processing of exogenous antigens transferred into the cell (internalized) in specialized antigen processing cells (APC). B lymphocytes internalize proteins in a unique way, capturing them by binding to cell surface immunoglobulin, namely, the B cell receptors.⁽³²⁾ Dendritic cells and macrophages are capable of internalizing not only extracellular pathogens but also particulate matter, resulting from cellular necrosis, immune complexes, complement-opsonized cell fragments, and apoptotic bodies, all additional sources of material foreign to the organism or of self-proteins to be presented by HLAII.⁽³³⁾

Novel forms of antigen capture recently identified include binding to DEC205, a receptor capable of transporting antigen directly to late endosomes in which the final assembly with HLAII is carried out;⁽³⁴⁾ trogocytosis, in which plasma membrane fragments are exchanged between cells;⁽³⁵⁾ intercellular transfer by exosomes,⁽³⁶⁾ also a source of tumor antigens to be targeted by cytotoxic T cells,⁽³⁷⁾ and autophagy.^(3,38) Autophagosomes as well as endocytosis of cytosolic and nuclear proteins (microautophagy) are important sources increasing the offer of proteins to be presented.⁽³⁾

Activation and ensuing maturation of the phagosome or autophagosome occurs when pathogen-associated or damage-associated molecular patterns (PAMPs and DAMPs, respectively) are recognized by specific Toll-like receptors (for example, TLR4 or TLR9)⁽³⁹⁾ and is necessary for fusion with lysosomes. During digestion by the lysosomal enzymes fragments of different sizes, usually around 25 amino acids and therefore longer than those produced by proteasomal degradation, are generated. Trimming will reduce these peptides to 14 or 15 residues.⁽⁴⁰⁾ Several factors will influence the peptides available for presentation. As expected, abundant proteins have increased chances of providing peptides. Lysosomal enzymes, like carboxypeptidase and cathepsins, cleave proteins preferably on dibasic arginine and lysine sites⁽⁴¹⁾ and peptides presented

by HLAII frequently carry a N-terminal proline due to N-aminopeptidase activity.⁽⁴²⁾ These peptides will be coupled to HLAII during the formation of a late endosomal compartment known as MIIC.⁽⁴³⁾

Peptide transport is quite different from the one described for HLAI, in spite of the fact that all MHC chains are synthesized and their $\alpha\beta$ heterodimers formed in the endoplasmic reticulum. We have seen that class I molecules receive their peptides while still in the reticulum being stabilized with help from the PLC. In contrast, class II molecules must be transferred to the phagolysosome for final assembly. To acquire the necessary stability for this transfer, coupling to a third protein, the invariant gamma chain takes place. In addition to directing the HLAII to the MIIC compartment,⁽⁴⁴⁾ part of the invariant chain covers the groove, blocking the access of TAP-transported peptides destined for class I presentation.⁽⁴⁵⁾

Once the endosomal compartment is reached, the invariant chain is digested by cathepsin B,⁽⁴⁶⁾ leaving behind only the fragment covering the groove known as CLIP (Class II – associated Invariant chain Peptide).⁽⁴⁷⁾ The substitution of any other peptide for CLIP is catalyzed by the class II heterodimer HLA-DM,^(48,49) which also acts as chaperone to class II molecules, maintaining the structure intact while the exchange takes place.⁽⁵⁰⁾ In B lymphocytes, HLA-DO further influences the efficiency of HLA-DM as a peptide acceptor by inhibiting its activity in neutral pH. Upon reaching the endolysosomal compartment in which pH is 4.5, HLA-DM function is restored opening way for binding of peptides from extracellular origin.⁽⁵¹⁾ The peptide exchange goes on until a high affinity peptide is bound to the groove, forming a stable complex that can be transported to the cell surface of the APC. The complex process of transporting the HLAII/peptide trimmer will need further assistance from tetraspanin family proteins CD63 and CD81⁽⁵²⁾ that assist in moving through the multilamellar and multivesicular MIIC endosomes.^(52,53)

ANTIGEN PRESENTATION: DIVERSITY AND EFFICIENCY OF THE TRIMOLECULAR COMPLEX

The concept of antigen presentation was initially outlined based on a series of studies carried out in the 1970 that opened way for the pioneering work done by Zinkernagel and Doherty⁽⁵⁴⁾ for which they received the 1996 Nobel Prize. Today, we know that T lymphocytes are capable of recognizing antigens solely when present

on the cell surface. These originate from all types of sources such as intracellular bacteria and viruses, by-products of cell metabolism, self and foreign proteins and lipids. All these antigens are captured, processed, and presented in the form of mainly peptide fragments by the HLA family of glycoproteins.

Overall, antigen presentation can explain different events such as thymic education, response against infection, transplant rejection, autoimmunity, and tumor immunity. It is therefore a highly relevant topic in immunology and still of great interest in research.

Recent studies have also clarified the role of peptide production and presentation on the quality of T lymphocyte responses.⁽⁵⁵⁾ According to the type of cell in charge of presenting antigen, the number of a specific peptide presented on each cell, the quantity and type of co-stimulatory molecules, the type and condition of the tissue where this cell located, the complexity and variety of the peptides presented, and the T cell receptor binding affinity for that particular peptide/HLA complex, the response elicited may result in different cytokine production profiles and specific response patterns by individual cells or subpopulations. Furthermore, these response patterns are influenced by the genetic background of each person.

Of note, when compared to the smaller closed groove of the HLAI the open HLAI groove is more permissive to peptide substitutions even when the molecule is already stable and anchored on the cell surface. In fact, as the usual peptides are longer than the class II groove, which accepts 9 amino acids in an extended conformation, flanking amino acids will be freely exposed on the surface.⁽⁵⁶⁾ It becomes thus understandable that recognition by the T cell receptor on the surface of the CD4+ T cells is very plastic and includes not only the borders of the HLA molecule but also regions of the peptides both inside and outside the groove.

In summary, phagocytic cells respond differentially to their internalized content, inducing inflammatory or suppressive responses locally. The central role in orchestrating humoral and cellular immune responses confers a unique importance to the HLAI/peptide complex to be recognized by the CD4+ T cells. Antigen recognition leads to activation, clonal expansion, and differentiation of lymphocytes involved in the process, increasing the population of effector lymphocytes and spurring maintenance of some long-lived cells that will form the immunological memory.

The sum of the gene polymorphism present in the molecules involved in adaptive immune responses and the diversity in the processing and peptide presentation, allied to the mechanisms of gene rearrangement that produce the highly diversified T cell repertoire translates into a unique and highly efficient system of antigen recognition and effective immune response.

Note: Due to publishing constraints innumerable references have not been included in this review of a topic under close examination for the past 40 years. We hope all researchers with significant contributions to the subject have been cited at some point of the text.

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