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Review

Autophagy-mediated antigen processing in CD4$^+$ T cell tolerance and immunity

Ludger Klein [a,*], Christian Münz [b,1], Jan D. Lünemann [b,*]

[a] Institute for Immunology, Ludwig-Maximilians-University, Goethestr. 31, 80336 Munich, Germany
[b] Institute of Experimental Immunology, University Hospital Zurich, Winterthurer Strasse 190, 8057 Zurich, Switzerland

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ABSTRACT

Macroautophagy, a homeostatic process that shuttles cytoplasmic constituents into endosomal and lysosomal compartments, has recently been shown to deliver antigens for presentation on major histocompatibility complex (MHC) class II. Autophagy-mediated antigen processing in thymic epithelial cells has been suggested to be involved in the generation of a self-MHC restricted and self-tolerant CD4$^+$ T cell repertoire. Furthermore, there is accumulating evidence that the up-regulation of autophagy by pattern-recognition receptor signaling represents an innate defense mechanism against intracellular pathogens. Thus, through linking pathogen breakdown with the presentation of pathogen-derived autophagy substrates on MHC class II, autophagy serves a dual function at the interface of the innate and the adaptive immune response.

1. Introduction

Autophagy is a homeostatic process by which cells recycle nutrients and degrade cytoplasmic constituents such as defective organelles and macromolecular aggregates for lysosomal degradation. There are at least three distinct pathways of autophagy: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy. Microautophagy is characterized by the uptake of cytoplasmic components at the lysosomal membrane via budding into the lysosome, through a poorly defined mechanism. Substrates for CMA carry signal peptides for sorting into lysosomes, similar to other protein transport mechanisms across membranes, and are directly imported into lysosomes through the LAMP-2a transporter [1,2], assisted by cytosolic and lysosomal HSC70 chaperones. Macroautophagy is the major route for lysosomal degradation of cytoplasmic constituents. During macroautophagy, cytosolic constituents including organelles are enclosed in a double-membrane vesicle, called autophagosome [3,4], which then fuses with late endosomal/lysosomal organelles for degradation of the inner autophagosomal membrane and its cargo. The resulting breakdown products of macromolecules are subsequently released back into the cytosol through permeases in the lysosomal membrane, where they can be reused for anabolic or catabolic reactions [5].

Studies on the molecular mechanisms of macroautophagy and its importance in protein metabolism [5] have set the stage to analyze its role in multiple biological processes including innate and adaptive immune responses. In keeping with its cellular clearance function, macroautophagy participates in limiting pathogen replication in host cells. In addition, macroautophagy delivers viral, parasitic, and bacterial antigens to late endosomal compartments, where macroautophagy substrates are then degraded by lysosomal hydrolases. The fusion vesicles between autophagosomes and late endosomes, the so-called amphisomes, display a multivesicular and multilamellar morphology reminiscent of major histocompatibility complex (MHC) class II containing compartments (MIICs) [6]. Indeed, studies in cell culture systems, including antigen presentation assays, co-localization studies and sequencing of MHC class II bound peptides, demonstrated that substrates of autophagy can be loaded onto MHC class II for CD4$^+$ T cell recognition. Animal models to monitor or genetically disrupt macroautophagy now provide the basis for elucidating the immunological relevance of autophagy in vivo.

As this review focuses on the role of autophagy in mediating CD4$^+$ T cell responses and in regulating CD4$^+$ T cell immunity through processing and presentation of intracellular antigens on

Abbreviations: MHC, major histocompatibility complex; DC, dendritic cells; APC, antigen presenting cell; TCR, T cell receptor; EBV, Epstein Barr virus; TEC, thymic epithelial cell; PRR, pattern-recognition receptors

* Corresponding authors. Fax: +49 89 5160 2236 (L. Klein); +41 44 635 6883 (J. Lünemann).
E-mail addresses: ludger.klein@med.lmu.de (L. Klein), jan.luenemann@usz.ch (J.D. Lünemann).
[1] Fax: +41 44 635 6883.

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MHC class II molecules, we will first outline the basic principles of antigen processing.

2. Antigen processing and presentation

T and B cells both express highly diverse receptors for antigen whose enormous variability is established through somatic rearrangement of gene fragments during early development of the respective lineage. Despite this similarity in the genetic makeup of the T and B cell receptor, there is a fundamental difference in how these two classes of lymphocytes recognize antigen: B cells directly recognize antigens through interaction of their receptor with free antigens or epitopes on the surface of supramolecular structures (e.g. cells, bacteria, viruses), whereby these epitopes can be made up of carbohydrates, lipids, proteins and even anorganic compounds. By contrast, T cells primarily recognize relatively small peptides that are generated by proteolytic breakdown of protein substrates. These peptide snippets are not recognized in free form, but have to be embedded in MHC molecules on the surface of cells.

MHC molecules come in two flavors, MHC class I and MHC class II. Both classes of molecules share as a characteristic feature of their tertiary structure a so-called peptide-binding groove, which consists of two alpha helices outlining the rims of the peptide-binding moiety and a beta sheet that forms the bottom of the groove. Despite the overall very similar structure of MHC class I and II molecules, the details of their tissue distribution, physiological function and how the peptides they present to T cells are generated, are remarkably distinct. For instance, MHC class I is expressed on essentially all tissues, whereas constitutive expression of MHC class II is restricted to so-called professional antigen presenting cells (APCs) of hematopoietic origin and epithelial cells of the thymus. Only upon exposure to interferon-γ (IFNγ), tissues other than these will up-regulate MHC class II expression. Furthermore, whereas peptide/MHC class I (pMHCI) complexes represent the ligands for the T cell receptor (TCR) of $CD8^+$ cytotoxic T cells, pMHCII complexes are recognized by $CD4^+$ helper T cells. This dichotomy in the recognition of pMHCI or II ligands by either cytotoxic or helper T cells, respectively, reflects the process of positive selection during T cell development in the thymus. This rather wasteful process ensures that immature T cells commit to the $CD8^+$ or $CD4^+$ lineage according to the principal capacity of their randomly rearranged TCR for MHC class I or II, respectively. As a result, cytotoxic T cells express the CD8 co-receptor, whereas helper T cells express the CD4 co-receptor, which interact with non-polymorphic regions of MHC class I or II, respectively.

The basic principles of how peptides embedded in MHC class I or II are generated have been well-established over the last two decades (Fig. 1) [7]. Thus, MHC class II bound epitopes are primarily generated through the proteolytic processing of proteins that reach endosomal/lysosomal compartments subsequent to having been taken up from the extracellular space. By contrast, MHC class I bound peptides are mostly derived from proteasomal substrates, that is, mis-folded cytoplasmic proteins that have been earmarked for degradation by ubiquitination.

At first glance, the paradigm that MHC class I or MHC class II bound peptides originate from topologically distinct sources represents an elegant way how the immune system copes with the challenge that the eradication of intra- or extracellular pathogens necessitates fundamentally different effector mechanisms. Thus, the control of viral infections requires that those cells that have been infected are eliminated by MHC class I-restricted $CD8^+$ cytotoxic T cells. By contrast, the clearance of extracellular pathogens such as most bacteria or helminths is largely dependent on antibody responses. In this case, MHC class II-restricted $CD4^+$ helper T cells fulfill a critical pacemaker function for humoral immune responses in the form of cytokines that orchestrate the efficient production of antibodies by B cells.

A closer look, however, renders an absolutely strict topological demarcation of the origin of MHC class I and MHC class II bound peptides epitopes rather unlikely. For example, the initiation of certain antiviral cytotoxic $CD8^+$ T cell responses is dependent on the MHC class I-restricted presentation of viral epitopes by dendritic cells (DCs), the major type of so-called "professional antigen presenting cells". Paradoxically, however, many viruses do not infect DCs, and yet elicit strong cytotoxic T cell responses. This observation underscores the physiological relevance of an "unconventional" MHC I loading pathway that involves the shuttling of exogenous material, in this case viral proteins, into the MHC class I loading pathway of DCs, a process termed cross-presentation [8]. Vice versa, early evidence for exceptions from the "rule" that MHC class II molecules are exclusively occupied by peptides of extracellular origin was provided by the sequencing of peptides eluted from MHC class II, revealing that around 20% of the identified epitopes originated from intracellular sources such as mitochondrial proteins, cytoskeletal proteins, metabolic enzymes, chaperones and nuclear proteins [9].

How do epitopes derived from cytoplasmic proteins gain access to MHC class II molecules? The candidate pathways implicated in so-called "endogenous MHC class II loading" can be grouped into two groups, depending on whether the intersection with the conventional exogenous MHC class II loading pathway occurs upstream or downstream of the proteolytic degradation of the substrate. The first category involves proteasomal antigen processing and subsequent TAP-mediated import of degradation products into the ER and thus may be best described as "spill-over" from the MHC class I pathway into MHC class II loading compartments [10]. The second category encompasses a rather heterogeneous group of mechanisms that deliver cytoplasmic material for lysosomal degradation, including chaperone-mediated autophagy and macroautophagy (reviewed in [11]). We will primarily focus on macroautophagy in this review, because this pathway was frequently suggested to deliver cytoplasmic material for MHC class II loading.

3. Autophagy substrates are loaded onto MHC class II molecules

More than a decade ago, the lab of Gitta Stockinger reported that treatment of macrophages and B cells with the macroautophagy inhibitor 3-methyl adenine (3-MA) prevented the presentation of an endogenously synthesized protein on MHC class II [12]. On this basis, these authors suggested that macroautophagy may shuttle cytosolic proteins into MHC class II loading compartments. In a subsequent study, Mautner and co-workers found that transfection of a renal carcinoma cell line with a model antigen resulted in the endogenous processing for MHC class II restricted presentation to $CD4^+$ T cells, and again, this process could be inhibited by 3-MA [13]. A caveat of both studies was that they involved the ectopic over-expression of the respective model antigens. The first study to document a role for macroautophagy in loading of a physiologically expressed cytoplasmic antigen dealt with the recognition of the nuclear antigen 1 (EBNA1) of Epstein Barr virus (EBV) by $CD4^+$ T cells. It was found that EBNA1 gains access to MHC class II in EBV transformed B cells [14], and that EBNA1 was detectable in autophagosomes when lysosomal proteolysis was inhibited [14]. Inhibition of macroautophagy by 3-MA or RNA interference directed against Atg12 strongly diminished recognition of these cell lines by EBNA 1 specific $CD4^+$ T cells [14]. In further support of a role for macroautophagy in endogenous MHC class II loading, recognition of tumor antigen derived epitopes on MHC class II after RNA transfection of DCs was reduced by 3-MA [15]. In support of