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Cathepsin S dominates autoantigen processing in human thymic dendritic cells

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ABSTRACT

The interaction of developing thymocytes with peptide-MHC complexes on thymic antigen presenting cells (APC) is crucial for T cell development, both for positive selection of "useful" thymocytes as well as negative selection of autoreactive thymocytes to prevent autoimmunity. The peptides presented on MHC II molecules are generated by lysosomal proteases such as the cathepsins. At the same time, lysosomal proteases will also destroy other potential T cell epitopes from self-antigens. This will lead to a lack of presentation on negatively selecting thymic antigen presenting cells and consequently, escape of autoreactive T cells recognizing these epitopes. In order to understand the processes that govern generation or destruction of self-epitopes in thymic APC, we studied the antigen processing machinery and epitope processing in the human thymus. We find that each type of thymic APC expresses a different signature of lysosomal proteases, providing indirect evidence that positive and negative selection of $CD4^+$ T cells might occur on different sets of peptides, in analogy to what has been proposed for $CD8^+$ T cells. We also find that myeloid dendritic cells (DC) are more efficient in processing autoantigen than plasmacytoid DC. In addition, we observed that cathepsin S plays a central role in processing of the autoantigens myelin basic protein and proinsulin in thymic dendritic cells. Cathepsin S destroyed a number of known T cell epitopes, which would be expected to result in lack of presentation and consequently, escape of autoreactive T cells. Cathepsin S therefore appears to be an important factor that influences selection of autoreactive T cells.

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1. Introduction

Although autoimmune diseases like multiple sclerosis (MS) and type I diabetes mellitus (T1D) occur with some frequency in the population, the vast majority of people possess an immune system that is both functional and self-tolerant. This state is largely due to two events early in T cell development. Positive selection of developing T cells in the thymus ensures that they are self-referential, i.e. they recognise antigen in the context of self-MHC (major histocompatibility complex) molecules. Negative selection or deletion of T cells strongly reacting to self-peptide-MHC complexes ensures that the T cell repertoire is largely self-tolerant, reducing the danger of autoimmunity [1].

The task of instructing developing T cells in this way falls to thymic antigen presenting cells (APC), namely cortical (cTEC) and medullary thymic epithelial cells (mTEC) and dendritic cells (DC). The different types of APC play different roles in the processes governing T cell development and selection [2]. Cortical TEC are both necessary and sufficient for positive selection and provide survival signals to thymocytes with a self-restricted T cell receptor (TCR) [3,4]. Having received these signals, thymocytes migrate from the cortex into the medulla where they interact with mTEC and DC. Both mTEC and DC can mediate negative selection, although mTEC appear to be less efficient [5,6], and expression of MHC II exclusively on DC was reported to be sufficient for effective deletion of autoreactive $CD4^+$ T cells [7]. Medullary TEC are specialised for "ectopic" or "promiscuous" expression of so-called tissue specific antigens (TSA). This gene expression pattern is partially controlled

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by AIRE and contributes to central tolerance towards self-proteins that are not ubiquitously expressed [8,9]. Recently, it has also been demonstrated that DC can take up TSA in the periphery, migrate to the thymus and induce clonal deletion of antigen-reactive thymocytes [10]. Further evidence exists that DC can also cross-present TSA expressed by mTEC, underscoring their importance for tolerance not only against ubiquitous self-antigens but also TSA [6,11]. Although the individual contributions of different types of APC are still a matter of debate and intense research, it is clear that they work together to ensure central tolerance towards a broad range of self-proteins [2].

The human thymus contains both conventional myeloid (mDC) and plasmacytoid dendritic cells (pDC). While the role of mDC in thymocyte selection is quite well understood, the function of pDC in this context has not been investigated in much detail. However, since they also express MHC II, they could in principle also be involved in $CD4^+$ T cell selection and there is evidence that in addition to mDC, thymic pDC can also promote the development of natural regulatory T cells [12].

The main factor determining thymocyte fate during selection is the interaction between the T cell receptor on thymocytes and the peptide-MHC complexes displayed by thymic APC [13]. Knowing how thymic APC handle different proteins to create the self-peptide matrix on which T cells are selected, is therefore crucial for understanding the mechanisms underlying self-tolerance and autoimmunity. Proteins typically enter the MHC II pathway via endocytosis or autophagy [14]. The peptides that are subsequently presented by MHC II molecules are generated by lysosomal proteases. Lysosomal proteases comprise the cathepsins (Cat) as well as asparagine endopeptidase (AEP). They can be further subdivided into cysteine, serine and aspartic proteases, depending on the amino acid residue which initiates proteolytic attack in the active site. AEP and most of the cathepsins (CatB, C, F, H, K, L, S, V, W and X) belong to the cysteine proteases, while CatD and E are aspartic, CatG and A serine proteases [15]. Although most of these proteases have rather broad cleavage specificities, the system is not entirely redundant. Mice lacking CatL, for example, have strongly impaired $CD4^+$ T cell selection, at least partially due to impaired invariant chain cleavage, but also to altered antigen processing resulting in an altered repertoire of positively selecting peptide ligands [16,17]. CatS knockout mice, although phenotypically normal, are resistant to experimental autoimmune myasthenia gravis and collagen-induced arthritis, while their wild type counterparts are susceptible [18,19].

The action of lysosomal proteases is required for releasing potential T cell epitopes from proteins processed in the MHC II pathway. Interestingly, it always seems to be the action of one or two specific endoproteases that dominate or “unlock” a certain antigen and make it accessible for further processing by other endo- and exopeptidases and binding to MHC II [20,21]. The unlocking step can critically influence the T cell response because proteolytic cleavage by the dominant protease(s) can either create or destroy potential T cell epitopes. AEP for example was shown to dominate processing of tetanus toxin and to be responsible for creating an immunodominant T cell epitope from the antigen [22]. In the case of myelin basic protein (MBP), one of the autoantigens in multiple sclerosis, AEP destroys the immunodominant MBP85-99 epitope in a B cell line and mouse thymic DC [23].

Very little is known about antigen processing in APC of the thymus. Most studies have been performed in mice and the few data from human thymus demonstrate that results from mouse experiments cannot be directly applied to the human system. For example, while CatL expression in mouse cTEC is required for positive selection of $CD4^+$ T cells [17], CatL is not expressed by human cortical epithelial cells. Instead, human cTEC express CatV, which is absent from mice, and seems to have overtaken the function of CatL in human cTEC [24]. Interestingly, CatV is overexpressed in thymi of patients with myasthenia gravis, suggesting that altered T cell selection due to altered antigen processing might be involved in development of this autoimmune disease [24].

While in the periphery destruction of a given epitope simply results in a failure to mount an immune response, lack of presentation of a potential T cell epitope in the thymus can lead to escape of autoreactive T cells into the periphery and autoimmunity, as has been shown in animal models for proteolipid protein in MS/EAE (experimental autoimmune encephalomyelitis) [25] and insulin in type 1 diabetes [26]. Both lack of expression or proteolytic destruction of an epitope will result in a failure to be presented. In order to understand the processes underlying creation or destruction of self-epitopes for T cell selection in the human thymus, we have characterised the MHC II antigen processing machinery in human thymic APC subsets and investigated autoantigen processing in thymic DC using MBP and proinsulin as a model antigens.

2. Materials and methods

2.1. Isolation of cells from the thymus

Thymi were obtained after parental consent following the Institutional Review Board guidelines from infants undergoing corrective cardiac surgery. Thymus tissue was finely cut and squeezed with a syringe. The remaining tissue pieces were digested with collagenase (1 mg/ml, Roche Diagnostics) and DNase (50 μg/ml, Roche Diagnostics) for up to 3 times for 45 min^{-1} h. APC were enriched from single cells suspension by a Percoll gradient (ρ = 1.07 g/ml; 30 min, 3500 g). The interphase contained the enriched APC. Subsequent isolation steps were performed with this fraction.

For isolation of myeloid DC, cells were labelled with CD11c-PE antibody and subsequently isolated using anti-PE magnetic beads (Miltenyi Biotec) or labelled with CD11c-FITC or -PE antibody and sorted (FACS Aria; Becton Dickinson) according to published methods [27]. Plasmacytoid DC were labelled with BDCA4-PE antibody (Mitenyi Biotec) and sorted or isolated using anti-PE magnetic beads. For isolation of thymic epithelial cells, cells were pre-enriched by depletion of CD45 high cells using anti-CD45 magnetic beads (Miltenyi Biotec) at one fifth of the recommended concentration. Cells were subsequently stained with antibodies against CD45 (Pacific Blue; DAKO), EpCAM (APC; Miltenyi Biotec) and CDR2 (FITC, the CDR2 clone was kindly provided by B. Kyewski, Heidelberg [28] and sorted as large $CD45^{-/low}$ $EpCAM^{hi}$ (medullary TEC) and $CD45^{-/low}$ $EpCAM^+CDR2^+$ (cortical TEC) as described [27].

2.2. Lysosomal extracts, in vitro processing and analysis of processing products

Lysosomal fractions were obtained from cells by differential centrifugation and hypotonic lysis as published [29]. Briefly, cell membranes were disrupted using a cell cracker. This procedure leaves organelles intact. Nuclei and cell membranes were pelleted by centrifugation at 10,000 g for 10 min. Lysosomes, endosomes and mitochondria were pelleted from the supernatant by centrifugation at 100,000 g for 5 min. Lysosomes were disrupted with distilled water and the contents recovered from the supernatant after 10 min centrifugation at 100,000 g. Substrate solutions (200 μg/ml MBP85-99 peptide, 40 μg/ml human recombinant MBP or proinsulin (kindly provided by Eli Lilly, Germany), 0.1 M citrate; pH 5.0 or 5.5, 2.5 mM DTT) were incubated at 37 °C with lysosomal fractions (0.2–0.5 μg total protein) or human recombinant CatS or CatD as indicated. Where indicated, protease inhibitors were added

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