



Letter to the Editor

A hypothesis—Peptide library generated by cathepsin S might be efficacious for allergen-specific immunotherapy*Keywords:*

Synthetic peptide vaccine
Allergen-specific immunotherapy
Cathepsin S

1. Introduction

Peptide-based therapeutic vaccines, harboring CD4⁺ T-cell epitopes, derived from the primary sequence of allergen proteins, have been used for allergen-specific immunotherapy (ASIT) over years [1]. Peptide vaccines are safer than traditionally used crude allergen extracts, as short linear peptide molecules generally lack the ability to cross link pre-existing immunoglobulin (Ig)-E on mast cells and basophiles in sensitized individuals. Meanwhile, the preserved T-cell epitopes possess the ability to induce protective immune responses [1]. Although the detailed immunologic mechanisms of ASIT have not been well understood, recent studies on ASIT by the using of peptide vaccines showed convinced outcomes: down-regulation of Th1 and Th2 cells response to allergen, together with up-regulated secretion of IL-10 [2]; induction of regulatory T cells [3] and modulation of the natural course of allergic disease.

It is clear that an effective therapeutic peptide vaccine should provide available epitopes to be loaded on MHCII alleles for activating specific CD4⁺ T cells [4], a lack of T-cell epitopes on such peptides would impair the efficacy of peptide vaccine. So, peptide vaccines could be made as a defined T-cell epitope. However, the polymorphism of MHCII molecules and the differences in specificity of the alleles often result in different individuals typically react to a different set of peptides [5]. What's more, within the defined 700 different MHCII alleles, only 3% of them had been sufficiently studied in epitope binding experiments [6]. Thus, further trial and error should be conducted on the development of representative allele-specific T-cell-epitope vaccines.

Other than the attempts of representative T-cell epitopes for ASIT, strategy of peptide vaccine design also had aimed at promiscuous peptide fragments. In that strategy, by the selection of breakpoint between the fragments outside known T-cell epitopes, the sequence of a given allergen protein was “cut” into several continuous ones, then these truncated sequences were synthesized together as a peptide library for ASIT [3,7]. In virtue of the peptide library harbors each part of its corresponding intact protein, it may represent the coverage of whole allele-specific epitopes as the native allergen protein does. However, the correct selection of such breakpoints is limited in the available information of epitopes in this protein. In the absence of such information, cutting at any objective selected breakpoints may also destruct potential epitopes. Whereas, for a given protein, the task of identifying specific CD4⁺ T-cell epitopes itself is tedious and is also restricted by

the polymorphism of MHCII molecules. How the rational selection of breakpoints could be achieved without any available epitope information to produce efficacious peptide library allowing broad population coverage? Here, we hypothesize that using cathepsin S (Cat S), a widely expressed endosomal protease in antigen presentation cells (APCs), in vitro degradation of target proteins may produce promiscuous peptides without destructing any potential CD4⁺ T-cell epitopes and the efficacy of the generated peptide library may have broader population coverage on different MHCII backgrounds.

To illustrate our hypothesis, it is necessary to review the general features in antigen processing pathway and some properties of Cat S in this pathway.

1.1. Overview of the general course in antigen processing and presentation pathway

Once foreign antigens are captured and endocytosed by APCs, they become localized in transport vesicles called endosomes. Endosomes are acid vesicles containing a large set of endosomal proteases. Along the endocytic route, endosomes become mature and luminal pH decreases, which provides an acid environment for endosomal proteases to degrade protein antigens into peptide fragments [8]. Upon the arrival of these transport vesicles at late endocytic multivesicular bodies (MVBs), namely MHCII-enriched compartment, matured polymorphic MHCII molecules begin to select loading their preferential peptides for presentation to CD4⁺ T cells [9].

In this scenario, it is obvious that generation of epitopes follows stepwise manner and is regulated by two factors: proteolytic degradation and MHCII selection. In terms of the fact that the proteases which involved in antigen degradation are homologously expressed among individuals, the reason accounting for the polymorphic epitopes is decided largely by the polymorphic selection of MHCII molecules among the previous generated peptide fragments. Based on this fact, we hypothesize that if a protease which is not only involved in antigen processing pathway in APCs, but also has the access to foreign antigen preceding the binding of MHCII molecules to peptides, would generate efficacious peptide library without destructing any potential CD4⁺ T-cell epitopes. Interestingly, there are increasing evidences showing that Cat S might be the best candidate in this peptide vaccine design strategy.

1.2. Evidence that peptide library generated by Cat S would harbor all potential CD4⁺ T-cell epitopes and be efficacious with broad population coverage regardless of the polymorphic MHCII context

In the MHC class II pathway, nascent MHC class II heterodimers are associated with an obligate chaperone, the invariant chain (Ii), which occupies the MHCII peptide-binding groove, preventing

pre-mature loading of peptides in the ER and functioning as a trafficking signal leading the entry of class II molecules into endocytic compartments where the antigen peptides are generated [10]. Degradation of Ii follows stepwise manner. Upon the arrival of MHCII molecules at their destination, namely MVBs, Ii will be degraded into CLIP, which is the final size of Ii and ready for exchange with antigenic peptides by the aids of peptide-exchanger, HLA-DM [11]. In normal processes of Ii degradation, function of Cat S is of great significance as its presence is required for the final formation of CLIP. In B cells and dendritic cells (DCs) deficient in Cat S, generation of CLIP is disrupted at the Ii-10 stage [12]; when Cat S is introduced in these cells degradation of Ii-10 occurs. Whereas Cat S is present in all endocytic compartments [13] and their proteolytic capacity is active even in less acidic endosomal compartments [14], such as early endosomes. So it is possible that the interaction between Cat S and protein antigens is earlier than mature MHCII polymorphic loading to generated peptide fragments.

Given the fact that Cat S is constitutively expressed in all known professional APCs and is expressed highly conserved among individuals [15], it could be speculated that peptide fragments generated from Cat S degraded protein allergen may act as pre-epitope peptides, which would have broad population as the native allergen does.

1.3. Evidence that CD4⁺ T-cell epitopes would not be destructed by Cat S-mediated degradation in normal endocytic pathway

In immature DCs, the activity of Cat S is blocked by a cysteine inhibitor, cystatin C [16]. When DCs receive environmental stimuli and become mature, cystatin C is down-regulated, and the activity of Cat S increase. Still, the inflammatory signal of IFN- γ also provides stimulation to indirectly up-regulate the expression level of Cat S mRNA [17]. These evidences collectively suggest that the proteolytic function of active Cat S is required in antigen processing pathway, and that foreign antigens endocytosed by APCs could not escape from Cat S-mediated degradation. Plus the fact that epitopes generated by endosomal proteases are resistant to degradation by the enzymes which generate them [15], it can be concluded that degradation of target proteins by Cat S would not impair any potential epitopes.

1.4. Evidence that Cat S would be competent to produce sufficient peptide fragments in various protein antigens

Cat S is a kind of endoprotease with broad substrate specificity and broad pH activity [14,18]. These properties of proteolytic capacity ensure that Cat S can generate not only peptide fragments from various protein allergens, but also sufficient peptides from a given allergen protein. These properties make it possible for Cat S to degrade and release sufficient and short peptide fragments from any target protein allergens.

Some evidences had directly revealed the roles of Cat S in the enhancement of epitope generation, as was shown in Chyi-Song Hsieh's study that the expression of Cat S could enhance the presentation of epitopes in model antigens [19]. Plüger et al. found that Cat S even has a specific role in generation of two epitopes in hen egg lysozyme [20].

2. Conclusions and further studies

These data are consistent with the hypothesis that Cat S might be a candidate in the peptide vaccine design strategy to produce efficacious peptide library for ASIT.

However, it is worthy to note that the reason why we choose Cat S as a candidate is not simply based on the distinct role of Cat S is required for the maturation of MHCII molecules, but for its

role of testifying the temporal order that in the natural course of antigen processing, Cat S-mediated antigen degradation is prior to MHCII molecules acquire their ability to bind peptides. Thus Cat S generated peptides may function as epitope precursors for MHCII selection. Based on this principle, we believe that any proteases both involved in antigen processing and obeying this temporal order would also be considered to choose in this vaccine design strategy. With the combination of more kinds of proteases, target protein antigen might be processed shorter and thus safer for ASIT.

To test our hypothesis, comparative experiments could be conducted to determine the efficacies in the induction of specific T cell hybridoma between Cat S generated peptides and native allergen protein. For a certain target allergen, the antigenicity among peptide fragments generated by Cat S could be tested by polyclonal antibodies against the target native allergen. Peptide fragments still having the capacity of IgE binding could be further degraded with other proteases chosen by the principle discussed above.

Acknowledgment

We thank Yuan-Jing Liang (Capital medical university, China) for English proofreading.

References

- [1] Larché M. Peptide immunotherapy for allergic disease. *Allergy* 2007;62(3):325–31.
- [2] Tarzi M, Klunker S, Texier C, Verhoef A, Stapel SO, Akdis CA, et al. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. *Clin Exp Allergy* 2006;36(4):465–74.
- [3] Verhoef A, Alexander C, Kay AB, Larché M. T cell epitope immunotherapy induces a CD4⁺ T cell population with regulatory activity. *PLoS Med* 2005;2(3):e78.
- [4] Larché M, Wraith DC. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat Med* 2005;11(4 Suppl.):S69–76.
- [5] Moudgil KD, Sekiguchi D, Kim SY, Sercarz EE. Immunodominance is independent of structural constraints: each region within hen eggwhite lysozyme is potentially available upon processing of native antigen. *J Immunol* 1997;159(6):2754–9.
- [6] Robinson J, Waller MJ, Parham P, de Groot N, Bontrop R, Kennedy LJ, et al. IMGT/HLA and IMGT/MHC: sequence databases for the study of the major histocompatibility complex. *Nucleic Acids Res* 2003;31(1):311–4.
- [7] Vrtala S, Akdis CA, Budak F, Akdis M, Blaser K, Kraft D, et al. T cell epitope-containing hypoallergenic recombinant fragments of the major birch pollen allergen, Bet v1, induce blocking antibodies. *J Immunol* 2000;165(11):6653–9.
- [8] Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. *Annu Rev Immunol* 2005;23:975–1028.
- [9] Kleijmeer M, Ramm G, Schuurhuis D, Griffith J, Rescigno M, Ricciardi-Castagnoli P, et al. Reorganization of multivesicular bodies regulates MHC class II antigen presentation by dendritic cells. *J Cell Biol* 2001;55(1):53–63.
- [10] Bush R, Cloutier I, Sékaly RP, Hämmerling GJ. Invariant chain protects class II histocompatibility antigen from binding intact polypeptides in the endoplasmic reticulum. *EMBO J* 1996;15(2):418–28.
- [11] Vogt AB, Kropshofer H. HLA-DM—an endosomal and lysosomal chaperone for the immune system. *Trends Biochem Sci* 1999;24(4):150–4.
- [12] Shi GP, Villadamos JA, Dranoff G, Small C, Gu L, Haley KJ, et al. Cathepsin S required for normal MHC class II peptide loading and germinal center development. *Immunity* 1999;10(2):197–206.
- [13] Driessen C, Bryant RA, Lennon-Duménil AM, Villadamos JA, Bryant PW, Shi GP, et al. Cathepsin S controls the trafficking and maturation of MHC class II molecules in dendritic cells. *J Cell Biol* 1999;147(4):775–90.
- [14] Brömme D, Steinert A, Friebe S, Fittkau S, Wiederanders B, Kirschke H. The specificity of bovine spleen cathepsin S. A comparison with rat liver cathepsin L and B. *Biochem J* 1989;264(2):475–81.
- [15] Chapman HA. Endosomal proteases in antigen presentation. *Curr Opin Immunol* 2006;18(1):78–84.
- [16] Abrahamson M. Cystatins. *Methods Enzymol* 1994;244:685–700.
- [17] Storm van's Gravesande K, Layne MD, Ye Q, Le L, Baron RM, Perrella MA, et al. IFN regulatory factor-1 regulates IFN- γ -dependent cathepsin S expression. *J Immunol* 2002;168(9):4488–94.
- [18] Kirschke H, Wiederanders B, Brömme D, Rinne A. Cathepsin S from bovine spleen. Purification, distribution, intracellular localization and action on proteins. *J Biochem* 1989;264(2):467–73.
- [19] Hsieh CS, deRoos P, Honey K, Beers C, Rudensky AY. A role for cathepsin L and cathepsin S in peptide generation for MHC class II presentation. *J Immunol* 2002;168(6):2618–25.
- [20] Plüger EB, Boes M, Alfonso C, Schröter CJ, Kalbacher H, Ploegh HL, et al. Specific role for cathepsin S in the generation of antigenic peptides in vivo. *Eur J Immunol* 2002;32(2):467–76.

Chaopin Li
Yuxin Jiang
Liangyi Li*

*Department of Human Parasitology, Wannan
Medical University, 10 Weiliu Road, Wuhu City,
Anhui Province 241002, China*

* Corresponding author. Tel.: +86 0553 3932482;
fax: +86 0553 3932589.
E-mail address: lly@email.com (L. Li)

8 November 2009
Available online 28 November 2009