

T-cell epitope mapping

Laurent Malherbe, PhD

Objective: To provide a general overview of T-cell epitope mapping and its relevance to physicians practicing allergy/immunology.

Data Sources: The PubMed database was searched for articles published from January 1, 1990, through December 31, 2008, for primary and review articles on T-cell epitope mapping. The keywords searched were *CD4 T cell*, *major histocompatibility complex class II*, *epitope mapping*, *methods*, and *allergen*.

Study Selection: Specific articles on commonly used mapping techniques were retrieved, along with articles on the use of epitope mapping in models of allergy.

Results: A synopsis of T-cell epitope mapping, with emphasis on the relevance to allergy is presented.

Conclusions: T-cell epitope mapping studies have already allowed the identification of several major T-cell allergens. Improvement in T-cell epitope mapping techniques and in computer algorithms capable of predicting peptides recognized by CD4 T cells will help refine our understanding of the immune responses to allergens and may also offer the possibility of peptide therapy in the future.

Ann Allergy Asthma Immunol. 2009;103:76–79.

INTRODUCTION

T cells recognize linear peptidic sequences (or epitopes) derived from complexed protein presented in the context of self major histocompatibility complex (MHC) protein. T-cell epitope mapping refers to the determination of the specific peptide sequence recognized by CD4 or CD8 cytotoxic T cells responding to a pathogen, a tumor, or an allergen. Since CD4 T cells play a central role in the pathophysiology of allergic responses, defining common allergen epitopes recognized by CD4 T cells is important clinically. It will help identify common traits among different T-cell allergens and enable the development of peptide immunotherapy. This article seeks to provide a description of the peptide recognized by allergen-specific CD4 T cells and a synopsis of the most common methods used in T-cell epitope mapping studies. The PubMed database was searched for articles published from January 1, 1990, through December 31, 2008, for primary and review articles on T-cell epitope mapping. The keywords searched were *CD4 T cell*, *major histocompatibility complex class II*, *epitope mapping*, *methods*, and *allergen*. Specific articles on commonly used mapping techniques were retrieved, along with articles on the use of epitope mapping in models of allergy.

CD4 T-CELL EPITOPE STRUCTURE

Once foreign antigens such as allergens enter the body by inhalation or ingestion, they are internalized by dendritic cells (DCs) that are present in all tissues in contact with the external environment. The main function of DCs is to present antigen to other cells of the immune system. DCs are efficient at processing protein antigen into fragments and displaying these peptidic fragments complexed with MHC class II on their membrane. They are also uniquely capable of migrating into the secondary lymphoid organs, where their peptide–MHC class II complexes are recognized by T-cell receptors expressed on the surface of CD4 T cells. CD4 T lymphocytes recognize antigens in the form of relatively short peptide fragments bound in the peptide-binding groove of MHC class II molecules. In contrast to ubiquitously expressed MHC class I molecules, expression of MHC class II molecules is limited to specialized antigen-presenting cells, such as DCs, B cells, and macrophages. MHC class II molecules expressed by these cells primarily sample protein breakdown products derived from their endosomal or lysosomal compartments.¹ Detailed peptide-binding² and peptide elution studies³ and crystallographic data of MHC-peptide complexes⁴ have led to a tremendous amount of information regarding the interaction of MHC class II molecules and their binding peptides.^{5,6} The binding groove in MHC class II molecules has open ends and therefore accommodates peptides of varying lengths (typically 12 to 26 amino acids) (Fig 1). Polymorphic pockets and depressions in the MHC binding groove interact with several side chains of the peptide core sequence, providing the specificity of the MHC-peptide interaction. The consensus core sequence of peptides binding to MHC class II is 9 amino

Affiliations: Blood Research Institute, Blood Center of Wisconsin, T-Cell Immunobiology Laboratory, Milwaukee, Wisconsin.

Disclosures: Author has nothing to disclose.

Funding Sources: National Institute of Allergy and Infectious Diseases and the American Cancer Society provided funding for this study.

Received for publication January 26, 2009; Received in revised form March 27, 2009; Accepted for publication April 6, 2009.

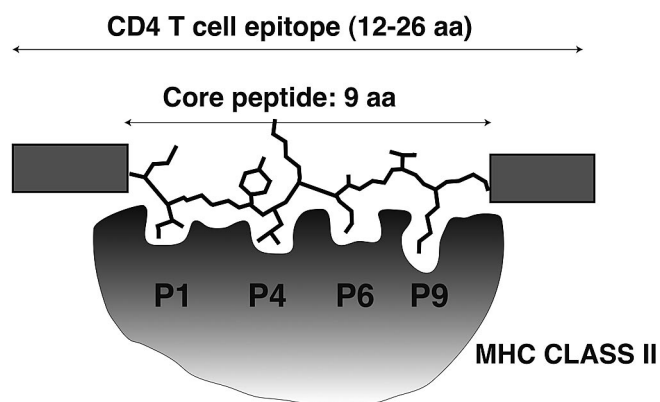


Figure 1. Structure of CD4 T-cell epitope bound to major histocompatibility complex (MHC) class II molecule. Black boxes represent amino acids present at the N-terminus and C-terminus of the core peptide (9 amino acids long). The interaction of the CD4 T-cell epitope and MHC class II is principally mediated by the interaction of the anchor residues at positions 1, 4, 6, and 9, with the corresponding pockets in the binding groove of the MHC class II molecules.

acids long with anchor residues at positions 1, 4, 6, and 9 (Fig 1).⁶

PEPTIDE DESIGN IN T-CELL EPITOPE MAPPING EXPERIMENTS

Data from peptide-binding studies have led to the development of computer-assisted algorithms, by which the sequences of large proteins can be scanned to predict individual peptides that might bind to distinct MHC molecules (eg, SYFPEITHI, Rankpep, Tepitope, ProPred).⁷⁻¹¹ Although these algorithms have successfully identified some allergen T-cell epitopes,^{12,13} they are limited to a subset of well-characterized MHC class II alleles and require knowledge of the restriction elements. T-cell epitopes as predicted by algorithms therefore still need to be confirmed by in vitro experimental data.¹⁴ Most T-cell epitope mapping studies use synthetic peptides spanning the length of the protein of interest to map regions recognized by T cells (Fig 2). Short peptides (8 to 10 amino acids) do not typically stimulate CD4 cells as efficiently as do peptides 12 or more amino acids in length.^{15,16} For CD4 mapping experiments, 12- to 20-mer peptides are therefore ideal in length. Each peptide overlaps by at least 3 to 10 amino acids to ensure that possible epitopes are not missed.¹⁷

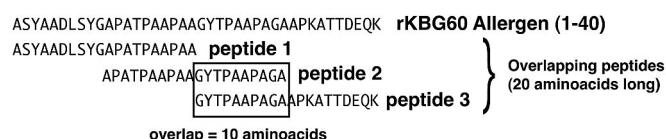


Figure 2. Schematic representation of a peptide scan covering the first 40 amino acids of the grass allergen rKBG60 with peptides 20 amino acids in length and overlap of 10 amino acids.³⁷

T-CELL EPITOPE MAPPING TECHNIQUES

The main problem in identifying which peptide is recognized by the CD4 T cells is the paucity of antigen-specific CD4 T cells present in the blood. A wide variety of methods have been proposed to circumvent this frequency issue. This article focuses on the most common methods used in T-cell epitope mapping studies, focusing on the specific challenge faced in identifying allergen T-cell epitopes.

T-CELL EPITOPE MAPPING USING PROLIFERATION ASSAY

The proliferation assay takes advantage of the fact that CD4 T cells undergo massive proliferation in response to their cognate antigen in vitro. In this assay, peripheral blood mononuclear cells (PBMCs) are stimulated with the antigenic peptides for 4 to 6 days, and the proliferative responses are evaluated by comparing the incorporation of tritiated thymidine in cultures performed with or without peptides.

Pros

This is an easy and relatively fast mapping technique.

Cons

The low frequency of allergen-specific CD4 T cells in PBMCs of atopic patients may not allow the detection of a significant proliferative response after 1 week in culture. Therefore, most mapping studies use allergen-specific T-cell lines or clones to map T-cell epitopes. These lines are generated by stimulating PBMCs in vitro with whole allergen protein for 5 days and further expanding the cells for 1 week with recombinant human interleukin (IL) 2.¹⁸ At this stage, allergen T-cell clones can be derived by limiting dilution. Allergen-specific T cells are seeded at 1 cell per well in microwells in the presence of irradiated allogeneic PBMCs (feeder cell), a mitogen (phytohemagglutinin), and recombinant human IL-2, and allergen T-cell clones are isolated from growing microculture after several weeks in culture.^{19,20}

T-CELL EPITOPE MAPPING BY FLOW CYTOMETRY

The attraction of using a flow cytometer for mapping epitopes lies in the number of different parameters that may be acquired in a single measurement (cell lineage and activation markers). The use of many parameters greatly enhances the resolution of the assay. Epitope mapping by flow cytometry was initially based on the detection of cytokine production by activated T cells after a short in vitro restimulation.²¹ This assay takes advantage of the fact that memory CD4 T cells rapidly release effector cytokines on antigen recognition.²² In the intracellular cytokine staining assay, PBMCs are stimulated for 6 hours with individual peptides²² in the presence of a Golgi inhibitor to prevent the cytokine release. PBMCs are then fixed, permeabilized, stained intracellularly for cytokine, and analyzed on a flow cytometer.

Pros

The biggest advantages of this approach are its unrivaled speed and its single-cell resolution.

Cons

Although this technique detects interferon γ -producing cells efficiently, the detection of IL-4 by flow cytometry is more difficult, probably reflecting the fact that IL-4 is secreted at a much lower level per cell. Two other flow cytometry-based mapping techniques have been recently developed that do not rely on the measurement of intracellular cytokines and that may thus be useful in mapping allergen T-cell epitopes. One flow cytometry assay analyzes T-cell proliferation by measuring the decrease of carboxyfluorescein diacetate succinimidyl ester (CFSE) staining in proliferating cells.²³ In this assay, PBMCs are first CFSE labeled before being cultured for 5 to 7 days with peptide *in vitro*. Flow cytometry is used to enumerate cells that have undergone proliferation (loss of CFSE fluorescence). The other assay uses MHC class II tetramers to map antigenic epitopes of known antigens through a novel peptide screening procedure known as tetramer-guided epitope mapping.^{24,25} In this assay, overlapping peptides spanning the protein of interest are divided into pools of 5 to 10 peptides, and each peptide pool is loaded onto soluble MHC class II molecules to generate pooled-peptide MHC class II tetramers. Pooled tetramers are then used to stain PBMCs that have been challenged previously with the appropriate antigen. Pooled tetramers that give positive staining are identified by flow cytometry, and peptides from positively staining pooled-peptide tetramers are then loaded individually onto the class II molecules and the staining repeated. Two prerequisites of this epitope-mapping approach are that the HLA phenotype of the individual is known and soluble HLA molecules of the restriction element of the T cells of interest are available.

T-CELL EPITOPE MAPPING USING ENZYME-LINKED IMMUNOSPOT (ELISPOT) ASSAYS

The ELISPOT assay also takes advantage of the fact that memory CD4 T cells secrete effector cytokines on antigen encounter, but it has proven more sensitive in detecting IL-4- and IL-10-producing cells. In ELISPOT assays, PBMCs are plated in the presence of peptide, and the production of secreted molecule is evaluated 24 hours after stimulation. ELISPOT plates are coated with anticytokine antibodies, allowing the capture of cytokines in the proximity of the secreting cell. In this way, each secreting cell leaves a trace (or spot) of its secretory activity. Each spot reflects the activity of a single cell. By far the most experience has been accumulated for both humans and mice using the interferon γ ELISPOT assay,²⁶ but IL-4 ELISPOT assays have also been used successfully to detect allergen-specific T_H2 cells from PBMCs of atopic patients.^{27,28}

Pros

Like the intracellular cytokine staining assay, the biggest advantages of this approach are its speed and its single-cell resolution.

Cons

Ex vivo detection of allergen-specific CD4 T cells could be difficult because of their low frequency in PBMCs. Cultured ELISPOT combines 1 round of *in vitro* stimulation with whole protein to enrich for allergen-specific CD4 T cells and the sensitivity of ELISPOT technique to map T-cell epitopes.²⁸

T-CELL EPITOPE MAPPING USING TRANSGENIC MICE EXPRESSING HLA

The paucity of circulating allergen-specific CD4 T cells in the peripheral blood of patients, the difficulty in long-term maintenance of human T-cell clones, and the presence of multiple HLA molecules in each individual make the determination of MHC restriction elements difficult, and many patients need to be analyzed before a clear pattern emerges. To circumvent these problems, transgenic mice, expressing individual human class II molecules in the absence of the endogenous mouse class II genes, have been used to characterize allergen epitopes presented by particular HLA class II molecules.²⁹ In this mapping technique, HLA transgenic mice are immunized subcutaneously with the protein allergen in complete Freund's adjuvant. Seven days later, draining lymph nodes are isolated and mechanically dispersed into a single-cell suspension. Lymph node cells of immunized HLA transgenic mice are used to map T-cell epitope by proliferation assay, intracellular staining assay, or ELISPOT.

Pros

The draining lymph nodes of immunized HLA transgenic mice provide a more abundant source of allergen-specific CD4 T cells to map T-cell epitopes than the PBMCs of atopic patients. All the epitopes detected are presented by 1 unique MHC class II molecule.

Cons

For every HLA specificity examined, a different HLA transgenic mouse is necessary.

CONCLUSION

T-cell epitope mapping studies have already allowed the identification of several major T-cell allergens, including pollen allergens (Art v1³⁰; Bet v1¹⁹), latex allergen (Hev b 5),³¹ house dust mite allergen (Der p 1),³² and cat allergen (Fel d 1).³³ Improvement in T-cell epitope mapping techniques and in computer algorithms capable of predicting peptides recognized by CD4 T cells will help refine our understanding of the immune responses to allergens and may also offer the possibility of peptide therapy in the future.³⁴⁻³⁶

REFERENCES

1. Jensen PE. Recent advances in antigen processing and presentation. *Nature Immunol.* 2007;8:1041-1048.

2. Hammer J, Valsasini P, Tolba K, et al. Promiscuous and allele-specific anchors in HLA-DR-binding peptides. *Cell*. 1993;74:197–203.
3. Chiciz RM, Urban RG, Lane WS, et al. Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. *Nature*. 1992;358:764–768.
4. Stern LJ, Brown JH, Jardetzky TS, et al. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature*. 1994;368:215–221.
5. Stern LJ, Wiley DC. Antigenic peptide binding by class I and class II histocompatibility proteins. *Structure*. 1994;2:245–251.
6. Rammensee HG, Friede T, Stevanović S. MHC ligands and peptide motifs: first listing. *Immunogenetics*. 1995;41:178–228.
7. Rammensee HG, Bachmann J, Emmerich NPN, Bachor OA, Stevanović S. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics*. 1999;50:213–219.
8. Reche PA, Glutting JP, Reinherz EL. Prediction of MHC class I binding peptides using profile motifs. *Hum Immunol*. 2002;63:701–709.
9. Reche PA, Glutting JP, Zhang H, Reinherz EL. Enhancement to the RANKPEP resource for the prediction of peptide binding to MHC molecules using profiles. *Immunogenetics*. 2004;56:405–419.
10. Sturniolo T, Bono E, Ding J, et al. Generation of tissue-specific and promiscuous HLA ligand databases using DNA microarrays and virtual HLA class II matrices. *Nat Biotechnol*. 1999;17:555–561.
11. Singh H, Raghava GPS. ProPred: prediction of HLA-DR binding sites. *Bioinformatics*. 2001;17:1236–1237.
12. de Lalla C, Sturniolo T, Abbruzzese L, et al. Cutting edge: identification of novel T cell epitopes in Lol p5a by computational prediction. *J Immunol*. 1999;163:1725–1729.
13. Brusica V, Petrovsky N. Bioinformatics for characterisation of allergens, allergenicity and allergic crossreactivity. *Trends Immunol*. 2003;24:225–228.
14. Richards KA, Chaves FA, Krafcik FR, et al. Direct ex vivo analyses of HLA-DR1 transgenic mice reveal an exceptionally broad pattern of immunodominance in the primary HLA-DR1-restricted CD4 T-cell response to influenza virus hemagglutinin. *J Virol*. 2007;81:7608–7619.
15. Gammon G, Geysen HM, Apple RJ, et al. T cell determinant structure: cores and determinant envelopes in three mouse major histocompatibility complex haplotypes. *J Exp Med*. 1991;173:609–617.
16. Gammon G, Shastri N, Cogswell J, et al. The choice of T-cell epitopes utilized on a protein antigen depends on multiple factors distant from, as well as at the determinant site. *Immunol Rev*. 1987;98:53–73.
17. Rodda SJ. Peptide libraries for T cell epitope screening and characterization. *J Immunol Methods*. 2002;267:71–77.
18. Ebner C, Schenk S, Najafian N, et al. Nonallergic individuals recognize the same T cell epitopes of Bet v 1, the major birch pollen allergen, as atopic patients. *J Immunol*. 1995;154:1932–1940.
19. Ebner C, Szépfalusi Z, Ferreira F, et al. Identification of multiple T cell epitopes on Bet v 1, the major birch pollen allergen, using specific T cell clones and overlapping peptides. *J Immunol*. 1993;150:1047–1054.
20. Jahn-Schmid B, Radakovics A, Lüttkopf D, et al. Bet v 1(142–156) is the dominant T-cell epitope of the major birch pollen allergen and important for cross-reactivity with Bet v 1-related food allergens. *J Allergy Clin Immunol*. 2005;116:213–219.
21. Kern F, Surel IP, Brock C, et al. T-cell epitope mapping by flow cytometry. *Nat Med*. 1998;4:975–978.
22. Waldrop SL, Davis KA, Maine VC, Picker LJ. Normal human CD4(+) memory T cells display broad heterogeneity in their activation threshold for cytokine synthesis. *J Immunol*. 1998;161:5284–5295.
23. Hoffmeister B, Kiecker F, Tesfa L, Volk HD, Picker LJ, Kern F. Mapping T cell epitopes by flow cytometry. *Methods*. 2003;29:270–281.
24. Kwok WW, Gebe JA, Liu A, et al. Rapid epitope identification from complex class-II-restricted T-cell antigens. *Trends Immunol*. 2001;22:583–588.
25. Yang JB, James EA, Huston L, Danke NA, Liu AW, Kwok WW. Multiplex mapping of CD4 T cell epitopes using class II tetramers. *Clin Immunol*. 2006;120:21–32.
26. Anthony DD, Lehmann PV. T-cell epitope mapping using the ELISPOT approach. *Methods*. 2003;29:260–269.
27. Ardern-Jones MR, Black AP, Bateman EA, Ogg GS. Bacterial superantigen facilitates epithelial presentation of allergen to T helper 2 cells. *Proc Natl Acad Sci U S A*. 2007;104:5557–5562.
28. Bateman EAL, Ardern-Jones MR, Ogg GS. Identification of an immunodominant region of Fel d 1 and characterization of constituent epitopes. *Clin Exp Allergy*. 2008;38:1760–1768.
29. Neeno T, Krco CJ, Harders J, Baisch J, Cheng S, David CS. HLA-DQ8 transgenic mice lacking endogenous class II molecules respond to house dust allergens: identification of antigenic epitopes. *J Immunol*. 1996;156:3191–3195.
30. Jahn-Schmid B, Kelemen P, Himly M, et al. The T cell response to Art v 1, the major mugwort pollen allergen, is dominated by one epitope. *J Immunol*. 2002;169:6005–6011.
31. de Silva H, Sutherland MF, Suphioglu C, et al. Human T cell epitopes of the latex allergen he v b 5 in health care. *J Allergy Clin Immunol*. 2000;105:S241–S241.
32. Yssel H, Johnson KE, Schneider PV, et al. T cell activation-inducing epitopes of the house dust mite allergen Der p 1. proliferation and lymphokine production patterns by Der p 1-specific CD4+ T cell clones. *J Immunol*. 1992;148:738–745.
33. Counsell CM, Bond JF, Ohman JL Jr, Greenstein JL, Garman RD. Definition of the human T-cell epitopes of Fel d 1, the major allergen of the domestic cat. *J Allergy Clin Immunol*. 1996;98:884–894.
34. Hoynes GF, Kristensen NM, Yssel H, Lamb JR. Peptide modulation of allergen-specific immune responses. *Curr Opin Immunol*. 1995;7:757–761.
35. van Neerven RJ, Ebner C, Yssel H, Kapsenberg ML, Lamb JR. T-cell responses to allergens: epitope-specificity and clinical relevance. *Immunol Today*. 1996;17:526–532.
36. Woodfolk JA. T-cell responses to allergens. *J Allergy Clin Immunol*. 2007;119:280–294.
37. Zhang L, Yang M, Chong P, Mohapatra SS. Multiple B- and T-cell epitopes on a major allergen of Kentucky Bluegrass pollen. *Immunology*. 1996;87:283–290.

Requests for reprints should be addressed to:

Laurent Malherbe, PhD
 Blood Research Institute
 Blood Center of Wisconsin
 T-Cell Immunobiology Laboratory
 PO Box 2178
 Milwaukee, WI 53201-2178
 E-mail: laurent.malherbe@bcw.edu