CHAPTER 15

Immunogenicity in Peptide-Immunotherapy: From Self/Nonself to Similar/Dissimilar Sequences

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Abstract

The nature of the relationship between an antigenic amino acid sequence and its capability to evoke an immune response is still an unsolved problem. Although experiments indicate that specific (dis)continuous amino acid sequences may determine specific immune responses, how immunogenic properties and recognition informations are mapped onto a non-linear sequence is not understood.

Immunology has invoked the concept of self/nonself discrimination in order to explain the capability of the organism to selectively immunoreact. However, no clear, logical and rational pathway has emerged to relate a structure and its immuno-nonreactivity. It cannot yet be dismissed what Koshland wrote in 1990: "Of all the mysteries of modern science, the mechanism of self versus nonself recognition in the immune system ranks at or near the top."[1]

This chapter reviews the concept of self/nonself discrimination in the immune system starting from the historical perspective and the conceptual framework that underlie immune reaction pattern. It also introduces future research directions based on a proteomic dissection of the immune unit, qualitatively defined as a low-similarity sequence and quantitatively delimitated by the minimum amino acid requisite able to evoke an immune response, independently of any, microbial or viral, "foreignness".

Introduction

Peptides and anti-peptide antibodies are widely used in biochemistry and molecular biology mostly for purification and characterization of specific oligopeptides and proteins, characterization of protein-protein, enzyme-substrate, or enzyme-inhibitor interactions, as well as for identification and mapping of the binding sites of antibodies. In addition, the last two decades have seen the exploitation of peptide antigens and anti-peptide antibodies in disease diagnosis and synthetic vaccine development. Previous and current clinical trials test a number of peptide-based vaccines against cancer[1-4] and both autoimmune and infectious diseases.[5-8] These vaccines suppose that short amino acid fragments derived from the parent protein antigen may induce or augment an immune response in cancer or, viceversa, alternatively the vaccines may neutralize autoreactive autoantibodies in autoimmune pathologies. As a matter of fact, peptide-immunotherapy appears able to obtain antibodies of predetermined specificity and without the complications associated with whole cells or entire protein vaccines.

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Multichain Immune Recognition Receptor Signaling: From Spatiotemporal Organization to Human Disease, edited by Alexander B. Sigalov.

Whatever the purpose of using peptide antigens and anti-peptide antibodies or whether trying to evoke or neutralize an immune response, success depends on the precise and exact identification of the antigenic peptide sequences at the root of an immune response. In this regard, a central concern is the understanding of the molecular basis of immunogenicity.

The Question: What Renders a Peptide Immunogenic?

We currently do not know why a peptide sequence is non-immunogenic, how the changing of only one amino acid residue can dramatically alter the peptide non-immunogenicity,[9] and when and where it arises the premises of the tight physico-chemical interaction between paratope and epitope. Empirically, epitope mapping shows the amino acid sequence interacting with the antibody under analysis. In abstract, we talk about the fine discrimination of the immune system's ability to sense and understand that specific single amino acid residue which is changed in the sequence. Experimentally, we are able to use harsh pH conditions and high salt reagents to break the strong bonds between the epitope and the paratope. But we do not know how the immunogenic epitope potency and the high paratope specificity originate.

Our ignorance is mainly due to (and partly justified by) the complexity of the system. As a general definition, the epitope is a set of atom groups in three-dimensional space that form the "target" of the immunoglobulin antigen binding site (the paratope). A typical epitope is roughly 5-6 residues long, but both trimer and octamer epitopes have been described.[10] The variability of the epitope length itself adds several orders of complexity to the analysis of epitope specificity. Indeed, peptide diversity is enormous and fits well with the enormous potential of antibody diversity. Using the 20 naturally occurring amino acids, one can generate about 3×10^6 different 5-mer peptides and about 2.5×10^{10} different 8-mer peptides.

Protein epitopes (or antigenic determinants) are classified into linear and nonlinear determinants. The latter are composed of noncontiguous residues that are not adjacent in the parent protein primary sequence but become so by three-dimensional folding. That makes practically infinite the possible determinant configurations, especially when considering a medium to high molecular weight protein. Moreover, numerically limited linear determinants might exist in a few configurations as components of linear determinants endowed with some mobility. Nonetheless, antibodies are exquisitely specific by hitting only a few of the numerous possible epitopic sequences.

The factors by which specific peptides are able to induce a B-cell response remain elusive.[11,12] A parallel question is present in our understanding in T-cell recognition: although the structural characteristics of the trimeric complex is clarifying[13,14] and we have learned that the TCRs recognize peptide-MHC with diagonal orientation and the CDR3 domains interact with the peptide bound to the MHC,[15,16] we remain ignorant of the functional process by which specific peptides are able to induce a T-cell response.

So, the inescapable question is: what characteristics render a peptide capable to evoke an immune response?

From a Historical Point of View—The Immune Response and Self/Nonself Sequences

The question of "what characteristics enable a structure to evoke an immune response" was easily answered in the nascent immunology of the late 19th-century concerned with understanding harmful infectious diseases.[17] In that context, immunology started as the study of defence mechanisms against the foreign pathogens. The patient as the attacked host became the "self" whose integrity had been threatened by external, foreign, nonself enemies. Slowly and tacitly the basis of the self/nonself dichotomy were dogmatically established in immunology. Potential immunogens were catalogued according to this self/nonself discrimination principle[18] with "nonself" strictly defined as belonging to a foreign organism, as opposed to "self", which were tolerated elements eliciting no response by being part of the organism itself.[19] The language of self and nonself had its foundations in a metchnikovian image of competitive struggle between organisms and infectious agents (e.g., bacteria and viruses)[20] and reflects the antinomy between benign and toxic, protection and damage,

internal and external.[21-23] In this perspective, intentionality and teleology became the molecular biochemistry of the immune response. The self became (and still is) a human category with ethical, political, psychological and existential meanings. The same immune system is viewed as 'recognizing', 'remembering', 'learning' and 'acting'—terms borrowed from the cognitive sciences.[18]

The immunological self/nonself antinomy became an example of "coincidentia oppositorum" by which everything could be intuitively explained, from cancer (tumor escape from immunosurveillance) to autoimmunity (self-defense excess). With the antibody molecule chemistry and pathology overlapped and the clear-cut physico-chemical coordinates that marked the three distinct domains of antigenicity, immunogenicity and pathogenicity fused together, surrounded by the emotional involvement of good against bad in a war-peace scenario.

This dominant self/nonself perspective remained unaltered during the century from Metchnikoff through Burnet[24,25] and still lingers.[26-28] Upon that metaphor, a theory of immunological tolerance was constructed that still dominates the field. Changes amount to little more than new terminology such as, Matzinger's danger model.[22,29] "Standing on the shoulders of the Self/Nonself",[29] the danger model proposes that antigen-presenting-cells are activated by danger/alarm signals from injured cells, such as those exposed to pathogens, toxins, or mechanical damage.

The danger model pari passu reproposes the Metchnikoff's overall representation, where the phagocyte is an agent[30] able to "sense" and "understand" the danger and, consequently, mount a response with a sense of independent arbitration.[17]

In this regard, Oldstone's molecular mimicry hypothesis, which defined molecular mimicry as similar structures—either linear amino acid sequences or their conformational foldings—shared by the host and virus, made significant scientific progress. The hypothesis suggested cross-reactivity between similar microbial determinants and host 'self' antigens as a pathogenic mechanism for autoimmune disease. In the hypothesis, the immune response against the determinant evokes a destructive tissue-specific immune response and the induction of cross-reactivity does not require a replicating agent, since the immune-mediated injury could occur after the immunogen has been removed—a hit-and-run event. The Oldstone's hypothesis marks a breaking point with the perspective of bad attacking spirits and good defensive intentions and introduces the immune response in molecular terms. For the first time in the immunology history, foreign entities have been reductionistically defined as bacterial or viral molecular sequences that mimic host molecular sequences.

The hypothesis has had an enormous impact on the science of the time and has greatly contributed to developing the sequence bioinformatic tools all of us utilize routinely. An intensive effort was undertaken in the attempt to validate the association of infectious agents with autoimmunity using molecular mimicry models to dissect the parameters required for the activation and association of virus-induced autoimmune disease. For decades the attention focused (and still focuses) on possible associations between infectious agents and autoimmunity. A list of examples includes, but are not limited to: Mycobacterium tuberculosis[33] and adjuvant arthritis; beta haemolytic streptococci and rheumatic fever;[34-36] herpes and autoimmune reactions against corneal tissues;[37] B3 coxsackieviruses and myocarditis;[38] Trypanosoma cruzi and Chagas' disease;[39] diverse viruses and multiple sclerosis;[40-44] Borrelia burgdorfii and Lyme arthritis;[45,46] and B4 Coxsackievirus, cytomegalovirus or rubella and type 1 diabetes.[47-52] However, many of the postulated associations remain unproven.[50,51,53]

Exempli gratia, the fact that the nitrogenase enzyme of K. pneumoniae, a bacterium present in the bowels of many individuals including ankylosing spondylitis patients, contains a 6-mer amino acid motif in common with HLA B27 protein sequence has repeatedly been reported as a model of molecular mimicry that might have a role in ankylosing spondylitis autoimmune disease. However, it is not a new observation that ankylosing spondylitis is mainly limited to the synovial joints of the spine, whereas HLA B27 molecules are expressed on almost all somatic cells.[54]

The weakness in the molecular mimicry hypothesis appears to be that molecular sequences are not analysed by themselves as a function of their own intrinsic qualities such as hydrophobicity/hydrophylicity, function, reactivity, 3-D conformation, masking (by glycosylation, polymerization, pairing to other molecules, etc.), spatiotemporal expression, quantitative level of expression, stability/

Table 1. From literature: examples of epitopic peptides characterized by being (or containing) sequences with low similarity to the host proteome

Protein	Amino Acid Position	Sequence*	Matches**	Proteome
Large tumour antigen (tag) of simian virus 40[67]	91-95	WEQWW	0	Murine
Duffy glycoprotein[70]	22-26	FEDVW	0	Murine
Receptor of vascular endothelial growth factor[71]	262-266	YPSSK	3	Murine
	256/257/261/313/315***	IDELT	2	Murine
p185HER2[80]	235-243	cCHEQCAag	0	Murine
Bovine leukemia virus transactivator protein tax[81]	261-280	HVWSSpqalqrflhdptltw	2	Murine
HIV gp41[82]	683-689	NWFDIt	0	Murine
Bordetella pertussis FIM2[83]	74-80	gRTPFIi	1	Human
Bordetella pertussis FIM3[83]	53-69	kvvqlpkISKNAlrndg	2	Human
	91-97	lkLYFEP	2	Human
Gluthathione-s-transferase from *Schistosoma bovis*[84]	58-67	iTDNHGHvkw	0	Murine
Leishmania infantum GRP94[85]	281-300	tqgvvkerrwtlvneNRPIW	0	Human
λ Repressor CII[86]	12-26	ledarrLKAIYekkk	1	Murine
Ovalbumin[87]	325-336	isqavhaaHAEINe	1	Murine
Toxic shock syndrome toxin-1[88]	47-56	fpSPYYSpaf	2	Murine
Staphylococcal enterotoxin B[88]	83-92	dvfgaNYYYQ	0	Murine
HOXD4 protein[89]		VYPWMK	0	Murine
α-subunit CK2[90]	319-324	MEHPYf	0	Murine
HLA class I H chain[91]	55-64	egpEYWDR(n/e)t	1	Murine
Cytochrome P4502D6[92]	193-212	RRFEYddprflrlldlaqeg	2	Human
Acetylcholinesterase[93]	112-119	tpvLVWIY	0	Murine
	143-151	rtvlVSMNY	2	Murine
	294-302	VFRFSfvpv	2	Murine
	332-341	kdegsYFLVY	2	Murine
	496-503	kapQWPPY	1	Murine
	523-532	glraqACAFW	0	Murine
Acetyl-Choline Receptor[94]	111-126	qytGHITWTppaifks****	0	Human
	122-138	aifkSYCEIlvthfpfd****	0	Human
	182-198	gwkhsvTYSCCpdtpy****	1	Human

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Table 1. Continued

Protein	Amino Acid Position	Sequence*	Matches**	Proteome
Myelin Basic Protein[95-97]	1-11	asqkrPSQRHg	1	Murine
	83-99	adpgsRPHLIrlfsrda	1	Murine
	70-89	tadPKNAWQD ahpadpgsrp	0	Human
Proteo-Lipid Protein [97]	139-151	chCLGKWlghpdk****	0	Murine
	178-191	FNTWTtcqsiafps****	1	Murine
Myelin Oligodendrocyte Glycoprotein[97]	1-22	gqfrVIGPRhpiralvgdevel	1	Murine
	92-106	deggFTCFFrDHSYQ****	0	Murine
Thyroglobulin[98]	2339-2358	qvaaltWVQTHirgfggdpr	0	Human
	2471-2490	pparalkRSLWVevdlligs	3	Human
	2651-2670	yefsrkvptfaTPWPDfvp	1	Human

*Low similarity 5-mers given in capital letters. **Matches: refer to the 5-mer in capital letters; correspond to the number of times a 5-mer occurs in the set of proteins that comprehensively constitutes the host proteome; calculated as already described in detail.[72-79] Low-similarity numerically defined as ≤ 3. ***Conformational epitope. ****All 5-mers forming the determinant have low similarity to the host proteome.

half-life time, proteolytic susceptibility, etc. The oldstonian analysis of the molecular sequences in the immunological context is still based mostly upon their derivance from bacterial or viral organisms.

From a Logical Point of View—The Immune Response and Similar/Dissimilar Sequences

Recently, it has been proposed that sequence similarity to the host proteome may modulate peptide immunogenicity.[55-58] The rationale is the following. If it is true that normal autoantigens are tolerated through the elimination of the antigen-reactive cells[59,60] and that the receptor repertoire must be purged of all antigen receptors that could possibly recognize self-antigens,[61-64] then it is logical to postulate that the sequences/patterns never or uniquely expressed in a proteome have more chances to escape the deletion process and, consequently, have more chances to induce an immune response.

But How to Define Sequence Similarity in the Immunological Context ?

Similarity between biological sequences is represented as sequence identity: the number of aligned positions where the corresponding characters (e.g., amino acids in proteins) are identical.[65] This protein similarity characterization by amino acid sequence comparison utilizes (multi)alignment programs and represents a very accurate method for predicting an evolutionary relationship among sequences.[66] High sequence identity (i.e., a high number of identical aligned amino acid residues) between two biological sequences indicates they belong to the same family. In other words, amino acid sequence similarity is a property that describes evolutionary history and whether biological sequences have a common ancestor.

In the immunological context, similarity analysis among biological sequences identifies amino acid groupings that represent rare or common sequences and, consequently, might or might not be considered as possible epitopes. To this aim, similarity search for immunogenic amino acid groupings (that we named Immunogenic Peptide Blocks, IPBs) utilizes perfect peptide match programs

and, by so doing, transforms sequence similarity from an evolutionary quality (when two sequences are compared point by point, i.e., amino acid by amino acid) into a mathematical quantity that describes "IPB percent identity" and can be numerically measured by the match number, i.e., the number of times that an IPB is present in the set of proteins analysed.

In such a context, the immunological significance of the IPB percent similarity to the host proteome primarily depends on the length definition of the shorter sequence that can constitute a linear determinant. Since literature data indicate five to six amino acids are sufficient minimal antigenic determinants,[67-71] IPB was defined as delimited by a minimal epitopic length of five amino acids. Therefore, immunologically the similarity between a pair of aligned biological sequences may be represented by the number of aligned IPBs (e.g., 5-mers in proteins) with perfect identity matching. Using this definition, the similarity level of a peptide sequence to a proteome is calculated as the number of times the peptide pentamers occur in the analysed proteome. More precisely, the similarity level of a peptide is zero when the 5-mers forming the peptide are absent in the proteome under analysis, whereas the similarity level of a peptide is high when its 5-mers are repeatedly represented in the protein set that comprehensively forms the proteome. As an important collateral notation, the relationship between peptide and proteome introduces the difference between similarity and redundancy, where similarity applies to peptide sequences from heterologous proteins and redundancy refers to autologous peptide sequences.

Browsing Through Literature: Similarity Level of Identified Epitopes

IPB similarity analysis has been successfully applied to define epitopic sequences in different experimental models.[72-79] In addition, the data obtained by analysing the scientific literature on identified epitopes are even more eloquent. Table 1 illustrates the concrete application of this IBP similarity rationale in analyzing the literature data. It shows how a first screening produced dozens of well-defined epitopic sequences that are or harbor IPB(s) with no or low similarity to the host proteome.

Concluding Remarks

In 1859 Darwin demonstrated that complex, gradual adaptation processes arise over time without outside agency and, in so doing, he demolished teleology in science. Nonetheless, today we still have an immunology science dominated by the teleology of intentionality: explaining immune reactions in terms of self entities against nonself enemies and interpreting immune processes as meditated actions against enemies and protective conduct towards self entities.

In this context, the development of high-throughput technologies and the nascent peptidomics research offer exciting new opportunities to comprehensively analyse peptides in the immune subsystem, that is to define the immuno-peptidome. The time for a more precise answer to the logical question, "what are the molecular features that make a peptide immunogenic?" appears closer. The time for a geometrical definition of the limits and intersections among the three distinct domains of peptide antigenicity, immunogenicity and pathogenicity is getting closer as well. The challenges for these goals lie in archiving and functionally relating the vast majority of data derived from immunoassay experiments and bioinformatic predictions into a coherent informational mass relevant to physio- and pathological processes. To this end, it will be necessary to establish universally accepted criteria for positive identification of immunoreactive peptides to design effective peptide-immunotherapies.

References

1. Koshland DJ Jr. Recognizing self from nonself. Science 1990; 248:4961.
2. Gjertsen MK, Bakka A, Breivik J et al. Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding RAS mutation. Lancet 1995; 346:1399-400.
3. Noguchi M, Kobayashi K, Suetsugu N et al. Induction of cellular and humoral immune responses to tumor cells and peptides in HLA-A24 positive hormone-refractory prostate cancer patients by peptide vaccination. Prostate 2003; 57:80-92.

4. Moulton HM, Yoshihara PH, Mason DH et al. Active specific immunotherapy with a beta-human chorionic gonadotropin peptide vaccine in patients with metastatic colorectal cancer: Antibody response is associated with improved survival. Clin Cancer Res 2002; 8:2044-2051.
5. Offner H, Vandenbark AA. Congruent effects of estrogen and T-cell receptor peptide therapy on regulatory T-cells in EAE and MS. Int Rev Immunol 2005; 24:447-477.
6. Herrington DA, Clyde DF, Losonsky G et al. Safety and immunogenicity in man of a synthetic peptide malaria vaccine against Plasmodium falciparum sporozoites. Nature 1987; 328:257-259.
7. El Kasmi KC, Muller CP. New strategies for closing the gap of measles susceptibility in infants: Towards vaccines compatible with current vaccination schedules. Vaccine 2001; 19:2238-2244.
8. Sabhanini L, Manocha M, Sridevi K et al. Developing subunit immunogens using B- and T-cell epitopes and their constructs derived from F1 antigen of Yersinia pestis using novel delivery vehicles. FEMS Immunol Med Microbiol 2003; 1579:1-15.
9. Wiens GD, Pascho R, Winton JR. A single Ala139-to-Glu substitution in the Renibacterium salmoninarum virulence-associated protein p57 results in antigenic variation and is associated with enhanced p57 binding to chinook salmon leukocytes. Appl Environ Microbiol 2002; 68:3969-3977.
10. Stephen CW, Helminen P, Lane DP. Characterisation of epitopes on human p53 using phage-displayed peptide libraries: Insights into antibody-peptide interactions. J Mol Biol 1995; 248:58-78.
11. van Regenmortel MHV. The recognition of proteins and peptides by antibodies. J Immunoassay 2000; 21:85-108.
12. van Regenmortel MHV. Antigenicity and immunogenicity of synthetic peptides. Biologicals 2001; 29:209-213.
13. Kellenberger C, Porciero S, Roussel A. Expression, refolding, crystallization and preliminary crystallographic study of MHC H-2K(k) complexed with octapeptides and non-apeptides. Acta Crystallogr D Biol Crystallogr 2004; 60:1278-1280.
14. Webb AI, Borg NA, Dunstone MA et al. The structure of H-2K(b) and K(bm8) complexed to a Herpes Simplex virus determinant: evidence for a conformational switch that governs T-cell repertoire selection and viral resistance. J Immunol 2004; 173:402-409.
15. Bankovich AJ, Garcia KC. Not just any T-cell receptor will do. Immunity 2003; 18:7-11.
16. Garcia KC, Adams EJ. How the T-cell receptor sees antigen—A structural view. Cell 2005; 122:333-336.
17. Tauber AI. The biological notion of self and nonself. In: Zalta EN, ed. The Stanford Encyclopedia of Philosophy. Spring 2006 Edition. (http:// plato.stanford.edu/ archives/spr2006/entries/biology-self/).
18. Tauber AI. Moving beyond the immune self? Semin Immunol 2000; 12:241-248.
19. Silverstein AM, Rose NR. On the mystique of the immunological self. Immunol Rev 1997; 159:197-206.
20. Tauber AI, Chernyak L. Metchnikoff and the origins of immunology: From metaphor to theory. Oxford: Oxford University Press, 1991.
21. Mitchison NA, Katz DR, Chain B. Self/nonself discrimination among immunoregulatory (CD4) T-cells. Semin Immunol 2000; 12:179-183.
22. Matzinger P. Tolerance, danger and the extended family. Annu Rev Immunol 1994; 12:991-1045.
23. Cohn M, Langman RE. The protecton: The evolutionarily selected unit of humoral immunity. Immunol Rev 1990; 115:1-131.
24. Burnet FM, Fenner F. The Production of Antibodies, 2nd edition. Melbourne: Macmillan and Co., 1949; 1-142.
25. Burnet FM. The concept of immunological surveillance. Prog Exp Tumor Res 1970; 13:1-27
26. Medzhitov R, Janeway CA Jr. Decoding the patterns of self and nonself by the innate immune system. Science 2002; 296:298-300.
27. Natarajan K, Dimasi N, Wang J et al. Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. Annu Rev Immunol 2002; 20:853-885.
28. Hickman HD, Luis AD, Buchli R et al. Toward a definition of self: Proteomic evaluation of the class I peptide repertoire. J Immunol 2004; 172:2944-2952.
29. Matzinger P. The Danger model: A renewed sense of self. Science 296; 2002:301-305.
30. Crist E, Tauber AI. The phagocyte, the antibody and agency: Contending turn-of-the-century approaches to immunity. In: Moulin AM, Cambrosio A, eds. Singular Selves: Historical Issues and Contemporary Debates in Immunology. Amsterdam: Elsevier, 2001:115-139.
31. Fujinami RS, Oldstone MB, Wroblewska Z et al. Molecular mimicry in virus infection: Crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments. Proc Natl Acad Sci USA 1983; 80:2346-2350.
32. Fourneau JM, Bach JM, van Endert PM et al. The elusive case for a role of mimicry in autoimmune diseases. Mol Immunol 2004; 40:1095-1102.

33. Van Bilsen JH, Wagenaar-Hilbers JP, Boot EP et al. Searching for the cartilage-associated mimicry epitope in adjuvant arthritis. Autoimmunity 2002; 35:201-210.
34. McDonald M, Currie BJ, Carapetis JR. Acute rheumatic fever: A chink in the chain that links the heart to the throat? Lancet Infect Dis 2004; 4:240-245.
35. Dinkla K, Rohde M, Jansen WT et al. Rheumatic fever-associated Streptococcus pyogenes isolates aggregate collagen. J Clin Invest 2003; 111:1905-1912.
36. Guilherme L, Cunha-Neto E, Coelho V et al. Human heart-infiltrating T-cell clones from rheumatic heart disease patients recognize both streptococcal and cardiac proteins. Circulation 1995; 92:415-420.
37. Panoutsakopoulou V, Sanchirico ME, Huster KM et al. Analysis of the relationship between viral infection and autoimmune disease. Immunity 2001; 15:137-147.
38. Kim KS, Hufnagel G, Chapman NM et al. The group B coxsackieviruses and myocarditis. Rev Med Virol 2001; 11:355-368.
39. Girones N, Cuervo H, Fresno M. Trypanosoma cruzi-induced molecular mimicry and Chagas' disease. Curr Top Microbiol Immunol 2005; 296:89-123.
40. Katz-Levy Y, Neville KL, Girvin AM et al. Endogenous presentation of self myelin epitopes by CNS-resident APCs in Theiler's virus-infected mice. J Clin Invest 1999; 104:599-610.
41. Kuchroo VK, Anderson AC, Waldner H et al. T-cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning and regulating the autopathogenic T-cell repertoire. Annu Rev Immunol 2002; 20:101-123.
42. Carrizosa AM, Nicholson LB, Farzan M et al. Expansion by self antigen is necessary for the induction of experimental autoimmune encephalomyelitis by T-cells primed with a cross-reactive environmental antigen. J Immunol 1998; 161:3307-3314.
43. Lang HL, Jacobsen H, Ikemizu S et al. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat Immunol 2002; 3:940-943.
44. Miller SD, Vanderlugt CL, Begolka WS et al. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. Nat Med 1997; 3:1133-1136.
45. Salazar CA, Rothemich M, Drouin EE et al. Human Lyme arthritis and the immunoglobulin G antibody response to the 37-kilodalton arthritis-related protein of Borrelia burgdorferi. Infect Immun 2005; 73:2951-2957.
46. Raveche ES, Schutzer SE, Fernandes H et al. Evidence of Borrelia autoimmunity-induced component of Lyme carditis and arthritis. J Clin Microbiol 2005; 43:850-856.
47. Atkinson MA, Bowman MA, Campbell L et al. Cellular immunity to a determinant common to glutamate decarboxylase and Coxsackie virus in insulin-dependent diabetes. J Clin Invest 1994; 94:2125-2129.
48. Bach JM, Otto H, Jung G et al. Identification of mimicry peptides based on sequential motifs of epitopes derived from 65-kDa glutamic acid decarboxylase. Eur J Immunol 1998; 28:1902-1910.
49. Hiemstra HS, Schloot NC, van Veelen PA et al. Cytomegalovirus in autoimmunity: T-cell crossreactivity to viral antigen and autoantigen glutamic acid decarboxylase. Proc Natl Acad Sci USA 2001; 98:3988-3991.
50. Horwitz MS, Bradley LM, Harbertson J et al. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. Nat Med 1998; 4:781-785.
51. Schloot NC, Willemen SJ, Duinkerken G et al. Molecular mimicry in type 1 diabetes mellitus revisited: T-cell clones to GAD65 peptides with sequence homology to Coxsackie or proinsulin peptides do not crossreact with homologous counterpart. Hum Immunol 2001; 62:299-309.
52. Uemura Y, Senju S, Maenaka K et al. Systematic analysis of the combinatorial nature of epitopes recognized by TCR leads to identification of mimicry epitopes for glutamic acid decarboxylase 65-specific TCRs. J Immunol 2003; 170:947-960.
53. Benoist C, Mathis D. Autoimmunity provoked by infection: How good is the case for T-cell epitope mimicry? Nat Immunol 2001; 2:797-801.
54. Benjamin R, Parham P. Guilt by association: HLA-B27 and ankylosing spondylitis. Immunol Today 1990; 11:137-142.
55. Willers J, Lucchese A, Kanduc D et al. Molecular mimicry of phage displayed peptides mimicking GD3 ganglioside. Peptides 1999; 20:1021-1026.
56. Natale C, Giannini T, Lucchese A et al. Computer-assisted analysis of molecular mimicry between HPV16 E7 oncoprotein and human protein sequences. Immunol Cell Biol 2000; 78:580-585.
57. Kanduc D. Peptimmunology: immunogenic peptides and sequence redundancy. Curr Drug Discov Technol 2005; 2:239-244.
58. Kanduc D. Defining peptide sequences: from antigenicity to immunogenicity through redundancy. Curr Pharmacogenomics 2006; 4:33-37.
59. Burnet FM. The Clonal Selection Theory of Acquired Immunity. Cambridge University Press, Cambridge: 1959; 1-209.
60. Burnet FM. Self and Not-Self. Cambridge University Press, Cambridge: 1969; 1-318.

61. Nossal GJ. How is tolerance generated? Ciba Found Symp 1987; 129:59-72.
62. Gonzalo JA, de Alboran IM, Kroemer G. Dissociation of autoaggression and self-superantigen reactivity. Scand J Immunol 1993; 37:1-6.
63. Touma M, Mori KJ, Hosono M. Failure to remove autoreactive Vbeta6+ T-cells in Mls-1 newborn mice attributed to the delayed development of B-cells in the thymus. Immunology 2000; 100:424-431.
64. Mapara MY, Sykes M. Tolerance and cancer: Mechanisms of tumor evasion and strategies for breaking tolerance. J Clin Oncol 2004; 22:1136-1151.
65. May AC. Percent sequence identity; the need to be explicit. Structure 2004; 12:737-738.
66. Park J, Karplus K, Barrett C et al. Sequence comparisons using multiple sequences detect three times as many remote homologues as pairwise methods. J Mol Biol 1998; 284:1201-1210.
67. Lindner K, Mole SE, Lane DP et al. Epitope mapping of antibodies recognising the N-terminal domain of simian virus large tumour antigen. Intervirology 1998; 41:10-16.
68. Reddehase MJ, Rothbard JB, Koszinowski UH. A pentapeptide as minimal antigenic determinant for MHC class I-restricted T-lymphocytes. Nature 1989; 337:651-653.
69. Hemmer B, Kondo T, Gran B et al. Minimal peptide length requirements for CD4(+) T-cell clones-implications for molecular mimicry and T-cell survival. Int Immunol 2000; 12:375-383.
70. Wasniowska K, Petit-LeRoux Y, Tournamille C et al. Structural characterization of the epitope recognized by the new anti-Fy6 monoclonal antibody NaM 185-2C3. Transfus Med 2002; 12:205-211
71. Lu D, Kussie P, Pytowski B et al. Identification of the residues in the extracellular region of KDR important for interaction with vascular endothelial growth factor and neutralizing anti-KDR antibodies. J Biol Chem 2000; 275:14321-14330.
72. Kanduc D, Lucchese A, Mittelman A. Individuation of monoclonal anti-HPV16 E7 antibody linear peptide epitope by computational biology. Peptides 2001; 22:1981-1985.
73. Mittelman A, Lucchese A, Sinha AA et al. Monoclonal and polyclonal humoral immune response to EC HER-2/neu peptides with low similarity to the host's proteome. Int J Cancer 2002; 98:741-747.
74. Lucchese A, Stevanovic S, Sinha AA et al. Role of MHC II affinity and molecular mimicry in defining anti-HER-2/neu MAb-3 linear peptide epitope. Peptides 2003; 24:193-197.
75. Kanduc D, Fanizzi FP, Lucchese G et al. NMR probing of in silico identification of anti-HPV16 E7 mAb linear peptide epitope. Peptides 2004; 25:243-250.
76. Mittelman A, Tiwari R, Lucchese G et al. Identification of monoclonal anti-HMW-MAA antibody linear peptide epitope by proteomic database mining. J Invest Dermat 2004; 123:670-675.
77. Dummer R, Mittelman A, Fanizzi FP et al. Nonself discrimination as a driving concept in the identification of an immunodominant HMW-MAA epitopic peptide sequence by autoantibodies from melanoma cancer patients. Int J Cancer 2004; 111:720-726.
78. Lucchese A, Mittelman A, Lin MS et al. Epitope definition by proteomic similarity analysis: identification of the linear determinant of the anti-Dsg3 MAb 5H10. J Transl Med 2004; 2:43.
79. Lucchese A, Willers J, Mittelman A et al. Proteomic scan for tyrosinase peptide antigenic pattern in vitiligo and melanoma. Role of sequence similarity and HLA-DR1 affinity. J Immunol 2005; 175:7009-7020.
80. Orlandi R, Formatici C, Menare S et al. X A linear region of a monoclonal antibody conformational epitope mapped on p185HER2 oncoprotein. Biol Chem 2005; 378:1387-1392.
81. Sakakibara N, Kabeya H, Ohashi K et al. Epitope mapping of bovine leukemia virus transactivator protein tax. J Vet Med Sci 1998; 60:599-605.
82. Cardoso RM, Zwick MB, Stanfield RL et al. Broadly neutralizing anti-HIV antibody 4E10 recognizes a helical conformation of a highly conserved fusion-associated motif in gp41. Immunity 2005; 22:163-173.
83. Williamson P, Matthews R. Epitope mapping the Fim2 and Fim3 proteins of Bordetella pertussis with sera from patients infected with or vaccinated against whooping cough. FEMS Immunol Med Microbiol 1996; 13:69-78.
84. da Costa AV, Lafitte S, Fontaine J et al. Definition and mapping of epitopes recognized by specific monoclonal antibodies to Schistosoma bovis 28 kDa glutathione S-ransferase: Relation with anti-egg viability immunity. Parasite Immunol 1999; 21:9-18.
85. Larreta R, Guzman F, Patarroyo ME et al. Antigenic properties of the Leishmania infantum GRP94 and mapping of linear B-cell epitopes. Immunol Lett 2002; 80:199-205.
86. Lai MZ, Huang SY, Briner TJ et al. T-cell receptor gene usage in the response to lambda repressor cI protein. An apparent bias in the usage of a V alpha gene element. J Exp Med 1988; 168:1081-1097.
87. Shimonkevitz R, Colon S, Kappler JW et al. Antigen recognition by H-2-restricted T-cells. II. A tryptic ovalbumin peptide that substitutes for processed antigen. J Immunol 1984; 133:2067-2074.
88. Pang LT, Kum WW, Chow AW. Inhibition of staphylococcal enterotoxin B-induced lymphocyte proliferation and tumor necrosis factor alpha secretion by MAb5, an anti-toxic shock syndrome toxin 1 monoclonal antibody. Infect Immun 2000; 68:3261-3268.

89. Burgess K, Han I, Zhang A et al. DiSSiMiL: Diverse Small Size Mini-Libraries applied to simple and rapid epitope mapping of a monoclonal antibody. J Pept Res 2001; 57:68-76.
90. Nastainczyk W, Issinger OG, Guerra B. Epitope analysis of the MAb 1AD9 antibody detection site in human protein kinase CK2alpha-subunit. Hybrid Hybridomics 2003; 22:87-90.
91. Perosa F, Luccarelli G, Prete M et al. Beta 2-microglobulin-free HLA class I heavy chain epitope mimicry by monoclonal antibody HC-10-specific peptide. J Immunol 2003; 171:1918-1926.
92. Kerkar N, Choudhuri K, Ma Y et al. Cytochrome P4502D6(193-212): A new immunodominant epitope and target of virus/self cross-reactivity in liver kidney microsomal autoantibody type1-positive liver disease. J Immunol 2003; 170:1481-1489.
93. Zhang XM, Liu G, Sun MJ. Epitopes of human brain acetylcholinesterase. Brain Res 2000; 868:157-164.
94. Deitiker P, Ashizawa T, Atassi MZ. Antigen mimicry in autoimmune disease. Can immune responses to microbial antigens that mimic acetylcholine receptor act as initial triggers of Myasthenia gravis? Hum Immunol 2000; 61:255-265.
95. He XL, Radu C, Sidney J et al. Structural snapshot of aberrant antigen presentation linked to autoimmunity: the immunodominant epitope of MBP complexed with I-Au. Immunity 2002; 17:83-94.
96. Crowe PD, Boehme SA, Wong T et al. Differential signaling and hierarchical response thresholds induced by an immunodominant peptide of myelin basic protein and an altered peptide ligand in human T-cells. Hum Immunol 1998; 59:679-689.
97. Dharmasaroja P. Specificity of autoantibodies to epitopes of myelin proteins in multiple sclerosis. J Neurol Sci 2003; 206:7-16.
98. Thrasyvoulides A, Sakarellos-Daitsiotis M, Philippou G et al. B-cell autoepitopes on the acetylcholinesterase-homologous region of human thyroglobulin: Association with Graves' disease and thyroid eye disease. Eur J Endocrinol 2001; 145:119-127.