

The role of autoantigens in autoimmune disease

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Many autoantigens have been identified in human patients and in rodent models. In numerous experimental settings, these autoantigens or related autoreactive lymphocytes can transfer autoimmunity. Although autoreactivity spreads to new epitopes during the course of disease, single-epitope-specific therapies show considerable efficacy in multi-epitope-induced models of autoimmunity. These observations may indicate that epitope-specific therapies operate at the level of regulating mechanisms of immune tolerance rather than exerting a direct effect on autoreactive T lymphocytes.

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Abbreviations

EAE	experimental autoimmune encephalomyelitis
GAD	glutamic acid decarboxylase
IDDM	insulin-dependent diabetes mellitus
MBP	myelin basic protein
MOG	myelin oligodendrocyte glycoprotein
PLP	proteolipid protein

Introduction

Immunology is predicated on the notion that an immune response is initiated when antigen selects reactive immunocytes and directs their clonal expansion and differentiation. This paradigm, when applied to autoimmune disease, has focused attention on the search for the antigen or epitope that initiates and/or perpetuates the autoimmune response. Indeed, the literature describes a plethora of candidate autoantigens in experimental animal models as well as in actual human autoimmune diseases. The search for the autoantigen(s) that initiate and/or perpetuate the autoimmune process is important from a practical viewpoint since this knowledge could be the focus of clinical intervention strategies that target the autoantigen-specific, pathology-inducing T cell. This review describes our current understanding of the identity and potential role of autoantigens in autoimmunity.

Identification of autoantigen candidates

Development of effective therapeutics for autoimmune diseases has been significantly impeded by our lack of knowledge concerning the nature of stimulants and/or events that initially precipitate these diseases. There have been suggestions that viral or other microbial infections may trigger the process of autoimmunity by initiating a response that is cross-reactive with a host autoantigen (for example, see [1–3]). Alternative theories for initiation of autoimmunity relate to the well-documented presence in

disease-free animals and humans of B cells and T cells with anti-self reactivity that are maintained in a nonpathogenic state via regulatory mechanisms that presumably break down with the development of autoimmune disease. Whatever causes the initial activation of autoreactive lymphocytes and/or their conversion to pathogenicity, it is likely that their continued stimulation is related to constant exposure to specific autoantigen(s).

Over the past decade, numerous approaches have led to the identification of many autoantigen candidates (reviewed in [4–6]; see also the review by Elson and Barker, this issue, pp 664–669), a proportion of which are summarized in Table 1. Autoantibodies from patients or animal models have been used to screen appropriate expression libraries that are derived from tissues stained by those autoantibodies, leading to identification of specific autoantigens ([7,8]; see also the review by Elson and Barker). The link of these autoantigens to disease has in some cases been established by showing that recombinant autoantigen, even if human in derivation, can frequently transfer related pathology to an appropriate normal animal. For example, the lesion of pemphigus vulgaris, a blistering skin disease with autoantibodies against the intracellular cement that holds epidermal cells together, was reproduced in infant mice by autoantibodies from human pemphigus patients [9].

The identification of autoantigens in T cell mediated autoimmune diseases such as multiple sclerosis and type 1 diabetes initially proceeded via the derivation from appropriate disease models of autoreactive murine T cell clones that were capable of transferring disease to untreated syngeneic mice, and then mapping the autoantigenic epitope identified by those T cells. Human T cells with similar specificities have been identified in autoimmune patient blood or sites of pathology [5,6,10–14]. However, demonstrating that these human autoantigens are pathogenic has been impeded by the fact that MHC restrictions prevent evaluating the ability of the human T cell clones to transfer pathology into conventional animals. Recently, HLA-transgenic RAG-2^{-/-} mice have been used as recipients for antigen-specific human autoreactive T cells, with some success in terms of disease transfer, implicating a pathogenic role for the autoantigen under study [15]. Studies of this type will be of considerable value in terms of evaluating the pathogenic roles of the multiple autoantigen candidates currently under investigation in T cell mediated autoimmune diseases.

Is autoimmunity initiated and/or perpetuated by recognition of a primary autoantigen?

Although multiple antigens can be associated with a particular autoimmune disease, the questions at issue are

Table 1**Some autoantigens in autoimmune diseases.**

Disease	Candidate autoantigens
IDDM	Pancreatic β -cell antigens, insulin, GAD and its isoforms
Multiple sclerosis	MBP, PLP, MOG
Rheumatoid arthritis	Collagen type II, human cartilage gp39 (HCgp39), gp130-RAPS
Scleroderma	Fibrillarin, small nucleolar protein (snoRNP)
Myasthenia gravis	Acetylcholine receptor (AChR)
Graves' disease	Thyroid stimulating factor receptor (TSH-R)
Systemic lupus erythematosus	Nuclear antigens, DNA, histones, glycoprotein gp70, ribosomes
Primary biliary cirrhosis	PDC-E2 (mitochondrial enzyme, pyruvate dehydrogenase dehydrolipoamide acetyltransferase)
Alopecia areata	Hair follicle antigens
Ulcerative colitis	Human tropomyosin isoform 5 (hTM5)

whether a primary antigen is responsible for the initiation of the autoimmune process, whether other autoantigen epitopes become involved in perpetuation of the disease, and whether disease treatment and cure will ultimately require precise identification of the initiating and/or perpetuating autoantigens.

To help unravel the potential role of autoantigen(s) in disease pathogenesis, we will first consider the development of the autoimmune response within the context of the pathophysiology of autoimmunity.

The most thoroughly studied models of spontaneous autoimmunity are the development of autoimmune diabetes in both the NOD mouse and the BB rat. In the NOD mouse, the development of disease in a population of animals, maintained under fixed environmental conditions, is highly predictable. Inflammation of the pancreatic islets develops around the time of weaning and intensifies as the animal ages. However, clinical disease is not seen before approximately 100 days of age. The development of clinical disease is asynchronous: some animals develop disease around 100 days of age whereas others may develop insulin-dependent diabetes mellitus (IDDM) 200 days, or more, later. The probability of individual animals developing disease remains relatively constant between 100 and 250 days of age but declines somewhat after that time. This asynchronous behavior of the population suggests that individual animals follow an idiosyncratic pathway towards the development of IDDM. Such idiosyncratic behavior of the population does not support the notion that

disease is initiated by an external immunological trigger, such as a viral infection. If this were the case, we would expect some synchrony within the colony. Moreover, in the case of the NOD mouse, the introduction of infectious agents into the colony tends to reduce the probability that individuals in the colony will develop clinical disease [16].

Another alternative, suggested by Yoon [17] and others [18], is that the stochastic expression of an endogenous retrovirus may precipitate IDDM by a process of molecular mimicry, or by damaging the pancreatic islet, thereby activating the autoimmune process. However, we have no evidence that the NOD islet is either fragile or immunogenic when placed in an immunocompetent environment (Figure 1). Nevertheless, there is a stochastic event associated with the disease process. This is seen at the level of inflammation associated with individual islets in the pancreas of disease prone individuals and in terms of the unpredictability of the time at which clinical disease will develop in individual animals.

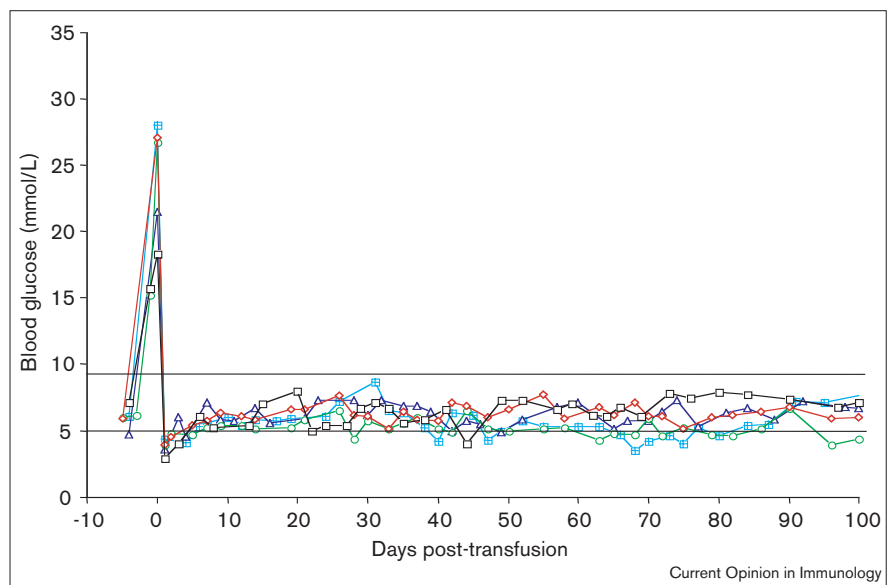
When the pancreas of disease prone animals is examined prior to the precipitation of IDDM, inflammatory cells are present in and around the pancreatic islets. The degree of β cell destruction can be quite variable between individual islets [19]. Some show no evidence of damage whereas others have collapsed into α -cell-containing pseudoislets [19]. Carnaud's laboratory [20] also described the initial autoimmune response to islet tissue to be a multicentric, asynchronous process rather than the simultaneous targeting of all the islet tissue by a systemic immune response. We must conclude therefore that the development of the autoimmune response is somehow regulated by events occurring in the microenvironment of the islet.

To summarize these considerations, it is likely that some immunogenic event develops in individual islets and is responsible for the initiation of the autoimmune response [20]; the stochastic element associated with the autoimmune insulinitis is consistent with the notion that expression of an endogenous virus could activate the autoimmune response. However, such correlations beg the question of causality: is it viral expression that triggers autoimmunity or does the development of autoimmunity activate an endogenous viral particle? The stability of the NOD islet in either a neutral or immunologically competent environment does not support the former contention. This heterogeneity in the autoimmune response to individual islets could reflect systemic changes in the state of autoimmunity. We will return to this question below when we consider the role that immune regulation plays in the breakdown of self-tolerance and the development of autoimmunity.

Although spontaneous models of autoimmune disease, such as the IDDM model just discussed, provide insight at a pathophysiological level, models of induced autoimmune disease provide direct access to the role and relationship of individual autoantigenic epitopes in those disease models.

Figure 1

Stability of NOD–Scid islets in (CBA × CBA) F_1 recipient mice. To determine whether the autoimmune response to NOD islet tissue is triggered by a change in the target islet tissue, NOD–Scid islets (450 islets per animal) were grafted to diabetic (streptozotocin-treated) (CBA × CBA) F_1 recipient animals ($n = 5$) on day 0. Normal islet function (assessed by measurement of blood glucose) was achieved and then maintained in these animals for more than 100 days, indicating that there is no intrinsic defect in the NOD islets that is responsible for the initiation of the autoimmune T cell response.



The rodent model of experimental autoimmune encephalomyelitis (EAE) has been studied extensively in this context. A phenomenon that is generically termed ‘epitope spreading’ has been observed when peptides of either myelin basic protein (MBP) or proteolipid protein (PLP) are used to initiate EAE in rats or mice [21]. Epitope spreading is the diversification of T and B cell responses to a particular antigen or group of antigens over time, where the specificity of determinants that are recognized increases from simple and restricted to complex and more diverse. Epitope spreading may occur either within a single antigen, within different antigens from a single macromolecular complex, or within different antigens that are not physically linked but colocalise to a particular anatomical site. Although the phenomenon of epitope spreading is more difficult to study in humans, numerous observations also suggest a diversification of epitope recognition in human autoimmune disease over time [22].

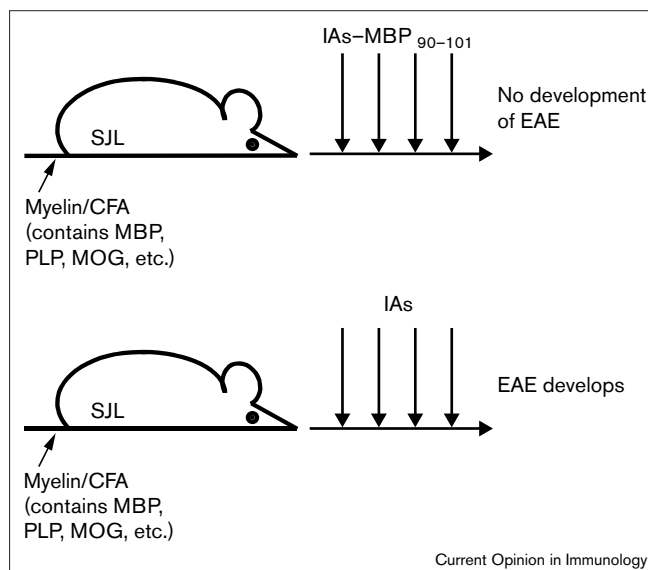
One important issue is whether the determinant spreading involves T cells activated in the peripheral organs or alternatively whether the diversification occurs after infiltration of the initiating T cells into the target organs, for example the central nervous system during EAE. It is possible that the expression of myelin antigens in the thymus and spleen may contribute not only to the induction of tolerance but also to determinant spreading; in this case, the outcome of *in vivo* diversification may ultimately be determined by the balance between Th1 and Th2 responses to antigenic determinants derived from myelin components. The potential contribution of epitope spreading to disease pathology involving cycles of relapsing followed by remitting episodes is of particular interest. In this context, it is possible that each relapse in cycling diseases like multiple sclerosis may reflect autoaggression to new epitopes that are exposed by determinant

spread. Several studies have attempted evaluation of this concept; however, conflicting results have been obtained. For example, several studies in EAE have shown that the progressive recruitment of T cells with new antigen specificity during epitope spreading contributes to the development of relapsing or chronic disease [23,24] whereas other laboratories have found that chronicity and/or relapsing is due to a continuous response focused on the dominant initiating determinant rather than to epitope spreading [25].

The fact that autoreactive T cells are part of the normal repertoire raises important questions as to the extent that determinant spreading depends on tissue damage, whether the T cells are activated in the periphery as a result of increased expression of certain antigens in lymphoid organs, whether the recruited T cells are sufficient to influence the outcome of the disease in a positive or negative way, and ultimately whether they do express a specific Th1, Th2 or Tr1 phenotype. Recent studies have demonstrated that some antigens, previously thought to be sequestered in the central nervous system, are also expressed in the thymus and peripheral lymphoid organs in both humans and mice [26–28]. For example, a structurally related MBP isoform, called golli-MBP, can be expressed in both the thymus and lymph node of susceptible mice and its expression is enhanced in lymph nodes of mice with relapsing EAE [27].

With regard to PLP expression, it has recently been shown that thymic expression of a shorter PLP isoform (DM20) is sufficient to induce tolerance to epitopes contained in this isoform but not to epitopes unique to the full-length PLP, such as the immunodominant PLP_{139–151} sequence [28,29]. Indeed, the fact that SJL mice contain a greatly expanded peripheral repertoire of T cells reactive to PLP_{139–151} appears

Figure 2



A single autoantigen complexed to MHC class II protects mice from EAE induced by multiple autoantigens. Treatment of SJL mice with recombinant, truncated, single-chain murine MHC molecules that are complexed to a peptide (IAs-MBP₉₀₋₁₀₁ complexes) prevents the development of EAE induced by injection of a whole-myelin homogenate (containing a mixture of autoantigens) in CFA (complete Freund's adjuvant). Vertical arrows indicate that recombinant complexes are given by multiple intravenous injections during the initial two weeks after disease induction.

to be due to the abundant thymic expression in this mouse strain of the truncated DM20 isoform of PLP, which lacks the PLP₁₃₉₋₁₅₁ and thereby fails to delete this reactivity [29].

The ability to detect responses to PLP₁₃₉₋₁₅₁ in lymph nodes of naïve SJL mice indicates that these T cells have escaped not only central tolerance but also peripheral tolerance. This suggests that specific MHC molecules are able to present selected epitopes of self-antigens that are not synthesized in the thymus. Furthermore, it implies the persistence and survival of autoreactive, nontolerant cells in the periphery and the existence of regulatory mechanisms that normally keep them 'in check'. Environmental factors, infectious agents, and so on, may shift this equilibrium toward the state of autoimmunity instead of ignorance, enabling the priming of self-reactive cells. It is therefore possible that higher expression of these antigens in the thymus might result in deletion whereas lower expression might enable positive selection of antigen reactive T cells [29-31]. Consequently, even healthy individuals may express a low-level response to autoantigens that can lead to the recruitment of appropriate regulatory T cells that are capable of controlling this response.

Epitope spreading does not compromise antigen-specific therapies in animal models of autoimmune disease

The preceding section on epitope spreading in autoimmune animal models raises speculation regarding the

possible therapeutic implications of this phenomenon, particularly in the context of antigen-specific therapies (see the review by Harrison and Hafler, this issue, pp 704-711). There is ample evidence, however, from this and other laboratories that epitope spreading does not compromise antigen-specific approaches to treatment of autoimmune disease in animal models. These observations question the mechanism(s) underlying these antigen specific therapies and render theories of modulated immunoregulatory events more likely than mechanisms of antigen-specific T cell apoptosis or anergy induction.

We have previously demonstrated that the administration of a complex of soluble native MHC class II noncovalently loaded with autoantigenic peptide renders specific autoreactive T cells functionally unresponsive and prevents the development of EAE in SJL mice [32,33]. Similar results have been reported in the case of autoimmune diabetes, using peptides derived from MHC class II molecule [34,35]. More recently, we have engineered a recombinant, truncated, single-chain, murine MHC-peptide complex (IAs-MBP₉₀₋₁₀₁; S Zhu *et al.*, unpublished data) to generate sufficient quantities of material to study the mechanism of induced unresponsiveness induced by complexes of MHC class II with peptide in rodent autoimmune models. Our data show that treatment with this complex of recombinant MHC class II with MBP₉₀₋₁₀₁ dramatically prevents mortality and paralysis that are induced by injecting SJL mice with an adjuvant-containing formulation of a whole-myelin homogenate containing a considerable mixture of autoantigens, including MBP, PLP, myelin oligodendrocyte glycoprotein (MOG) and so on (see Figure 2). Preliminary analyses have indicated that treated animals fail to exhibit T cell reactivity to PLP as well as the MBP epitope contained in the complex of MHC class II with peptide.

Other examples also indicate that antigen-specific therapies utilizing single epitope treatments are efficacious in complex models that are induced by an array of autoantigens. Recent data from Santamaria's laboratory [36] indicate that a single autoantigenic peptide can significantly reduce the probability that NOD mice will spontaneously develop clinical diabetes in a model that is believed to invoke pathogenic responses to a variety of autoantigens. Such animals are functionally 'tolerant' but show evidence of a nondestructive autoimmune response in their pancreas, an observation that is consistent with an effector mechanism targeting immunoregulatory events rather than deletion of autoreactive T cells [36]. Yet another example of an efficacious antigen-specific therapy in disease that is caused by an alternative autoantigen is the recent report that intranasal administration of human cartilage glycoprotein 39 (HCgp39) antigen protects rodents from collagen-induced arthritis [37]. Although the mechanistic basis for the protection from autoimmunity that is achieved by the administration of single-epitope therapies is not clear at this stage, preclinical studies of this type

establish that epitope spreading is not a barrier to this therapeutic approach and encourage continued clinical validation of this treatment strategy.

Is autoimmunity caused by a primary pathogenic autoantigen or by a breakdown in immune regulation?

Numerous mechanisms of immune tolerance are believed to regulate T cell responses to self antigens. These include the clonal deletion and clonal anergy induction of antigen-specific autoreactive T cells, as well as bystander suppression mediated by regulatory T cells. The latter mechanism(s) is reviewed extensively by Roncarolo and Levings (this issue, pp 676–683). As discussed above, pre-clinical studies demonstrating efficacy of individual autoantigen-based therapeutic approaches in animal models induced by multiple autoantigens are best explained if one considers that these strategies somehow operate by preventing breakdown of immune regulation and/or induction of bystander suppression. Although it is unclear what precipitates the proposed failure of immune regulation in autoimmune disease, one could envision a variety of inducants, including an array of discrete autoantigens liberated as a result of microbial encounter, all being capable of non-specifically altering the balance of immune regulation.

In seeming contrast to these suggestions of a relatively promiscuous role for autoantigens in disease induction, several recent studies have rekindled debate regarding the existence of a primary pathogenic autoantigen in autoimmune disease. Particularly compelling is the report of Yoon *et al.* [38] identifying a glutamic acid decarboxylase (GAD) isoform as the critical autoantigen in type 1 diabetes. In this study, the role of pancreatic GAD expression in the development of IDDM was evaluated by generating transgenic NOD mice that expressed a GAD antisense gene exclusively in β -islet cells such that expression of both GAD isoforms was prevented in these cells but not in the brain. The results showed that in animals that efficiently expressed the antisense transgene there was no β -islet GAD expression and the mice remained free of diabetes. In contrast, mice that poorly expressed the transgene, or that expressed a transgene with irrelevant information, became diabetic. Although interpretation of these data has been heavily debated in the immunological community, the simplest interpretation undoubtedly invokes a critical role for GAD isoforms in the development of IDDM.

Another intriguing study, from Wong *et al.* [39], has refocused attention on insulin as a critical autoantigen in IDDM. In this report, a pancreatic-islet cDNA library was screened to identify the autoantigen target of a highly pathogenic CD8⁺ effector T cell. The identified epitope was nearly identical to the insulin epitope recognized by previously identified pathogenic CD4⁺ T cells. These data would suggest that rodent IDDM can be induced by T cells specific for an insulin epitope, presumably without any requirement for the critical role of GAD that was postulated

by Yoon *et al.* [38]. From a clinical perspective, it may not matter if we identify 'the' critical primary autoantigen involved in disease pathogenesis if antigen-specific therapeutic strategies operate at a level of maintaining or restoring the regulatory mechanisms that govern the process of immune tolerance.

Conclusions

Extensive investigation has revealed the existence of a plethora of autoantigens, both human and mouse, that specifically interact with autoantibodies or autoreactive T cells. Rodent autoantigens or autoreactive lymphocytes can frequently transfer disease in animal models, and in some cases human autoantibodies or their corresponding autoantigens have transferred related pathology to mice.

Dissection of the pathophysiology of murine autoimmunity has identified a phenomenon whereby autoreactivity spreads to multiple autoantigenic epitopes during the course of autoimmune disease. Nevertheless, preclinical data evaluating antigen-specific therapeutic strategies clearly indicate the efficacy of individual-epitope treatments in complex autoimmune models. Integration of these apparently contradictory observations into a single unifying hypothesis is possible if one invokes the mechanisms of immune regulation that govern immune tolerance, and presumably breakdown in autoimmunity, as a central consideration. In this context, we would postulate that antigen-specific therapies for autoimmune disease are able to treat autoimmunity despite epitope spreading because they prevent the breakdown of the regulatory mechanisms governing immune tolerance. How this might be achieved mechanistically awaits experimental analysis, as does clinical validation of antigen-specific therapeutic efficacy.

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