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Efficacy of *HLA-DRB1*03:01* and *H2E* transgenic mouse strains to correlate pathogenic thyroglobulin epitopes for autoimmune thyroiditis

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Abstract

Thyroglobulin (Tg), a homodimer of 660 kD comprising 2748 amino acids, is the largest autoantigen known. The prevalence of autoimmune thyroid disease, including Hashimoto's thyroiditis and Graves' disease, has provided the impetus for identifying pathogenic T cell epitopes from human Tg over two decades. With no known dominant epitopes, the search has long been a challenge for investigators. After identifying *HLA-DRB1*03:01* (HLA-DR3) and $H2E^b$ as susceptibility alleles for Tg-induced experimental autoimmune thyroiditis in transgenic mouse strains, we searched for naturally processed T cell epitopes with MHC class II-binding motif anchors and tested the selected peptides for pathogenicity in these mice. The thyroiditogenicity of one peptide, hTg2079, was confirmed in DR3 transgenic mice and corroborated in clinical studies. In $H2E^b$-expressing transgenic mice, we identified three T cell epitopes from mouse Tg, mTg179, mTg409 and mTg2342, based on homology to epitopes hTg179, hTg410 and hTg2344, respectively, which we and others have found stimulatory or pathogenic in both DR3- and H2E-expressing mice. The high homology among these peptides with shared presentation by DR3, $H2E^b$ and $H2E^k$ molecules led us to examine the binding pocket residues of these class II molecules. Their similar binding characteristics help explain the pathogenic capacity of these T cell epitopes. Our approach of using appropriate human and murine MHC class II transgenic mice, combined with the synthesis and testing of potential pathogenic Tg peptides predicted from

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computational models of MHC-binding motifs, should continue to provide insights into human autoimmune thyroid disease.

Keywords

Autoimmune thyroiditis; HLA-DR3; H2E; transgenic mice; thyroglobulin peptides

1. Introduction

Murine experimental autoimmune thyroiditis (EAT), a T cell-mediated, organ-specific autoimmune disease, manifests many defining characteristics of Hashimoto's thyroiditis (HT). Upon immunization with a self antigen, mouse thyroglobulin (mTg), EAT shows typical mononuclear cell infiltration resulting in thyroid follicular destruction, autoantibody production and T cell proliferative response to mTg. Since the early '70s, EAT has served as a proven prototype for studies in other autoimmune diseases, with respect to major histocompatibility complex (MHC) (*H2*)-linked susceptibility [1], autoreactive T cell activation to syngeneic mTg, unencumbered by adjuvant [2], and regulatory T cell (Treg) control of self tolerance which can be augmented by either exogenous or endogenous mTg, as reviewed recently [3].

After locating the MHC-based susceptibility to *H2A* by using immune response (I)-region gene recombinant mouse strains [4,5], we made use of new advances in MHC *H2* (mouse) [6] and *HLA* (human) [7] class II transgenic technology to demonstrate the feasibility of clarifying the role of MHC class II allelic polymorphism in encoding susceptibility. Indeed, $H2Aa^kAb^k$ transgene from EAT-susceptible $H2^k$ mice rendered resistant strain, B10.M ($H2^f$), susceptible to EAT induction [8]. Similarly, *HLA-DRA/DRB1*03:01* (HLA-DR3) transgene also enabled B10.M mice to develop EAT [9]. Moreover, the use of double transgenic mice made it possible to study the positive and negative influences of gene complementation between *H2A* and *H2E* [8,10,11], as well as *HLA-DR* and *HLA-DQ* [12]. Such studies were facilitated by the targeted mutation of H2Aβ chain [13]; this strategy created an Ab^0 strain to serve as transgene recipient background, thereby obviating pairing with endogenous MHC allele which could hinder data interpretation. By the same token, an *H2Ea* transgene permitted the expression of normally absent H2E molecules for comparative study [14].

Mouse studies have proven invaluable in directing research avenues for human investigations. However, because both EAT-susceptible and -resistant strains, while expressing H2A molecules, do not necessarily co-express H2E molecules, confusion has arisen as to the appropriateness of EAT as a reliable model for HT, particularly relating to pathogenic peptide studies or predictions [15]. However, as both H2A and H2E molecules participate in presenting peptides and shaping the T cell receptor (TCR) repertoire that comprises autoreactive T cells and Tregs, it is essential to dissect the relative contribution of H2E molecules in the presence, as well as absence, of H2A molecules. Such analysis is critical in view of the high homology between H2E and HLA-DR [16] and between mTg and human (h) Tg [17]. This review summarizes studies in the past 10 years testing predicted DR3- and H2E-binding Tg peptides, some of which share sequence homology between hTg and mTg, for pathogenicity in HLA-DR3 and H2E transgenic mice.

2. MHC class II gene control of susceptibility in EAT and HT

2.1. HLA-DR3 as a susceptibility determinant

HLA alleles are frequently associated with genetic predisposition for autoimmunity. In particular, *HLA-DRB1* polymorphisms frequently correlated with HT, though there was

much controversy as to which particular *HLA-DRB1* allele was most associated due to *HLA-DR* and *-DQ* linkage disequilibrium, limitations in HLA typing technology, and the presence of non-MHC genes in the *HLA* complex [9,18]. In brief, we found that HLA-DR3, but not *HLA-DRB1*15:02* (DR2β chain) nor *DRB1*04:01* (DR4β chain) transgenic mice were permissive for both hTg and mTg induction of EAT as illustrated in a recent review [12]. Since both DR2β and DR4β chains required the presence of an *H2Ea* gene (the *HLA-DRA* gene equivalent) for expression, unlike DR3 transgenic mice where a *HLA-DRA* gene fragment was coinjected, we reaffirmed the resistance alleles by introducing each into a recombinant strain [19]. B10.RFB3 mice harbor an EAT resistance A^f allele and an *H2Eb* pseudogene, and could accept the DR2 or DR4 β-chains with $H2Ea^k$ transgene without endogenous H2E protein expression. In addition to using EAT-resistant mice as early transgene recipients, we routinely used Ab^0 mice without H2A expression. The important role of DR3 has been corroborated by genetic analysis in HT patients with or without the co-existence of type 1 diabetes [20]. Interestingly, similar corroborative studies exist in Graves' disease (GD) patients [21] and our murine GD model in which hyperthyroidism and thyroiditis were induced in DR3 transgenic mice after immunization with thyrotropin receptor (hTSHR) DNA [22].

To promote autoimmunity, our murine HT model and many of our Treg and pathogenic Tg epitope studies (see Sect. 3.1) were carried out in Ab^0 mice on an autoimmune-prone, nonobese diabetic (NOD) background. These $DR3^+$ Ab^0/NOD mice developed EAT after immunization with either mTg or hTg, which reaffirmed DR3 as a susceptibility determinant on a different genetic background [23]. We also showed that autoimmune-prone mice depleted of Tregs developed more severe thyroiditis after immunization with hTg only [3], since the strong thyroid pathology after mTg immunization made it difficult to discern possible exacerbation due to Treg depletion [3,23].

Although Ab^0 mice have been successfully used by us and others to study HLA class II transgenic mice for autoimmune susceptibility (as reviewed by Mangalam *et al*. [24]), these mice still possess functional *Aa* and *Eb* genes. To eliminate "potential" contribution of these genes, DR3 transgenic mice were also generated in mice lacking all conventional class II gene expression (AE^0 mice) [25]. Preliminary data show that these $DR3^+$ AE^0/B10 mice developed thyroid infiltration after immunization with hTg or mTg, and Treg depletion resulted in more severe thyroiditis after immunization with mTg only (Fig. 1). The weak thyroid pathology from hTg immunization may reflect the lack of autoimmune-prone NOD background genes which promote EAT pathology.

2.2. $H2E^b$ allele as a susceptibility determinant

Mouse strains either express only H2A class II molecules, or they express H2E in the presence of H2A. Fortuitously, when a monomorphic *H2Ea* transgene was introduced into Ab^0 mice, enabling $H2E^b$ expression, to serve as control for studies on *DRB1* polymorphism (see Sect. 2.1), we uncovered a novel $H2A^-E^+$ model that is strongly susceptible to heterologous (human, porcine, or bovine) Tg-induced, but not mTg-induced, EAT [10]. Moreover, while Treg depletion enhanced hTg-induced thyroid destruction, these mice remained unresponsive to mTg immunization, indicating that Treg influence does not supersede class II-based susceptibility [26,27]. Interestingly, the $H2^b$ haplotype has been categorized as EAT-resistant, based on class II *H2A* genes [4]. However, if *H2E* genes were used as sole classifier, $H2E^b$ mice would be considered susceptible in that they are susceptible to heterologous Tg induction, considered sufficient in studies of other autoimmune diseases. Moreover, since H2E and DR molecules are highly homologous, as with mTg and hTg, and DR molecules are always expressed in humans, studies of H2E-binding Tg peptides have provided invaluable insight into the pathogenic peptides for EAT and HT (see Sect. 4).

2.3. Class II transgenes as a tool to examine gene interactions

Whereas single *HLA* class II transgenic mice can pinpoint susceptibility alleles in autoimmune thyroiditis, co-existing class II genes, unavoidable in polygenic humans and some mouse strains, also participate in shaping the TCR repertoire and influencing disease manifestations. In double transgenic mice, it is possible to examine combinations of at least two class II alleles. We have observed that *HLA-DQA1*03:01/DQB1*03:02* (HLA-DQ8) transgene alone is permissive for mild hTg-induced thyroiditis, but ameliorates DR3-mediated severity in DR3/DQ8 double transgenic Ab^0/NOD mice [28]. In contrast, the $DQ8^+$ mice are resistant to mTg-induced EAT, and yet its co-expression with DR3 transgene has no effect on thyroiditis severity. mTg is known to harbor more immunogenic T cell epitopes than heterologous Tg and usually induces more extensive thyroid destruction [29]. With collagen-induced arthritis (CIA), *DRB1*04:02* expression also inhibited DQ8-mediated disease [30].

The availability of *H2Ea* transgenic mice has been invaluable in studies of mutual influences between *H2A* and *H2E* genes. In autoimmune diseases, the expression of H2E molecules has led to reduced severity of type 1 diabetes in NOD mice [31], of autoimmune thyroiditis in EAT-susceptible B10.S mice [8] and of myasthenia gravis in B10 mice [32]. Using our novel model wherein only $H2E^b$ molecules are expressed and mediate only heterologous Tg-induced EAT, co-expression of H2A molecules in E^+ B10 ($H2A^+E^+$) mice also reduced thyroiditis severity [10]. Using hTg to represent heterologous Tg, we compared hTg and mTg induction of EAT on EAT-resistant B10 ($H2^b$) background, with or without H2E molecule expression and with or without Treg depletion [26,27]. In all three strain combinations, A^+E^-, A^-E^+, and A^+E^+, Treg depletion increased thyroiditis incidence and severity without changing class II restriction; A^+E^- mice responded only to mTg immunization, A^-E^+ mice to hTg immunization, and A^+E^+ mice to both hTg and mTg induction. Surprisingly, A^+E^+ mice are susceptible to both Tgs without the need for Treg depletion. While the response to hTg was expected due to the presence of E^b molecules, the response to mTg without Treg depletion was unexpected, because A^b is normally a resistance allele.

To determine how $H2E^b$ molecule expression could enable $H2A^b$ molecules to present mTg peptides, we hypothesized that $H2E^b$-derived peptides may block mTg peptide presentation by interfering with clonal deletion of autoreactive T cells during T cell ontogeny, enabling their escape to the periphery. The possibility of such interactions has been reported. For example, a DR4-derived peptide has been shown to be presented by DQ8 molecules in DR4/DQ8 double transgenic mice, thereby suppressing DQ8-mediated CIA induction [30]. The finding of an H2E-derived 17-mer peptide (Eα52-68) that binds to ~15% of A^b molecules with high affinity [33] was particularly germane to study in this strain. Accordingly, seven putative A^b-binding Tg peptides, together with Eα52-68, were synthesized and tested [11]. Fig. 2A shows the presence of Eα52-68/A^b complex in our mouse strain. Eα52-68 competitively reduced the proliferative response of mTg- and two 16-mer mTg peptide-primed lymph node cells from A^+E^- mice in vitro, designated mTg1677 and mTg2342. Fig. 2B shows that the proliferative response to mTg1677 was competitively blocked by Eα52-68. Such blocking by H2E-derived peptides of the peptide-binding cleft of many H2A molecules, enabling autoreactive T cells to enter the periphery without the supervision of peptide-specific Tregs, could explain the pathogenicity of mTg or particular peptides. Since the Eα52-68 sequence is conserved in humans and mice, this peptide (or other conserved self-MHC peptides) could contribute to the editing of the TCR repertoire, both autoreactive and regulatory, thereby affecting susceptibility or resistance in autoimmune diseases. Only by the use of single and double class II transgenic mice could these mechanistic studies be realized.

3. Prediction and characterization of pathogenic peptides

Systematic experimental screening of MHC ligands and T cell epitopes is impractical because of the large number of peptide sequences as well as the diversity of MHC molecules. Combined with laboratory experimentation, computational models improve the cost and efficiency of identifying T cell epitopes [34,35]. Computational methods have proven useful in identification of T cell epitopes on autoantigens in both human [36–38] and animal models [39,40]. Similar approaches have been used to identify T cell epitopes and validate them in *HLA* transgenic mice. Examples include identification of HLA-A*02:01 self-epitopes in proinsulin [41], and HLA-DRB1*03:01 in Tg (Sect. 3.1) [42].

3.1. Studies in HLA-DR3 transgenic mice

3.1.1. Predicting putative DR3-binding peptides—Autoantibodies targeting Tg are markers of autoimmune thyroiditis in humans, but can also be found in healthy subjects. Studies in susceptible mouse strains have shown that immunization with hTg- or mTg-derived T cell epitopes can induce autoimmune thyroiditis in susceptible mice (as reviewed in [43]). Characterization of these peptides and their specific interactions are necessary for understanding and deciphering mechanisms of autoimmune pathogenesis in EAT-susceptible mouse strains, and they serve as model organisms for study of autoimmune thyroiditis in humans. Tg is the largest autoantigen known, longer than 2700 amino acids and systematic experimental screening would be prohibitively expensive. We used computational methods of prediction for identifying candidate HLA-DR3-binding, thyroiditogenic T cell epitopes followed by experimental validation to demonstrate the utility of this approach. The process involved collection of putative DR3-binding peptides, alignment of these peptides using binding motif anchors, training of an artificial neural network, and finally prediction of peptides from hTg for HLA-DR3 binding. The HLA-DR3 binding motif has the form [LIFMV]-X-X-D-X-[KREQN]-X-X-[YLF] [44]; the anchor residues are indicated in bold in Table 1. The prediction method was described in detail in [36]. The 50 predicted peptides were synthesized and tested in HLA-DR3 transgenic mice for their thyroiditogenic capacity [42].

3.1.2. Testing HLA-DR-binding peptides for pathogenicity—To identify potential T cell epitopes responsible for stimulating pathogenic T cells, we first immunized $DR3^+$ Ab^0 mice with hTg before screening for the in vitro proliferative response of spleen cells to hTg-derived 15–23-mer peptides [42]. We reasoned that, by screening cells from native hTg-immunized mice, the stimulatory peptides were more likely to represent epitopes naturally processed by antigen-presenting cells (APC), in contrast to “cryptic” epitopes, which might be stimulatory after direct immunization with the peptide, but not stimulatory for hTg-immunized cells [45]. Of the 39 hTg peptides, 8 were stimulatory for hTg-primed cells based on IFN-γ secretion. Of these 8, 4 (hTg181, 418, 1518 and 2079) consistently stimulated and expanded hTg-primed cells (Table 1). The most immunogenic was hTg2079, which consistently expanded sufficient numbers of hTg-primed cells for adoptive transfer of thyroiditis to naïve mice. Direct immunization with hTg2079 led to thyroid destruction in 71% of mice, and in vitro hTg2079-activated cells from hTg2079-primed mice transferred thyroiditis [42].

The four peptides mentioned above were stimulatory for $DR3^+$ hTg-primed cells on either the B10 or NOD background, but not for $DQ8^+$ mice, supporting the DR3 restriction of these predicted peptides [42]. However, the co-expression of DQ8, which is moderately permissive for hTg-induced EAT and reduces thyroiditis in hTg-immunized $DR3^+DQ8^+$ mice [28], led to a decreased proliferative response to DR3 peptides (Fig. 3A). This may be

one of the several potential mechanisms for DQ8 modulation of hTg-induced thyroiditis in DR3/DQ8 double transgenic mice.

We also tested $DR3^+$ mice in the absence of any conventional H2 class II molecules ($DR3^+$ AE^0 mice, see Sect. 2.1). Consistent with the proliferation data from $DR3^+$ Ab^0/B10 and Ab^0/NOD mice [42], hTg-primed $DR3^+$ AE^0 mice responded the best to hTg2079 (Fig. 3B). In line with a lack of discernible Treg contribution in hTg-induced thyroiditis (see Fig. 2), Treg depletion prior to hTg immunization neither 1) increased the proliferative response to any of the peptides, nor 2) resulted in the identification of more stimulatory peptides (data not shown).

The weak pathology seen with hTg-immunization, with or without Treg depletion (see Fig. 1), suggested that the ability to induce EAT with hTg peptides would be difficult to achieve, especially when the DR3 transgene is on the AE^0 background. Given the moderate severity of mTg-induced EAT in these mice without Treg depletion, we examined the mTg sequences of some of our stimulatory hTg peptides identified earlier. We chose mTg179 because: 1) hTg179 stimulated hTg-primed cells from $DR3^+$ AE^0 mice (Fig. 3B); 2) hTg181, which shared the same DR3-binding motif as hTg179, had proven weakly pathogenic in $DR3^+$ Ab^0/NOD mice (see above); and 3) mTg179 was thyroiditogenic in A^-E^+ Ab^0/B10 mice (Sect. 3.2.2) [46]. As shown in a preliminary experiment (Fig. 4), immunization of $DR3^+$ AE^0 mice with mTg179 was thyroiditogenic in 4/6 mice. Furthermore, both the severity and incidence were increased after Treg depletion, indicating that these mice may be useful for screening pathogenic Tg epitopes.

3.2. Studies in $H2E^b$ transgenic mice

3.2.1. Predicting putative $H2E^b$-binding peptides—An accurate model based on advanced techonologies, such as quantitative matrices, neural networks, or support vector machines, was not available. The alternative method of predicting $H2E^b$-binding peptides was through identifying the presence of binding motifs. The $H2E^b$-binding motif has the form [WFYILV]-x-x-[LIFSA]-x-[QNASTHRE]-x-x-[KR] [44]. The 21 peptides predicted as potential $H2E^b$ binders, which include the presence of three or four anchor residues, were tested for pathogenicity in transgenic mouse models.

3.2.2. Testing $H2E^b$-binding peptides for pathogenicity—The *$H2E^b$* ($H2A^-E^+$) transgenic model that ostensibly distinguishes self from nonself by mediating susceptibility to heterologous Tg, but not mTg, enabled us to first search for pathogenic hTg-derived peptides, using the computer-generated, putative H2E-binding peptides [47,48]. hTg410 and hTg2344, listed in Table 1, appeared to be naturally processed peptides in that they stimulated hTg-primed T cells and activated such cells for adoptive transfer of thyroiditis to naive recipient mice. The thyroiditogenic peptides also generated cytotoxic T cells that mediated killing of target cells loaded with intact hTg or hTg-derived peptides either from hTg-primed or peptide-primed cells [3]. Next, we examined what mTg epitopes were being recognized by hTg- or peptide-primed cells to mediate thyroid destruction in the mouse thyroid. Using three hTg peptides as a guide, we synthesized corresponding peptides on mTg. As listed in Table 1, mTg179 and mTg409 each contained 4/4 MHC-binding residues, equivalent to corresponding hTg179 and hTg410. Both mTg peptides induced thyroiditis in A^-E^+ mice [46], and the peptide-primed T cells mutually cross-reacted to peptide pairs. Interestingly, as described earlier (Sect. 3.1.2), hTg179/hTg181 and hTg418 were also stimulatory in hTg-primed $DR3^+$ mice (Fig. 3). Moreover, mTg179 induced thyroiditis with CFA as adjuvant, and Treg depletion further exacerbated thyroiditis incidence and severity in these mice (Fig. 4.).

4. EAT as a model for HT is reaffirmed by comparing HLA-DR and H2E class II presentation of pathogenic Tg peptides

EAT exhibits several defining features of HT: mononuclear cell infiltration leading to thyroid destruction, autoantibody production, and T cell proliferation to Tg. Moreover, the use of HLA-DR3 and $H2E^b$ transgenic mice enabled the definitive demonstration of each as a susceptibility determinant for HT [9] and GD [22], and for hTg-induced EAT [10,47], respectively (Sect. 2). The role of DR3 as a susceptibility allele has also been corroborated in HT and GD [49]. We further identified several naturally processed, hTg- and mTg-derived peptides in DR3 and $H2E^b$ single transgenic mice without the complicating presence of other class II genes, and characterized a select few for thyroiditogenic activity (Sect. 3). We observed similar amino acid sequences presented by DR3 and $H2E^b$ molecules, and interestingly also by $H2E^k$ molecules (mTg179 and hTg2340) (Table 2), which are co-expressed with $H2A^k$ from an EAT-susceptible strain [1,50]. In view of the high homology between *HLA-DRA/DRB1* and *H2Ea/Eb* genes, encoding similar MHC-binding motifs by certain *H2E* alleles is not surprising. Moreover, when these $H2E^b$ and $H2E^k$ alleles are aligned with the DR3 allele, as reported by Menconi *et al*. [15], the similarities of the binding grooves are clearly evident, especially in contrast to the $H2E^d$ allele, co-expressed with $H2A^d$ from an EAT-resistant strain [1] (Table 3). These findings indicate that studies of pathogenic peptides from either hTg or mTg, as predicted from MHC-binding motifs in the mouse, could have relevance to human thyroiditis, provided that thyroiditogenic testing in appropriate *H2Eb* allelic strains was carried out.

In contrast, a different conclusion was reached by Menconi *et al*., using *H2E* gene sequencing data from EAT-susceptible and -resistant strains for MHC-binding pocket analysis [15]. They followed the successful strategy of sequencing *HLA-DRB1* genes of 94 HT patients and 153 control subjects, which led to the identification of six positions (see Table 3) where amino acid differences were significantly associated with either the HT or control group. Unfortunately, some of the premises for segregating susceptible vs. resistant alleles for the various mouse strains were not in accordance with known mouse immunogenetics, resulting in inappropriate interpretations. Some of these are: 1) Segregation of susceptible and resistant strains, which the authors utilized, has been shown to be based on *H2A* genes [4,5] and not *H2E* genes, whereas *H2E* genes and *HLA-DR* genes are better correlated as we discussed above; 2) 15 of the 22 strains listed should not be considered independent, as they actually belong to only two haplotypes (10 $H2^k$, 5 $H2^b$), and thus they share the same two class II *H2Eb* genes. A few haplotypes were mislabeled and therefore misgrouped. For example, there were no actual $H2^a$ haplotype strains, which would have been susceptible; instead, one was PL/J ($H2^u$), which is EAT-resistant [51], and the other, DBA/1 ($H2^q$), is EAT-susceptible [1]; and 3) Many strains do not express natural H2E molecules due to an *H2Ea* gene defect, and their *H2Eb* genes have no role in antigen-presentation or shaping of the TCR repertoire. More importantly, as this review has discussed, some H2E molecules, when expressed, promote autoimmune thyroiditis by interfering with clonal deletion of autoreactive T cells, while others inhibit thyroiditis by a yet-to-be defined mechanism (Sect. 2.3). The use of H2A KO mice with independent expression of H2E molecules, coupled with pathogenic peptide analysis, provides a strategy to increase our understanding of the genetic regulation of pathogenic mechanisms.

Already, the use of HLA-DR3 transgenic mice has led to the identity of hTg2079 with pathogenic potential [42], which was confirmed by others [52]. Moreover, the validity of this peptide was corroborated by data from the clinic. Cells from patients with autoimmune thyroid disease proliferated in vitro to hTg2079 [52]. When natural, HLA-DR-associated peptides from Graves' disease-affected tissues were characterized by mass spectrometry, 7 peptides were derived from Tg [53], one of which was hTg2079 with the same length and

exact sequence. Thus, as we have shown in *HLA-DRA/DRB1*03:01* transgenic mice, this peptide was observed to bind to DR3. Clearly, in both mice and patients, a naturally processed peptide can be characterized with screening based on MHC-binding motifs. Taken together, these points demonstrate the high importance of studying the appropriate MHC molecule.

5. Perspectives

Important inroads from the past several decades have improved our understanding of *HLA* associations with autoimmune diseases, especially once the mechanism of T cell recognition of MHC-presented peptides was discovered. For more than 15 years, MHC class II transgenic technology has been an invaluable tool. This is particularly important since manipulation of *HLA* genes cannot be performed in humans. In addition, the *HLA-DR* and -*DQ* linkage disequilibrium complicates the dissection of individual allelic contributions. Introduction of transgenes into mouse strains is an important approach to single out specific class II genes for disease association. The insertion of additional, specific class II genes provides a strategy to study possible gene interactions, which may protect or exacerbate autoimmune conditions. Moreover, since we have demonstrated that Treg control does not supersede MHC class II restriction for EAT [27], the prediction and characterization of pathogenic peptides can be approached by utilizing MHC-binding motifs, as we have reported for HLA-DR3 and H2E class II molecules [42,46]. However, all these studies would not have been possible without the collaborative efforts from those with in-depth knowledge of mouse immunogenetics to ensure that proper control mice were included to validate the findings. To put this in perspective, the novel $H2A^{-}E^{+}$ transgenic model on the *b* haplotype background, which is susceptible to EAT induction by heterologous hTg, but not mTg, was uncovered only when $H2E^{b}$ molecules were expressed in the absence of $H2A^{b}$ molecules ($E^{+}Ab^{0}$ mice) to provide control mice for our studies on *HLA-DRB1* polymorphism. The definitive contribution of *HLA-DRB1*03:01* to EAT susceptibility was observed as a transgene, and the finding helped alleviate the controversy that then-existed with patient studies for HT association [9]. $DR3^{+}$ mice also developed GD-like syndrome when immunized with hTSHR DNA [22]. A number of corroborating studies have been reported for humans [20,49,53].

In this review, we have summarized parallel studies of pathogenic hTg and mTg peptides selected according to MHC-binding pockets of HLA-DR3 and $H2E^{b}$ molecules (Table 1). The nearly identical pathogenic peptides presented by HLA-DR3 and $H2E^{b}$, as well as $H2E^{k}$, molecules (Table 2) led us to compare the binding motifs among the three. Indeed, the three susceptibility alleles encode remarkably similar binding pocket residues on their β-chains, in contrast to the $H2E^{d}$ allele from an EAT-resistant haplotype (Table 3). Such studies have provided the impetus for developing more accurate HLA-binding algorithms, enabling faster and more precise identification of targets of autoimmunity. Several servers are now available for predicting MHC class I and class II epitopes [54–57]. These resources will enable the transition from slower identification of individual targets to rapid high-throughput mapping of complete target sets. However, these target sets will still need to be tested in animals. Thus, our approach of using appropriate human and murine MHC class II transgenic mouse models, combined with the synthesis and testing of potential pathogenic Tg peptides predicted from computational models of MHC-binding motifs, will continue to provide critical insights into human autoimmune thyroid disease.

This paper is part of a special series that honors a senior contributor to the autoimmunity arena, Chella S. David. We take this opportunity to acknowledge with special gratitude our long-term, continuous collaboration over a span of 32 years with the first joint publication in 1979 [5], that continues without interruption to the present. The editorial board of the

Journal of Autoimmunity/Autoimmunity Reviews publishes special issues once or twice a year to honor either great figures in autoimmunity and/or special topics. In the past we have honored Professor Ian Mackay, Susumu Ikehara, Noel Rose and Harry Moutsopoulos, and have attempted to include within those issues topics of particular interest to autoimmunologists [62–66]. This issue and the paper herein reflect the journal's attempt to provide contemporary data on cutting edge issues that relate to the treatment as well as the geoepidemiology of autoimunity [67–77]. Finally, the editors thank Chella David for this opportunity and for his warmth and generosity to researchers throughout the world.

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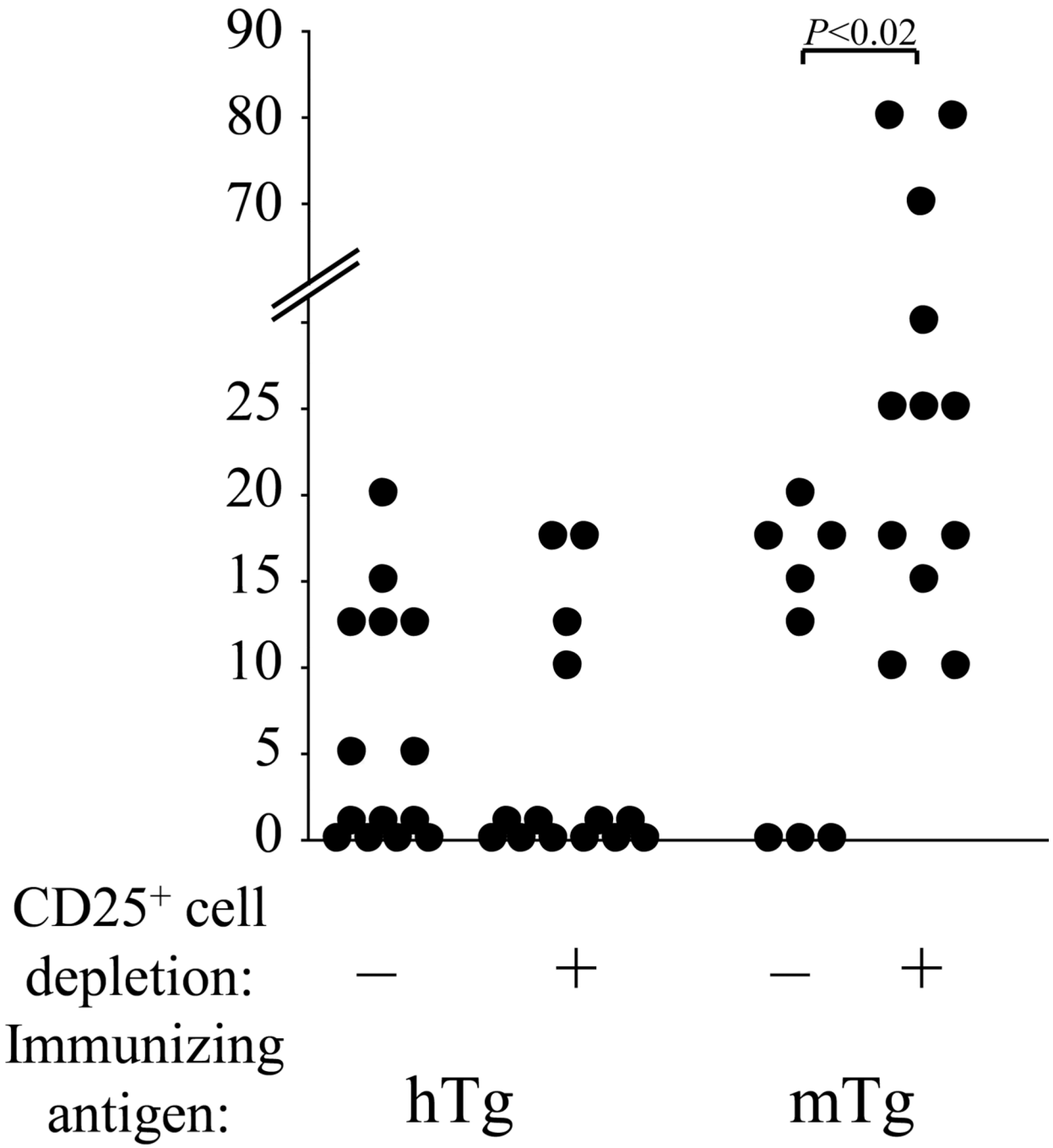

Fig. 1.

Depletion of $CD4^+CD25^+$ regulatory T cells exacerbates mTg-induced, but not hTg-induced, thyroiditis. $DR3^+$ AE^0 mice were given 1 mg anti-CD25 i.v. on days −14, −10, immunized i.v. with 40 μg mTg or 100 μg hTg + 20 μg LPS on days 0, 7 and sacrificed on day 28 or 35.

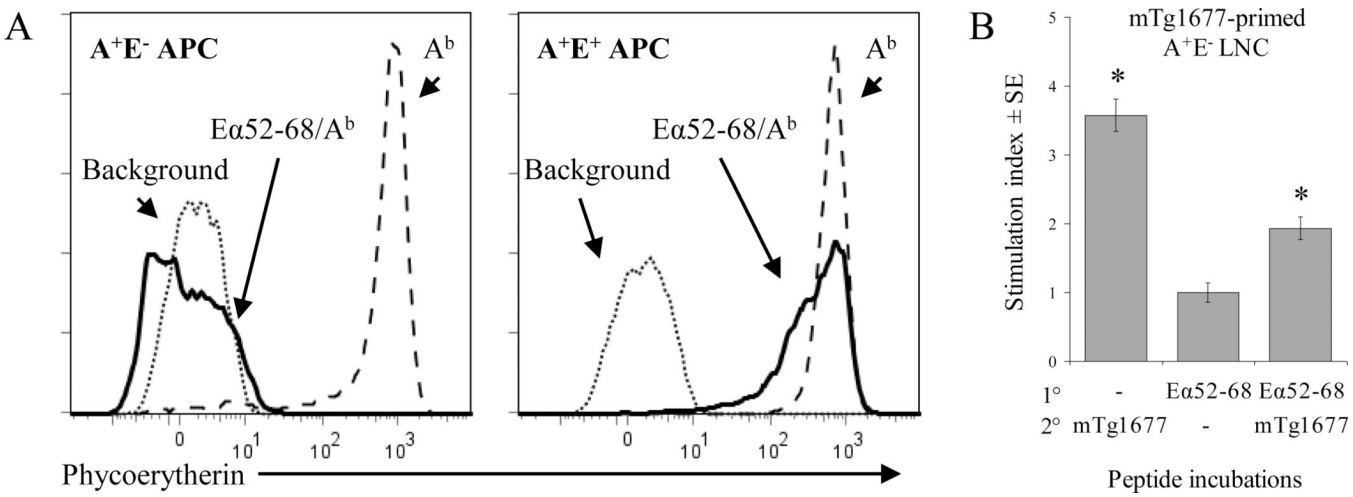

Fig. 2.

H2E MHC class II-derived peptide Eα52-68 blocks presentation of an mTg peptide by H2A^b. (*A*) B220-gated APC from A$^+$E$^-$ (left) or A$^+$E$^+$ (right) spleen cells were FACS analyzed for expression of Eα52-68/A^b (mAb Y-Ae, thick line), or total A^b (mAb Y-3P, dotted line) as a control. The dashed line shows background fluorescence. (*B*) A$^+$E$^-$ mice were immunized with peptide mTg1677 in CFA and lymph nodes were removed on day 10. On day of culture, irradiated spleen cells were incubated for 1 h with or without Eα52-68 (1° incubation, 10 μg/ml) and washed. The spleen cells were subsequently incubated for another hour with mTg1677 (2° incubation, 10 μg/ml) and washed. These pulsed APC were then added to cultures (4×10^5/well) along with mTg1677-primed LNC from immunized mice (6×10^5/well) for 5 days. Proliferation was assessed by [^{3}H]thymidine incorporation (background mean±SE cpm: 1200±160). *, $P<0.01$ (Modified and reproduced by permission from Brown et al. [11]. ***Copyright 2008. The American Association of Immunologists, Inc.***).

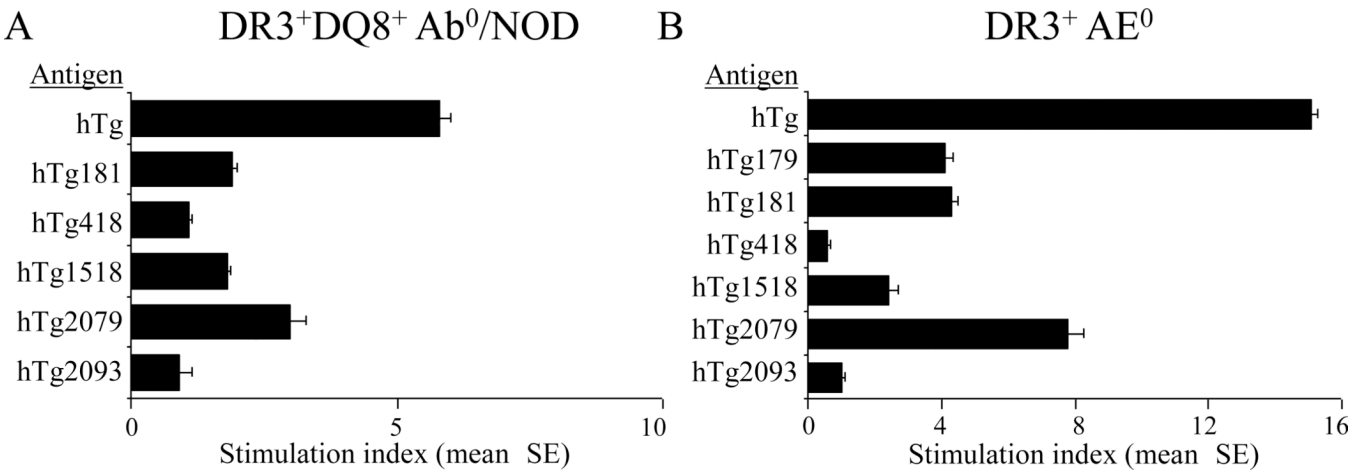

Fig. 3.

Proliferation of hTg-primed mouse spleen cells to hTg-derived DR3 peptides. $DR3^{+}DQ8^{+}$ Ab^{0}/NOD (A) or $DR3^{+}$ AE^{0} (B) mice were immunized with 100 μg hTg followed 3 hours later by 20 μg LPS (days 0, 7). When mice were sacrificed 4–5 weeks later, spleen cells (6×10^{5}/well) were cultured for 4 days with hTg (40 μg/ml) or hTg peptides (5 μg/ml). Proliferation was assessed by [^{3}H]thymidine incorporation (background mean±SE cpm: A, 7280±400; B, 13320±4120).

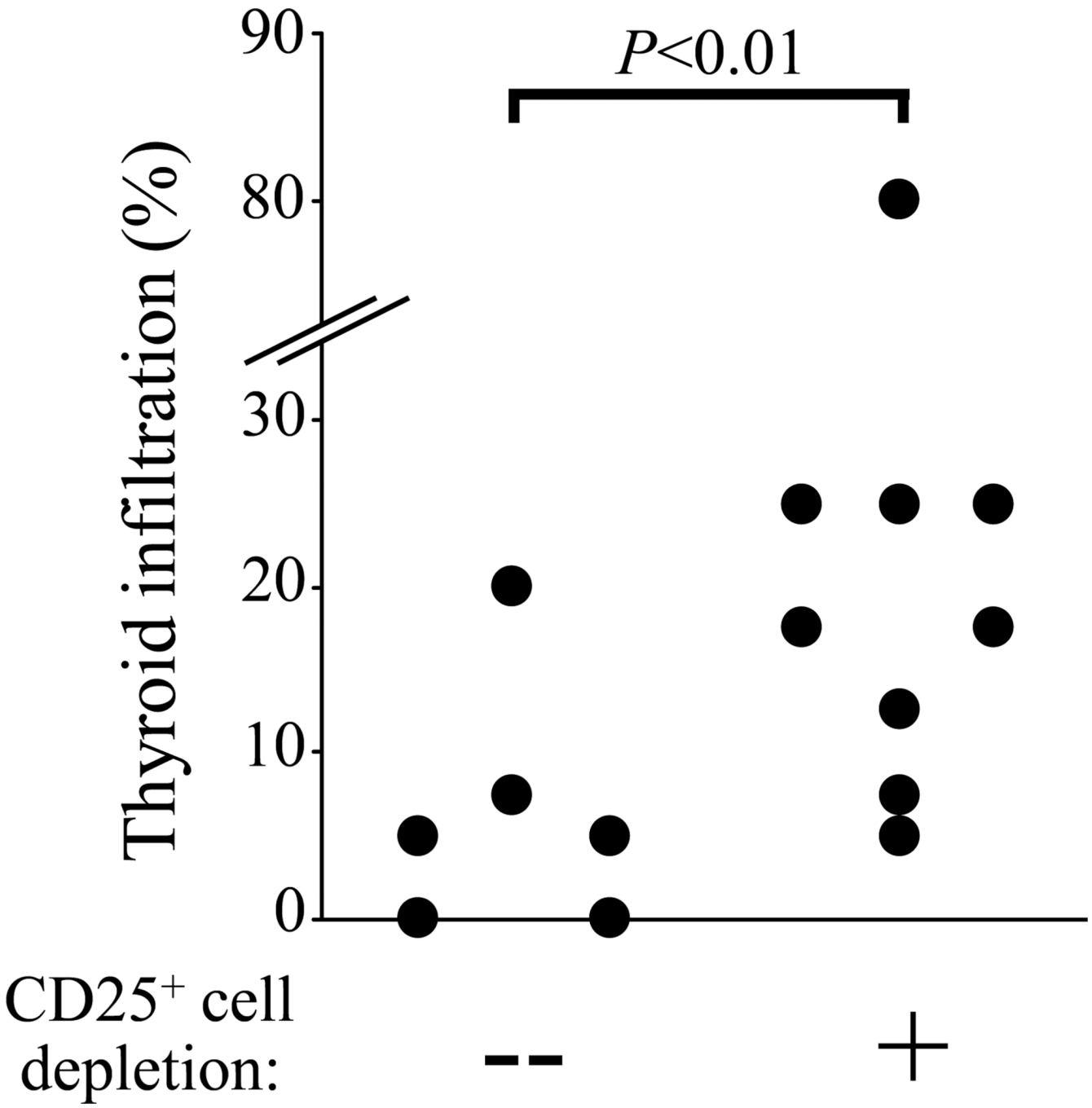

Fig. 4.

mTg179 is pathogenic for $DR3^{+}$ AE^{0} mice and causes a higher incidence and more severe thyroid destruction after $CD4^{+}CD25^{+}$ regulatory T cell depletion. Mice were given 0.5 mg anti-CD25 i.v. on days −14, −10, immunized i.v. with 100 μg mTg179 in CFA on days 0, 7 and sacrificed on day 29.

Table 1

Amino acid sequence and binding motif of antigenic, synthetic Tg peptides presented by MHC class II HLA-DR and H2E molecules

MHC Class II molecule	Name[a]	Peptide length	Amino acid sequence[b]
DR3	hTg179	19	NTTDM**M**IF**D**L**V**HS**Y**NRFPD
	mTg179	19	NTTDM**M**IF**D**L**I**HN**Y**NRFPD
	hTg181	15	TDM**M**IF**D**L**V**HS**Y**NRF
	hTg418	15	EN**L**LK**E**A**I**RA**I**FPSR
	hTg1518	15	GSGKA**F**CV**D**G**E**GR**R**L
	hTg2079	15	LSS**V**VV**D**P**S**IR**H**FDV
H2E[b]	hTg179	19	NTTDMMI**F**DL**V**H**S**YN**R**FPD
	mTg179	19	NTTDMMI**F**DL**I**H**N**YN**R**FPD
	hTg410	15	QSQQ**F**SV**S**E**N**LL**K**EA
	mTg409	15	HYQR**L**SE**S**R**S**LL**R**EA
	hTg1241	15	GP**L**IC**S**L**E**SG**R**WESQ
	hTg2344	16	**L**TW**V**Q**T**HI**R**GFGGDPR

[a] hTg/mTg indicates peptide derivation and number indicates position of first amino acid in Tg sequence.

[b] Bolded/underlined amino acid indicates predicted MHC class II anchor residue.

Table 2

Amino acid sequences of Tg peptides presented by both MHC class II HLA-DR and H2E molecules

Species	Residues	Amino Acid Sequence	Class II presentation
hTg	181-195	TDMMIFDLVHSYNRF	DR3[a] [42]
hTg	179-197	NTTDMMIFDLVHSYNRFPD	DR3[a], H2E[b] [3]
mTg	179-197	NTTDMMIFDLIHNYNRFPD	DR3[a], H2E[b] [3,46]
mTg	179-194	NTTDMMIFDLIHNYNR	H2E[k] [58,59]
hTg	410-424	QSQQFSVSENLLKEA	H2E[b] [3,46,48]
mTg	409-423	HYQRLSESRSLLREA	H2E[b] [3,46]
hTg	418-432	ENLLKEAIRAIFPSR	DR3[a] [42]
hTg	2340-2359	QVAALTWVQTHIRGFGGDPR	DR3 [60], H2E[k] [61]
hTg	2344-2359	LTWVQTHIRGFGGDPR	H2E[b] [3,46,48]

[a]This review

Table 3

Comparison of amino acid residues of select HLA-DRβ and H2Eβ chains[a]

DR/H2E haplotype	26[b]	30	47	70	71	74
HLA-DR3[c]	Y[d]	Y	F	Q	K	R
H2E^b	L	Y	F	Q	K	E
H2E^k	L	Y	F	Q	K	E
H2E^d	F	F	Y	D	A	S

[a] The amino acids at these positions of the HLA-DRβ protein were found to be significantly associated with either HT patients or controls by Menconi *et al*. [15]

[b] Amino acid position of the mature HLA-DRβ/H2Eβ chain.

[c] Haplotype of the MHC protein. HLA-DR3 refers to the *DRB1*03:01* allele.

[d] Identity of amino acid at given position.