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Suppressive Effect of Glutamic Acid Decarboxylase 65-Specific Autoimmune B Lymphocytes on Processing of T Cell Determinants Located Within the Antibody Epitope

Juan Carlos Jaume,*† Sarah Louise Parry,§ Anne-Marie Madec,¶ Grete Sønderstrup,§ and Steinunn Baekkeskov2*‡

Type 1 diabetes is a T cell-mediated disease in which B cells serve critical Ag-presenting functions. In >95% of type 1 diabetic patients the B cell response to the glutamic acid decarboxylase 65 (GAD65) autoantigen is exclusively directed at conformational epitopes residing on the surface of the native molecule. We have examined how the epitope specificity of Ag-presenting autoimmune B cell lines, derived from a type 1 diabetic patient, affects the repertoire of peptides presented to DRB1*0401-restricted T cell hybridomas. The general effect of GAD65-specific B cells was to enhance Ag capture and therefore Ag presentation. The enhancing effect was, however, restricted to T cell determinants located outside the B cell epitope region, because processing/presentation of T cell epitopes located within the autoimmune B cell epitope were suppressed in a dominant fashion. A similar effect was observed when soluble Abs formed immune complexes with GAD65 before uptake and processing by splenocytes. Thus, GAD65-specific B cells and the Abs they secrete appear to modulate the autoimmune T cell repertoire by down-regulating T cell epitopes in an immunodominant area while boosting epitopes in distant or cryptic regions. The Journal of Immunology, 2002, 169: 665–672.
study using GAD65 autoantibody-positive sera from type 1 diabetic patients found an enhancement of presentation of a T cell determinant residing in aa 274–286 when GAD65 immune complexes rather than Ag alone were fed to peripheral blood APCs (18). The epitope specificity of a soluble Ab that binds to an Ag before uptake via the FcR can also affect the processing and presentation of T cell epitopes and either suppress or boost a particular epitope (15, 16). While boosting effects may be difficult to separate from the general enhancement of uptake of immune complexes compared with free Ag, relative suppressing effects are more easily demonstrated. For instance, a mouse mAb to thyroglobulin was found to exert a relative suppression on the presentation of a nondominant epitope in this molecule (19). Thus, Ab specificities are likely to modulate the T cell epitopes made available by all three APCs: macrophages, dendritic cells, and B lymphocytes.

Recently, we have mapped in detail the GAD65-specific epitopes recognized by human mAbs derived from type 1 diabetic patients and shown that almost the entire surface of native folded GAD65 is targeted by human autoimmune B cells (20). In this study we assess how the epitope specificities of four corresponding EBV-transformed DRB1*0401-positive B cell lines affect immunodominant T cell epitopes generated from regions within and outside the B cell epitope region. Furthermore, we have studied how soluble Abs from the same B cell lines affect T cell epitopes presented by DRB1*0401-positive splenocytes.

Materials and Methods

**EBV-B cell lines and Abs**

The derivation of EBV-transformed, GAD65-specific, monoclonal B cell lines DPA, DPB, DPC, and DPD from an HLA-DRB1*0401-positive type 1 diabetic patient was described previously (21). The B cell lines have a stable production of IgG1 in tissue culture (22), and the epitopes have been mapped using homologous scanning mutagenesis, taking advantage of the lack of reactivity of the human mAbs with the highly homologous isoform GAD65, determined by surface plasmon resonance on BIAcore, is 0.11 nM for DPA, 9.45 nM for DPB, 0.31 nM for DPC, and 2.79 nM for DPD (A.-M. Madec, unpublished observations). The B cell lines were grown in RPMI 1640 medium (Life Technologies/BRL, Gaithersburg, MD) containing 10% heat-inactivated FCS, 2 mM l-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 50 µM 2-ME (tissue culture medium). Abs were purified from culture supernatants by G4B fast flow (Sigma-Aldrich, St. Louis, MO) affinity chromatography. The eluate was dialyzed against PBS. The human mAb concentration was determined using an IgG ELISA kit (Roche, Mannheim, Germany).

**T cell hybridomas**

The derivation of HLA-DRB1*0401-restricted, GAD65-specific T cell hybridomas from mice expressing HLA-DRB1*0401, human CD4 as transgenes on the I-Ab + background was described previously (22). The hybridomas 91, 103, and 81 recognizing aa 271–285, 481–495, and 556–570 in GAD65, respectively, were used in this study. A fourth, HLA-DRB1*0401-restricted, GAD65-specific T cell hybridoma, recognizing aa 115–130 (23), was donated by Dr. L. Wicker (Sharp and Dohme Research Laboratories, Merck, Rahway, NJ). T cell hybridomas were maintained in tissue culture medium (see above).

**GAD65 protein**

Human GAD65, expressed in *Saccharomyces cerevisiae* and purified as described (24), was donated by Drs. M. Powell, B. Rees-Smith, and J. Furmaniak (FIRS Laboratories, Cardiff, U.K.). The purified protein was conformationally stable and was recognized by a series of human mAbs recognizing conformational epitopes in GAD65, including the mAbs used in this study (results not shown).

**Ag presentation assays**

**B cells as APCs.** EBV-transformed, GAD65-specific B cell lines were incubated with graded amounts of Ag for 1 h on ice and then washed twice in tissue culture medium to remove unbound Ag. Ag-loaded B cells (3 × 10^6) were added to each well of a round-bottom 96-well plate containing DPA, DPB, DPD, DPD, and DPC, specific T cell hybridomas in a final volume of 150 µl tissue culture medium. The cells were incubated for 24 h (37°C, 4% CO_2_). The HLA-DRB1*0401-positive human EBV-transformed B cell line, Priess (25), which does not express a GAD65-specific IgG, was used as a negative control. IL-2 secretion was measured using a sandwich immunoassay and a streptavidin-europium detection system (26). Experiments were repeated a minimum of three times in triplicate wells.

**Splenocytes as APCs.** Preformed immune complexes were generated by incubating 1.5 µg/ml purified human mAbs and graded amounts of Ag (0.03–3.0 µg/ml) in a round-bottom 96-well plate for 1 h at room temperature before addition of APCs. Splenocytes were prepared from HLA-DR A1*0101/+, B1*0401/+, hCD4/+, I-Ab/- transgenic mice (23) and used as APCs. Splenocytes (3 × 10^5 cells/well in 50 µl tissue culture medium) were incubated with graded amounts of Ag alone, peptide alone, or the preformed immune complexes for 1 h before addition of T cell hybridomas (1.5 × 10^4 cells in 100 µl tissue culture medium were added per well). After 24-h incubation, the samples were processed for measurement of IL-2 secretion as described above.

**FcR blockade**

The rat anti-mouse FcR mAb 2.4G2 (IgG2b) was used to block FcRII receptor uptake of immune complexes. The mAb was purified from supernatants of HB197 cells (American Type Culture Collection, Manassas, VA) using G4B Fast Flow (Sigma) column affinity chromatography and added to splenocyte experiments (10 µg/ml) using preformed immune complexes as Ag.

**Results**

**Localization and overlap of B and T cell epitopes in GAD65**

The four EBV-transformed monoclonal HLA-DRB1*0401-positive monoclonal B cell lines used in this study recognize four distinct conformational epitopes residing on the surface of the GAD65 molecule (20). Fig. 1 shows the linear and spatial relationships between the B cell epitopes of these four lines compared with four immunodominant GAD65 T cell epitopes recognized by HLA-DRB1*0401-restricted T cell hybridomas (22, 23). DBP and DPD B cells recognize distinct conformational epitopes in the N-terminal domain of GAD65, which were mapped to aa 39–173 and 96–173, respectively (20). The determinant of the T35 T cell hybridoma (aa 115–130) overlaps or is adjacent to the DPB and DPD epitopes. The third B cell line, DPA, recognizes a conformational epitope residing in the C-terminal domain of GAD65, involving residues in aa 483–499 and 556–585. In particular, H568 is critical for binding (20). The epitopes of two of the T cell hybridomas, HY103 (aa 481–495) and HY81 (aa 556–570), overlap or are proximal to the DPA epitope. The fourth B cell line, DPC, recognizes a conformational epitope residing in the middle domain of GAD65 and involving aa 231–234 and 366–413. The epitope of the fourth T cell hybridoma, HY91 (aa 271–285), localizes to the same domain, but does not overlap with the DPC epitope (Fig. 1). Thus, in contrast to the DPA, DBP, and DPD epitopes, the DPC epitope does not overlap with a T cell determinant recognized by the panel of T cell hybridomas. In the three dimensional model of the middle and C-terminal regions of GAD65 (20), the HY103 and HY81 epitopes are exposed on the surface of the GAD65 dimer, while the HY91 epitope is at the interface between the middle and C-terminal domains, which form a sandwich in each monomer (Fig. 2). It is therefore buried in the native GAD65 molecule. A three-dimensional model of the N-terminal region is not available. However, in the predicted secondary structure of GAD65 (20) the determinant of the T35 T cell hybridoma (aa 115–130) resides in an amphipathic α helix, the hydrophobic phase of which is likely to be exposed on the surface of native GAD65 (20).
Inhibition of presentation of GAD65 T cell epitopes in the mIg binding site of autoimmune B cells

We evaluated the ability of the DPA, DPB, DPC, and DPD B cell lines to process and present GAD65 to the four T cell hybridomas used in this study (Fig. 3). The Priess B cell line, which shares the HLA-DRB1*0401 haplotype with the DP-B cells, but does not recognize GAD65 epitopes, was used as a control APC. All the DP-B cell lines elicited similar IL-2 responses from the DRB1*0401-restricted, GAD65-specific T cells used in this study when synthetic GAD65 peptides were used as an Ag, indicative of a similar expression level of HLA-DRB1*0401 on their surface. The Priess cell line presented synthetic GAD65 peptides to DRB1*0401-restricted GAD65-specific T cells more efficiently than any of the DP-B cell lines (results not shown), consistent with a higher expression level of HLA-DRB1*0401 on its surface. The Priess cell line has been shown to nonspecifically (pinocytosis) take up GAD65 at high concentrations and process it for presentation to T cells.

FIGURE 1. Location of GAD65-specific epitopes recognized by autoimmune B and T cell lines. A, Location of autoimmune epitopes in the primary amino acid sequence of the N-terminal (upper panel), middle (middle panel), and C-terminal (lower panel) domains of GAD65. The epitopes recognized by DPA and DPC B cell lines were mapped to the indicated regions by domain swapping and homologue-scanning mutagenesis, taking advantage of the high homology between GAD65 and GAD67 in the middle and C-terminal domains and the fact that none of the DP Abs recognizes GAD67 (20). Because of the low degree of homology between GAD65 and GAD67 in the N-terminal domain, mapping of DPB and DPD was undertaken by domain swapping and deletion mutagenesis, but was not amenable to homologue-scanning mutagenesis and is therefore less detailed (20). Three of the four immunodominant T cell epitopes (HY103, HY81, and T35) reside either in or adjacent to a B cell epitope region. Predicted α helices, which may represent potential contact points between complementarity-determining regions of Igs and Ag are underlined (20). B, Location of autoimmune epitopes in a schematic representation of the three-dimensional model of GAD65. The relative location of B (ovals) and T (rectangles) cell epitopes in the N-terminal (light gray), middle (black), and C-terminal (dark gray) domains are shown. The HY91 (peptide 271–285) rectangle is hatched to indicate its location on the opposite face of the middle region.

FIGURE 2. Location of T cell epitopes in a three-dimensional model of the middle and C-terminal regions of the GAD65 dimer. A, A view of a space fill model of the GAD65 dimer showing the location of middle and C-terminal epitopes recognized by the T cell hybridomas used in this study. The monomer on the left has the middle domain in front and the C-terminal domain behind it. The two monomers are related by a P21 symmetry (20). Therefore, the monomer on the right has the C-terminal domain in front and the middle domain behind it. The HY81 and HY103 epitopes in the C-terminal domain (peptides 556–570 and 481–495, respectively) are exposed on the surface of the dimer. In contrast, the HY91 epitope residing in the middle domain (peptide 271–285, green) is buried in the interface between the middle and C-terminal domains. B, A view of the same model, where the C-terminal region has been removed from the monomer on the right to reveal the location of the aa 271–285 epitope.
presentation to T cells (27) (Fig. 3). Because of the enhanced uptake of GAD65 via mIg, the DP cell lines would be expected to process and present whole GAD65 more efficiently than Priess unless the bound Ig exerts an inhibiting effect on a particular epitope. Consistent with this prediction, the DPC cell line, which binds to a conformational epitope distant from all the T cell epitopes recognized by our panel of T cell hybridomas, elicited severalfold higher IL-2 levels than Priess from the four T cell hybridomas following incubation with whole GAD65 (Fig. 3 and Table I). Similarly, DPA, DPB, and DPD elicited severalfold higher IL-2 responses than Priess from T cell hybridomas recognizing T cell epitopes outside their Ab binding region (Fig. 3 and Table I). By contrast, the presentation of T cell epitopes residing in or adjacent to the Ab epitope was either blocked or inhibited. Thus, presentation of the aa 556–570 peptide, which resides in its Ab binding site, at all concentrations (0.03–3 μg/ml). C, Presentation of the aa 481–495 peptide to the T cell hybridoma HY103. Proliferative responses of HY103 are observed at 0.1 μg/ml GAD65 Ag presented by DPB, DPC, and DPD B cells. Presentation of this peptide is suppressed by DPA compared with the other DP B cells and is only moderately enhanced compared with Priess B cells. D, Presentation of the aa 115–130 peptide to the T cell hybridoma T35. Proliferative responses of T35 are observed at 0.1 μg/ml Ag presented by DPA and DPC. Presentation of this peptide is blocked, however, by DPB cells and suppressed by DPD cells, whose epitopes reside in the same region.

**FIGURE 3.** GAD65-specific B cells suppress presentation of T cell epitopes residing in their Ab binding site. IL-2 secretion by DRB1*0401-restricted mouse T cell hybridomas in response to presentation of human recombinant GAD65 protein by DRB1*0401-positive EBV-transformed B cells from a diabetic patient. Each panel shows IL-2 production by a T cell clone in response to GAD65 presentation by four GAD65-specific B cell lines and the control Priess B cell line. A, Presentation of the aa 271–285 epitope to the T cell hybridoma HY91. All the human B cell lines expressing GAD65-specific IgG enhance presentation of the 271–285 peptide compared with the Priess B cell line. IL-2 secretion by HY91 is observed at 0.1 μg/ml GAD65 Ag presented by DPA and DPB and at 0.2 μg/ml Ag presented by DPC and DPD B cells. DPC B cells induce IL-2 production by HY91 in a dose-response manner, but the maximal doses of Ag produce a lower response than with the other DP B cell lines. B, Presentation of the aa 556–570 epitope to the T cell hybridoma HY81. Proliferative responses of HY81 are observed at 0.1 μg/ml GAD65 Ag presented by DPA, DPB, and DPD B cells. In contrast, DPA B cells completely fail to present this peptide, which resides in its Ab binding site, at all concentrations (0.03–3 μg/ml). C, Presentation of the aa 481–495 peptide to the T cell hybridoma HY103. Proliferative responses of HY103 are observed at 0.1 μg/ml Ag presented by DPB, DPC, and DPD B cells. Presentation of this peptide is suppressed by DPA compared with the other DP B cells and is only moderately enhanced compared with Priess B cells. D, Presentation of the aa 115–130 peptide to the T cell hybridoma T35. Proliferative responses of T35 are observed at 0.1 μg/ml Ag presented by DPA and DPC. Presentation of this peptide is blocked, however, by DPB cells and suppressed by DPD cells, whose epitopes reside in the same region.
Similar results were obtained with the DPB and DPD B cell lines. DPB presented the T cell epitope 115–130, which resides within or adjacent to its Ab epitope, at a similar level as Priess, and presentation was effectively inhibited compared with that of DPA and DPC (Fig. 3D). Yet DPB was able to present the middle and C-terminal domain epitopes efficiently (Fig. 3, A–C, and Table I). Similarly, presentation of the 115–130 epitope by DPD was moderately improved compared with Priess, but poor compared with

![Figure 4](http://www.jimmunol.org/Downloadedfrom)

**Figure 4.** Soluble Abs suppress presentation of T cell epitopes residing in their binding site. IL-2 secretion by DRB1*0401-restricted mouse T cell hybridomas in response to presentation of GAD65 by DRB1*0401-positive mouse splenocytes. Each panel shows the proliferation of a T cell clone in response to presentation of the GAD65 Ag alone or in an immune complex with one of the four GAD65-specific mAbs DPA, DPB, DPC, or DPD. A, Presentation of aa 271–285 to T cell hybridoma HY91. Incubation of splenocytes with immune complexes with each of the mAbs enhance presentation of this epitope compared with Ag alone. B, Presentation of aa 556–570 to the T cell hybridoma HY81. The DPB, DPC, and DPD Abs, which bind to regions distant from this T cell epitope, enhance its presentation compared with Ag alone. In comparison, presentation of the 556–570 determinant is suppressed by the DPA Ab, whose epitope encompasses the peptide. IL-2 secretion is only detected when Ag is in excess of the DPA Ab. C, Presentation of the aa 481–495 peptide to the T cell hybridoma HY103. The DPB, DPC, and DPD Abs, epitope of which are distant from this peptide, enhance its presentation compared with Ag alone. However, presentation is relatively suppressed by DPA, which binds to the same region. D, Presentation of the aa 115–130 peptide to the T cell hybridoma T35. The DPA and DPC Abs, the binding sites of which are distant from this peptide, enhance its presentation. In contrast, no enhancement is observed with the DPB Ab whose epitope is in the same region as the peptide. IL-2 responses are only induced in conditions of Ag excess. The DPD Ab, which binds an epitope shifted downstream compared with DPB, shows less enhancement at high Ag concentrations than DPA and DPC, but is less suppressive than DPB.

<table>
<thead>
<tr>
<th>B Cell</th>
<th>T35 (115–130)</th>
<th>HY91 (271–285)</th>
<th>HY103 (481–495)</th>
<th>HY81 (556–570)</th>
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<td>Priess</td>
<td>+</td>
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<tr>
<td>DPA (483–499)</td>
<td>+</td>
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<tr>
<td>DPC (231–234)</td>
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<td>+</td>
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<td>DPD (96–173)</td>
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Table I. Summary of the effects of autoimmune GAD65-specific B cell lines on presentation of four different T cell determinants
DPA and DPC (Fig. 3D). In contrast, DPD presented the middle and C-terminal domains at similar levels as DPC and severalfold more efficiently than Priess (Fig. 3, A–C, and Table I).

In summary, the autoimmune GAD65-specific B cell lines appear to efficiently present T cell epitopes residing outside their Ab epitope region, while presentation of T cell determinants that reside within the Ab-bound region of GAD65 is suppressed in a dominant fashion (Table I).

Ab binding and presentation of immune complexes by macrophages and dendritic cells

We next analyzed whether the inhibiting effect of the DPA, DPB, and DPD B cell lines on processing and presentation of T cell epitopes located within their Ab-bound GAD65 regions was also relevant when immune complexes were taken up, processed, and presented by APCs like macrophages and dendritic cells. For these experiments purified GAD65 or GAD65 in an immune complex with DPA, DPB, DPC, or DPD Abs was incubated with HLA-DRB1*0401-positive splenocytes, which were then used to stimulate the T cell hybridomas. The binding of immune complexes by the FcRII receptor on macrophages enhances their uptake compared with Ag alone (17).

Compared with incubation with Ag alone, the incubation of splenocytes with GAD65 in an immune complex with DPA, DPB, DPC, or DPD Abs resulted in a severalfold enhanced stimulation of the HY91 hybridoma, recognizing the 271–285 peptide (Fig. 4A). Similarly, the presentation of peptides 115–130, 481–495, and 556–570 was severalfold enhanced following incubation of splenocytes with GAD65 in an immune complex with purified DP Abs, which do not recognize epitopes that overlap with those peptides (Fig. 4). In contrast, loading of a complex between GAD65 and DPA Ab, which recognizes an epitope overlapping with the 481–495, and 556–570 peptides, resulted in an inhibition of their presentation to levels similar to that with Ag alone (Fig. 4, B and C), with a more complete inhibition of epitope 556–570. Likewise, loading of a complex between GAD65 and either DPB or DPD Abs, which recognize a B cell epitope overlapping with or adjacent to the 115–130 epitope, resulted in an inhibition of its presentation compared with DPA and DPC Abs, with DPD Ab inhibiting presentation more completely than DPD (Fig. 4D). Thus, purified DPA, DPB, and DPD Abs in an immune complex with GAD65 inhibited presentation of T cell epitopes by macrophages in a similar manner as when the corresponding B cells were used for Ag presentation.

Abs enhance uptake via the FcRII on macrophages. To confirm that the stimulating effect on outlying epitopes observed in the experiments with soluble Abs is dependent on an enhanced uptake via the FcRII molecule, the experiments described above were repeated in the presence of an FcRII Ab, which blocks uptake by the FcIIA, but not by pinocytosis. In the presence of the FcRII Ab, none of the DP Abs in an immune complex with GAD65 enhanced presentation to T cell hybridomas compared with Ag alone (Fig. 5 and results not shown), suggesting that the general boosting effect of soluble DPA, DPB, DPC, and DPD on T cell epitopes outside the Ab binding site is the result of increased Ag capture via the FcRII.

Discussion

The results presented here suggest that autoimmune GAD65-specific B cells and the Abs they secrete may play a major role in shaping the autoimmune T cell response by specifically suppressing presentation of T cell determinants residing within Ab-bound regions. Abs effects on uptake are generally positive whether expressed on the surface of Ag-presenting B cells or forming soluble immune complexes that are taken up by FcR on APCs. However, the expected increase in Ag presentation is only observed for T cell

![FIGURE 5](image.png)

FIGURE 5. Enhancement of Ag presentation by soluble Abs is blocked by an Ab to FcRII. IL-2 secretion by DRB1*0401-restricted mouse T cell hybridomas in response to presentation of GAD65 alone or GAD65 immune complexes by DRB1*0401-positive mouse splenocytes in the presence of an Ab to FcRII. Each panel shows IL-2 secretion of a T cell clone in response to presentation of GAD65 Ag alone or in an immune complex with one of the four GAD65-specific mAbs: DPA, DPB, DPC, or DPD. A. Presentation of the aa 556–570 peptide to T cell hybridoma HY81. The enhancement of presentation of this peptide observed with DPB, DPC, and DPD immune complexes compared with Ag alone (Fig. 4B) is eliminated when FcRII is blocked. B. Presentation of the aa 115–130 peptide to T cell hybridoma T35. The enhancement of presentation of this peptide observed with DPA and DPC and to a lesser degree DPD immune complexes compared with Ag alone (Fig. 4D) is eliminated when FcRII is blocked.
determinants located outside the region captured by Ab. In contrast, T cell determinants located within or adjacent to the B cell epitope are specifically suppressed, and the suppressive effect dominates. One possible explanation for this effect is that binding of a GAD65 autoantibody to its epitope is stable throughout the acidic environment of Ag processing and MHC class II loading compartments, resulting in masking of T cell determinants residing in the region. While the binding affinities of the DP Abs varied 86-fold between DPA (highest) and DPB (lowest; see Materials and Methods), both mAbs and the corresponding cell lines effectively blocked the presentation of T cell determinants located within the Ab epitope. Thus, T cell epitope suppression appears to occur over a wide range of Ab affinities.

The findings of epitope suppression are consistent with earlier studies in which tetanus toxin-specific mlg as well as the corresponding soluble Abs suppressed the processing and presentation of T cell determinants within the footprint of the Ab (15, 16). In previous studies a detailed mapping of the B cell epitopes in tetanus toxin was not available. Instead, the suppressed T cell determinants were found to be localized within the Ab footprint, i.e., the Ag fragment that is protected by Ab during limited proteolysis and usually encompasses a larger area than the Ab epitope. In one case, the Ab footprint included both a T cell epitope that was suppressed and an epitope that was enhanced by Ab binding (16). Based on these results, Smitse et al. (16) proposed a model in which theprocessing and MHC class II loading of a T cell epitope residing in the actual Ab contact site (epitope) are suppressed, while the loading of a nearby epitope residing inside the footprint but outside the actual binding site is favored. The present study has identified three cases of effective suppression of immunodominant DRB1*0401-restricted T cell determinants residing within or adjacent to regions constituting the epitope of human monoclonal GAD65 autoantibodies arising in type 1 diabetes. We did not identify a T cell epitope whose processing/presentation were relatively enhanced by Ab binding to the epitope area, but the possibility is not excluded by this limited set of B cell lines and T cell hybridsomas.

Both immunization of DRB1*0401-positive transgenic mice (22, 23) and derivation of human T cell lines from human patients (28) have identified the aa 271–285 determinant as perhaps the most immunodominant DRB1*0401-restricted T cell epitope in the GAD65 molecule. In the three-dimensional model of the middle and C-terminal regions of GAD65, the 271–285 epitope is buried in the native folded molecule. This is in contrast to both the aa 481–495 and 556–570 T cell epitopes, which are exposed on the surface. More than 95% of type I diabetic patients exclusively make autoantibodies to conformational epitopes on the surface of the native GAD65 molecule (Ref. 20 and references therein). The aa 271–285 T cell epitope does not, therefore, appear to reside in the Ab binding site of a typical autoimmune B cell in type 1 diabetes. For example, while the epitopes of two other human mAbs derived from diabetic patients, MICA 6 and MICA 10, have been mapped close to the 271–285 T cell epitope (242–282 region), both Abs bind the surface of native intact GAD65, suggesting that the buried 271–285 residues are not part of the epitope. Furthermore, in experiments using splenocytes as APCs, MICA 10 in an immune complex with GAD65 does not suppress presentation of the 271–285 determinant (W. Richter, J. C. Jaume, S. L. Parry, G. Sonderstrup, and S. Baekkeskov, unpublished observations). We hypothesize that the absence of B cell reactivity to the aa 271–285 region, and the consequent absence of suppression of processing and presentation of T cell determinants in this region by B cells and APCs may contribute to the immunodominance of this epitope.

Taken together, our results suggest that autoimmune GAD65-specific B cells not only serve a critical function as APCs in type 1 diabetes, but also play a major role in shaping the epitope specificity of the autoimmune T cell response to GAD65. Thus, autoimmune GAD65-specific B cells and the Abs they secrete appear to modulate the autoimmune T cell repertoire by down-regulating T cell epitopes in an immunodominant area while boosting epitopes in distant regions, providing a mechanism for autoimmune T cell epitope spreading.

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References


