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Differential Presentation of Glutamic Acid Decarboxylase 65 (GAD65) T Cell Epitopes Among HLA-DRB1*0401-Positive Individuals

Helena Reijonen, John F. Elliott, Peter van Endert, and Gerald Nepom

Glutamic acid decarboxylase 65 (GAD65) is one of the major autoantigens in type 1 diabetes. We investigated whether there is variation in the processing of GAD65 epitopes between individuals with similar HLA backgrounds and whether the processing characteristics of certain immunogenic epitopes are different in distinct APC subpopulations. Using DR401-restricted T cell hybridomas specific for two immunogenic GAD65 epitopes (115–127 and 274–286), we demonstrate an epitope-specific presentation pattern in human B-lymphoblastoid cell lines (B-LCL). When pulsed with the GAD protein, some DRB1*0401-positive B-LCL, which presented GAD65 274–286 epitope efficiently, were unable to present the GAD65 115–127 epitope. However, all B-LCL presented synthetic peptides corresponding to either GAD epitope. In addition, when pulsed with human serum albumin, all cell lines gave equal stimulation of a DR4-restricted human serum albumin-specific T hybridoma. GAD65-transfected cell lines displayed the same presentation phenotype, showing that lack of the presentation of the 115–127 epitope was not due to inefficient uptake of the protein. Blood mononuclear adherent cells, B cells, or dendritic cells derived from the same individual displayed the same presentation pattern as observed in B cell lines, suggesting that the defect most likely is genetically determined. Therefore, individual differences in Ag processing may result in the presentation of distinct set of peptides derived from an autoantigen such as GAD65. This may be an important mechanism for the deviation of the immune response either into a regulatory pathway or into an inflammatory autoimmune reactivity.


T cell responses directed against various islet cell Ags, particularly glutamic acid decarboxylase 65 (GAD65), have frequently been demonstrated in patients with insulin-dependent diabetes (IDDM) (1–5). Studies using peptide Ags derived from the GAD65 sequence show that the T cell-proliferative response is very heterogeneous: multiple different peptide epitopes have been described, and responses occur in about 50% of patients and also occur in some unaffected relatives of diabetic patients. The link between T cell reactivity to GAD65 and pathogenic events in IDDM is not clear. It is likely that T cell responses to islet Ags such as GAD65 represent amplification events in a cumulative immune response; whether a specific T cell determinant from GAD65 is presented by risk-associated HLA class II molecules, which might skew the T cell response into a disease-accelerating direction in some individuals, is not known. The limited set of peptides processed from GAD65 that are immunogenic and the selection of epitopes determined by binding to MHC class II molecules are primary determinants of potential immunogenicity that vary between different individuals. Differences in Ag processing may result in the presentation of certain epitopes at the expense of the others, and by that mechanism influence the specificity and magnitude of Ag-specific T cell response.

T cell reactivity to several GAD65 epitopes mediated by various DR molecules has been demonstrated both in IDDM patients and normal subjects (6–9) as well as DR401-transgenic mice (10, 11). In the nonobese diabetes mouse model, T cell responses have been described initially confined to a limited region of the GAD molecule, followed by intramolecular spreading of the T cell response to additional GAD peptides (12). Whether a similar epitope spreading plays a role in the progression of human IDDM is not known. The islet inflammation may be initiated by one APC population, but further diversified and amplified by another. The efficiency of HLA class II-restricted presentation has been demonstrated to vary depending upon the APC type (13). Molecular mechanisms behind these differences are the ability to internalize the Ag and as well as distinct Ag processing regulated by proteases and chaperones. Changes in Ag processing may lead to a shift in the peptide repertoire generated, such that a novel peptide becomes a dominant epitope presented by HLA class II. Therefore, during disease progression new APCs with different processing characteristics may be recruited into the inflammation site and T cell responses to additional epitopes may be generated.

Our aim was to identify first whether there is interindividual variation, presumably on a genetic basis, in the capacity to process and present epitopes generated from intact GAD65 protein, and second, whether such differences would be present in all cell lineages used as APCs. Both of these issues may arise from variation in the specific types of proteases and chaperones used during Ag processing and presentation. We analyzed processing and presentation of two immunogenic epitopes of GAD65 using DR401-restricted murine T cell hybridomas specific for peptides 274–286 and 115–127, respectively. In a previous study, these peptides were shown to be immunogenic in the context of HLA-DR401 in

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3 Abbreviations used in this paper: GAD65, glutamic acid decarboxylase 65; B-LCL, B-lymphoblastoid cell line; HSA, human serum albumin; IDDM, insulin-dependent diabetes.
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B10.M/DR4 transgenic mice (11). It is interesting to note that epitopes overlapping with the ones in this study have been shown to be recognized also by human T cells. Endl et al. (6) described reactivity for GAD65 peptide 266–285, with T cells from a newly diagnosed DR401-positive IDDM patient. Another group demonstrated a cytotoxic HLA-A*0201-restricted T cell response for GAD 114–123 epitope naturally processed in target cells in one prediabetic and two IDDM subjects (14). In this study, we report a differential Ag presentation pattern for these two epitopes among APCs derived from DRB1*0401-positive individuals.

Materials and Methods

Cells

B-LCLs were the following: BpH (DRB1*0401, 0301), BrDe (DRB1*0401,0701), EmWi (DRB1*0401, 0301), JeBa (DRB1*0401, 0801), RePo (DRB1*0401, 0301), Bsmi (DRB1*0401 homozygous), and Priess (DRB1*0401 homozygous). The cells were obtained from diabetic patients (BpH, BrDe, JeBa), subjects positive for autoantibodies against multiple β cell Ags (EmWi, RePo), and normal subjects (Bsmi, Priess). The first two groups were also positive for GAD65 autoantibodies. Three of these individuals (EmWi, JeBa, RePo) were available for subsequent blood drawing at the Department of Endocrinology at Virginia Mason, and from one subject frozen PBMC had been collected earlier (BrDe). Fresh PBMC used in APC subpopulation experiments were obtained from the volunteers mentioned above as well as one healthy subject (SAM, Wallac, Turku, Finland) diluted 1:1000 in assay buffer (Wallac) and incubated at 25°C for 30 min. Plates were washed five times as before, and enhancement buffer (Wallac) was added. Plate was incubated 15 min at 25°C, and fluorescence was measured using a DELFIA 1232 fluorometer (Wallac). The sp. act. of the europium-streptavidin was calibrated to be 1466 fluorescence units per femtomole of biotin bound.

GAD65 recombinant retrovirus-producing cell lines and infection of B-lymphoblastoid cells

GAD65-expressing B cell lines were generated by retroviral-mediated gene transfer, as previously described (17). Briefly, GAD65-cDNA fragment was cloned into the parental retroviral construct pLNCX2. GAD protein-producing cell lines were generated by introduction of the retroviral constructs into the amphotropic packaging line PE501. Next day supernatants from transfected PE501 cells were used to infect PG13 fibroblasts in the presence of polybrene (4 μg/ml) in RPMI 1640 with 10% FCS. Infected PG13 fibroblasts were selected under a selective medium containing G418 (1 mg/ml), isolated, expanded, and determined for a viral titer. Virus-producing cells (5 × 10^7) were plated and cocultivated with B-lymphoblastoid cells (2 × 10^6) in 60-mm plates in the presence of polybrene (4 μg/ml). The nonadherent B cells were harvested after 24 h of coculture and were grown for another 24 h before placing them in a selective medium containing G418 (1 mg/ml). GAD65 expression was detected on a Western blot and RIA (18).

T cell proliferation assay

In some experiments, B-LCLs were first fixed with 1% paraformaldehyde (1%/25°C) and then pulsed with Ag to avoid possible degradation or toxicity of the protein expression system, as described earlier (12), and the other one was obtained from Sf9 insect cells infected with recombinant baculoviruses; this preparation had a purity of 95% estimated by FACScan. CD19-negative unbound cells were allowed to adhere to a plastic plate for 2 h at 37°C, after which the attached cells were released by trypsinization. Average 75% of recovered adherent cells were CD19-negative. To generate dendritic cells, the adherent fraction of PBMC was cultured for 7 days in the presence of GM-CSF (600 U/ml) and IL-4 (400 U/ml).

Antigens

Two different rGAD65 protein preparations were used in most experiments. One was produced in Escherichia coli expression system, as described earlier (12), and the other one was obtained from Sf9 insect cells infected with recombinant baculoviruses; this preparation had a purity of ~95% (8). HSA protein was purchased commercially (Sigma, St Louis, MO). GAD peptides 115–127 (MNILLQYVVKSFD) and 274–286 (IAFTSEHSHFSLK) were respectively, peptide-binding assays were performed as described earlier (15).

Flow-cytometric analysis

mAb binding to live cells was detected by flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). The following affinity-purified, isotype-specific mouse mAbs were used to stain cell surface molecules: L243 (anti-DR, hybridoma purchased from American Type Culture Collection, Rockville, MD), SPLV3 (anti-DQ, obtained from DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA), NFLD.D1 (anti-DR04, Dr. Sheila Drover, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada), anti-CD83 (Dr. Thomas Tedder, Duke University Medical Center, Durham, NC), anti-CD40, anti-CD86, anti-CD1a, anti-CD3, anti-CD4, anti-CD8, anti-CD14-FITC (PharMingen, San Diego, CA), anti-CD52 FITC, and anti-CD19 PE (Becton Dickinson). Isotype-matched control Abs were used to detect background staining. Binding of unlabeled Abs was detected using FITC-conjugated goat anti-mouse Ig.

Results

Differential presentation of GAD65 epitopes by DRB1*0401-positive B-LCLs

Three DR401-positive B-LCLs pulsed with intact rGAD65 protein efficiently processed and presented both GAD 115–127 and 274–286 epitopes, but four other DR401 B-LCLs displayed an epitope-specific presentation pattern: the GAD 274–286 epitope was efficiently presented to the T33.1 hybridoma, whereas no stimulation of the T cell hybridoma T35 specific for GAD 115–127 was detected (Fig. 1, A and B). The level of presentation of the hybridomas was dependent on the GAD65 concentration. When 25

Binding assays with paraformaldehyde-fixed cells

Peptide-binding assays were performed as described earlier (15). Briefly, HLA class II homozygous EBV-transformed human B cell lines (1 × 10^6) or transfected type 1 bare lymphocyte syndrome cell line (a gift from Janet Lee (16)) expressing a single HLA class II allele were fixed with 0.5% paraformaldehyde before incubation with 10 μM biotinylated peptide in 50 mM Tris, pH 7.5, 5 mM EDTA, 1 mM iodoacetamide, 1 mM benzamidine, and 1 mM pefabloc for 24 h at 37°C. Cells were washed twice in PBS before lysis in 0.5% Nonidet P-40, 0.15 M NaCl, 50 mM Tris (pH 8), 1 mM pefabloc, 1 μg/ml leupeptin, and 1 μg/ml pepstatin A. The lysates were cleared by centrifugation 15 min, 20,000 g, and supernatants were transferred to 96-well plates precoated with either 10 μg/ml of SPVL1 mAb (anti-DQ) or L243 mAb (anti-DR) and blocked with 5% FCS in PBS. The 50 μl samples were diluted 1:1 on the plate in 0.02% n-dodecyl-β-maltoside (for HLA-DQ) or 0.75% n-octyl-β-d-glucopyranoside (for HLA-DR) in 50 mM Tris, pH 8, for neutralization, and complexes were captured overnight at 4°C. The wells were then washed five times with PBS containing 0.2% Tween-20 and europium-labeled streptavidin (Wallac, Turku, Finland) diluted 1:1000 in assay buffer (Wallac) and incubated at 25°C for 30 min. Plates were washed five times as before, and enhancement buffer (Wallac) was added. Plate was incubated 15 min at 25°C, and fluorescence was measured using a DELFIA 1232 fluorometer (Wallac). The sp. act. of the europium-streptavidin was calibrated to be 1466 fluorescence units per femtomole of biotin bound.
mg/ml GAD65 was used to pulse the APCs, both hybridomas were efficiently stimulated. HLA-DRB1*0401 homozygous cell lines (Priess and Bsm) presented GAD65 274–286 epitope well at lower concentration than the heterozygous cell lines. Similarly, GAD65 115–127 was most efficiently presented by Priess, in contrast to Bsm, which displayed the presentation-deficient phenotype at all concentrations tested.

One possible explanation for the deficient presentation phenotype might have been a lack of essential factors in the processing machinery. This was investigated by testing the capacity of these cell lines to present another protein Ag, exogenously added HSA (Fig. 1C). All cell lines presented the HSA Ag equally well at several concentrations between 1–6 mg/ml, excluding the possibility of a general inability of processing exogenous Ags. Fixed cells did not present GAD protein to both T-35 and T-33.1 hybridomas, indicating that the presentation of epitopes from GAD65 protein required intracellular processing (data not shown).

GAD65 epitopes are presented by all B-LCLs
All B cell lines (fixed and unfixed) efficiently presented peptides corresponding to the particular epitopes, demonstrating that the peptide-DR401 complex can interact with the specific hybridoma and induce stimulation (Fig. 2). DR401 homozygous cell lines (Priess and Bsm) presented the most efficiently at low peptide concentration: <10 μM for the GAD 274–286 peptide and <1 μM for the GAD 115–127 peptide. This result also demonstrated that T cell hybridoma T-35 specific for the epitope 115–127 produced more IL-2 when stimulated by its cognate peptide at low concentration than did T cell hybridoma T-35 with peptide 274–286 under identical conditions, indicating that the deficiency to present the 115–127 epitope is not due to a lower sensitivity of the T35 hybridoma. The lower stimulation of T cell hybridomas by the heterozygous cell lines was not due to peptide stealing, because the other class II molecules present on those cells were not good binders for the given peptides (data not shown); differences may be a result of a higher density of the equivalent class II molecules on the cell surface of the homozygous cells.

Same presentation pattern occurs in the GAD65-transfected cell lines
To investigate the processing pattern in a quantitative manner, we stably transfected the APCs (Priess, Bsm) with rGAD65 cDNA. The intracellular expression of GAD65 was equivalent in both cell lines, as detected by RIA and Western blot (data not shown). However, the processing failure in terms of the GAD115–127 epitope was observed in Bsm, whereas Priess-transfected cells could present the relevant endogenous epitope (Fig. 3).

Presentation of at least one epitope in each of these transfected cells demonstrated that the HLA class II pathway is intact, even though GAD65 is now an endogenous Ag. The presentation of the 274–286 epitope via class II endogenous pathway is similar in both transfected cell lines, whereas the presentation of the 115–127 peptide is not, indicating that the processing-deficient phenotype of the Bsm cells, shown in Fig. 1, was not due to more inefficient uptake of exogenously added GAD65.

Differential presentation of GAD65 epitopes is not dependent on APC lineage
To address the question of whether lack of processing of the GAD65 115–127 epitope would be displayed in primary APCs and different APC lineages, Ag presentation was tested in distinct cell populations. PBL were obtained from the same four subjects from whom we had B cell lines (BrDe, EmWi, JeBa, and Repo used in the experiments presented above) and one healthy new subject as...
a control (Sam). This group represents both presentation phenotypes. When GAD65 protein was presented by these primary peripheral lymphocytes, the epitope-specific presentation phenotype was not altered (Fig. 4). To determine whether a distinct APC subpopulation would be capable of presenting the subdominant epitope 115–127, B cells and adherent cells were differentially separated from fresh peripheral blood. One-half of the adherent cells were subjected to GM-CSF and IL-4 in culture for 7–10 days to generate a population of dendritic cells. A population positive for CD40, DR, DQ, CD86, and CD1a; low for CD83; and negative for CD14, CD4/8, CD25, and CD19 was obtained. Once again, the epitope-specific presentation pattern did not change in any of these APC types, nor in dendritic cells, which have the greatest capacity to present Ag (Fig. 5). Most of the experiments were performed using two concentrations of GAD65 at optimal dose-response range. An example of GAD65 dose response in all APC lineages derived from Repo is shown in Fig. 5. Both hybridomas, T33.1 and T35, were highly stimulated by all APCs when pulsed with GAD65 p274–286 and p115–127 peptides, respectively (data not shown).

The effects of protease inhibitors on the presentation of GAD65 epitopes

To explore the mechanism behind the presentation-deficient phenotype, B cell lines were incubated in the presence of protease inhibitors, leupeptin and pepstatin A, for 20–24 h before the subsequent pulsing with GAD65 protein. The effect of the protease treatment on GAD65 epitopes was similar in all cell lines: leupeptin inhibited the responses, whereas pepstatin A augmented the response. However, the presentation of GAD65 115–127 was not rescued by either of the protease inhibitors (Fig. 6). A submaximal concentration of GAD65 (5–15 µg/ml) was used in these assays for each cell line and both T cell hybridomas, allowing the detection of either augmentation or inhibition of the epitope processing. To evaluate potential nonspecific toxicity of protease inhibitors at high concentrations, three concentrations of leupeptin (40, 100, and 200 µg/ml) and pepstatin A (10, 25, and 50 µg/ml) were used to define a dose-response effect. The highest concentration that gave complete inhibition or the strongest augmentation was tested for toxicity on the APCs pulsed with a synthetic peptide. Protease inhibitor treatment had no effect on peptide presentation (data not shown).
Discussion

We report differences between DRB1*0401-positive individuals in the processing of epitope 115–127 from human rGAD65, showing an epitope-specific deficiency in the processing of an important IDDM autoantigen.

Epitope GAD65 115–127 was not processed and presented by four of seven B-LCLs derived from different DR401-positive individuals. The differences in the presentation cannot be due to differences in the ability to internalize an Ag, first, because the other GAD65 epitope 274–286 was presented equally well by all APCs, and second, because the same processing pattern was observed in GAD65-transfected cell lines. The expression level of GAD65 in transfected cell lines was quantitated showing a comparable high level of expression in both APC phenotypes. We also tested the presentation of HSA to see whether the cell lines with defective processing of GAD65 epitope also displayed an inefficient processing of epitopes from other Ags. This was not the case, because all APCs induced similar stimulation of an HSA-specific T hybridoma, ruling out the possibility of a global defect in the processing machinery. Fixed cells efficiently presented peptides, but did not present the whole GAD65 Ag, which shows that the inability to present GAD65 115–127 epitope is at the processing level. Titration of the synthetic peptides corresponding to the T cell epitopes is also a good indication of the sensitivity of T hybridomas. Both T hybridomas were stimulated in a dose-dependent manner, and at low peptide concentration T-35 hybridoma specific for GAD65 115–127 was induced to produce more IL-2 than T-33.1 hybridoma, demonstrating that the processing difference was not due to a lower sensitivity of the T-35 hybridoma.

One hypothesis for the processing deficiency in the distinct cell lines is an inefficient loading of peptide onto a particular MHC class II, if in the peptide-loading compartment the other class II and possibly class I molecule(s) have a higher binding affinity to a given peptide (19–21). In our study, some of the cell lines were
HLA identical, but to exclude the possibility of epitope stealing, peptide binding was measured for all of the other HLA-DR or DQ molecules present on the B-LCLs. None of the other HLA class II molecules displayed a higher affinity for the p115–127 or p274–286 than DR401 molecule. Because HLA-A*0201 has been shown to bind and present an almost completely overlapping naturally processed GAD peptide 114–123 (14), all of the cell lines were tested for the positivity of HLA-A*02. Four of them were positive for HLA-A*02 (Bsm, BrDe, JeBa, Priess), but no correlation between the processing phenotype and genotype was observed. The processing phenotype did not associate with IDDM in our study, but this small number of subjects tested does not exclude a possible relationship with disease progression. Five subjects in our study were positive for GAD65 autoantibodies. Ag-specific IgG on the surface of B cells facilitate Ag uptake and may modulate the transportation and processing of Ag. However, in our study, GAD65 autoantibody positivity did not correlate with the epitope-specific presentation phenotype.

Another possible explanation for the lack of presentation of the 115–127 epitope is an inefficiency in the generation of that particular epitope in the processing compartment, or loading of the peptide onto MHC class II molecules. In an earlier study (11) when GAD-transfected Priess was used as a source of APC, more IL-2 was secreted by the T cells recognizing GAD65 274–286 compared with those recognizing GAD65 115–127. This might have been due to a lower expression level of GAD65 in these particular Priess-transfected cells than in our GAD65 transfecants. In a long-term culture without the G418 selection, the number of cells expressing high levels of GAD65 decreases. In this study, the level of GAD65 expression was quantitated from the cell pellets by RIA. However, consistent with the previous observation, a lower stimulation of T-35 than T-33.1 hybridoma was seen in response to a low concentration of exogenously added protein, as shown in Fig. 1. Therefore, in all experiments, a high enough concentration of exogenous GAD65 protein (15–25 μg/ml, depending on the APC) was added to ensure maximal presentation of the 115–127 epitope. At this concentration, the cells capable of presenting both epitopes induced a high IL-2 production in both T-35 and T-33.1 hybridomas.

We wanted to investigate whether the differential processing was characteristic only in one APC population, B cells, or whether it could be seen across the APC lineages. Cell proteases play a key role in Ag processing, and it has been shown that distinct APC lineages differ in their ability to process T cell epitopes from a single Ag (22, 23). Distinct APCs also internalize Ag by different mechanisms, and some chaperones are found only in certain APC populations, such as the recently described DO molecule in B cells (24). To test this hypothesis, we separated distinct APC populations, monocytes, and primary B cells, from peripheral blood from the same four subjects and one healthy control representing both processing phenotypes observed in B-LCL. In addition, we generated dendritic cells from adherent mononuclear cells, which allowed us to investigate the presentation of the GAD65 protein in this most efficient APC population. The dendritic cells generated from monocytes using GM-CSF and IL-4 have properties of immature dendritic cells (high expression of CD1a), and they have maximal capacity to capture and process an Ag (25–27). Upon maturation, the population of dendritic cells, characterized by CD83 expression (28), loses their Ag-processing capacity, but becomes efficient stimulators of T cells. Our results showed that all APC populations derived from the same individual displayed the same epitope-specific presentation pattern as observed in B-LCL. Although our results do not define the precise molecular basis for the defect in the processing of the GAD65 115–127 epitope in some individuals, the consistent presentation pattern through the APC lineages strongly suggests that the defect is most likely genetically determined.

One hypothesis for the differential processing is a lack of a chaperone essential for the presentation of the given epitope. Dependence of chaperones in terms of T cell epitope generation, class II intracellular trafficking, and peptide loading can vary in distinct processing compartments (29–32), where different epitopes can be generated from a single Ag (25–28). It is also possible that other competing peptides with higher affinity are generated. Different epitopes within one Ag may vary in their sensitivity to proteases and processing requirements. Certain epitopes may be processed in early endosomes, while others may require processing in a more acidic late endocytic compartment. Some studies have implicated distinct processing mechanisms for different epitopes (29–31). Walker et al. (33) tested a number of B-LCL for their ability to present keyhole limpet hemocyanin (KLH). The observation was that three cell lines presented KLH to only one particular KLH-specific T cell hybridoma, but not to the others. A differential ability to process various distinct Ags has been observed also in la-transfected fibroblasts (34–36). Michalek et al. (37) provided evidence that genetically identical APC clones processed differently the same immunodominant T cell determinant from native OVA. These findings all suggest that different epitopes, even from a single protein Ag, may vary in their dependence on particular
processing compartments or chaperones, such as DM or DO, that contribute to Ag-processing mechanisms.

Distinct APCs may vary in their processing capacity, resulting in the destruction of a GAD65 T cell epitope or alternatively generation of a competing epitope hindering the binding of GAD 115–127 onto the DRB401 molecule. Different epitopes may be involved in the processing of GAD epitopes, and their relative deficiency or excess could result in a skewing of the T cell determinants generated in the endosomes. Vidard et al. (38) showed that poor presentation of some epitopes from OVA to specific T cell hybridomas was augmented by leupeptin treatment, whereas other determinants from the same Ag were inhibited. Other protease inhibitors had a complex pattern of inhibition/summation of presentation of these epitopes. In one recent study using experimental leishmaniasis as a model, treatment with a specific cathepsin B inhibitor modulated the polarity of TH differentiation from Th2 to Th1 by altering the Ag processing (39). In our study, leupeptin inhibited the processing of both GAD 274–286 and GAD 115–127 epitopes, whereas pepstatin A augmented the presentation, suggesting that same type proteases are involved in at least part of the processing of both epitopes. On the other hand, leupeptin inhibits serine and thiol proteases, and pepstatin A inhibits several acid proteases, in particular aspartate proteases; therefore, the proteases involved in the processing of the distinct GAD65 epitopes may be different, but still affected by the same inhibitors. More specific protease inhibitor studies are needed to answer this question.

Leupeptin inhibits cathepsin B protease, which appears to cut preferentially after two contiguous basic amino acid residues. Interestingly, the GAD274–286 region is preceded by an Arg-Lys dibasic sequence providing a possible cut site for cathepsin B. A similar dibasic proteolytic site is located three amino acid residues downstream of the 115–127 region in GAD65. The positioning of dibasic motifs within a protein Ag could be of crucial significance in determining the length of the processed peptide. The flanking residues have been shown to modulate binding to MHC class II molecule as well as T cell interaction (40). In addition, cathepsin B mediates II degradation in APCs (41). Because in our study both epitopes were sensitive to leupeptin, it is unlikely that cathepsin B provides a mechanism for the differential presentation pattern of GAD65 epitopes, and consistent with this interpretation, we observed no correlation in the processing of the invariant chain in the distinct B cell lines (data not shown).

We have described an epitope-specific difference in the presentation of two distinct immunogenic epitopes from GAD65. The difference suggests either quantitative or qualitative variation in the intrinsic ability of APCs from different individuals to process and present epitopes from this important diabetes autoantigen. This pathway of immunomodulation may be crucial for the promotion of autoimmunity mediated by determinant spreading or the selection of dominant epitope reactivity in the process leading to clinical IDDM.

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