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Identification of immunodominant T cell epitopes of human glutamic acid decarboxylase 65 by using HLA-DR(α1*0101,β1*0401) transgenic mice

(insulin-dependent diabetes mellitus/autoantigens/epitope mapping/T cell hybridomas)

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ABSTRACT **Glutamic acid decarboxylase isoform 2 (GAD65; EC 4.1.1.15) has been identified as a key target autoantigen of insulin-dependent diabetes mellitus (IDDM). IDDM is genetically associated with the major histocompatibility complex (MHC), and particular alleles from the HLA-DQ and HLA-DR loci contribute to disease. Among DR4 subtypes, HLA-DRB1*0401, HLA-DRB1*0402, and HLA-DRB1*0405 alleles lend susceptibility, while HLA-DRB1*0403 confers protection. We have utilized HLA-DR(α1*0101,-β1*0401) (hereafter referred to as DR0401), human CD4, murine class II null triple transgenic mice and recombinant GAD65 to generate T cell hybridomas, and we have used overlapping sets of peptides to map the immunodominant epitopes of this autoantigen. We have identified 10 immunogenic regions for GAD65, of which 6 are recognized by multiple hybridomas. These epitopes are also generated by human antigen-presenting cells and their presentation is DR0401 restricted, as shown by the use of typed human lymphoblastoid cell lines and antibody blocking experiments. Immunodominant GAD65 epitopes defined in transgenic mice correspond to GAD65 regions previously shown to elicit T cell responses specifically in DR0401 IDDM patients, underscoring the validity of this approach. Interestingly, although the major epitopes contain DR0401 binding motifs, one of the epitopes contains a DR0405 motif.**

Susceptibility to insulin-dependent diabetes mellitus (IDDM), though multifactorial, is markedly influenced by the major histocompatibility complex (MHC) class II genotype (1). The strongest associations of IDDM susceptibility in Caucasians are with the HLA-DQ loci, specifically with alleles that carry a neutral residue (Val, Ala, or Ser) at position 57 of the DQ β chain, such as the HLA-DQB1*0302 allele, while other alleles encoding an Asp residue at the same position are negatively related to IDDM susceptibility (1). In addition, 90% of patients with IDDM express HLA-DR3 or HLA-DR4 (2), and three separate studies have recently shown an association between certain HLA-DR4 alleles and IDDM. In populations of Mexican Americans (3), Sardinians (4), and Belgians (5), it was shown that HLA-DRB1*0405 lends susceptibility to IDDM, while HLA-DRB1*0403 confers a protective effect. HLA-DRB1*0405 increases the effect of HLA-DQB1*0302 when found on the same haplotype, while HLA-DRB1*0403 acts in a dominant manner to confer protection (4, 5), even when linked to DQB*0302 with the DRB1*0301-DQB1*0201 susceptibility haplotype present on the other chromosome. In northern Europeans HLA-DRB1*0401 and DRB1*0402 are also associated with disease. In relative terms, the contribution of HLA-DRB1*04 alleles to disease is in the following order: HLA-DRB1*0405, *0402, *0401, *0404, *0403, and *0406 (6).

Although these associations have been known for a number of years, the molecular or biochemical basis for such HLA associations remains unclear. MHC class II molecules function at the level of both positive and negative selection of the T cell repertoire in the thymus, but HLA-DQ and HLA-DR molecules also select and present peptides to CD4$^+$ T cells in the periphery, and they may play a role in IDDM through the presentation of islet cell specific peptides to pathogenic T cells. Due to the presence of autoantibodies and T cell reactivity in patients, several β-islet cell proteins have been identified as potential disease targets (7). Eighty percent of prediabetic individuals followed in families with a history of diabetes, and most recent onset diabetic patients, have serum autoantibodies directed against glutamic acid decarboxylase (GAD; EC 4.1.1.15) (8). Furthermore, half of new-onset IDDM patients have T cell proliferative responses to GAD (9, 10). Finally, GAD isoform 2 (GAD65) has been used in tolerogenic regimens to prevent disease in susceptible nonobese diabetic (NOD) mice (11–13).

The identification of the immunodominant epitopes of target autoantigens presented by the different HLA molecules is a key step toward delineating the role of MHC molecules in disease, as well as providing reagents for diagnosis and possible therapies for IDDM. Such efforts are difficult in human subjects due to the paucity of circulating autoreactive T cells in peripheral blood of patients, and the difficulty in long term maintenance of human T cell clones. In addition, the presence of multiple HLA molecules in each individual makes the determination of MHC restriction elements difficult, and many patients need to be analyzed before a clear pattern emerges.

To circumvent these problems, we have utilized HLA-DR(α1*0101,β1*0401) (hereafter referred to as DR0401) transgenic mice to isolate T cell hybridomas, and a rapid screening assay to map the immunodominant epitopes recognized by these hybridomas. We have defined the immunodominant epitopes for GAD65 and demonstrated that these epitopes are processed by human antigen-presenting cells (APCs) and presented in an HLA-DR4-restricted manner.

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Abbreviations: APCs, antigen-presenting cells; EBV, Epstein–Barr virus; FACS, fluorescence-activated cell sorter; GAD, glutamic acid decarboxylase; GAD65, GAD isoform 2; HAT, hypoxanthine/aminopterin/thymidine; hCD4, human CD4; IL-2, interleukin 2; MHC, major histocompatibility complex; TCR, T cell antigen receptor; DR040*n*, HLA-DR(α1*0101,β1*040*n*).

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Moreover, some of these epitopes have also been found to elicit T cell responses in DR0401 IDDM patients.

MATERIALS AND METHODS

Generation of HLA-DR Transgenic Mice. All animals used in this study were bred and maintained at the research animal facility at Stanford University. DR0401 mice co-expressing human CD4 (hCD4) were generated as described previously (14). Transgenic mice expressing DR0401 and hCD4 on the DBA/1J background were mated with I-A$\beta^{0/0}$ mice [obtained from D. Mathis and C. Benoist, Strasbourg (15)]. Offspring were screened for HLA-DR and hCD4 expression by fluorescence-activated cell sorter (FACS) analysis of peripheral blood mononuclear cells, using anti-HLA-DR (L243) and anti-hCD4 antibodies (Beckton Dickinson Immunocytometry Systems). DR0401, hCD4, I-A$\beta^{+/0}$ mice were backcrossed to I-A$\beta^{0/0}$ mice and the DR4^{+}, I-A$\beta^{0/0}$ progeny were intercrossed to generate mice also homozygous for DR0401 and hCD4.

Expression and Purification of Recombinant Human GAD. Human cDNA for GAD65 was obtained as two fragments (A. Lernmark, University of Washington, Seattle). These fragments were joined and a 6-His sequence was introduced immediately after the last codon of the open reading frame by using standard cloning techniques. This *Not*I–*Eco*RI cassette was subcloned into the pVL1393 baculoviral vector (Invitrogen).

This vector was used to generate recombinant baculovirus, using standard methods, and GAD65 was expressed in Sf9 insect cells. Purification was achieved by Ni^{2+} chelate affinity chromatography of a cleared lysate obtained from guanidine hydrochloride-lysed cells infected for 3 days. Preparations were separated by SDS/PAGE and the specific band was visualized by KCl staining and purified to homogeneity by electroelution. Purity was checked by analytical SDS/PAGE and Western blot analysis using anti-GAD antibody (G6 mAb from S. Baekkeskov, University of California at San Francisco).

Generation of T Cell Hybridomas. The DR0401, hCD4, I-A$\beta^{0/0}$ (C-line) mice were immunized in the footpads and at the base of the tail with 50 μg of gel-purified protein in incomplete Freund's adjuvant (IFA; Difco). These mice were homozygous for the HLA-DR4 transgene and had a peripheral CD4^{+} T cell count between 20% and 30%. After 10 days, the draining lymph nodes were removed. Lymph node cells were resuspended at 10^7 cells per ml in complete medium [RPMI 1640 medium (Bethesda Research Laboratories) containing 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml streptomycin, 50 mM 2-mercaptoethanol, and Hepes] and restimulated *in vitro* for 3 days with 10 μg/ml gel-purified human GAD65. At the end of the stimulation period, cells were purified by Ficoll separation, washed, and cultured overnight with interleukin 2 (IL-2)-conditioned medium. Cells were fused to the T cell antigen receptor (TCR) $(\alpha\beta)^-$ variant of the BW5147 cell line at a ratio of 1:3 in 50% polyethylene glycol by standard techniques and plated out in flat-bottomed 96-well plates. Hypoxanthine/aminopterin/thymidine (HAT) selection medium was added at days 2 and 3 after plating, and the plates were incubated for 10 days. Growing hybridomas were expanded to 24-well plates prior to testing.

Generation of Overlapping Peptides. A set of 115 overlapping peptides (15-mers) spanning the entire human GAD65 sequence were synthesized using the PIN synthesis strategy (Chiron Mimotopes, Clayton, Australia; peptides are named by putting "p" before the position numbers). These peptide sequences were shifted by a frame of 5 amino acids, thus generating all possible peptide 10-mers of GAD65. Two control peptides were also synthesized to verify purity and assess yield. Peptides were resuspended in PBS at a concentration of 1 mg/ml and stored at −20°C.

Testing Hybridoma Reactivity by Using a Europium-Based IL-2 Immunoassay. *APCs.* Transgenic mouse spleen cells or Epstein–Barr virus (EBV)-transformed lymphoblastoid cells were washed in HAT medium (complete medium with HAT) prior to irradiation (spleen cells, 3,000 rads; EBV-transformed cells, 12,000 rads). Antigen or peptides were added to the APCs (3×10^5 transgenic cells or $1–3 \times 10^5$ EBV-transformed cells added per well in a final volume of 50 μl), in round-bottomed 96-well plates. Plated cells were incubated with antigen while T cell hybridomas were prepared.

Antigen. Recombinant antigen was isolated by Ni^{2+} columns followed by gel purification. Gel-purified proteins were resuspended in PBS and used at 5–10 μg/ml. Individual peptides and pools were used at a final concentration of 1–2 μM. Overlapping peptides were pooled into 10 pools of 10–12 consecutive peptides for primary screening.

T cell hybridomas. T cell hybridomas were "washed" by aspiration of culture medium and resuspension in fresh HAT medium. To each well, 15×10^4 cells in 100 μl were added to the APCs.

IL-2 immunoassay. This was performed according to the protocol described in (16). Briefly, an IL-2 sandwich immunoassay format was used with a streptavidin–europium detection system. The resulting fluorescence was read using an LKB–Wallac (Gaithersburg, MD) fluorescence plate reader.

RESULTS

Mapping of Immunogenic Regions. We initially utilized DR0401, hCD4 transgenic mice on DBA/IJ or NOD backgrounds. Since complete Freund's adjuvant has been shown to affect the quality of the immune response (17), mice were immunized with antigen in incomplete Freund's adjuvant. The majority of hybridomas isolated from these mice were I-A restricted. To resolve this, we subsequently introduced the I-Aβ null mutation into the HLA-DR4 transgenic mice, to generate mice expressing DR0401 as the predominant MHC class II species; H-2q haplotype mice (DBA) do not express the I-Eα chain and so lack cell surface I-E molecules (18), but chimeric DRA*0101, I-Eβ heterodimers are expressed in these mice, albeit at low levels (less than 10% as assessed by FACS and the fact that less than 10% of specific hybridomas detected are restricted to the chimeric molecule—see below). FACS analysis of peripheral blood lymphocytes from nontransgenic I-A$\beta^{+/0}$, or HLA-DR4, I-A$^{0/0}$ mice in the presence or absence of hCD4 revealed that expression of homozygous DR0401, hCD4 transgenes was required to reconstitute the peripheral T cell compartment with CD4 T cells at levels comparable to nontransgenic I-A$\beta^{+/0}$ mice (Fig. 1).

Of 1,000 hybridomas, 216 were found to be specific for GAD65 by using splenic APCs from DR0401, hCD4, I-A$\beta^{0/0}$ mice. GAD65-specific hybridomas were then tested against pools of overlapping GAD65 peptides consisting of 10 or 12 consecutive peptides, using DR0401 human lymphoblastoid cells as APCs. Ninety-four hybridomas responded to a single GAD65 peptide pool. A small number (14 of 96 tested) of hybridomas were found to recognize EBV-transformed cells pulsed with whole GAD65 protein, but failed to respond to peptide pools, possibly reflecting the presence of an immunogenic minor contaminant in the gel-purified GAD65 used for immunization, and primary screening.

Each peptide pool-reactive T cell hybridoma was tested against individual peptides from the same pool. Fig. 2 *A* and *B* shows the experimental analysis performed for one representative hybridoma, specific for p271–285; this form of analysis was carried out for every single hybridoma. For each of the 10 identified epitopes, we verified MHC restriction by antibody blocking experiments. As shown in Fig. 2*C*, anti-DR antibodies

Generating the DRB1*0401 repertoire

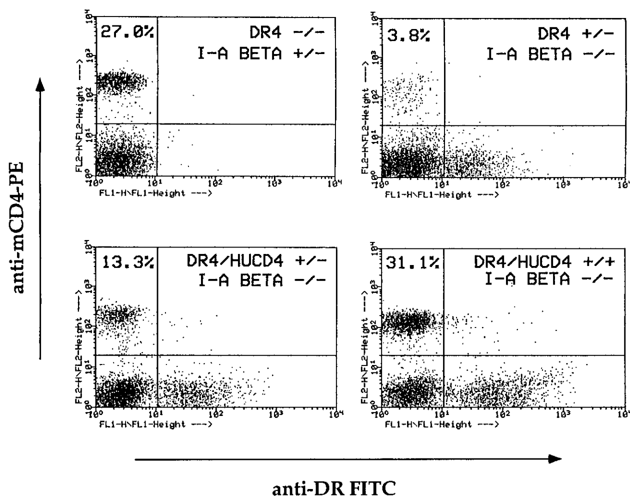

FIG. 1. The repertoire of mouse T cells in DR(α*0101,β*0401) transgenic mice. FACS analyses of peripheral blood lymphocytes from HLA-transgenic mice of different genetic background. Cells were stained using mouse CD4, hCD4, and human DR-specific reagents in standard protocols. FITC, fluorescein isothiocyanate; PE, phycoerythrin. (*Upper Left*) $CD4^+$ T cell levels of progeny of DBA/1J mice backcrossed once with I-A$\beta^{0/0}$ mice (27% $CD4^+$ T cells). (*Upper Right*) Analysis for a DR0401 mouse without hCD4 on an I-A$\beta^{0/0}$ background (3.8% $CD4^+$ T cells). (*Lower Left* and *Right*) Heterozygous (13.3% $CD4^+$ T cells) and homozygous (31.1% $CD4^+$ T cells) HLA-DR, hCD4 mice, respectively. The DR4 and hCD4 are part of a single transgene in the C-line mice.

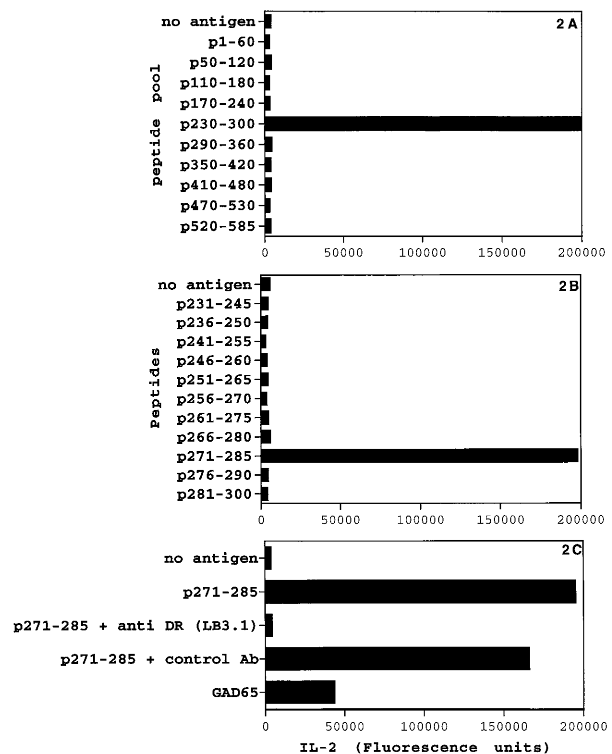

FIG. 2. Representative plot to demonstrate definition of epitope sequence. The analysis for hybridoma 216 is shown. IL-2 production was measured as fluorescence units. Analysis was carried out using DR0401-positive EBV-transformed cells as APCs. (*A*) Hybridoma 216 responds specifically to a pool containing peptides in the region p230–300. (*B*) Hybridoma 216 recognizes only p271–285 within that pool. (*C*) The T cell response is DR0401 restricted as shown by specific blocking of response in the presence anti-HLA-DR antibodies.

completely blocked the T cell response, whereas an isotype-matched control antibody had no effect on the antigen-specific response of the T cell hybridoma. We also found that multiple hybridomas responded to the intact, recombinant GAD65 protein when presented by human EBV-transformed cells as APCs (Fig. 2*C*). These data demonstrate that each epitope we have identified can also be generated and presented by human cells. The 10 DR0401-restricted peptide epitopes determined using these transgenic mice are listed in Table 1.

A small number (less than 10%) of T cell hybridomas specific for GAD65 (p246–260, p476–490) were reactive with transgenic murine cells as APCs, but not with EBV-transformed human cells as APCs. These T cell hybridomas were a result of presentation of the GAD65 peptides by DRA/I-Eβ chimeric molecules as confirmed in independent experiments using APCs from DRA1*0101 single transgenic mice (data not shown).

Mapping of Core Epitope Sequences. For each of the immunodominant peptide epitopes, a set of truncated peptides were used in a comparative analysis to define the core epitope sequences necessary for stimulation of the T cell hybridomas. This is illustrated for p271–285 in Fig. 3. The hybridomas that recognize p271–285 were found to respond to either p266–280 and/or p271–285, or p271–285 and p276–290; in each case p271–285 elicited the stronger response. The truncated peptide analyses revealed that one set of hybridomas responds to LIAFTSEHS as a core epitope, while the other recognizes the FTSEHSHFS sequence. Hybridoma 91 (Fig. 3*A*) is an example of a hybridoma that specifically recognizes the former sequence. Removal of Ser-281 diminishes the response significantly, while removal of Leu-273 eradicates the response completely (Fig. 3*A*). Peptides containing the FTSEHSHFS sequence elicit no response with this hybridoma. Similarly, hybridoma 63 (Fig. 3*B*) is an example of a hybridoma that recognizes FTSEHSHFS. Loss of Phe-276 or Ser-284 leads to total loss of response to these peptides and thus defines these residues as the N- and C-terminal boundaries of the epitope (Fig. 3*B*). Again peptides containing LIAFTSEHS only do not elicit a response in this subset of hybridomas, demonstrating the precision of this mapping and the fine specificity of the response.

Other epitope core sequences were mapped in a similar manner; analysis with hybridoma 55 was used to assess the core epitope in region p116–130 (Fig. 3*C*). Peptides beginning at VMN . . . elicit the strongest response, thus favoring MNILLQYVV as the major epitope sequence in this region.

Table 1. Immunogenic peptides identified in this study

Position	Sequence	No. of hybridomas
116–130	**MNILLQYVV**KSFDRST	8
271–285	PR**LIAFTSEHS**HFSL	31*
	PRLIA**FTSEHSHFS**L	
356–370	KYKIWMHVDAAWGGG	1
376–390	KHKWKLSGVERANSV	1
481–495	L**YNIIKNREG**YEMVF	19
511–525	P**SLRTLEDNEE**RMSR	8
546–560	SYQPLGDKVNFFRMV	1
551–565	GDKVN**FFRMVISNPA**	14†
556–570	**FFRMVISNPAA**THQD	
566–580	ATHQDIDFLIEEIER	1

For each peptide, the sequence and the number of responding hybridomas is shown. Boldface sequences mark experimentally defined core immunogenic sequence.

*Refers to p271–285, which contains both epitopes.

†All 14 hybridomas respond to p551–565 and p566–580.

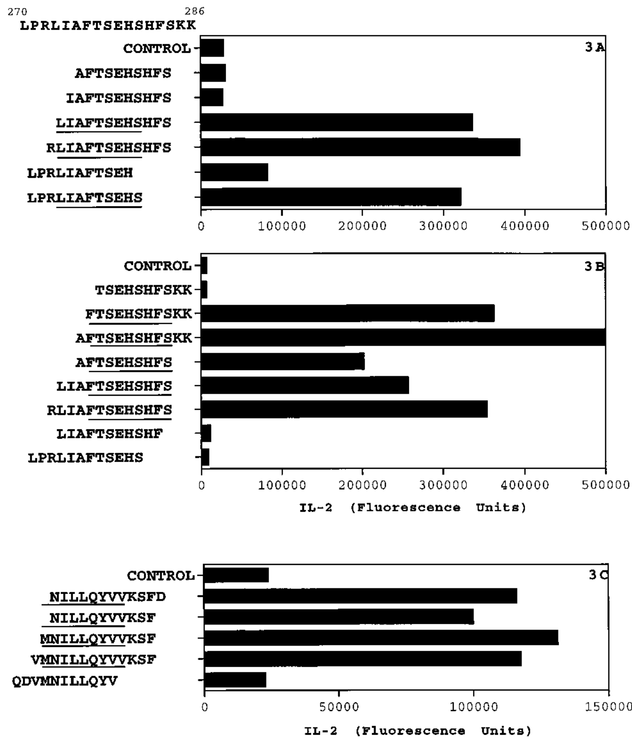

FIG. 3. Representative plots to demonstrate minimization analysis of epitope sequence. The specific epitope regions were mapped utilizing a set of variant peptides. These were designed to truncate the N and C termini of the putative core epitope as shown. The protocol used was as described for Fig. 2. (*A*) Hybridoma 91 recognizes LIAFTSEHS within p271–285. (*B*) Hybridoma 63 recognizes FTSEHSHFS within p271–285. (*C*) Hybridoma 55 recognizes (M)NILLQYVV within p116–130.

However, loss of Met-115 does not affect peptide recognition, and this may be due to a weak contribution by the methionine to the binding of this particular peptide to the MHC molecule. Peptides truncated from the C terminus of this peptide clearly define Val-123 to be essential for T cell recognition by T cell hybridomas, thus mapping Val-123 as P9 (position 9 of the MHC binding motif) and the core epitope sequence as p115–123 (Fig. 3*C*).

All hybridomas responding to p481–495 showed an increase in response to p478–492, thus suggesting a core motif of LYNIIKNREG. This includes a DR0401 motif (YNIIKNREG) and a DR0405 motif (LYNIIKNRE) (19). Using N- and C-terminal-truncated peptides revealed a critical need for Gly-490. At the N terminus, the removal of Leu-481 diminishes the response, but the removal of Tyr-482 abrogates it completely. Thus it is likely that the epitope in this region consists of YNIIKNREG with Leu-481, at position P1, optimizing binding.

For region p551–570 all hybridomas recognized p551–565 and p556–570 with equal intensity. Since both peptides contain a DR0401 binding motif, at an equally terminal position, it is likely that the core epitope sequence is FRMVISNPA. Indeed, a minimal peptide consisting of FFRMVISNPAA elicits a strong response in this subset of hybridomas.

All five p511–525-responding hybridomas tested recognized the 11-mer peptide SLRTLEDNEER. A summary of mapped core epitope sequences is shown in Fig. 4 and in boldface letters in Table 1.

DISCUSSION

We have identified six immunodominant regions of human GAD65 which are processed and presented by DR0401 molecules. These sequences are marked on the human GAD65 sequence shown in Fig. 4.

The DR0401 and DR0405 peptide binding motif-containing sequences of GAD65, as defined by Rammensee *et al.* (19) and identified using PMOTIF software (http://alces.med.umn.edu/pmotif.html), are also summarized in Fig. 4. The DR0401 peptide binding motif in ref. 19 contains a polar, charged, or aliphatic residue at position 7 and a polar, aliphatic, or Lys residue at position 9. As defined by Taylor (20), this should strictly preclude the presence of Gly at position 9; however, elution of peptides from DR0401 molecules clearly includes some with a Gly at position 9 (19). Thus, we used a motif less stringent for positions 7 and 9 to identify other "permissive" motif-containing peptides. It is clear that although three of the

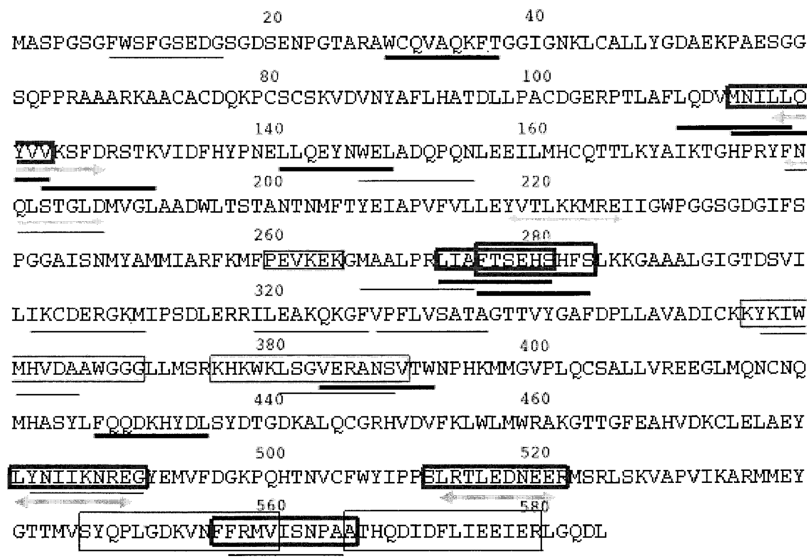

FIG. 4. Summary of identified immunodominant epitopes of GAD65. Boxed sequences represent mapped immunogenic sequences. Thick-lined boxes correspond to immunodominant regions; thin-lined boxes correspond to single-hybridoma-recognized regions. The PEVKEK sequence refers to the area of homology between GAD65 and Coxsackie viral sequence. The black underlining shows DR0401 peptide binding motif sequences (thin line, permissive motifs; thick line, strong motifs) and gray arrowed underlining indicates DR0405 sequences (thin line, permissive motifs; thick line, strong motifs) as predicted using PMOTIF software and a motif from ref. 17.

six major epitopes contain strong DR0401 binding motifs, two correspond to "permissive" sequences and one has no DR0401 binding motif. However, this epitope (p511–525) does contain a DR0405 peptide binding motif (see below).

Seven additional DR0401 motif-containing regions in GAD65 do not elicit T cell responses in DR0401 transgenic mice. Peptide binding motifs are based on peptide elution studies, or screening of phage display libraries (21), and so are biased toward identifying abundant or high-affinity peptides. Since peptides with a relatively low affinity for MHC molecules, such as Ac1–11 of myelin basic protein, have been demonstrated to be pathogenic, the above results are not unexpected, but serve to highlight the need for caution in utilizing pools of predicted HLA-binding peptides in isolating autoreactive T cells from patients, or for the identification of autoantigens, as previously postulated (22). The approach of using HLA-transgenic mice as a means to identify T cell epitopes may have the advantage of identifying a broader range of immunogenic T cell epitopes. Because murine T cell hybridomas can interact with human APCs of appropriate MHC genotype it is also possible to ensure that all identified immunogenic peptide epitopes are indeed processed by the human antigen-processing machinery; this should be another applied criterion for using peptide epitopes to test for responses in patients.

Wicker *et al*. (23) have recently identified p115–127 and p274–286 as immunogenic epitopes in their HLA-DR4 transgenic mice, using HLA-binding studies to predict immunogenic peptides. Three of the immunodominant epitopes reported here map within these two regions (p115–123, p273–281, and p276–284). In addition, two of the immunodominant epitopes reported by Endl *et al.* (24), p271–285 and p551–570, have previously been shown to be recognized by T cells isolated from a recent-onset DR0401 diabetic patient. For p271–285, Endl *et al*. fine mapped the epitope to be LIAFTSEHS, which contains a DR0401 binding motif. However, our results suggest that an additional T cell response may be directed against FTSEHSHFS (p276–284) which is in the same region. Lohmann *et al* (25, 26) also reported proliferative T cell responses to peptide region p473–555 in IDDM patients specifically. This region corresponds to epitopes (p481–495, p511–525) identified in our transgenic mice.

These data taken together underscore the validity of using HLA-transgenic mice to map immunodominant epitopes, and they also suggest that the potential differences between human and mouse antigen-processing and -presenting systems are minimal. We are in the process of testing the full panel of immunodominant GAD65 peptides identified here in patients with recent-onset IDDM. Preliminary data have shown that IDDM patients with a positive T cell proliferative response to whole GAD65 protein mount recall T cell proliferative responses to several of the immunodominant GAD65 peptides described here (M.C., S.D.P., and G.S.-M., unpublished results).

It is interesting that three of the immunodominant regions for GAD65 contain DR0405 motifs, and for p511–525, the DR0405 motif is the only known motif present. Since DR0401, DR0403, and DR0405 molecules are different at several amino acid positions in the peptide binding groove region, it was expected that these different HLA-DR4 subtypes would bind a different set of peptides. Indeed, peptide elution analysis showed such a difference between DR0401 and DR0405, with the latter showing a preference for peptides with an Asp, Glu, or Gln residue at position 9. This is probably due to the lack of Asp at position 57 of the DRB*0405 chain, allowing a charge interaction between the peptide and Arg-76 of DRA*0101, which normally forms a salt bridge with Asp-57 and thus also contributes to the stability of the MHC–peptide complex. The fact that p511–525 contains a DR0405 peptide binding motif raises the possibility that although some peptides may be immunogenic specifically for one allele, others may be immunogenic for several related alleles. A comparative analysis of epitope mapping in DR0403 and DR0405 transgenic mice should shed further light on this issue.

It has also been suggested that different alleles contribute to disease in opposing terms due to the difference in the peptides bound by them (27). This could translate into a different disease pathology due to a differential ability of the peptide sets to drive Th1 or Th2 responses (28). This may be due to the difference in the affinity of peptide binding or to an effect on the surface half-life of the MHC–peptide complex (29). In addition, MHC class II molecules play an important role in TCR repertoire selection in the thymus, which is also likely to have a major effect on immune response in patients with autoimmune diseases. Because humans have both HLA-DR and HLA-DQ genes participating in shaping the TCR repertoire, we are in the process of cross-breeding HLA-DR and HLA-DQ transgenic mice to develop transgenic mice with a T cell repertoire comparable to that of humans.

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