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T-cell reactivity to GAD65 peptide sequences shared with coxsackie virus protein in recent-onset IDDM, post-onset IDDM patients and control subjects

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Summary GAD65 is one of the major autoantigens associated with insulin-dependent diabetes mellitus (IDDM). The two peptides p17 and p18 of GAD65 that share sequence similarity with coxsackie virus (amino acid sequence identity: PEVKEK) appeared to be the major determinants of GAD65 recognized preferably by T cells from new-onset IDDM patients and their first degree relatives. In contrast, in our study unrelated control subjects frequently recognized the two GAD peptides (55 %, 16/29), similar to first degree relatives (41 %, 12/29) and IDDM patients post-onset (68 %, 15/22). However, recent-onset IDDM patients, responded less frequently (25 %, 4/16) compared with IDDM patients post-onset ($p < 0.03$) or unrelated control subjects (borderline significant) confirming previous observations in humans and NOD mice that T-cell reactivity to GADp17/p18 at diabetes onset is decreased. Moreover, this study demonstrated a positive correlation of T-cell proliferation to GAD p17 (amino acid 247–266) and p18 (amino acid 260–279) with simultaneous responses to both peptides in 13 % of all subjects tested ($n = 97$) ($p < 0.001$). T-cell proliferation to GAD p17 was higher than to p18 in recent-onset diabetic patients, first degree relatives and unrelated control subjects ($p < 0.02$, $p < 0.004$, $p < 0.002$, respectively). However, in post-onset IDDM patients, the two peptides were recognized equally well. Our results show that T-cell reactivity to GAD65 peptides homologous with coxsackie protein is very frequently observed, but not primarily associated with IDDM. The temporary decline of T-cell proliferation is not associated with the beta-cell destruction process, but with clinical manifestation. The positive correlation of reactivity to the two peptides in the viral motif implicates that PEVKEK is an immunogenic epitope. [Diabetologia (1997) 40: 332–338]

Keywords Autoreactivity, autoimmunity, human T-cells, GAD65, GAD autoantibodies, insulin-dependent diabetes, molecular mimicry.

Insulin-dependent diabetes mellitus (IDDM) is the result of a genetically associated autoimmune mediated process in which the insulin-producing pancreatic beta cells are thought to be destroyed by autoreactive T cells [1, 2]. The disease process is accompanied by autoreactive T cells and autoantibodies to various islet antigens such as GAD (glutamic acid decarboxylase) [3–5], insulin [6, 7], islet cells [8], tyrosine phosphatase (ICA512, 37K) [9–11], ICA69 [12–13] and 38k [14, 15].

Human GAD65 is mainly expressed in the central nervous system where it functions as the biosynthesizing enzyme of the inhibitory neurotransmitter gamma-aminobutyric acid. It is expressed at lower levels in alpha and beta cells in the islets of Langerhans of fetal and postnatal human pancreata, in islets of infants and to a lesser extent in islets of adults [16]. GAD is a major autoantigen in human IDDM and in

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Abbreviations: GAD, Glutamic acid decarboxylase; SI, stimulation index; IDDM, insulin-dependent diabetes mellitus; NOD, non-obese diabetic; PBMC, peripheral blood mononuclear cells.

non-obese diabetic (NOD) mice [17]. Autoreactive T cells directed to GAD65 are detectable early in the life of NOD mice and injection of GAD65 or GAD65 peptides (i. v., intrathymic, intranasal) [18–20] can delay or even prevent diabetes in NOD mice. Furthermore, GADp17 specific T cells derived from islet infiltrates of NOD mice are capable of transferring diabetes into healthy recipients [21]. In humans, autoantibodies to GAD65 precede diabetes [3, 22, 23] and appear in 64–84 % of newly diagnosed patients [3, 24], whereas less than 4 % of unrelated healthy control subjects are GAD65 antibody positive [25]. Autoreactive peripheral blood mononuclear cells (PBMC) of recent-onset diabetic patients and first degree relatives recognize the two isoforms GAD67 and GAD65 [26, 27]. T cell responses to GAD67 have been shown to be inversely related to GAD autoantibodies [28], whereas for GAD65 a positive relation between antibodies and T-cell recognition has been described [29]. The two GAD65 peptides designated p17 and p18 with significant sequence homology to the P2-C protein of coxsackie B virus appeared to be the major determinants of GAD65 recognized by new-onset IDDM patients and first degree relatives [30]. Although these peptides overlap by the 6 amino acids "PEVKEK" which are completely homologous in GAD65 and coxsackie B4 protein (but not in GAD67), PBMC proliferation was detected in either one of the peptides only. Responses to p18 appeared to be associated with HLA-DR4. T cells of unrelated healthy control subjects did not recognize GAD p17 or p18.

If indeed PBMC responses to these two GAD peptides are primarily measurable in prediabetic subjects and to a lesser extent at clinical onset of IDDM, such reactivity would be a useful predictive marker [17, 31]. To verify this predictive potential, we tested and extended our study to IDDM patients after disease onset to assess whether the decline in PBMC responsiveness was associated with beta-cell loss, or alternatively, would present a temporary dip in reactivity.

Subjects and methods

Probands. Informed consent was obtained from all subjects after the nature of the procedure was explained. Peripheral blood (35 ml) was drawn by venipuncture. Sixteen children (mean age 12 ± 4, range 6–17 years) with newly diagnosed IDDM from Denver, Colorado, USA were tested within 3 weeks after initiation of insulin therapy. Twenty-two age-matched IDDM patients (12 ± 3, 5–20 years) were investigated, who were treated with human insulin and had manifest diabetes for 6–12 months. Additionally, 29 unrelated healthy control subjects (22 ± 9, 5–42 years) and 13 first degree relatives of IDDM patients (24 ± 12, 8–43 years) with at least two islet antibodies (IAA, GAD65, ICA) considered to be relatives at increased risk [23] and 19 first degree relatives (22 ± 14, 7–49 years) with less than two antibodies (low-risk relatives) were investigated. The blood was used for investigation of lymphocyte autoreactivity, autoantibodies and HLA typing.

Determination of GAD autoantibodies. GAD65 antibodies were determined using a radiobinding assay as described previously [32]. The 99th percentile of normal range (index) was 0.032. In the Immunology of Diabetes Society Combinatorial Autoantibody Workshop (held in Orvieto, Italy, 1995) the assay showed 99.0 % specificity and 83.7 % sensitivity [33, 34].

Determination of HLA. HLA-DQAl was determined by PCR based techniques using the Ampli Type TM HLA DQalpha Forensic DNA Analysis kit (Perkin, Elmer, Branchburg, NY, USA) according to the manufacturer's instructions. HLA-DQBl was determined as described previously [35]. HLA-DR was assigned by linkage disequilibrium.

Antigens. The GAD65 peptides (purchased from Molecular Resources Center, National Jewish Hospital, Denver, Col., USA) from no. 17 (amino acid 247–266) and no. 18 (amino acid 260–279) derived from the human sequence of GAD65 [30] were tested at 5, 15 and 50 μg/ml. Tetanus toxoid (Wyeth Labs, Pearl River, NY, USA) was dialysed and tested at a concentration of 1 Limes Floculation/ml.

Lymphocyte stimulation assay. The T-cell proliferation assay was performed as described previously, modified by using autologous serum [36]. In brief, freshly isolated 150000 PBMC were cultured in round-bottomed 96-well plates for 6 days at 37 °C, 5 % CO_2 in 150 μl Iscove's modified Dulbecco's medium (Gibco BRL, cat. no. 430-2200, Gaithersburg, M. D., USA) containing 10 % autologous, normoglycaemic serum. Autologous serum was chosen to keep the in vitro experiments as close as possible to the situation in vivo. The PBMC were incubated in triplicate wells in the presence of either human GAD65 peptides, tetanus toxoid, interleukin-2 (IL-2) (10 % Lymphocult T; Biotest, Dreieich, Germany) or in medium alone.

After 5 days 10 μl Hank's Balanced Salt Solution (Gibco BRL) containing 0.5 μCi (= 37 kBq) [^{3}H]thymidine (NEN, Dupont, Boston, Mass., USA) was added per well and the incubation was continued for 18 h. Cultures were harvested and [^{3}H]thymidine incorporation was measured by liquid scintillation counting. The results of lymphocyte proliferation are expressed as Stimulation Indices (SIs) using the medians of a triplicate (cpm in the presence of antigen divided by cpm in the absence of stimulus [medium]). An SI of at least three was considered positive.

Statistical analysis

The magnitude of T-cell proliferation of the different groups was compared using Mann Whitney-U and Wilcoxon rank sum test, the frequencies of positive T cell responses were compared using Fisher's exact test. The relation between age, T cell proliferation and antibody responsiveness was determined by Rank Spearman correlation. Statistical analysis was performed with GraphPad InStat using Graph Pad computer software (Graph Pad Inc, San Diego, CA, USA).

Results

T-cell response to interleukin-2 and tetanus toxoid. Only experiments with PBMC proliferating to IL-2 were included in the analyses. T-cell proliferation to tetanus toxoid served as a diabetes unrelated control.

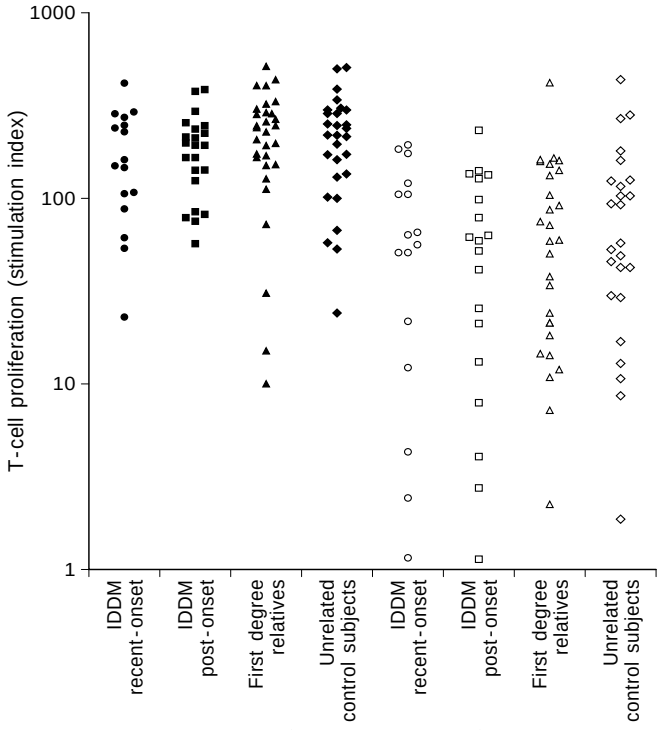

Fig. 1. T-cell proliferation (Stimulation index) to interleukin-2 (closed symbols) and tetanus toxoid (open symbols)

The groups did not differ in PBMC responsiveness to IL-2 or tetanus toxoid (Fig. 1). SIs obtained with tetanus toxoid were 10–15 times higher compared to responses to GAD65 peptides and reached similar levels as in previous studies, in which pooled human serum was used [36, 37].

T-cell recognition of GAD65 no. 17 and no. 18. Freshly isolated PBMC of IDDM patients, first degree relatives and unrelated healthy control subjects were cultured with GADp17 (247–266) and p18 (260–279) at three different concentrations. No particular concentration of p17 or p18 was preferentially recognized. Therefore, optimal T-cell responses of individual subjects to GADp17 and p18 were analysed further. T-cell responses to GADp17 and p18 were not related to age.

T-cell responses directed to p17 and p18 were found in all groups (Fig. 2). Recent-onset IDDM patients (25%, 4/16) recognized the two peptides less frequently than IDDM patients post onset (68%, 15/22) ($p < 0.03$) or unrelated control subjects (55%, 16/29) ($p = 0.05$). First degree relatives at increased risk responded (46%, 6/13), similar to 37% of relatives at low risk who recognized the peptides (6/16).

T-cell proliferation to p17 and p18 was positively correlated ($p < 0.001$) (Fig. 2). A simultaneous T-cell response (SI ≥ 3) to p17 and p18 was detected in 13.4% (13/97) subjects of whom five were IDDM patients post-onset ($n = 22$) and five were unrelated control subjects ($n = 29$). Only one recent-onset IDDM patient ($n = 16$) and two first degree relatives ($n = 29$) recognized p17 and p18 simultaneously (Fig. 2). Of all subjects tested 33% (32/97) recognized exclusively one of the two GAD peptides.

T-cell proliferation to p17 was higher than to p18 in recent-onset IDDM patients ($p < 0.02$), first degree relatives ($p < 0.004$) and unrelated healthy control subjects ($p < 0.002$) (Fig. 2). In contrast, T-cells from IDDM patients post-onset recognized the two peptides equally well.

GAD65 autoantibodies. Recent-onst IDDM patients were positive for GAD65 autoantibodies in 43.8% (7/16), post-onset IDDM patients in 72.7% (16/22), whereas all unrelated control subjects were GAD65 autoantibody negative (0%, 0/27, 2 not detected). In the group of first degree relatives, 37.9% (11/29) were GAD65 autoantibody positive. Thirteen first degree relatives were positive for at least two antibodies (IAA, ICA, GAD) and therefore defined "at high risk" [34]. GAD65 autoantibody titres were not related to age ($p = 0.626$).

T-cell proliferation to GADp18 but not to p17 was positively correlated with GAD65 autoantibodies investigating IDDM patients and first degree relatives ($p < 0.03$) or IDDM patients only ($p < 0.04$) (Fig. 3). Of all subjects tested, simultaneous T-cell responses and GAD65 autoantibodies were measured in 12.0% (9/75) for p17 and in 10.7% (8/75) for p18.

Table 1. Frequency of HLA-DQBl in IDDM patients, first degree relatives and unrelated control subjects. X is not HLA-DQB1 *0201, y is not HLA-DQB1 *0302

HLA DQB1 Group	Risk alleles DQB1 *0201, *0302			Protective alleles DQB1 *0602, *0603, *0301	
	0201/0302	0302/x	0201/y	2 protective alleles	at least 1 protective allele
IDDM patients	9/31 29%	6/31 19%	6/31 19%	0/31 0%	9/31 29%
First degree relatives	3/27 11%	5/27 19%	8/27 30%	1/27 4%	6/27 22%
Unrelated control subjects	1/27 4%	2/27 7%	5/27 19%	6/27 22%	17/27 63%

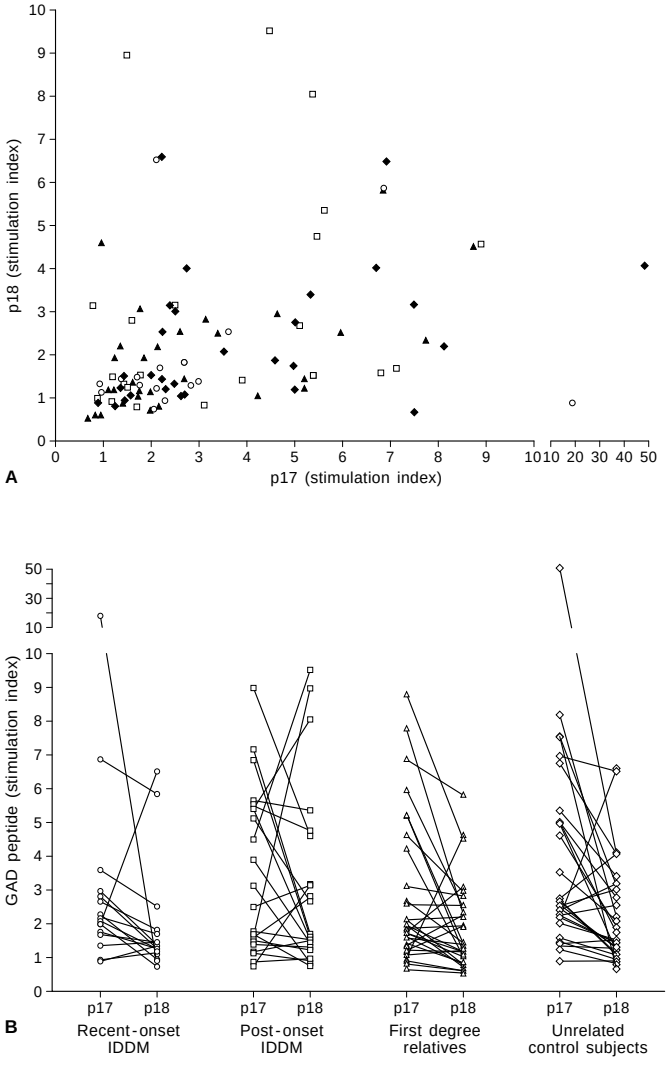

Fig. 2 A, B. Correlation of T-cell responses to GAD65 p17 and GAD65 p18. T-cell proliferation of first degree relatives at low risk and at increased risk did not differ and were pooled. **A** Positive correlation of T-cell responses to GAD65 p17 and GAD65 p18 ($p < 0.001$). ○, recent-onset IDDM patients; □, post-onset IDDM patients; ▲, first degree relatives; ◆, unrelated control subjects. **B** T-cell proliferation to GAD65 p17 were higher compared with T-cell responses to GAD65 p18 in recent-onset IDDM patients ($p < 0.02$), in first degree relatives ($p < 0.004$) and in unrelated control subjects ($p < 0.002$)

HLA determination. HLA-DQAl and Bl were determined and HLA-DR was assigned by linkage disequilibrium. As expected, recent-onset IDDM patients, patients with IDDM for 6–12 months and first degree relatives more often possessed the high-risk HLA-DQBl alleles (*0302 or *0201) and less frequently the protective alleles (DQBl*0602, DQBl*0602, DQBl*0301) than healthy unrelated control subjects (Table 1).

T-cell recognition of GAD65 p17 or p18 was not associated with a particular DQ haplotype. Of those subjects where T cells recognized p18 ($n = 23$) 47.8% were negative for HLA-DR4 which is different from the previously described HLA-DR4 association of T-cell responses to p18 [30].

Discussion

It has been hypothesized that the pathogenesis of IDDM may involve molecular mimicry of GAD65 and a coxsackie B protein [30, 38, 39]. The GAD65 amino acid region 247–279 and the P2-C protein of coxsackie virus B are 79% conserved and share 42% amino acid identity [30]. Peptides p17 and p18 span this region of sequence similarity and overlap in the PEVKEK region, which is 100% identical in GAD65 and P2-C-protein [30]. We analysed T-cell proliferation to GAD65 peptides p17 (amino acid 247–266) and p18 (amino acid 260–279) in recent-onset IDDM patients, IDDM patients post-onset, first degree relatives and control subjects.

Our study confirms the previous finding that T-cell reactivity is measurable in prediabetic subjects and to a lesser extent at clinical onset of IDDM, with virtually identical fractions [30]. However, we additionally found frequent T-cell recognition to p17/18 in unrelated control subjects (55% responders) which demonstrates that the two peptides are very immunogenic, but that PBMC proliferating to p17 or p18 are not associated with autoimmune diabetes and cannot be used as predictive markers. Although molecular mimicry to coxsackie B virus cannot be excluded by our observations, the link to the pathogenesis of IDDM at the T-cell level is unclear. It seems unlikely that differences between Atkinson et al. [30] and our assay conditions are responsible for the discrepant results in unrelated control subjects, since the frequency of T-cell responses of recent-onset IDDM patients and relatives was very similar in both assays. One likely explanation would be differences in the control groups tested. However, the different ages of the control groups (29 years [30] compared to 22 years (our study)), are unlikely to explain the different results, because T-cell responses to GAD p17/p18 tested by us were not related with age. Our observation that T-cell proliferation to islet antigen is not exclusive to IDDM patients is in concordance with investigations of other antigens such as insulin secretory granules [36], ICA69, lactoglobulin [40] and insulin [41, 42].

Recent-onset IDDM patients responded less frequently to GAD p17/p18 than IDDM patients post-onset ($p < 0.03$) and unrelated control subjects (borderline significant, $p < 0.06$), supporting the notion that T-cell reactivity to GAD p17/p18 is decreased at diabetes onset [18, 30]. This temporary decline in responsiveness at diabetes onset is distinct from T-cell responses to other antigens. T-cell proliferation to insulin secretory granules [36], ICA69 and insulin (N. C. Schloot, unpublished data) were found to be increased in recent-onset IDDM patients and

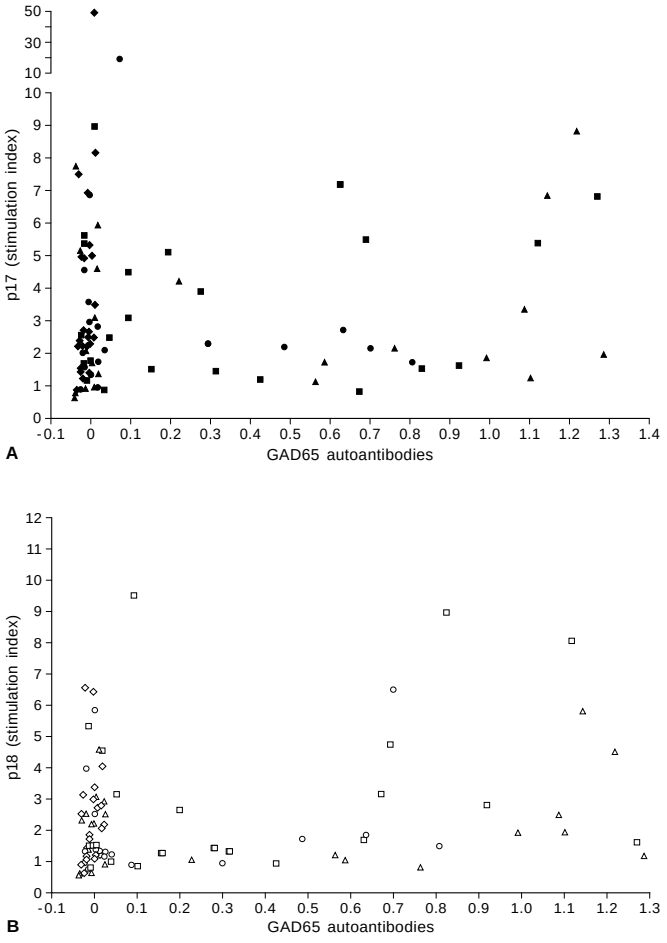

Fig. 3 **A** Relation of GAD65 antibodies and cellular recognition of p17. ●, recent-onset IDDM patients; ■, post-onset IDDM patients; ▲, first degree relatives; ◆, unrelated control subjects. **B** Relation of GAD65 antibodies and cellular recognition of p18 ($p < 0.04$ for IDDM patients with or without first degree relatives). ○, recent-onset IDDM patients; □, post-onset IDDM patients; △, first degree relatives; ◇, unrelated control subjects

decreased in IDDM patients post-onset and healthy control subjects. However, only longitudinal investigations of subjects in the prediabetic period, at the time of diabetes onset and after disease manifestation can validate these observations for GAD p17/p18.

Furthermore, T-cell responses to p17 and p18 were positively rather than inversely correlated ($p < 0.01$). Since the viral epitope is the only shared region between the two peptides, this positive correlation emphasizes PEVKEK as an immunogenic T-cell epitope. In addition to the simultaneous responses to p17 and p18, single peptide responses in our subjects were observed in 33.0% (22/97 for p17, 10/97 for p18) suggestive of the involvement of several epitopes. Proliferative responses to p17 or p18 in our experiments were not associated with HLA, in contrast to the previously described association of HLA-DR4 with T-cell proliferation to p18 [30]. T-cell responses to p17 were higher than to p18 in recent-onset IDDM patients, first degree relatives and unrelated control subjects ($p < 0.02$, $p < 0.004$, $p < 0.002$, respectively). Interestingly, investigations in NOD mice and humans have indicated likewise that GAD p17 is better recognized by T cells than p18 [18, 30]. IDDM patients post-onset however, recognized both GAD peptides equally well.

T-cell proliferation to GAD p17 was not correlated with GAD65 autoantibodies whereas GAD p18 did show a positive correlation with GAD65 autoantibodies ($p < 0.04$). Simultaneous appearance of T-cell proliferation to the GAD peptides p17/p18 and GAD65 autoantibodies was observed in 12% of all subjects tested (primarily consisting of IDDM patients post-onset), similar to studies investigating T-cell responses to whole GAD and GAD autoantibodies [29]. However, the two (linear) peptides tested with T cells represent only a short portion of GAD65 and a relation with GAD65 autoantibodies which are directed to several confirmational epitopes [38, 43] should be interpreted with caution.

The PEVKEK region of P2-C of coxsackie sharing homology with GAD65 is also present to a similar degree in a number of other species of animal and human picornaviruses [44]. Therefore, it will be important to test the viral equivalent of PEVKEK in similar studies to evaluate whether T cells can distinguish between the various viral proteins. Clearly, the NOD mouse model illustrates that IDDM can develop in the absence of coxsackie virus infection, which leaves endogenous as well as other unknown environmental factors as candidates for the initiation or prolongation of the beta-cell destruction process.

Our results demonstrate that T-cell reactivity to GAD65 peptides homologous with coxsackie protein is frequently found in IDDM patients, relatives and healthy unrelated control subjects. These observations together with the positive correlation of T-cell reactivity to GAD p17 and p18 emphasize that the viral motif PEVKEK is an immunogenic epitope, but that T-cell responses directed to this region are not necessarily indicative of IDDM. Similar to studies in NOD mice, T-cell responses to GAD p17 and p18 are decreased at diabetes onset with better recognition of GAD p17 than p18. The temporary decline of T-cell proliferation is not associated with the beta-cell destruction process, but with clinical manifestation, since after diabetes manifestation T-cell responses are increased and furthermore appear qualitatively altered. Further longitudinal characterization of GAD- and GAD peptide-reactive lymphocytes regarding epitope recognition, cytokine production and T-cell receptor utilization are warranted to characterize T-cell autoimmunity to GAD with regard to IDDM in more detail.

References

1. Atkinson MA, Maclaren NK (1994) The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 331: 1428–1436
2. Eisenbarth GS (1986) Type I diabetes mellitus. A chronic autoimmune disease. N Engl J Med 314: 1360–1368
3. Atkinson MA, Maclaren NK, Scharp DW, Lacy PE, Riley WJ (1990) 64,000 Mr autoantibodies as predictors of insulin-dependent diabetes. Lancet 335: 1357–1360
4. Christie M, Landin-Olsson M, Sundkvist G, Dahlquist G, Lernmark A, Baekkeskov S (1988) Antibodies to a Mr-64,000 islet cell protein in Swedish children with newly diagnosed type 1 (insulin-dependent) diabetes. Diabetologia 31: 597–602
5. Gerling I, Baekkeskov S, Lernmark A (1986) Islet cell and 64K autoantibodies are associated with plasma IgG in newly diagnosed insulin-dependent diabetic children. J Immunol 137: 3782–3785
6. Palmer JP, Asplin CM, Clemons P et al. (1983) Insulin antibodies in insulin-dependent diabetes before insulin treatment. Science 222: 1337–1339
7. Riley WJ, Atkinson MA, Maclaren NK (1988) Insulin autoantibodies in pre-diabetes. Adv Exp Med Biol 246: 45–51
8. Bottazzo GF, Florin-Christensen A, Doniach D (1974) Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet II: 1279–1282
9. Payton MA, Hawkes CJ, Christie MR (1995) Relationship of the 37,000- and 40,000-M(r) tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA512). J Clin Invest 96: 1506–1511
10. Christie MR, Genovese S, Cassidy D et al. (1994) Antibodies to islet 37k antigen, but not to glutamate decarboxylase, discriminate rapid progression to IDDM in endocrine autoimmunity. Diabetes 43: 1254–1259
11. Gianani R, Rabin DU, Verge CF et al. (1995) ICA512 Autoantibody radioassay. Diabetes 44: 1340–1344
12. Miyazaki I, Cheung RK, Gaedigk R et al. (1995) T cell activation and anergy to islet cell antigen in type I diabetes. J Immunol 154: 1461–1469
13. Roep BO, Duinkerken G, Schreuder GMT, Kolb H, De Vries RRP, Martin S (1996) HLA associated inverse correlation between T-cell and antibody responsiveness to islet autoantigen in recent-onset insulin dependent diabetes mellitus. Eur J Immunol 26: 1285–1289
14. Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark A (1982) Autoantibodies in newly diagnosed diabetic children immunoprecipitate specific human islet cell proteins. Nature 298: 167–169
15. Roep BO, Arden SD, De Vries RRP, Hutton JC (1990) T-cell clones from a type-I diabetes patient respond to insulin secretory granule proteins. Nature 345: 632–634
16. Mally MI, Cirulli V, Otonkoski T, Soto G, Hayek A (1996) Ontogeny and tissue distribution of human GAD expression. Diabetes 45: 496–501
17. Ellis TM, Atkinson MA (1996) The clinical significance of an autoimmune response against glutamic acid decarboxylase. Nature Medicine 2: 148–153
18. Kaufman DL, Clare-Salzler MJ, Tian JD et al. (1993) Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature 366: 69–72
19. Tisch R, Yang XD, Singer SM, Liblau RS, Fugger L, McDevitt HO (1993) Immune response to glutamic acid decarboxylase correlates with insulitis in non-obese diabetic mice. Nature 366: 72–75
20. Tian J, Atkinson MA, Clare-Salzler M (1996) Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. J Exp Med 183: 1561-1567
21. Schloot N, Gottlieb P, Wegmann D (1996) Characterization of diabetogenic, GAD65-specific T cell clones established from NOD mice. Diabetes 45: 307 (Abstract)
22. Wang Y, Hao L, Gill RG, Lafferty KJ (1987) Autoimmune diabetes in NOD mouse is L3T4 T-lymphocyte dependent. Diabetes 36: 535–538
23. Bingley PJ, Christie MR, Bonifacio E et al. (1994) Combined analysis of autoantibodies improves prediction of IDDM in islet cell antibody-positive relatives. Diabetes 43: 1304–1310
24. Christie MR, Brown TJ, Cassidy D (1992) Binding of antibodies in sera from type 1 (insulin-dependent) diabetic patients to glutamate decarboxylase from rat tissues – vidence for antigenic and non-antigenic forms of the enzyme. Diabetologia 35: 380–384
25. Hayball JD, Robinson JH, Ohehir RE, Verhoef A, Lamb JR, Lake RA (1994) Identification of two binding sites in staphylococcal enterotoxin-B that confer specificity for TCR V(beta) gene products. Int Immunol 6: 199–211
26. Atkinson MA, Kaufman DL, Campbell L (1992) Response to peripheral-blood mononuclear cells to glutamate decarboxylase in insulin-dependent diabetes. Lancet 339: 458–459
27. Honeyman MC, Cram DS, Harrison LC (1993) Glutamic acid decarboxylase 67-reactive T cells: a marker of insulin-dependent diabetes. J Exp Med 177: 535–540
28. Harrison LC, Honeyman MC, De Aizpurua H (1993) Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin-dependent diabetes. Lancet 341: 1365–1369
29. Ellis TM, Darrow B, Campbell L, Atkinson MA (1995) Inverse relationship between humoral and cellular immune responses to glutamate decarboxylase 65 (GAD) and insulin in IDD. Diabetes 44s: 52A (Abstract)
30. Atkinson MA, Bowman MA, Campbell L, Darrow BL, Kaufman DL, Maclaren NK (1994) Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. J Clin Invest 94: 2125–2129
31. Atkinson M, Leslie D (1994) Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin-dependent diabetes – Comment. J Endocrinol Invest 17: 581–584
32. Yu L, Chase HP, Falorni A, Rewers M, Lernmark A, Eisenbarth GS (1995) Sexual dimorphism in transmission of expression of islet autoantibodies to offspring. Diabetologia 38: 1353–1357
33. Yu L, Gianani R, Eisenbarth GE (1994) Quantitation of glutamic acid decarboxylase autoantibody levels in prospectively evaluated relatives of patients with type 1 diabetes. Diabetes 43: 1229–1233
34. Verge CF, Gianani R, Kawasaki E et al. (1996) Number of autoantibodies (against insulin, GAD or ICA512/IA2) rather than particular autoantibody specificities determines risk of type 1 diabetes. J Autoimm 9: 379–383
35. Bugawan TL, Erlich HA (1991) Rapid typing of HLA-DQB1 DNA polymorphism using nonradioactive oligonucleotide probes and amplified DNA. Immunogenetics 33: 163–170
36. Roep BO, Kallan AA, Duinkerken G et al. (1995) T-cell reactivity to beta-cell membrane antigens associated with beta-cell destruction in IDDM. Diabetes 44: 278–283

37. Nell LJ, Thomas JW (1983) The human immune response to insulin. J Immunol 131: 701–705
38. Kaufman DL, Erlander MG, Clare-Salzler MJ et al. (1992) Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest 89: 283–292
39. Hyoty H, Hiltunen M, Knip M (1995) A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. Diabetes 44: 652–657
40. Vaarala O, Klemetti P, Savilahti E, Reijonen H, Ilonen J, Akerblom H (1996) Cellular immune response to cow's milk β-lactoglobulin in patients with newly diagnosed IDDM. Diabetes 45: 178–182
41. Keller RJ (1990) Cellular immunity to human insulin in individuals at high risk for the development of type I diabetes mellitus. J Autoimmun 3: 321–327
42. Durinovic-Bello I, Hummel M, Ziegler AG (1996) Cellular immune response to diverse islet cell antigens in IDDM. Diabetes 45: 795–800
43. Richter W, Shi YG, Baekkeskov S (1993) Autoreactive epitopes defined by diabetes-associated human monoclonal antibodies are localized in the middle and C-terminal domains of the smaller form of glutamate decarboxylase. Proc Natl Acad Sci USA 90: 2832–2836
44. Argos P, Kamer G, Nicklin MJH, Wimmer E (1984) Similarity in gene organization and homology between proteins of animal picornavirus and a plant comovirus suggest common ancestry of these virus families. Nucleic Acids Res 12: 7251–7267