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Relating homology between the Epstein-Barr virus BOLF1 molecule and HLA-DQw8 β chain to recent onset Type 1 (insulin-dependent) diabetes mellitus

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Summary. A role for the Epstein-Barr virus in initiating Type 1 (insulin-dependent) diabetes mellitus has been proposed since Epstein-Barr virus BOLF1(497–513) *AVTPL* RIFIVP*PAAEY* has an 11 amino acid identity with HLA-DQw8β(49–60) *AVTPL GPPAAEY*. Rabbit antisera to the BOLF1(496–515) peptide crossreacted with the homologous DQw8β (44–63) peptide but not with the related DQw7β(44–63) peptide, which differed from the DQw8 peptide only in an ALA to ASP substitution in position 57. Antisera to DQw8β(49–60) reacted with the DQw8β(44–63) peptide and BOLF1 (496–515), but not with DQw7β(44–63). The antiserum to the BOLF1 peptide bound to denatured class II major histocompatibility complex β chains from Epstein-Barr virus-transformed DQw8-positive lymphocytes in an immunoblotting analysis. Epstein-Barr virus antibodies were detected at equal frequencies and similar titres in sera of 30 patients with Type 1 diabetes (16 of 30; 63%) and in sera of 20 non-diabetic control subjects (13 of 20; 65%). Sera from diabetic patients did not bind to DQw8β (44–63) or BOLF1(496–515) peptides. From these data we conclude that there is no simple relationship between serological evidence of Epstein-Barr virus infection and crossreactions between homologous Epstein-Barr virus and class II major histocompatibility complex peptides.

Key words: Type 1 (insulin-dependent) diabetes mellitus, molecular mimicry, Epstein-Barr virus, class II MHC molecules, EBV BOLF1

The molecular mimicry hypothesis for the origin of autoimmune disease proposes that sequence homology between a protein of a pathogen and an organ-specific, self protein can trigger an autoimmune response [1]. Evidence for this hypothesis is found in peptide homologies between *Klebsiella pneumoniae* nitrogenase and HLA-B27 in the case of ankylosing spondylitis [2], and between adenovirus E1B protein and α-gliadin in coeliac disease [3]. For Type 1 (insulin-dependent) diabetes mellitus, acute coxsackie virus B4, persistent cytomegalovirus, and rubella virus infections may precede the clinical onset [4–7], but diabetes-eliciting sequences of these viruses have not been proposed. Environmental factors must contribute to the pathogenesis of Type 1 diabetes, since the concordance rate for the disease in monozygotic twins is about 35% [8]. Genetics also influence the incidence of the disease, since certain class II major histocompatibility complex (MHC) alleles are closely linked to disease susceptibility [9]. The role of those alleles of class II MHC antigens, especially of HLA-DQ, in the pathogenesis of Type 1 diabetes remains obscure. HLA-DR4 is in linkage disequilibrium with two different alleles of HLA-DQ, -DQw7, and -DQw8, of which the DQw8 allele is increased among Type 1 diabetic patients [10]. Within the first domain, these two β-chain alleles differ only by 4 amino acids at positions 13, 26, 45, and 57 and it has been suggested that the residue in position 57 may be particularly important [10, 11].

Linkages between MHC alleles and diabetes could reflect either MHC restriction of presentation of foreign antigens, and homologous autoantigens, or presentation of a segment of the MHC molecule itself. Homologies between antigens and peptides forming the antigen-binding site (desetope) of class II MHC molecules have been suggested as leading to tolerance [12]. Highly conserved, basic dipeptide sites about the class II MHC desetopes could lead to excision of such segments of those molecules and presentation to the immune system (L. J. Thomas, V. Lam, D. Kostyal, R. E. Humphreys, unpublished observations). In looking for an infectious pathogen which might initiate Type 1 diabetes, Dyrberg et al. found that the Epstein-Barr virus (EBV) BOLF1(497–513) sequence *AVTPL* RIFIVP*PPAAEY* is homologous to HLA-DQw8 or 3.2β (49–60) *AVTPL GPPAAEY* [13]

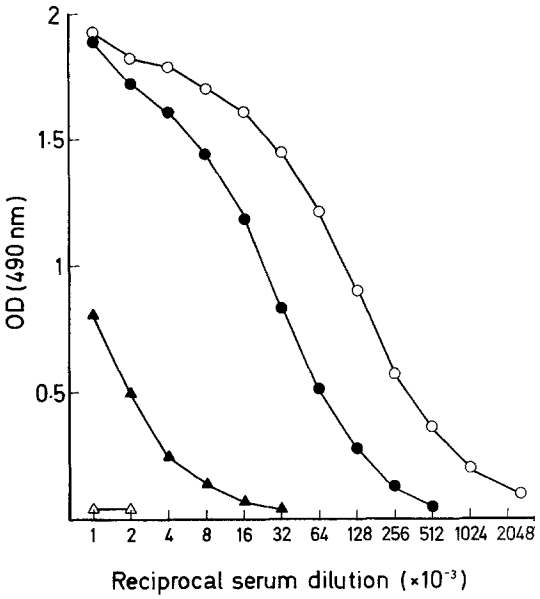

Fig. 1. Immunological crossreactivity of antiserum R883 to BOLF1 (496–515) with several peptides. Peptides absorbed to the ELISA plate: ○—○ BOLF1(496–515), ●—● HLA-DQw8β (44–63), ▲—▲ HLA-DQw7β(44–63), △—△, irrelevant peptide (papilloma virus E2 protein(76–84) (CVLHLESLKD))

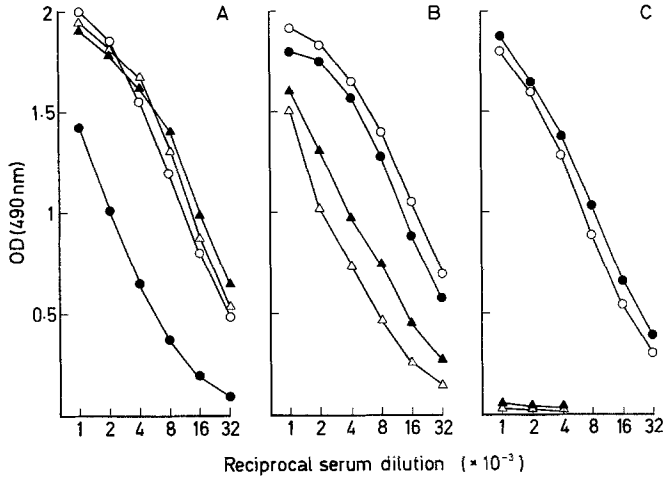

Fig. 2 A–C. Differential binding of rabbit anti-peptide sera to HLA-DQ β-chain molecules. Peptides absorbed in ELISA plates: **A** HLA-DQw7β (44–63); **B** HLA-DQw8β (44–63); **C** BOLF1 (496–515). Antisera to: HLA-DQw7β (49–60), △—△ R1626, ▲—▲ R1627; HLA-DQw8β(49–60), ●—● R1630, ○—○ R1631

(Table 1). BOLF1 is a gene for a hypothesized product of unknown function from BamHI restriction endonuclease fragment O, leftward open reading frame in the B95-8 EBV genome [14]. Furthermore, HLA-DQw8β (53–60) brackets the 57th residue which is strongly linked to diabetes susceptibility (VAL^{57}, SER^{57}, ALA^{57}) or resistance (ASP^{57}) [10, 11]. That DQβ-chain sequence, including the diabetes-resistance-correlated ASP^{57}, is at the end of a helix forming one wall of the antigen-binding site (desetope) [15]. We tested whether serological crossreaction occurred between the homologous sequences of DQw8β and BOLF1, and we analysed sera of recent onset, Type 1 diabetic patients for the presence of EBV antibodies.

Materials and methods

Peptide syntheses and immunizations

Peptides were synthesized by a solid-phase method [16] and purified by step gradient elution from a reverse-phase column in 2% acetic acid with increasing concentrations of acetonitrile. The identity of each peptide was confirmed by analyses of amino acid composition and sequence. Antisera to DQw7β(49–60), DQw8β(49–60) [17] and BOLF1(496–515) were obtained after immunization of rabbits with keyhole limpet haemocyanin-coupled peptides. Peptide-specific antibodies to BOLF1(496–515) were purified by affinity chromatography on peptide-coupled Affi-Gel 10 (BioRad, Richmond, Calif., USA) [18]. The peptide-bound gel was incubated with antiserum, washed with 50 mmol/l Hepes buffer, 1 mmol/l CHAPS, pH 7.5, and the specific antibodies were eluted in 1 mol/l acetic acid and neutralized.

ELISA

Microtitre plates were incubated with 5 µg/ml peptide in 50 mmol/l Na_2CO_3/$NaHCO_3$ buffer, pH 9.6, overnight at room temperature and were washed three times in phosphate-buffered saline solution (PBS) with 0.1% Tween 20 [19]. The plates were incubated with antisera or sera diluted in PBS containing 1% BSA and 0.1% Tween 20, at room temperature for 1.5 h, washed and incubated with peroxidase-conjugated $F(ab')_2$ goat-anti-rabbit IgG or goat-anti-human IgG (Zymed, South San Francisco, Calif., USA) for 1.5 h at room temperature. After washing, substrate (0.4 mg/ml 0-phenyldiamine and 0.03% hydrogen peroxide) was added. The reaction was stopped after 30 min with 2 mol/l H_2SO_4 and the absorbance was measured at 490 nm. For competitive ELISA experiments, peptides were added to antisera prior to addition of those mixtures to peptide-coated, microtitre plates [19].

Immunoblotting assays

EBV-transformed lymphoblasts (10^7 cells) were homogenized in 10 mmol/l Hepes buffer, pH 7.4, containing 0.25 mol/l sucrose, 10 mmol/l benzamidine, 0.1 mmol/l p-chloromercuriphenyl sulfonic acid and 0.5% (weight/volume) aprotinin, and were centrifuged at 4 °C for 30 min at 35000 × *g* [17]. The particulate fraction was solubilized for 2 h at 4 °C in 10 mmol/l Hepes, pH 7.4, with 150 mmol/l NaCl, 10 mmol/l benzamidine, 0.63% (weight/volume) aprotinin and 2% Triton X-114. The lysate was centrifuged for 15 min at 4 °C at 10000 × *g* to remove nuclei and insoluble particles, and the supernatant was layered on a Hepes buffer with 6% sucrose [20]. The gradient was incubated at 30 °C for 5 min, and amphiphilic membrane proteins were obtained by centrifugation at 1 500 × *g* for 3 min [20]. The detergent-phase proteins were denatured under reducing conditions, subjected to SDS 10% polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose filters. For immunostaining, nitrocellulose strips were incubated with antisera, washed, incubated with $F(ab')_2$ goat-anti-rabbit IgG-alkaline-phosphatase (Zymed), washed, and incubated in substrate buffer (150 mmol/l NaCl, 5 mmol/l $MgCl_2$, 0.33 mg/ml nitroblue tetrazolium and 0.17 mg/ml 5-bromo-4-chloro-3-indolyl phosphate) [17].

Patients and control subjects

For the first study, sera were obtained from 10 Swedish children who were within 10 days of the diagnosis of Type 1 diabetes and were therapeutically plasmapheresed. Age, sex, and DR histotype are reported in Table 2 for each patient. In the second study, sera were obtained from 20 recent-onset, diabetic Swedish children and from

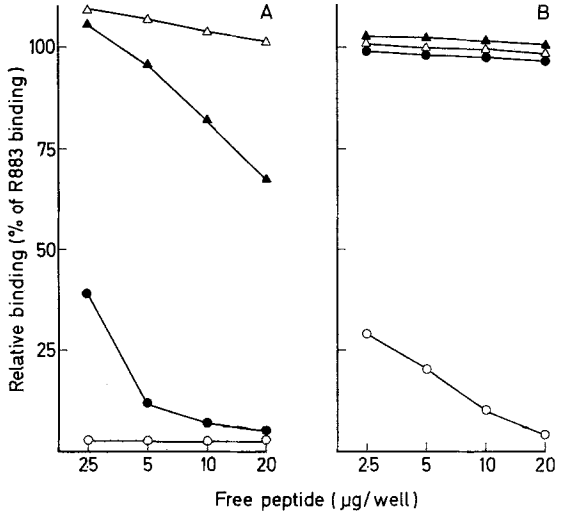

Fig. 3 A, B. Binding of anti-BOLF1(496–515) serum R883: competition analysis with free peptides. Peptides absorbed to ELISA plate: **A** HLA-DQw8β (44–63); **B** BOLF1(496–515). Competing peptides: ●—● HLA-DQw8β (44–63); ○—○ BOLF1(496–515); ▲—▲ HLA-DQw7β (44–63); △—△ irrelevant peptide (papilloma virus E2 protein (76–84))

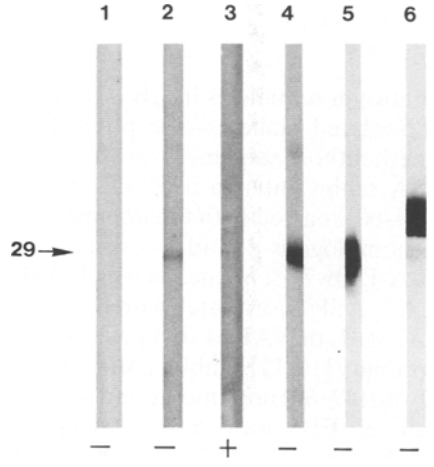

Fig. 4. Immunoblot analysis of EBV-transformed HLA-DQw8-positive lymphocytes. Binding of anti-BOLF1(496–515) serum R883 (diluted 1:25) to 29 kilodalton protein. *(1)* preimmune serum, *(2)* post-immune serum, *(3)* post-immune serum with excess of free BOLF1 (496-515) peptide, *(4)* R1630 antiserum (diluted 1:100) to HLA-DQw8β (49–60), *(5)* monoclonal antibody to HLA-DR/DQ β chains, *(6)* monoclonal antibody to HLA-DR α chain

20 non-diabetic Swedish children who were matched with the patients for age, sex, and year of presentation. In addition, sera from the following individuals were screened for antibodies to BOLF1 (496–515) and DQwβ (44–63): 45 patients between 10 and 20 years with Type 1 diabetes with less than 3 months duration of diabetes when the sample was obtained at the Steno Memorial Hospital, Gentofte; 10 adults with Type 1 diabetes of 2–5 years duration, and 10 adult patients with a disease duration of 8–20 years and 10 age-matched control subjects.

Assays of EBV antibodies

Antibodies to EBV-associated early antigen (EA) [21, 22], viral capsid antigen (VCA) [23], and nuclear antigen (EBNA) [24] were quantitated by indirect immunofluorescence. Fluorescein isothiocyanate (FITC)-conjugated anti-human IgG or IgM antibodies (Cappel, Malvern, Penn., USA) were used for the EA and VCA determinations. Antibodies to EBNA were determined by a three-stage, anti-complement immunofluorescence assay [25].

Assays for pancreatic islet cell antibodies (ICA) and insulin autoantibodies (IAA)

Beta cell-specific autoantibodies, ICA, were assayed by a two-colour immunofluorescence technique in which a patient's serum was incubated with slides of human pancreatic tissue in the presence of a monoclonal antibody to proinsulin. FITC-labelled, anti-human IgG and Texas red-labelled, anti-mouse IgG served as second antibodies. ICA titres from serial dilutions of the sera were converted to Juvenile Diabetes Foundation (JDF) units with a standard curve generated from the JDF International reference sera [26, 27]. IAA was determined in a radioligand binding assay [28]. The BOLF1 (496–515) peptide was ^{125}I-labelled by the chloramine-T method [29] and separated from free iodide by step gradient elution from a reverse phase column. Serum was incubated for 60 min at 4 °C with ^{125}I-BOLF1(496–515) at 250000 cpm/sample in a final volume of 150 μl. That mixture was incubated for 30 min 4 °C with protein A-Sepharose, 5 mg/sample, and immune complexes were isolated by centrifugation, washed twice, and the precipitated radioactivity was counted.

Results

In a comparison of the sequence of HLA-DQw8β to proteins of the Protein Identification Resource [30], a region was found with 11 of 17 residues shared with an EBV protein, BOLF1 [13], (Table 1). To determine whether these sequences crossreacted serologically, antisera to each peptide were tested by ELISA methods. Anti-BOLF1 (496–515) bound to the BOLF1 and DQw8β (44–63) peptides with a high titre, but to the DQw7β (44–63) peptide with a much lower titer, and not at all to an irrelevant peptide, papilloma virus, E2 protein (76–84) (Fig. 1). Antisera to DQw8β (49–60) bound DQw7β (44–63) (A), DQw8β (44–63) (B), and BOLF1(496–515) (C) (Fig. 2). In contrast, antisera to DQw7β (49–60) bound the DQw7β (44–63) and DQw8β (44–63) peptides, but not the BOLF1 (496–515) peptide. The binding of the BOLF1 antisera to DQw8β (44–63) was inhibited by DQw8β (44–63) and by the BOLF1 peptide, but not by the DQw7β or the irrelevant papilloma virus peptide (Fig. 3). The binding of anti-BOLF1 serum to BOLF1(496–515) was inhibited by the BOLF1 peptide, only. Affinity-purified antibodies to BOLF1(496–515) showed the same binding characteristics as did the antiserum (data not shown).

Antisera from three of the four rabbits immunized with the BOLF1 peptide immunoblotted to a 29 kilodalton (kd) protein, presumably the class II MHC β chain, from HLA-DQw8-positive, transformed lymphoblasts (Fig. 4). The binding to the 29 kd protein, was inhibited by BOLF1(496–515) or DQw8β (44–63) peptides, but only partly by DQw7β (44–63) or the irrelevant papilloma virus peptide. Similarly, affinity-purified antibodies to the BOLF1 peptide showed specific binding to the 29 kd protein in immunoblotting analysis (data not shown).

In order to determine whether recently diagnosed Type 1 diabetic patients had been infected with EBV, we tested sera from 10 children treated with plasmapheresis

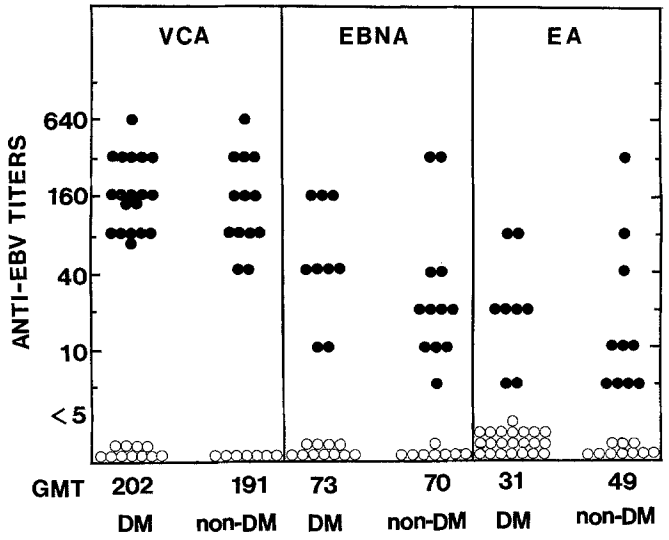

Fig. 5. Epstein-Barr virus (EBV) antibodies in patients' sera with or without Type 1 (insulin-dependent) diabetes mellitus. DM, Type 1 diabetes mellitus (insulin-dependent); non-DM, control; GMT, geometric mean titre; VCA – viral capsid antigen; EBNA – EBV nuclear antigen; EA – early antigen

at the time of clinical onset. These sera (Nos. 1–10 of Table 2) each contained EBV antibodies, as shown in IgG VCA titres ranging from 1:80 to 1:640. Since IgM anti-VCA antibodies, which are detected in acute stages of the infection, were not detected, the patients were considered to have past, but not acute or early convalescent phase infections as seen in the course of infectious mononucleosis [31]. An IgG anti-EA titre $\geq 1:20$ was detected in only sera of patients with acute or chronic EBV infection [31]. One serum (No. 8) had abnormally high titres to VCA and EA, indicating a late stage of acute infection or a chronic infection.

In light of the concordance between EBV infection and diabetic status in these 10 patients, additional sera from diabetic patients and control subjects were tested (Nos. 11–50 of Table 2). EBV antibodies to both VCA and EBNA were detected, respectively, in 9 of 20 and 12 of 20 sera of the patients and control subjects. Anti-EA antibodies ($\geq 1:20$) were detected in 6 of 30 (20%) or 3 of 20 (15%) of the patients and control subjekts, respectively (Table 2 and Fig. 5). These anti-EA-positive individuals (No. 8, 15, 24, 27, 30, 34, 36, 38, and 39) must have had acute or chronic EBV infections, but no IgM anti-VCA antibody was detected (Table 2). There was no correlation between diabetic status and infection with EBV (Fig. 5). Also, among diabetic patients with EBV antibodies, there was no correlation between ICA and EBV antibodies (Table 2).

The presence of anti-BOLF1(496–513) antibodies in sera of diabetic patients was tested for by an ELISA. The binding to BOLF1(496–515) and DQw8β(44–63) induced by sera at a final dilution of 1:24, from 10 diabetic patients with a disease duration of 2 to 5 years (absorbance at 492 nm, mean ± SD: 0.076 ± 0.038 and 0.071 ± 0.037) and from 10 patients with 8 to 20 years disease duration (0.061 ± 0.019 and 0.059 ± 0.016 respectively) was not different from that induced by 10 control sera to the same

Table 1. Homologous amino acid sequences between an Epstein-Barr virus BOLF1 and HLA-DQ β-chain molecules

Protein	Residues	Amino acids
EBV BOLF1	496–516	D*AVTPL*RIFIV*PPPAA* *EY*EQV
HLA-DQw8 βchain	43– 63	DVGVYR*AVTPLGPPAAEY*WNS
HLA-DQw7 βchain	43– 63	DVEVYR*AVTPLGPPD*A*EY*WNS

peptides (0.107 ± 0.067 and 0.066 ± 0.042, respectively) or to uncoated ELISA plates (0.091 ± 0.056). The sera of 45 recent onset diabetic children were also tested for binding to ^{125}I-BOLF1(496–515) in a radioligand assay. At a final serum dilution of 1:25 the mean binding (275 ± 22 cpm) was not different from the non-specific binding of the radiolabelled peptide to protein A-Sepharose (276 ± 10 cpm). In contrast, rabbit antiserum to BOLF1(496–515) at a final dilution of 1:1000 bound the radiolabelled peptide (35,219 cpm). This binding was specific since incubation with an excess of unlabelled BOLF1(496–515) reduced to 195 cpm.

Discussion

Finding a homology between sequences in EBV BOLF1 and the HLA-DQw8β-related diabetes-susceptible DQ allele, led us to test whether those sequences crossreacted serologically. In ELISA rabbit antisera to BOLF1(496–515) and to DQw8β(49–60) reacted with the immunizing peptide and with the homologous peptide, but not with the closely related HLA-DQw7β sequence in which ASP is substituted for ALA^{57}. ASP^{57} correlates with diabetes resistance, while ALA, SER, or VAL at that position relate to diabetes susceptibility [10, 11]. Rabbit anti-BOLF1 (496–515) or anti-DQw8β(49–60) immunoblotted to a denatured DQw8β-positive, EBV-transformed, lymphoblast protein of the molecular weight (29 kD) of class II MHC β chain. There was no evidence for recognition of native class II MHC β chains by anti-BOLF1(496–515) in immunofluorescence or immunoprecipitation assays. Also, antibodies to DQw8β(44–63) or BOLF1(496–515) peptides were not detected in sera of patients with acute onset or long-term Type 1 diabetes. These studies demonstrated clearly the potential for immunological recognition of sequence homology and discrimination of the ALA→ASP substitution in position 57. Homologous sequences have been found in class II MHC β chains and some viruses which infect humans. The IE-2 region of cytomegalovirus shares a five amino acid segment with a conserved region of the first domain of HLA-DR β-chain, showing immunological crossreactivity [32]. This homology might enhance graft rejection when cytomegalovirus infection follows organ transplantation. Other homologies between rubella virus [33] and EBV [34, 35], and HLA-DR and -DQ β-chain sequences have been suggested to lead to Type 1 diabetes and rheumatoid arthritis, respectively. Human immunodeficiency virus (HIV) gp41 (838–844) shares 4 of 6 amino acids with HLA-DR(19–25) and HLA-DQβ(19–25) [36]. Since antibodies from

Table 2. Epstein-Barr virus (EBV) antibodies in subjects with and without newly diagnosed, Type (insulin-dependent) diabetes mellitus

Test No.	Serum No.	Age	Sex	DR Histotype	Type 1 diabetes (days)	EBV VCA IgG	EBV VCA IgM	EBV EA IgG	EBV EBNA	EBV Ab	64 kD Ab	ICA Ab	Insulin autoantibodies % binding	Insulin autoantibodies Displaced binding (cpm)
1	618	12	M	3/4	4	80	<5	<5	n.d.	+	+	pos	1.3	25
2	652	14	M	3/4	7	160	<5	<5	n.d.	+	+	neg	1.5	50
3	859	12	M	3	6	160	<5	<5	n.d.	+	−	pos	1.6	44
4	595	13	F	3/4	2	80	<5	<5	n.d.	+	+	pos	1.5	52
5	626	15	M	3/4	5	80	<5	<5	n.d.	+	+	pos	1.6	104
6	677	11	F	3/4	6	80	<5	<5	n.d.	+	+	pos	1.8	112
7	687	10	F	3/4	7	80	<5	<5	n.d.	+	+	pos	2.8	312
8	714	16	M	3/4	7	640	<5	20	n.d.	+	+	pos	1.9	119
9	830	12	F	3/4	9	160	<5	<5	n.d.	+	+	neg	1.7	93
10	920	13	M	1/4	5	160	<5	<5	n.d.	+	−	pos	1.6	32
11	727	10	M	n.d.	−	<5	<5	<5	<5	−	n.d.	neg	n.d.	n.d.
12	735	8	M	n.d.	−	160	<5	<5	20	+	n.d.	neg	n.d.	n.d.
13	738	13	F	n.d.	−	320	<5	5	40	+	n.d.	neg	n.d.	n.d.
14	739	11	F	n.d.	−	80	<5	<5	20	+	n.d.	neg	n.d.	n.d.
15	742	14	F	n.d.	−	40	<5	40	20	+	n.d.	neg	n.d.	n.d.
16	744	11	M	n.d.	−	<5	<5	<5	<5	−	n.d.	neg	n.d.	n.d.
17	801	9	M	n.d.	+	<5	<5	<5	<5	−	n.d.	pos	1.7	142
18	802	5	M	n.d.	+	<5	<5	<5	<5	−	n.d.	neg	11.6	2216
19	803	14	M	n.d.	+	<5	<5	<5	<5	−	n.d.	neg	1.3	34
20	804	14	F	n.d.	+	320	<5	<5	160	+	n.d.	pos	1.4	35
21	805	7	F	n.d.	+	160	<5	5	40	+	n.d.	pos	5.5	907
22	806	2	F	n.d.	+	<5	<5	<5	<5	−	n.d.	neg	16.6	3286
23	807	13	M	n.d.	+	<5	<5	<5	<5	−	n.d.	pos	1.5	36
24	808	8	M	n.d.	+	80	<5	80	10	+	n.d.	pos	1.5	36
25	809	15	M	n.d.	+	<5	<5	<5	<5	−	n.d.	neg	1.7	95
26	810	12	F	n.d.	+	<5	<5	<5	<5	−	n.d.	neg	1.6	47
27	812	12	M	n.d.	+	320	<5	20	10	+	n.d.	pos	10.5	1953
28	813	12	M	n.d.	+	<5	<5	<5	<5	−	n.d.	pos	1.8	75
29	815	11	M	n.d.	+	<5	<5	<5	<5	−	n.d.	neg	1.8	86
30	816	8	M	n.d.	+	320	<5	80	160	+	n.d.	pos	4.2	564
31	817	13	M	n.d.	+	<5	<5	<5	<5	−	n.d.	neg	1.7	77
32	818	7	F	n.d.	+	<5	<5	<5	<5	−	n.d.	?	3.5	501
33	819	6	F	n.d.	+	160	<5	<5	40	+	n.d.	pos	1.5	25
34	821	3	M	n.d.	+	160	<5	20	40	+	n.d.	pos	11.3	2103
35	822	10	F	n.d.	+	320	<5	5	40	+	n.d.	neg	1.7	112
36	823	14	F	n.d.	+	320	<5	20	160	+	n.d.	pos	1.7	60
37	837	10	M	n.d.	−	320	<5	5	10	+	n.d.	pos	2.2	132
38	839	4	M	n.d.	−	320	<5	80	10	+	n.d.	neg	1.8	46
39	855	3	F	n.d.	−	640	<5	320	10	+	n.d.	neg	1.4	14
40	892	7	M	n.d.	−	<5	<5	<5	<5	−	n.d.	neg	2.3	166
41	894	13	F	n.d.	−	<5	<5	<5	<5	−	n.d.	neg	1.7	103
42	898	11	F	n.d.	−	80	<5	10	40	+	n.d.	neg	1.6	89
43	899	13	F	n.d.	−	160	<5	10	320	+	n.d.	neg	1.5	22
44	902	13	F	n.d.	−	80	<5	10	20	+	n.d.	neg	1.5	48
45	903	11	F	n.d.	−	40	<5	<5	5	+	n.d.	neg	1.6	78
46	905	8	M	n.d.	−	80	<5	5	<5	+	n.d.	neg	1.6	26
47	906	13	F	n.d.	−	160	<5	5	320	+	n.d.	neg	1.4	21
48	912	8	F	n.d.	−	<5	<5	<5	<5	−	n.d.	neg	1.5	−7
49	919	9	M	n.d.	−	<5	<5	<5	<5	−	n.d.	neg	1.7	114
50	1047	14	F	n.d.	−	<5	<5	<5	<5	−	n.d.	neg	1.3	35

n.d. = not determined; VCA = viral capsid antigen; EBNA = nuclear antigen

some patients with AIDS reacted with the class II MHC β peptide and intact class II MHC molecules, the reaction of anti-HIV antibodies with self class II MHC antigens was proposed to alter class II MHC-mediated immune responses, contributing to immunodeficiency [36, 37]. Proliferative responses of normal $CD4^+$ T cells to tetanus toxoid and alloantigens were inhibited by antisera to class II MHC molecules [37]. In our study, diabetic patients did not demonstrate antibodies to the unfolded peptides or denatured, electrophoresed class II MHC β chain molecules. The lack of such serological recognition of denatured determinants did not exclude serological recognition of native determinants, or of excised fragments by T cells.

If EBV were the principal, predisposing pathogen for Type 1 diabetes, as one might suppose from our serological study of the homologous epitopes in EBV BOLF1 and some class II MHC β chains, then one might expect increased titres of anti-EBV antibodies in the sera of patients with acute onset, Type 1 diabetes. We therefore as-

sayed sera from 10 children with acute onset Type 1 diabetes at the onset of their disease. All of those patients had EBV antibodies. We extended this study to coded samples of 20 additional patients at the onset of diabetes and 20 age- and sex-matched children without diabetes. These latter 40 sera demonstrated no correlation between EBV infection and diabetes. The failure to relate serological evidence of EBV to Type 1 diabetes indicates that there is no simple relationship between EBV infections and the homologies and crossreactions which exist between some EBV proteins and class II MHC molecules. Further studies of concident cases and matched control subjects from one geographical area might be required to disprove, conclusively at an epidemiological level, the hypothesis that EBV infection is causally related to Type 1 diabetes. Furthermore, studies of T cell reactivity might reveal immunogenic determinants not seen with sera, or more sensitive serological measurements, perhaps for additional determinants might be required.

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