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Antibody reactivity to an Epstein–Barr virus BERF4-encoded epitope occurring also in Asp-57 region of HLA-DQ8 β chain

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SUMMARY

A five amino acids-long sequence (GPPAA) in the region of the 57th amino acid of HLA-DQ8 β chain, which seems to be important in defining the risk for type 1 diabetes, occurs also in the BERF4-encoded EBNA3C protein of Epstein-Barr virus (EBV) in six successive repeats. The antigenicity of this region was analysed using synthetic peptides containing different modifications of the GPPAA sequence. Two of the seven individuals who had acute EBV infection produced antibodies against an EBV-derived peptide (GPPAAGPPAAGPPAA) paralleling the EBNA2 antibodies. These two cases also contracted type 1 diabetes immediately after the infection. High antibody levels against this peptide were found in a total of 12% of EBV^+ individuals, and in most cases antibodies remained at high levels for several years. Human sera as well as affinity-purified antibodies specific for the GPPAAGPPAAGPPAA peptide reacted also with shorter peptide analogues (GPPAAGPPAA and GPPAA), as well as with peptides containing the surrounding motifs from DQ8 β chains. However, none of these antibodies bound to denatured DQ8 β chains in immunoblotting. The charge of the 57th amino acid modulated the antigenicity of this epitope, as peptides from Asp-57-negative DQ molecules were reactive, while peptides from Asp-57-positive DQ molecules were not. The responsiveness was seen in both HLA-DQ8-positive and -negative subjects as well as in type 1 diabetic individuals. The results suggest that some individuals who carry the GPPAA sequence in their HLA-DQ molecule recognize this epitope in EBV. This phenomenon may have potential importance in EBV-induced immune abnormalities, although cross-reactivity against DQ molecules could not be demonstrated in the present study.

Keywords Epstein–Barr virus molecular mimicry HLA molecules type 1 diabetes

INTRODUCTION

Immunological cross-reactivity between viral and host proteins is one possible mechanism by which viruses could induce autoimmunity (so called molecular mimicry). Up to 5% of all MoAbs originally raised against viral antigens cross-react also with uninfected tissues [1]. Relatively long homologous amino acid sequences have been found in evolutionarily unrelated proteins which sometimes induce cross-reactivity [2–5]. Recently, a five amino acids long homology sequence, GPPAA, was identified in both Epstein–Barr virus (EBV) BERF4 coding EBNA3C protein and DQ8 allele of HLA-DQ β chain [6]. In EBV this sequence occurs in six successive repeats, and in DQ β chain it includes the 57th amino acid, a residue suggested to be of special importance in defining the risk for type 1 diabetes [6–8].

As in several other autoimmune diseases, the risk for type 1 diabetes is connected with certain genes in the HLA region. The genes coding for class II HLA alleles DR3 and DR4 are associated with increased risk, while DR2 seems to be protective. Recent studies have suggested that the strongest risk is mediated by genes in the DQ region which code for phenotypes

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DQ8 (in linkage with DR4) and DQ2 (in linkage with DR3). The 57th amino acid in the β chain of DQ molecule seems to be important in defining this risk, as aspartic acid in this position has been connected with low risk and other amino acids with high risk phenotypes [7]. As the GPPAA sequence is identical in EBV and in the risk type DQ8 molecule there is a possibility that EBV infection could induce cross-reactivity against cells positive for DQ8 and have relevance in the pathogenesis of type 1 diabetes. In theory, such cross-reactivity could disturb the interaction between DQ8 molecules and T cell receptors when the activation of T cells against antigens presented by DQ8 molecules may become impaired.

Our hypothesis was that by using synthetic peptides we can demonstrate the immunogenicity of this epitope in man, and that the antibody response may become induced by EBV infection. We also postulated that this response may influence the pathogenesis of type 1 diabetes, and that this can be shown by demonstrating differences in the antibody responses in healthy and type 1 diabetic individuals that were linked with the HLA type and the exact amino acid sequence of the epitope.

SUBJECTS AND METHODS

Peptides

The amino acid sequence from EBV EBNA3C protein encoded by the BERF4 open reading frame was synthesized by Cambridge Research Biochemicals (Cambridge, UK). This soluble synthetic peptide contained three repeats of the homology sequence GPPAA and one additional cysteine at the carboxy terminus (GPPAAGPPAAGPPAAC, EBV-BERF4 peptide, Table 1a). Another EBV-derived synthetic peptide (EQNQEQKRAAQRAAGC, encoded by the BALF4 open reading frame, Table 1b) was used as a control peptide. All other peptides were synthesized by Epitope Scanning Kit (Cambridge Research Biochemicals) by coupling the carboxy terminus of the peptide on activated beta-alanine conjugated polyethylene rods using 9-fluorenylmethyloxycarbonyl/t-butyl protected amino acids [9]. These pin-adhering peptides contained the residues around the 57th amino acid from DQ β chain having aspartic acid, alanine, valine or serine at position 57 as well as a control peptide derived from DR molecule (Table 1c).

Table 1 Synthetic peptides used in the present work. Soluble peptides are presented in **a** and **b** representing three repeats of the homologous GPPAA sequence occurring in Epstein–Barr virus (EBV) BERF4 encoded protein (**a**) and a control peptide from BALF4-encoded EBV protein (**b**). Pin-adhering peptides are presented in **c** corresponding to one to three repeats of the homologous amino acids (GPPAA) or corresponding amino acids from other DQ β chains, as well as a longer sequence containing the amino acids around the homology sequence in different DQ molecules

a EBV BERF4-peptide, GPPAAGPPAAGPPAAC

b EBV BALF4- peptide, EQNQEQKRAAQRAAGC

c HLA haplotype and allele	Corresponding peptides	Asp-57 +/−
DR4-DQ8	GPPAA×1-3	−
DQB1*0302	GPPAAEY×1-3	
	PPAAEY×1-3	
	GPPAAEYWNSQ	
	PLGPPAAEYWN	
	VTPLGPPAAEY	
	TPLGPPAAEYWN	
DR3-DQ2	GLPAA×1-3	−
DQB1*0201	TLLGLPAAEYW	
DR1-DQ5	GRPVA×1-3	−
DR13(DR6)-DQ6	TPQGRPVAEYW	
DQB1*0501		
DQB1*0604		
DR16(DR2)-DQ5	GRPSA×1-3	−
DQB1*0502	TPQGRPSAEYW	
DR4-DQ7	GPPDA×1-3	+
DR5-DQ7	TPLGPPDAEYW	
DQB1*0301		
DR15(DR2)-DQ6	GRPDA×1-3	+
DR14(DR6)-DQ6	TPQGRPDAEYW	
DQB1*0602		
DQB1*0503		
DR8-DQ4	GRLDA×1-3	+
DQB1*0402	TPLGRLDAEY	
DRB1† (control)	RPDAE×1-3	

The 57th amino acids are underlined.

† Several alleles, e.g. DRB10101, 1501, 0301, 0401.

Sera

Three sources of serum samples were used. Series 1 consisted of healthy individuals including laboratory personnel ($n = 133$) and their children ($n = 29$) from the Universities of Oulu and Tampere. Series 2 was a family series containing 20 families participating in the nationwide Childhood Diabetes in Finland (DiMe) study and having more than three children, at least one of whom was diabetic [10]. Serum samples taken at the diagnosis of diabetes in the index case were available from all children. From the parents serum samples were taken at a later time. Twenty-six per cent of these children, but none of the parents, were diabetic. For the family study a total of 31 families, 20 from DiMe (series 2) and 11 from laboratory personnel (series 1) were available. Series 3 contained 54 newly diagnosed type 1 diabetic children and their age-, sex- and residence area-matched controls which were collected in the Department of Paediatrics, University of Tampere [11]. Controls were from the same hospital as patients, and included children who were admitted for minor surgical operations and had no autoimmune diseases or malignancies. In addition, sera from 53 diabetic children of DiMe study not included in the family series and sera from seven individuals with serologically confirmed (EBV IgM-positive) acute EBV infection were analysed.

Antibody analyses

Antibody levels against the soluble EBV peptides were analysed from all these samples using ELISA. The microtitre plates (Nunc Immunoplate, Glostrup, Denmark) were coated with uncoupled EBV BERF4 and BALF4 peptides in 2·5 μg/ml concentration in PBS using an overnight incubation at 4°C. The serum samples were diluted 1:50 in PBS containing 1% bovine serum albumin (BSA), 0·5 M NaCl and 0·05% Tween 20 and incubated overnight at 4°C. Peroxidase-conjugated anti-human IgG antibodies (Dako, Copenhagen, Denmark) and *o*-phenylendiamine-dihydrochloride substrate (OPD; Fluka AG, Buchs, Switzerland) were used in subsequent steps. The enzyme reaction was stopped with 1 N H_2SO_4 and the colour intensity recorded at 492 nm using Titertek Multiskan spectrophotometer (Eflab, Helsinki, Finland). The specificity of antibody binding to BERF4 peptide was evaluated by inhibiting this reaction using corresponding peptide or the control BALF4 peptide in different concentrations (0·45–60 μg/ml) in the serum diluent.

IgG and IgM class antibodies against EBV viral capsid antigen (VCA) were analysed using commercial enzyme immunoassay kits (Du Pont Company, Billerica, MA) as previously described [11]. Coded sera were analysed in duplicate. Antibody levels were expressed as enzyme immunoassay units (EIU) which show relative antibody activity of the sample compared with positive and negative control sera [12]. Limit value of 10 EIU in EBV VCA antibodies was used as an indicator of previous EBV infection (seropositivity) according to the producer's instructions. In the BERF4 peptide ELISA the cut-off value of 30 EIU was selected on the basis that all VCA antibody-negative sera gave absorbances under this value. Poly-L-proline (Sigma Chemical Co., St Louis, MO) was used for the EBNA2 (EBV nuclear antigen 2) antibody analyses as previously described [13].

The reactivity of selected sera against pin-adhering peptides was tested following the principles presented above [5,11]. The sera were diluted 1:200 in PBS containing 1% BSA, 1% ovalbumin and 0·05% Tween 20 and incubated for 2 h at 37°C. After washings with 0·05% Tween 20-PBS peroxidase conjugated anti-human IgG was incubated for 1 h at 37°C in 1:6000 dilution. Tetramethylbenzidine (TMB; Kierkegaard & Perry Labs, Gaithersburg, MD) was used as the enzyme reaction substrate. The reaction was stopped with 1 N HCl and the colour intensity was recorded at 450 nm.

HLA determinations

HLA-A, -B, -C and -DR antigens were defined using standard two-stage-microlymphocytotoxicity test. HLA-DQB1 gene allelism was studied using restriction fragment length polymorphism (RFLP) and oligonucleotide hybridizations. RFLP was done with IVS-I specific probe and BamHI digestion and oligonucleotide typing with seven oligonucleotide probes specific for gene segment coding for the 57. and flanking amino acids. The procedures have been described in detail earlier [14,15].

Preparation of antisera

New Zealand white rabbits were immunized by BERF4 peptide coupled to BSA with 0·2% glutaraldehyde [16]. Four s.c. injections with 2 week interval were used, the first one in Freund's complete adjuvant (FCA) and the rest in Freund's incomplete adjuvant (FIA), and the animals were bled 1 week after the last injection. The rabbit sera and one highly BERF4 peptide antibody-positive human serum were affinity purified using cyanogen bromide-activated Sepharose 4B column (Pharmacia, Uppsala, Sweden) coupled with BERF4 peptide via the amino terminus. Ammonium sulphate-precipitated serum immunoglobulins were passed through the column and after washings with PBS (pH 7·2) and 0·1 M glycine (pH 2·3) specific antibody activity was eluted with 8 M urea (pH 1·5). Collected fractions were rapidly neutralized with 1 M Tris buffer and their specificity was ascertained in ELISA using BERF4 peptide as well as irrelevant mumps virus antigen or BSA–BSA conjugate as antigens.

Immunoblotting

Immunoblotting was done as previously described using EBV-transformed B lymphocytes from HLA-DQ8$^+$ donor as the source of DQ molecules [8]. Briefly, after homogenization ($20–40 \times 10^6$ cells), nuclei and unbroken cells were removed by centrifugation for 3 s in microcentrifuge and the supernatant was further centrifuged at 35000 ***g*** for 30 min at 4°C. The amphiphilic membrane proteins were next enriched from Triton X-114-treated pellet fraction by centrifugation over a 6% sucrose cushion [17]. Detergent phase proteins were recovered as an oily droplet at the bottom of the tube. Proteins were then denatured under reducing conditions by boiling for 3 min at 90 mM Tris-HCl, 17% sucrose, 3% SDS, and 10 mM dithiothreitol and subjected to SDS–PAGE (10% gel, 10 μg proteins/lane).

After the electrophoresis the proteins were electroblotted onto nitrocellulose membranes. The membranes were blocked in 5% non-fat dry milk–PBS solution. The binding of human and rabbit antibodies to DQ β chain was evaluated in different serum dilutions using commercially available MoAbs against HLA-DQ/DR β chain as a positive control antibody (Dako M 775) and peroxidase-conjugated anti-human IgG (Dako P214), anti-rabbit IgG (Dako P217) or anti-mouse immunoglobulin (Dako P161) as secondary antibodies.

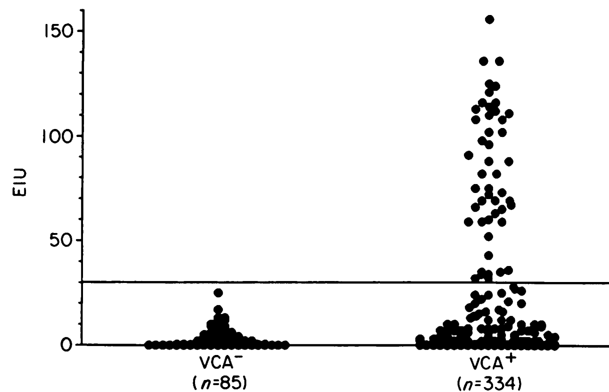

Fig. 1. The distribution of antibody levels to Epstein–Barr virus (EBV) BERF4 peptide in ELISA (EIU) in EBV viral capsid antigen (VCA)-seropositive and seronegative human sera. The cut off value of 30 EIU (indicated by a horizontal line) was used as a limit of responsiveness.

Statistical analysis

χ^2 test, Fisher's exact test and Student's *t*-test were used.

RESULTS

IgG class antibody levels to the EBV peptide GPPAAGP-PAAGPPAA (BERF4 peptide) were found in 12% of EBV$^+$ individuals, but in none of the EBV$^-$ individuals (Fig. 1). Antibodies were usually found in high titres and binding was specific as the reactivity was completely abolished when BERF4 peptide was added to the serum diluent (Fig. 2). Antibody reactivity was also analysed in seven individuals with serologically confirmed acute EBV infection, two of whom developed type 1 diabetes soon (some months) after the infection. Antibodies were found only in these two diabetic cases (Fig. 3). Antibody levels decreased rapidly after the infection, paralleling the response against EBNA2 protein. In another series containing five healthy individuals, who were found to have antibodies to this peptide and from whom follow-up samples were available, antibodies remained at high levels throughout the 5-year follow-up period.

Responsiveness against BERF4 peptide was clustered in certain families as seen in the analysis of EBV VCA-seropositive family members. In families where one or both parents (nine and two couples, respectively) were BERF4 peptide antibody-positive, the number of children who had these antibodies

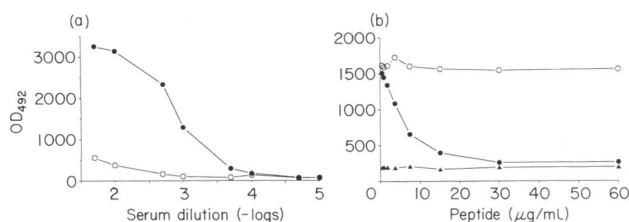

Fig. 2. The binding of human sera to soluble BERF4 peptide in ELISA. (a) The antibody dilution curves of one antibody-positive serum (●) and one antibody-negative serum (○) to BERF4 peptide. (b) The inhibitory effect of different BERF4 and control peptide concentrations (μg/ml in serum diluent) on the binding of human serum antibodies to BERF4 peptide. ●, Positive+BERF4; ○, positive+control; ▲, negative +BERF4.

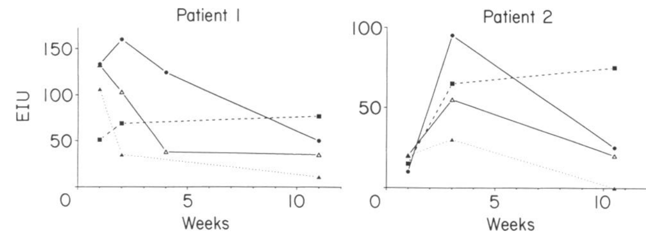

Fig. 3. Antibody reactivity of sera from two newly diagnosed type 1 diabetic subjects against Epstein–Barr virus (EBV) viral capsid antigen (VCA) and EBNA2 proteins as well as BERF4 peptide (EIU) indicating acute/recent EBV infection. The first sample was taken within 1 week after the diagnosis of diabetes. ■, VCA IgG; ▲, VCA IgM; ●, EBNA2 IgG; △, BERF4 peptide IgG.

(10/17, 59% and 2/5, 40%, respectively) was higher than in families in which both parents (15 couples) were peptide antibody-negative (12/45, 27% of children had peptide antibodies, $P<0.05$). However, antibody-positive children were also found in some families with seronegative parents.

Sera which reacted with the uncoupled BERF4 peptide in ELISA reacted also with the corresponding pin-adhering peptides GPPAAGPPAAGPPAA, GPPAAGPPAA, and even with the exact DQ homology sequence GPPAA (Fig. 4). These sera reacted also with peptides deduced from other Asp57-negative DQ molecules with alanine, valine or serine at the 57th position

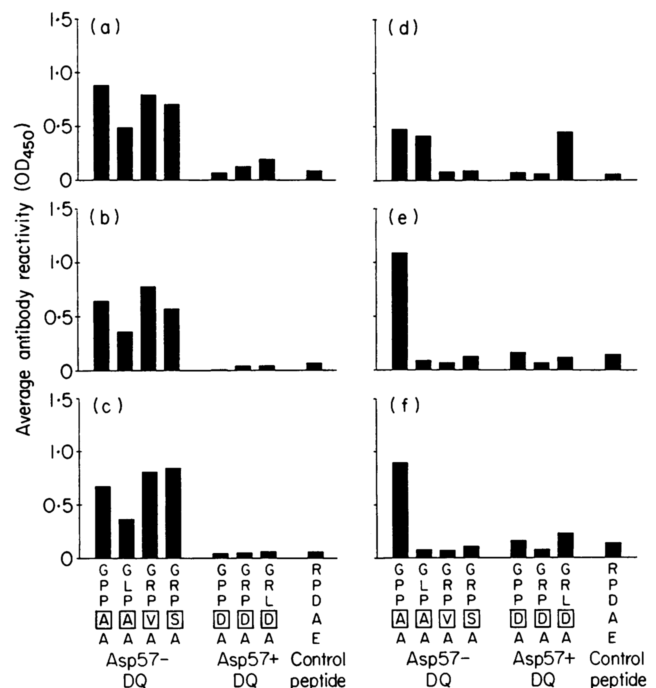

Fig. 4. Antibody reactivity of human antibodies to pin-adhering peptides consisting of one (a, d), two (b, e) or three (c, f) successive repeats of amino acids 54 to 58 from different DQ β chain haplotypes as presented in category c in Table 1. The reactivity of three BERF4 peptide antibody-positive human sera from which were subtracted that of three Epstein–Barr virus (EBV)-seronegative and BERF4 antibody-negative human sera is presented in a–c. The reactivity of affinity-purified BERF4 peptide-specific antibodies from one of the seropositive individuals is presented in d–f. Each bar indicates the mean level of reactivity (ELISA absorbance OD_{450}) to one synthetic peptide.

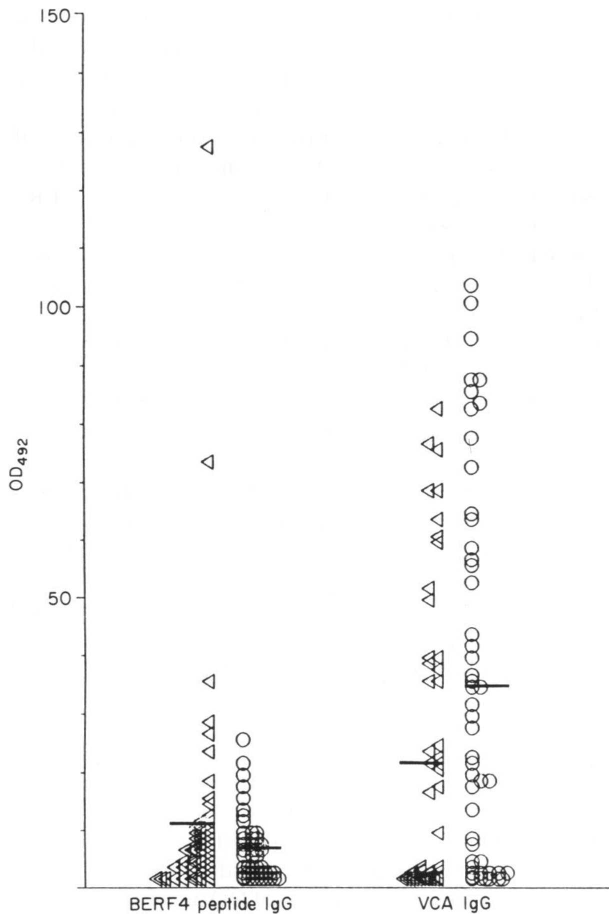

Fig. 5. IgG class antibody levels (EIU) to BERF4 peptide and Epstein–Barr virus (EBV) viral capsid antigen (VCA) in 54 type 1 diabetic children (▽) and their matched controls (○). The mean level is presented as a horizontal line. The difference in VCA antibodies between patients and controls is statistically significant in paired *t*-test ($P<0{\cdot}02$).

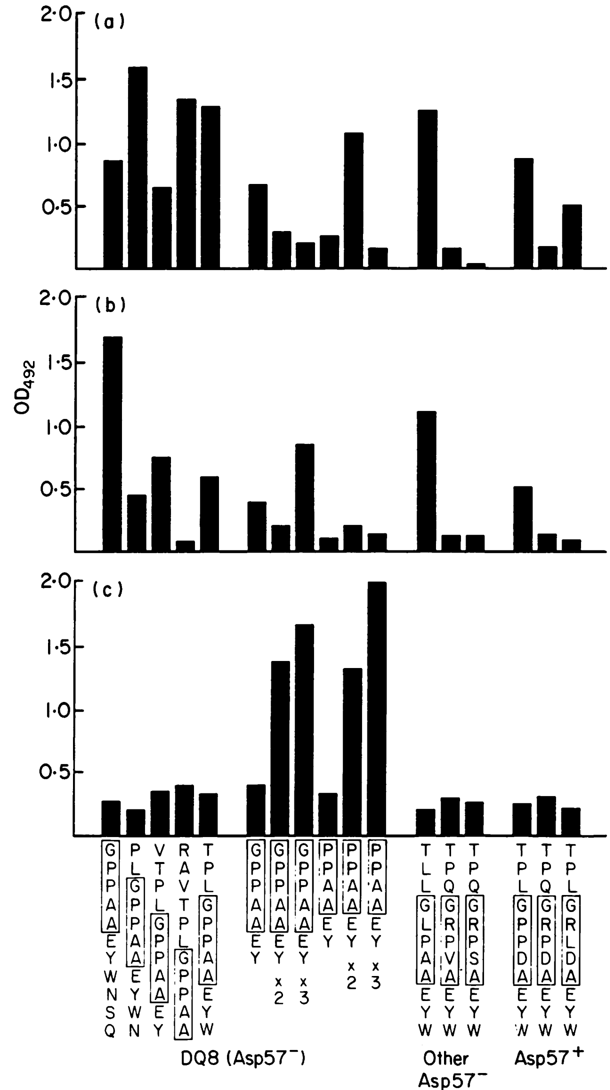

Fig. 6. Antibody reactivity of human and rabbit sera to pin-adhering peptides carrying the motifs surrounding the Asp-57 region of β chain of different HLA-DQ molecules. (a) Mean reactivity of three BERF4 peptide-positive human sera from which were subtracted that of three antibody-negative sera. (b) The reactivity of affinity-purified BERF4 peptide-specific antibodies from the seropositive individual. (c) The reactivity of rabbit hyperimmune serum raised against the BERF4 peptide from which was subtracted that of the preimmune serum. Amino acid sequences and corresponding DQ type are indicated.

(Fig. 4). In contrast, the reactivity was much lower if the peptides had Asp in position 57 corresponding to the non-risk DQ allele. Antibodies purified from one human serum using affinity column with BERF4 peptide coupled in Sepharose 4B reacted with all variants of GPPAA sequence. These antibodies did not, however, bind to peptides deduced from other DQ molecules than DQ8 (Fig. 4).

From the 53 VCA$^+$ HLA typed individuals 18 (34%) were DR4-positive. Among these 22% had antibodies to BERF4 peptide which was not different from the 26% of DR4-negative subjects. The strong association between HLA-DR4 and DQ8 antigens suggests that many of the DR4-positive subjects were DQ8-positive as well, and that also DQ8-positive individuals are able to produce antibodies against this epitope. This was confirmed when DQ typing was done in 34 VCA$^+$ subjects using DNA hybridization methods. Antibodies to BERF4 peptide were found in 21% of the DQ8-positive (3/14) and in 45% of the DQ8-negative (9/20) subjects, indicating no statistically significant difference between the groups. The antibody response was thus found also in subjects who had the specific epitope on their DQ β chain.

High antibody levels against the BERF4 peptide were found also in type 1 diabetic subjects irrespective of their HLA-DR type. In the case-control comparison (series 3) including both EBV$^+$ and EBV$^-$ young children, 6% of newly diagnosed type 1 diabetic subjects (3/54) had BERF4 peptide antibodies, compared with none of the matched controls (NS), while the response against EBV VCA antigen was significantly lower in patients than controls (Fig. 5). If only VCA-positive diabetic patients were included, 13% (3/24) were BERF4 peptide antibody-positive.

To analyse the ability of the homology sequence to induce cross-reactive antibodies, rabbits were immunized with the BERF4 peptide. Hyperimmune serum had high antibody titres (end-point dilution 2×10^{-6}) and the binding could be inhibited completely by adding this peptide to the serum diluent. Rabbit antibodies also reacted with pin-adhering GPPAA peptide

repeats, but not with peptides deduced from other DQ molecules than DQ8. Cross-reactivity was studied using synthetic peptides containing different regions around the GPPAA sequence in DQ8 β chain (Fig. 6). Human sera bound to several such DQ peptides, and affinity-purified antibodies particularly to the GPPAAEYWNSQ peptide from DQ8 as well as to TLLGLPAAEYW peptide from DQ2 molecules, which both are Asp-57-negative. Rabbit serum, however, reacted only with DQ8 peptides which closely resembled the BERF4 peptide sequence (GPPAAEY and PPAAEY repeats, Fig. 6). The binding of rabbit and human antibodies to the β chain of DQ8 molecules was tested in immunoblotting under reducing conditions. No specific binding was observed, even though the control antibody specific for DQ β chain showed clear positive staining.

DISCUSSION

In the present study 12% of healthy EBV VCA-positive individuals were found to have antibodies against a linear peptide epitope in EBV BERF4-encoded EBNA3C protein. Antibodies against this epitope were seen early after EBV infection, paralleling the response against EBNA2 protein [13]. However, considerable individual variation was observed: some individuals did not produce antibodies against this epitope during acute EBV infection at all, some became antibody negative within some months after the infection, and in some, high antibody levels persisted for several years.

Interestingly, individuals having antibodies to the BERF4 peptide were clustered in certain families. This could reflect the role of genetic control of antibody responsiveness against this EBV epitope, but the appearance of non-responders among the children of responder parents rules out the simple one gene associated inheritance. Responsiveness was not related to the presence or absence of HLA-DR4 allele, and high antibody levels were also found in individuals with the HLA-DQ8 phenotype. However, in DQ8-positive individuals the antibodies were slightly less frequent than in DQ8-negative individuals, suggesting that the immune responsiveness may be suppressed in individuals who carry the GPPAA sequence in their HLA-DQ molecules. On the other hand, strain difference of EBV may also explain the clustering of responsiveness in certain families, since the GPPAA sequence is absent from some EBV strains like the prototype-B strain of EBV [18–20].

As this epitope (GPPAA) occurs also in the β chain of HLA-DQ molecules, there is a possibility that EBV infection could induce immunological cross-reactivity against DQ. This may have relevance particularly in the pathogenesis of type 1 diabetes, since the sequence is the same as in the risk type DQ8 molecules including alanine at position 57 of DQ β chain. In fact, the charge of 57th amino acid in DQ β chain had clear influence on antibody binding, as peptides having other residues than Asp (i.e. Val, Ala or Ser) at this position were highly reactive, whereas Asp-positive peptides were not. These differences in antibody binding may reflect the effect of the 57th amino acid on the tertiary structure of this region.

Previous studies have indicated that EBV infections might have a role in autoimmune diseases such as rheumatoid arthritis [21]. EBV infections have also been described in association with a rapid onset of symptoms of type 1 diabetes [22–24). The peak age of EBV seroconversion in Finland is between the age of 1 and 4 years, and another smaller peak is seen around puberty. About 50% of Finnish children have had EBV infection by the age of 5 years and 90–95% of the adult population are EBV$^+$ [25]. The incidence peaks of type 1 diabetes are at the age of 6 years and around puberty [26]. In newly diagnosed diabetic children EBV VCA antibody titres are lower than in controls, suggesting abnormalities in EBV-specific immunity in type 1 diabetic subjects [11]. This may make comparisons of BERF4 peptide antibody levels between diabetic subjects and healthy individuals difficult to interpret. There was a slight excess of antibody-positive cases among type 1 diabetic subjects, as 13% of the seropositive patients and none of the controls had antibodies against the peptide. Furthermore, the two individuals who developed antibodies during acute EBV infection contracted type 1 diabetes soon after the infection, while the five otherwise healthy individuals with acute EBV infection did not produce antibodies against this epitope.

As HLA-DQ8$^+$ type 1 diabetic subjects are able to produce antibodies against this EBV epitope, the possibility that these antibodies could cross-react with DQ molecules and have pathogenic significance should be considered. In theory, cross-reactive antibodies against DQ could disturb the delicate interaction between T cell receptors and peptides presented by these molecules. This, in turn, could lead to impaired suppression of T cell clones specific for critical β cell antigens as, at least on certain occasions, T suppressor cells are activated preferentially by antigens presented by DQ molecules [27, 28].

Cross-reactivity at peptide levels was demonstrated as both human sera and affinity-purified antibodies specific for the BERF4 peptide reacted not only with the exact GPPAA homology sequence but also with peptides carrying additional motifs from the surrounding region of DQ molecules. However, human or rabbit antibodies against the BERF4 peptide did not react with denatured DQ8 molecules in immunoblotting. Thus, in the present study we were not able to demonstrate cross-reactivity against DQ molecules. This does not, however, exclude the possibility that binding to this DQ epitope may occur in certain conditions *in vivo*, and further studies are needed to elucidate this question in more detail.

Recently, Sairenji *et al.* reported the identity of an EBV BOLF1 protein-derived peptide (AVTPLRIFIVPPAAEY) and DQ β chain (AVTPLGPPAAEY) [29]. This sequence contains the same 57th amino acid as the BERF4 peptide, but most other residues are different. It was antigenic in rabbits but antibodies were not found in man. GPPAA sequence is also present in herpes simplex virus type 1 and in cytomegalovirus, as well as in such human proteins as transforming growth factor, steroid hormone receptors, excision repair protein precursor and in some animal proteins like a DNA-binding protein and insulin-like growth factor binding protein, but the biological significance of these homologies are not known [30–36]. On the other hand, sequence similarity between the area around the 57th amino acid of DQ β chain and BSA has also raised speculation in connection with type 1 diabetes [37].

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