Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 329 (2005) 356–361
BBRC
www.elsevier.com/locate/ybbrc

Analysis of eluted peptides from type 1 diabetes-susceptible HLA class II molecules identified novel islet protein, heparin/heparan sulfate-interacting protein

Koji Nakanishi [a,c,*], Yasuhiko Komatsu [d,1], Noriaki Kogawa [d,2], Hiroshi Matsushita [b,c]

[a] Department of Endocrinology and Metabolism, Toranomon Hospital, Tokyo, Japan
[b] Department of Pathology, Toranomon Hospital, Tokyo, Japan
[c] Okinaka Memorial Institute for Medical Research, Tokyo, Japan
[d] Pharmaceuticals and Biotechnology Laboratory, Japan Energy Corporation, Saitama, Japan

Received 19 January 2005

Abstract

Identification of peptides derived from pancreatic islet and presented by type 1 diabetes-susceptible MHC class II molecules has great significance to elucidate the pathogenesis of type 1 diabetes. A bulk culture of Epstein–Barr virus-transformed B-cells, which were established from a 22-year-old type 1 diabetic woman with HLA-DR4 and -DQw8, was pulsed with the homogenate of a human embryonic pancreas-derived cell line 1B2C6, and another culture was not pulsed with antigen. Peptide fractions were obtained by treatment of affinity-purified HLA-DR and -DQ molecules with 0.1% trifluoroacetic acid, and were subjected to reverse-phase high performance liquid chromatography (RP-HPLC). The RP-HPLC profiles of peptides derived from DR molecules revealed three peaks that specifically appeared after pulsing, but no such peaks were obtained from DQ molecules. From one of these three peaks, a peptide that consisted of 14 amino acids (AKSXNHTXXNQXRK, where X represents the undetermined amino acids) was identified. This peptide was derived from heparin/heparan sulfate-interacting protein (HIP). Immunostaining of pancreatic sections using antiserum for HIP peptide revealed exclusive staining of the islets. Thus, HIP was identified as an islet protein naturally processed and presented by HLA-DR4 molecules.

Keywords: Type 1 diabetes; MHC class II; HLA-DR4; Peptide; Islet

Type 1 diabetes is caused by the autoimmune destruction of pancreatic β-cell and T-cell-mediated autoimmunity has been proposed to underlie this β-cell destruction [1]. The major histocompatibility complex (MHC), especially HLA class II (DR and DQ), most strongly confers the susceptibility to type 1 diabetes [1]. HLA-DR4-DQA1*0301-DQB1*0302 and HLA-DR3-DQA1*0501-DQB1*0201 are susceptible haplotypes in Caucasians, and HLA-DR4-DQA1*0301-DQB1*0401, HLA-DR4-DQA1*0301-DQB1*0302, and HLA-DR9-DQA1*0301-DQB1*0303 are susceptible haplotypes in Japanese [2]. However, the molecular mechanisms by which these HLA class II molecules cause the autoimmune destruction of pancreatic β-cells remain unclear.

MHC class II molecules present antigens to CD 4 T-cells as form of peptides bound to the peptide-binding groove of these molecules, which is composed of α-helixes of polymorphic α and β chains of MHC class II molecules [3]. Since the targets of autoimmunity are thought to exist in the pancreatic β-cells or islets in type 1 diabetes, identification of antigenic peptides derived from the pancreatic islet and presented by type 1

* Corresponding author. Fax: +81 3 3582 7068.
E-mail address: koji01@toranomon.gr.jp (K. Nakanishi).
[1] Present address: Advanced Life Science Institute, Inc., Saitama, Japan.
[2] Present address: Sumitomo Pharmaceuticals, Takarazuka, Japan.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.01.144

diabetes-susceptible MHC class II molecules has great significance to elucidate the pathogenesis of type 1 diabetes. For this purpose, three types of investigational approaches have been tried alone or in combination. The first approach is assessment of affinity between MHC class II molecules and synthetic peptides derived from established autoantigens recognized by autoantibodies such as glutamic aid decarboxylase (GAD) 65, insulin, and tyrosine phosphatase-like molecules IA2 [4–6]. However, this approach is biased towards assessment of peptide-binding only and does not take into account the effects of antigen processing. The second approach is to identify antigenic peptides by screening antigen-specific T cell lines with a set of overlapping peptides spanning the entire sequence of native protein [7–10], or alternatively by screening peptide-specific T-cell lines using T-cell proliferation assay performed with antigen presenting cells expressing native protein [11]. In this approach, antigen or peptide-specific T-cell lines were obtained by in vitro stimulation of peripheral blood mononuclear cells [7] or by immunizing the mouse transgenic for human MHC class II alleles [8,9,11] with known autoantigens. Disadvantage of theses approaches is that the sources of peptide antigens are limited to specific molecules such as GAD65, insulin, and IA2. Search for antigenic peptides should be performed from entire β-cell or islet in the form of naturally processed and presented antigens. The third approach is direct elution of antigenic peptides from MHC class II molecules on the cell surface [12]. Although naturally processed and presented peptides can be obtained by this method, management and analysis of complex peptide fractions is troublesome. In the present study, to overcome these obstacles, we tried to obtain islet-derived antigenic peptides in the naturally processed and presented form by comparing the high performance liquid chromatography (HPLC) patterns of peptides eluted from the MHC class II molecules of cultured B-cells pulsed with the homogenate of a fetal islet-derived cell line and unpulsed cells.

Materials and methods

Bulk culture of B-cells from a type 1 diabetic patient. After obtaining informed consent, fresh heparinized blood was obtained from a type 1 diabetic patient at 2 months after the diagnosis. She was 22 years old and showed hyperglycemia with ketosis at the onset. Insulin therapy was started soon after the onset. Antibodies to GAD65 have been positive from the onset to the present (8 years after the onset). HLA-A, -B, -C, and -DR antigens were typed by the microcytotoxicity test, and HLA-DR and -DQ alleles were typed by the polymerase chain reaction-restriction fragment length polymorphism method [13,14]. This study was approved by the Toranomon Hospital Committee on Human Research.

Peripheral blood mononuclear cells (PBMCs) were isolated on density gradients (Mono-poly resolving medium, Dainippon Pharmaceutical, Osaka, Japan) and were washed in culture medium [RPMI 1640 medium containing 20% fetal bovine serum (FBS), 50 μg/ml kanamycin, and 200 ng/ml cyclosporine A]. After adjusting the density to 2×10^6 cells/ml in a volume of 800 μl, 200 μl of the culture supernatant of Epstein–Barr virus (EBV)-producing B95-8 cells, obtained from American Type Culture Collection (ATCC), was added. Then the EBV-infected PBMCs were cultured in a round-bottomed tube for 3 weeks with 1/2-volume medium exchange twice a week, after which the EBV-transformed B-cells were expanded by consecutive 2-fold dilution with culture medium. For the antigen-pulse experiment, EBV-transformed B-cells were grown up to 1.2×10^{10} cells (10 L) using roller bottles in RPMI 1640 medium supplemented with 20% FBS and 50 μg/ml kanamycin.

Antigen pulsing, purification of HLA-DR and -DQ molecules, and analysis of peptides eluted from HLA class II molecules. The antigen source used to pulse B-cells was the homogenate of a human embryonic pancreas-derived cell line 1B2C6, which was obtained from Riken Bioresource Center (Cell No. RCB0794) (http://www.brc.riken.go.jp/), cultured in Ham's F-10 medium supplemented with 20% FBS. Homogenates of 1B2C6 cells were prepared by sonication in phosphate-buffered saline (PBS). One set of 10 L culture of EBV-transformed B cells (1×10^6 cells/ml at final concentration) was pulsed twice with the homogenate of 1B2C6 cells (1.6×10^4 cells/ml at final concentration) at 5 days and 1 day before harvesting, and the other set was not pulsed with antigens. Purification of HLA-DR and -DQ molecules and bound peptides was performed by the modification of a previously described method [15]. Both pulsed and unpulsed EBV-transformed B-cells were lysed at a concentration of 2×10^8 cells/ml in PBS, pH 7.0, containing 1% Nonidet P-40 (NP-40), 25 mM iodoacetamide, 0.04% NaN_3, and 1 mM pefabloc SC (Roch Applied Science, Mannheim, Germany), incubated for 15 min on ice, and centrifuged at 100,000*g* for 20 min. Monoclonal antibodies specific for HLA-DR and HLA-DQ were purified using a protein A column (AffinityPak Immobilized Protein A, Pierce, Rockfoed, IL) from the ascites of BALB/c mice and BALB/c nu/nu mice transplanted intraperitoneally with Antibody 2.06 and IVD12 murine hybridomas, respectively, both of which were purchased from the ATCC. HLA-DR and -DQ molecules were purified from the supernatant of NP-40 lysate on immunoaffinity columns in which the above monoclonal antibodies were coupled to Actigel ALD (Sterogene, Carlsbad, CA). After the supernatant was applied, the columns were washed sequentially with 10 column volumes of lysis buffer, detergent buffer (10 mM Tris–HCl, pH 8.3, 1% NP-40, and 150 mM NaCl), Tris buffer (10 mM Tris–HCl, pH 7.3, 150 mM NaCl), and low salt buffer (10 mM Tris–HCl, pH 7.3), and then HLA class II (DR or DQ) molecules and bound peptides were eluted with 10 column volumes of 0.1% trifluoroacetic acid (TFA). SDS–PAGE was performed to confirm the presence of HLA-DR and -DQ molecules in the eluates. The protein concentration was determined by the BCA assay (Pierce).

Eluates containing HLA-DR and -DQ molecules were lyophilized, dissolved in 2 ml of 0.1% TFA, and incubated at 70 °C for 15 min. The low molecular weight (LMW) fractions were isolated by centrifugation through an Ultracent 30 ultrafiltration filter (molecular weight cut-off = 30,000, Tosoh, Tokyo, Japan), after which the filtrates were lyophilized. The LMW fractions were dissolved in 100 μl of 0.1% TFA, subjected to reverse-phase (RP)-HPLC using a Shiseido Capcell Pak C 18 column (UG120 Å 5 μm, 1.5 mmϕ × 150 mm/L), and eluted with a gradient of acetonitrile (2–60%) containing 0.1% TFA at 0.2 ml/min. The absorbance of the eluate was monitored at 215 nm. By comparing the RP-HPLC patterns derived from pulsed and unpulsed samples, peaks specifically emerged by the pulse with the homogenate of 1B2C6 cells were identified and subjected to amino acid sequence analysis by employing Edman degradation chemistry (Takara, Kusatsu, Japan). The peptides thus obtained were aligned to regions of proteins stored in the database using the BLAST network at the National Center for Biotechnology Information [16].

Generation of antiserum to a synthetic peptide, Western blotting, and histological examination. A synthetic peptide of AKSKNHTTHNC (obtained from Qiagen, Tokyo, Japan) was conjugated via its

sulfhydryl residue to keyhole limpet hemocyanin (KLH) with sulfo-succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (Pierce). Antiserum was raised against this peptide by immunizing Harteley guinea pigs with the peptide-KLH conjugate according to the standard protocol.

Lysates of EBV-transformed B cells and those of 1B2C6 cells, which were prepared in the same way, were subjected to 15% SDS–PAGE (10 μg protein/lane) and then transferred to a polyvinylidine difluoride (PVDF) membrane. The membrane was blocked with 3% bovine serum albumin and 3% polyvinylpyrrolidone K-30 dissolved in Tris-buffered saline with Tween (TBST; 10 mM Tris–HCl, pH 8.0, 150 mM NaCl, and 0.05% Tween 20). Then immunoblotting was done with antiserum for the synthetic peptide (dilution 1:500), control guinea pig serum (dilution 1:500), and anti-α-tubulin monoclonal antibody (Monosan, Uden, Netherlands, dilution 1:5000). Subsequently, the membrane was probed with horseradish peroxidase (HRP)-conjugated rabbit anti-guinea pig IgG (Dako, Glostrup, Denmark, dilution 1:10,000) and visualized with an ECL-Plus Western blotting detection system (Amersham-Pharmacia Biotech, Buckinghamshire, UK).

Sections (5 μm) were cut from formaldehyde-fixed paraffin-embedded specimens of human pancreas, and were stained by an indirect immunoperoxidase technique using the above-mentioned antiserum for the synthetic peptide or control guinea pig serum (dilution 1:400) followed by biotinylated rabbit anti-guinea pig IgG antibodies (Zymed, San Francisco, CA, dilution 1:500) and streptavidin–HRP (Pierce, dilution 1:5000).

Results

This patient had the following HLA type: A 2/31, B 51/48, C −/−, DR4 (DRB1*0405/*0407), and DQw8 (DQA1*03-DQB1*0302/ DQA1*03-DQB1*0302). Sixty micrograms of HLA-DR molecules and 47 μg of HLA-DQ molecules were obtained from the unpulsed culture of 1.2×10^{10} EBV-transformed B-cells, while 990 μg of HLA-DR molecules and 133 μg of HLA-DQ molecules were obtained from the culture of 1.2×10^{10} EBV-transformed B-cells pulsed with the homogenate of 1B2C6 cells. Many peaks were revealed by RP-HPLC analysis of the peptides derived from HLA-DR and HLA-DQ molecules in both pulsed and unpulsed cultures (Figs. 1A and B). Although no pulse-specific peak was observed in RP-HPLC analysis of the peptides derived from HLA-DQ molecules (Fig. 1B), RP-HPLC analysis of the peptides derived from HLA-DR molecules revealed three peaks that specifically appeared after pulsing (Fig. 1A). Although no phenylthiohydantoin-amino acids were released from peaks 1 and 3 (shown in Fig. 1A) during sequencing, a peptide from peak 2 (Fig. 1A) was successfully sequenced. This peptide consisted of 14 amino acids: AKSXNHTXXNQXRK (where X represents undetermined amino acids). A homology search using the BLAST network showed that this peptide completely coincided with residues 2–15 of human homologue of ribosomal protein L29 [17] or heparin/heparan sulfate-interacting protein (HIP) [18] (Table 1). When we tentatively aligned this peptide with the previously reported peptide motifs of HLA-DRB1*0405 [19] and HLA-DRB1*0407 [20](Table 1), it did not coincide with the reported peptide motifs of either molecule at relative positions 1 and 4, but did coincide at relative positions 6 and 9 (Table 1).

Western blotting of the lysates of 1B2C6 cells and EBV-transformed B cells showed the expression of HIP proteins by both cells (Fig. 2). Immunostaining using antiserum raised against obtained HIP peptide showed exclusive and homogeneous staining of the islets in the pancreatic sections (Fig. 3), while immunostaining using control guinea pig serum showed no staining of the islet.

Discussion

It is unknown what kind of peptides are presented on neighboring antigen-presenting cells by type 1 diabetes-susceptible MHC class II molecules when pancreatic

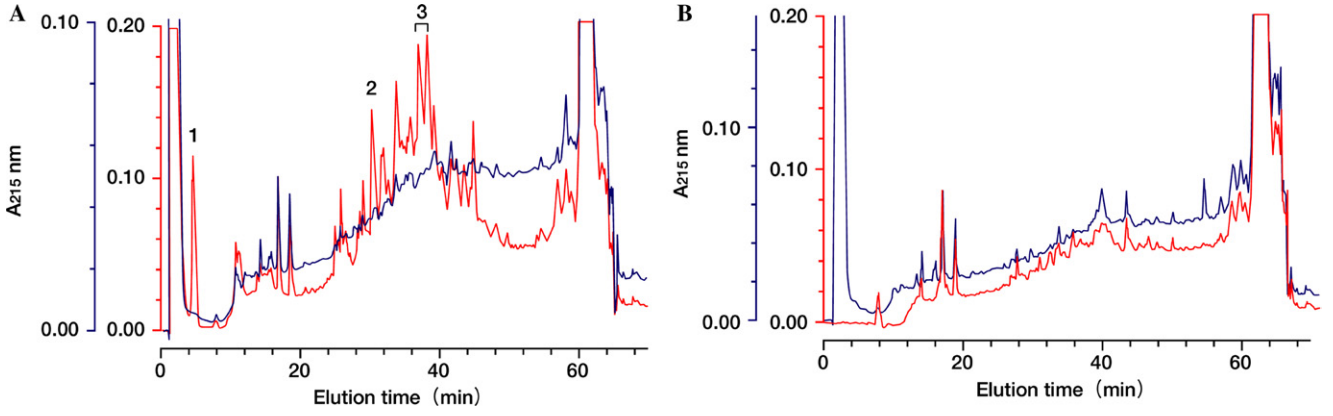

Fig. 1. RP-HPLC analysis of peptides eluted from HLA-DR molecules (A) and HLA-DQ molecules (B). HLA-DR and -DQ molecules were purified from EBV-transformed B cells, which were derived from a newly diagnosed type 1 diabetic patient, and were pulsed with the homogenate of 1B2C6 cells (red lines) or were unpulsed (blue lines). Although no difference was noted in the RP-HPLC profile of the peptides derived from HLA-DQ molecules, three peaks (1–3 in A) was specifically emerged after pulsing in the RP-HPLC profile of the peptides derived from HLA-DR molecules. These three peaks were subsequently sequenced. Please note the difference in the scales of A_{215} nm between pulsed (red lines) and unpulsed (blue lines) conditions.

Table 1
Tentative alignment of the newly isolated peptide with the peptide motifs of HLA-DRB1*0405 and HLA-DRB1*0407

	Relative position									Source (reference)	Residues
	1	2	3	4	5	6	7	8	9		
Anchor or preferred residues in HLA-DRB1*0405	**F**, **Y**			V, I		N, S	Pol[a]		**D**, E		
	W, V I, L M			L, M D, E		T, Q K, D	Chag[b] Ali[c]		Q		
Anchor or preferred residues in HLA-DRB1*0407	**F**, **Y**			**A**		N, T			**Q**		
	W			V, K		D, S			N		
Sequenced peptide	A K S		X N	H	T	X	X	N	**Q**	X R K	
Homologous peptide	A K S		K N	H	T	**T**	H	N	**Q**	S R K	RPL29 [17] or HIP [18] 2–15

Amino acids are expressed as one-letter codes. X denotes undetermined amino acids. RPL 29, ribosomal protein L29; HIP, heparin/heparan sulfate-interacting protein. Anchor or preferred residues in HLA-DRB1*0405 and -DRB1*0407 were quoted from references [19] and [20], respectively.
[a] Polar amino acid.
[b] Charged amino acid.
[c] Aliphatic amino acid.

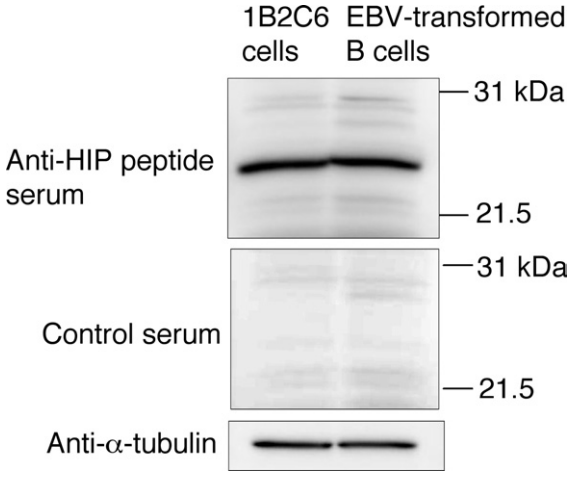

Fig. 2. Western blotting of lysates of 1B2C6 cells and EBV-transformed B cells using guinea pig anti-HIP peptide serum, control guinea pig serum, and anti-α-tubulin antibody. HIP was detected in lysates of both cells.

β-cells are destroyed. To address this issue, we created an in vitro model using bulk culture of EBV-transformed B-cells with type 1 diabetes-susceptible HLA class II alleles as the antigen-presenting cells and a human embryonic pancreas-derived cell line 1B2C6 as the source of islet antigens. 1B2C6 cells originally secreted insulin and glucagon, but lost this ability after several passages (Hiroshi Ishikawa, personal communication). On the basis of the reproducibility of peptide profiles eluted from each HLA-DR allele [21], our strategy was to obtain unique RP-HPLC peaks in the condition of antigen pulse by subtracting the unpulsed RP-HPLC profile from the pulsed profile, as suggested by Chicz and Urban [22]. We found three such RP-HPLC peaks, and identified a peptide presented by HLA-DR molecules but not HLA-DQ molecules. Restriction of islet cell antigen (GAD65)-specific T cell lines by HLA-DR molecules rather than HLA-DQ molecules, was reported previously [7]. In this study, the increase of expression after antigen pulsing itself was striking for HLA-DR (about 17-fold) compared with HLA-DQ (about 3-fold).

The peptide that we detected had a length of 14 amino acids, corresponding to the usual length of peptides eluted from MHC class II molecules [19]. Tentative alignment of this peptide showed that its motif did not closely resemble those of peptides eluted from HLA-DR 4 (DRB1*0405 and DRB1*0407) molecules, especially in that it did not have a hydrophobic residue at relative position 1 [19,20]. Since this peptide was partially matched with the reported peptide motif only at relative positions 6 and 9 [19,20], it may be possible that this peptide was bound to MHC molecule by partially filling the peptide-binding groove as reported in murine MHC class II molecule, I-A^d [23]. Little is known, however, about the peptide motifs that arise when exogenous antigens are pulsed and MHC expression is up-regulated as in our experiment. Thus, binding of this peptide with MHC molecules may not be so strong, but the peptides with strong binding do not necessarily induce T-cell recognition in vivo [24]. Although the β_4 product DRB4*0101 (DR53) is coexpressed with the β_1 product DRB1*0405 or DRB1*0407, its effect on the analyzed peptides seemed to be small or negligible because of the abundant expression of β_1 molecule compared with the other β molecules [25,26].

The source of this peptide was identified as heparin/heparan sulfate-interacting protein (HIP) [18] or ribosomal protein L29 [17] by a homology search. HIP plays a role in the implantation of embryo [18], modulation of

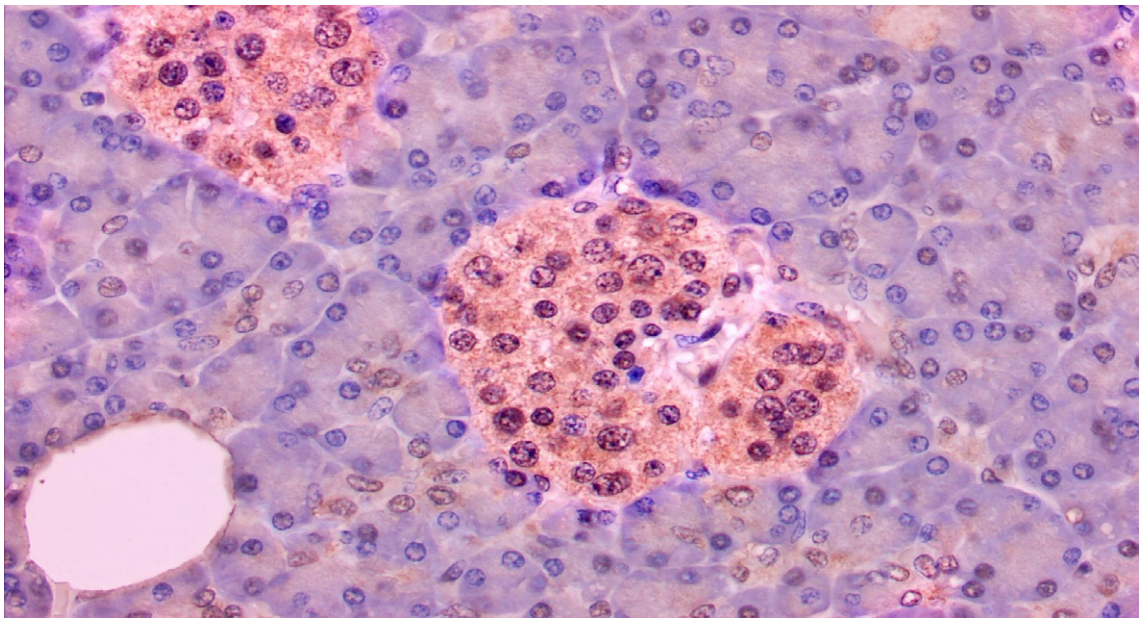

Fig. 3. Immunohistochemical staining of a human pancreatic section for heparin/heparan sulfate-interacting protein (HIP) (original magnification 200×). Islets are homogeneously stained.

coagulation activity [27], and metastasis of cancer [28]. It is expressed at different levels by a variety of human cell lines and normal tissues [18]. In fact, we found that both 1B2C6 cells and EBV-transformed B cells expressed HIP by Western blot analysis (Fig. 2). Since it specifically emerged after pulsing with the homogenate of 1B2C6 cells, one possibility is that HIP protein was incorporated into EBV-transformed B-cells, processed, and presented by HLA-DR molecules, while the other possibility is that upregulation of HLA-DR molecules induced by pulsing with the homogenate of 1B2C6 cells caused EBV-transformed B-cells to present an intrinsic HIP peptide that is seldom presented in the natural state. In either case, destruction of 1B2C6 cells (islet cells) was associated with HIP peptide presentation by HLA-DR molecules on neighboring B-cells (antigen-presenting cells). On the other hand, the expression of HIP in pancreatic sections was exclusively observed in the islets. Thus, even if the HIP peptide in MHC groove was derived from EBV-transformed B-cells, T-cell activation against islets may occur in the pancreas. However, demonstration of HIP-reactive T-cells is needed to provide support for this speculation. Although preliminary T-cell proliferation assays were performed on the patient from whom the EBV-transformed B-cells were derived using HIP peptide and HIP protein expressed in *Escherichia coli* as antigens at 1 year and 7 years after the onset of diabetes, respectively, we failed to show a proliferative response of T-cells (Komatsu and Nakanishi, unpublished data). Further analysis of T-cell responses in newly diagnosed type 1 diabetic patients is needed to determine the role of this peptide in cellular autoimmunity.

In conclusion, by analyzing peptides eluted from type 1 diabetes-susceptible HLA class II molecule, HLA-DR4, we identified a novel islet protein, heparin/heparan sulfate-interacting protein (HIP).

Acknowledgments

This work was supported by grants from the Japanese Ministry of Education, Science, and Culture (Grant Nos. 07807098, 09671086, 12671130, and 14571117), and by a grant from Takeda Science Foundation. We thank Dr. Hiroshi Ishikawa for permission to use 1B2C6 cells, Mr. Hiroyuki Morita for his generous help in coordinating our research team, and Miss Fumie Takano for her secretarial work. Part of this work was presented at the 40th Annual Meeting of the European Association for the Study of Diabetes (Munich, Germany, September 5–9, 2004).

References

[1] R. Tisch, H. McDevitt, Insulin-dependent diabetes mellitus, Cell 85 (1996) 291–297.

[2] K.S. Rønningen, A. Spurkland, B.D. Tait, B. Drummond, C. Lopez-Larrea, F.S. Baranda, M.J. Menendez-Diaz, S. Caillat-Zucman, G. Beaurain, H.-J. Garchon, J. Ilonen, H. Reijonen, M. Knip, B.O. Boehm, C. Rosak, C. Löliger, P. Kühnl, T. Ottenhoff, L. Contu, C. Carcassi, M. Savi, P. Zanelli, T.M. Neri, K. Hamaguchi, A. Kimura, R.P. Dong, N. Chikuba, S. Nagataki, C. Gorodezky, H. Debaz, C. Robles, H.B. Coimbra, A. Martinho, M.A. Ruas, J.A. Sachs, M. Garcia-Pachedo, A. Biro, A. Nikaein, L. Dombrausky, T. Gonwa, C. Zmijewski, D. Monos, M. Kamoun, Z. Layrisse, M.C. Magli, P. Balducci, E. Thorsby,

HLA class II associations in insulin-dependent diabetes mellitus among Blacks, Caucasoids, and Japanese, in: K. Tsuji, M. Aizawa, T. Sasazuki (Eds.), HLA 1991. Proceedings of the Eleventh International Histocompatibility Workshop and Conference, Oxford university press, Oxford, 1992, pp. 713–722.

[3] C.A. Janeway, P. Travers, M. Walport, M. Shlomchik, Immunobiology. The Immune System in Health and Disease, fifth ed., Garland Publishing, New York, 2001.

[4] A. Geluk, K.E. van Meijgaarden, N.C. Schloot, J.W. Drijfhout, T.H.M. Ottenhoff, B.O. Roep, HLA-DR binding analysis of peptides from islet antigens in IDDM, Diabetes 47 (1998) 1594–1601.

[5] E. Harfouch-Hammoud, T. Walk, H. Otto, G. Jung, J.-F. Bach, P.M. van Endert, S. Caillat-Zucman, Identification of peptides from autoantigens GAD65 and IA-2 that bind to HLA class II molecules predisposing to or protecting from type 1 diabetes, Diabetes 48 (1999) 1937–1947.

[6] T.P. Astill, R.J. Ellis, S. Arif, T.I.M. Tree, T.M. Peakman, Promiscuous binding of proinsulin peptides to type 1 diabetes-permissive and -protective HLA class II molecules, Diabetologia 46 (2003) 496–503.

[7] J. Endl, H. Otto, G. Jung, B. Dreisbusch, F. Donie, P. Stahl, R. Elbracht, G. Schmitz, E. Meinl, M. Hummel, A.-G. Ziegler, R. Wank, D.J. Schendel, Identification of naturally processed T cell epitopes from glutamic acid decarboxylase presented in the context of HLA-DR alleles by T lymphocytes of recent onset IDDM patients, J. Clin. Invest. 99 (1997) 2405–2415.

[8] A.E. Herman, R.M. Tisch, S.D. Patel, S.L. Parry, J. Olson, J.A. Noble, A.P. Cope, B. Cox, M. Congia, H.O. McDevitt, Determination of glutamic acid decarboxylase 65 peptides presented by the type I diabetes-associated HLA-DQ8 class II molecule identifies an immunogenic peptide motif, J. Immunol. 163 (1999) 6275–6282.

[9] J. Liu, L.E. Purdy, S. Rabinovitch, A.M. Jevnikar, J.F. Elliott, Major DQ8-restricted T-cell epitopes for human GAD65 mapped using human CD4, DQA1*0301, DQB1*0302 transgenic IA^{null} NOD mice, Diabetes 48 (1999) 469–477.

[10] C.-C. Chao, H.-K. Sytwu, E.L. Chen, J. Toma, H.O. McDevitt, The role of MHC class II molecules in susceptibility to type I diabetes: identification of peptide epitopes and characterization of the T cell repertoire, Proc. Natl. Acad. Sci. USA 96 (1999) 9299–9304.

[11] L.S. Wicker, S.-L. Chen, G.T. Nepom, J.F. Elliott, D.C. Freed, A. Bansal, S. Zheng, A. Herman, Å. Lernmark, D.M. Zaller, L.B. Peterson, J.B. Rothbard, R. Cummings, P.J. Whiteley, Naturally processed T cell epitopes from human glutamic acid decarboxylase identified using mice transgenic for the type 1 diabetes-associated human MHC class II allele, DRB1*0401, J. Clin. Invest. 98 (1996) 2597–2603.

[12] M. Peakman, E.J. Stevens, T. Lohmann, P. Narendran, J. Dromey, A. Alexander, A.J. Tomlinson, M. Trucco, J.C. Gorga, R.M. Chicz, Naturally processed and presented epitopes of the islet cell autoantigen IA-2 eluted from HLA-DR4, J. Clin. Invest. 104 (1999) 1449–1457.

[13] K. Nakanishi, T. Kobayashi, T. Murase, T. Nakatsuji, H. Inoko, K. Tsuji, K. Kosaka, Association of HLA-A24 with complete β-cell destruction in IDDM, Diabetes 42 (1993) 1086–1093.

[14] M. Ota, T. Seki, H. Fukushima, K. Tsuji, H. Inoko, HLA-DRB1 genotyping by modified PCR-RFLP method combined with group-specific primers, Tissue Antigens 39 (1992) 187–202.

[15] E-P. Reich, H. von Grafenstein, A. Barlow, K.E. Swenson, K. Williams, C.A. Janeway Jr., Self peptides isolated from MHC glycoproteins of non-obese diabetic mice, J. Immunol. 152 (1994) 2279–2288.

[16] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, Basic local alignment search tool, J. Mol. Biol. 215 (1990) 403–410.

[17] P.T.W. Law, S.K.W. Tsui, W.Y. Lam, S.C.W. Luk, D.M. Hwang, C.C. Liew, C.Y. Lee, K.P. Fung, M.M.Y. Waye, A novel cDNA encoding a human homologue of ribosomal protein L29, Biochim. Biophys. Acta 1305 (1996) 105–108.

[18] S. Liu, S.E. Smith, J. Julian, L.H. Rohde, N.J. Karin, D.D. Carson, cDNA cloning and expression of HIP, a novel cell surface heparan sulfate/heparin-binding protein of human uterine epithelial cells and cell lines, J. Biol. Chem. 271 (1996) 11817–11823.

[19] H-G. Rammensee, T. Friede, S. Stevanović, MHC ligands and peptide motifs: first listing, Immunogenetics 41 (1995) 178–228.

[20] M.P. Davenport, A. Godkin, T. Friede, S. Stevanović, A.C. Willis, A.V.S. Hill, H.-G. Rammensee, A distinctive peptide-binding motif for HLA-DRB1*0407, an HLA-DR4 subtype not associated with rheumatoid arthritis, Immunogenetics 45 (1997) 229–232.

[21] R.M. Chicz, R.G. Urban, J.C. Gorga, D.A.A. Vignali, W.S. Lane, J.L. Strominger, Specificity and promiscuity among naturally processed peptides bound to HLA-DR alleles, J. Exp. Med. 178 (1993) 27–47.

[22] R.M. Chicz, R.G. Urban, Analysis of MHC-presented peptides: applications in autoimmunity and vaccine development, Immunol. Today 15 (1994) 155–160.

[23] C.A. Scott, P.A. Peterson, L. Teyton, I.A. Wilson, Crystal structures of two $I\text{-}A^{d}$-peptide complexes reveal that high affinity can be achieved without large anchor residues, Immunity 8 (1998) 319–329.

[24] B.T. Wipke, S.C. Jameson, M. Bevan, E.G. Pamer, Variable binding affinities of listeriolysin O peptides for the $H\text{-}2K^{d}$ class I molecule, Eur. J. Immunol. 23 (1993) 2005–2010.

[25] G. Andersson, L. Andersson, D. Larhammar, L. Rask, S. Sigurdardóttir, Simplifying genetic locus assignment of HLA-DRB genes, Immunol. Today 15 (1994) 58–62.

[26] T. Friede, V. Gnau, G. Jung, W. Keilholz, S. Stevanović, H.-G. Rammensee, Natural ligand motifs of closely related HLA-DR4 molecules predict features of rheumatoid arthritis associated peptides, Biochim. Biophys. Acta 1316 (1996) 85–101.

[27] S. Liu, F. Zhou, M. Höök, D.D. Carson, A heparin-binding synthetic peptide of heparin/heparan sulfate-interacting protein modulates blood coagulation activities, Proc. Natl. Acad. Sci. USA 94 (1997) 1739–1744.

[28] D. Marchetti, S. Liu, W.C. Spohn, D.D. Carson, Heparanase and a synthetic peptide of heparan sulfate-interacting protein recognize common sites on cell surface and extracellular matrix heparan sulfate, J. Biol. Chem. 272 (1997) 15891–15897.