with disease duration and age of disease onset in type 1 diabetes, suggesting that the CXCL10 level may reflect "disease activity". Thus, serum CXCL10 appears to be a useful marker in type 1 diabetes, and its level may be used to define disease progression.

Role of the CXCL10/CXCR3 system in type 1 diabetes

As mentioned above, serum CXCL10 levels in type 1 diabetics are higher than those in healthy controls, but what does this mean in terms of pathogenesis? In the NOD model of type 1 diabetes, serum CXCL10 levels seem to correlate with high levels of CXCR3 (and high CXCL10) in pancreatic lymph nodes, where T cells are educated [4]. Moreover, longitudinal analysis of serum CXCL10 levels during the disease course indicated that the CXCL10 level seems to predict the onset of diabetes. Taken together, serum CXCL10 levels reflect an accumulation of CXCR3-positive T cells in pancreatic lymph nodes [4], a phenomenon that occurs just before the onset of diabetes.

These data provoke the following question: could the disease course be altered favorably, if the CXCL10/CXCR3 system were blocked by neutralizing CXCL10 antibodies? We tested this hypothesis and found that administration of CXCL10 monoclonal antibodies to a cyclophosphamide-induced NOD diabetes model resulted in a deceleration of diabetes onset [5]. Moreover, induction of anti-CXCL10 antibodies by a gene transfer system in young NOD mice even resulted in disease suppression [21]. These results indicate that blocking the CXCL10/CXCR3 system can be an effective intervention strategy to suppress diabetes onset both in the malignant phase [22], i.e. the active disease phase, and in the benign phase, i.e. the less active phase.

Interestingly, when both CXCL10 and CXCR3 were blocked, no difference in immunological responses, i.e. in cytokine profile, was found. Instead, a difference in pancreatic beta-cell proliferation was discovered. This means that beta-cells were significantly increased by a blockade of the CXCL10/CXCR3 system. It is of note that these interventions were performed before, rather than after, diabetes onset. We do not know, whether or not this approach is similar effective when applied in the hyperglycemic state.

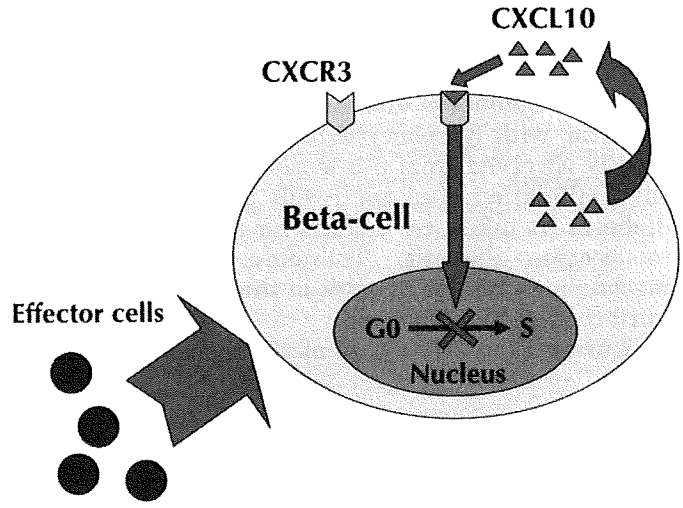

Figure 2. Suppression of beta-cell proliferation by the CXCL10/CXCR3 system. The destruction of beta-cells by effector cells results in CXCL10 production in beta-cells. CXCL10 binds to CXCR3 on beta-cells, and suppresses beta-cell proliferation in an autocrine manner. Blocking this cycle by CXCL10 neutralizing antibody results in beta-cell proliferation.

There is evidence that the effect relates to the beta-cell regeneration capacity. It has recently been shown that an interaction between CXCL10 and Toll-like receptor 4 (TLR4) can result in suppression of beta-cell proliferation [23]. As CXCL10 and CXCR3 are co-expressed in pancreatic beta-cells [5], and CXCL10 expression increases as insulitis progresses [4], we believe that, beside its role in the immune response, the CXCL10/CXCR3 system is also critical for the suppression of pancreatic beta-cell proliferation (Figure 2).

Concluding remarks

It is commonly assumed that both immune regulation and beta-cell regeneration are required to cure type 1 diabetes [24]. Some studies have suggested that the CXCL10/CXCR3 chemokine system plays a critical role in the autoimmune process and in beta-cell destruction in type 1 diabetes. Blocking the CXCL10 chemokine in new onset diabetes seems to be a possible approach for treatment. In combination with another regulatory intervention strategy, such as GAD autoantigen sensitization, this approach could contribute to a curative treatment for type 1 diabetes. We envisage that further research into the CXCL10/CXCR3 system will enable to develop a new and effective therapy.

Conflict of interest statement: Dr. Akira Shimada (M.D., Ph.D.) has research grants from Eli Lilly. All other authors declare that they have no conflict of interests.

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Clinical Immunology (2010) xx, xxx–xxx

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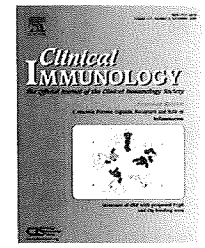

Administration of a determinant of preproinsulin can induce regulatory T cells and suppress anti-islet autoimmunity in NOD mice

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Received 18 September 2009; accepted with revision 24 February 2010

KEYWORDS
Diabetes;
Immunotherapy;
Insulin;
Leader peptide;
NOD;
Regulatory T cell

Abstract Antigen-specific immunotherapy is expected to be an ideal strategy for treating type 1 diabetes (T1D). We investigated the therapeutic efficacy of a peptide in the leader sequence of preproinsulin, which was selected because of its binding affinity to the MHC I-A^{g7} molecule. Preproinsulin-1 L7–24 peptide (L7–24) emulsified in Freund's incomplete adjuvant was administered subcutaneously to NOD mice. Administration of L7–24 increased the proportion of regulatory T cells in the spleen. Splenocytes of NOD mice immunized with this peptide secreted IL-4 and IL-10 in response to L7–24. This peptide also significantly prevented the development of diabetes and cured some newly diabetic NOD mice without recurrence. L7–24 peptide, which has a high affinity for pockets of I-A^{g7}, induced regulatory T cells and showed therapeutic effects. This peptide may provide a new approach for developing antigen-specific immunotherapy for autoimmune diabetes.

Introduction

Type 1 diabetes (T1D) is an organ-specific autoimmune disease characterized by progressive loss of β cells [1]. Autoreactive T cells that react with β cell antigen play a major role in β cell destruction. Several treatments have been attempted to suppress these autoreactive T cells. Cyclosporin slows the autoimmune process and improves residual β cell function, although the positive effect is transient and the drug cannot be applied in clinical practice because of adverse side effects [2,3]. So far, treatment with anti-CD3 monoclonal antibodies seems to be the most effective [4,5], but the preventive effect is limited and this drug may cause general immune suppression.

Administration of autoantigen is expected to become a therapy to establish self-tolerance without severe adverse effects. A recent study demonstrated that subcutaneous injection of glutamic acid decarboxylase (GAD), an important autoantigen in the development of T1D, contributes to the preservation of residual insulin secretion in patients

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doi:10.1016/j.clim.2010.02.016

Please cite this article as: T. Arai, et al., Administration of a determinant of preproinsulin can induce regulatory T cells and suppress anti-islet autoimmunity in NOD mice, Clin. Immunol. (2010), doi:10.1016/j.clim.2010.02.016

Table 1 Sequence of L7–24, B9–23, and HEL10–23.

L7–24	FLPLLA**L**LA**L**W**E**PKPTQA
B9–23	SHLV**E**ALYLVCG**E**RG
HEL10–23	AAMKRHGL**D**NYRGY

Amino acids that have a high affinity for pockets of MHC I-A^{g7} are shown with thick characters.

with alrecent-onset T1D, although it cannot halt the disease process [6].

Several autoantigens involved in the development of T1D have been identified, and there is increasing evidence that insulin is the primary autoantigen among them [7 9]. However, randomized controlled clinical trials of insulin have shown that it cannot delay or prevent the development of T1D [10,11], possibly because of the limited antigen dose, the timing of administration, or a critical epitope in the C-peptide or leader sequence.

In our study, we focused on a determinant of a signal peptide as a new candidate for immunotherapy. Mice have two preproinsulin genes, and the preproinsulin-1 L7–24 peptide (L7–24) was selected as the therapeutic peptide because these amino acids match the unique pockets of MHC I-A^{g7} of NOD mice [12] and peptidase cleavage sites. The peptide was administered to NOD mice to evaluate its protective effects on the development of diabetes.

Materials and methods

Mice

NOD/Shi/Kbe mice were maintained in the Institute for Experimental Animals, Kobe University School of Medicine. Insulin 2 knockout NOD mice were a gift from Dr. George Eisenbarth at the Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center. All experiments were done using female mice. All animals were housed in specific pathogen-free facilities and handled under the Guidelines for Animal Experimentation of Kobe University School of Medicine.

Peptides

HPLC-purified (>95%) peptides were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Amino acid sequences of the synthesized peptide used in our study were as

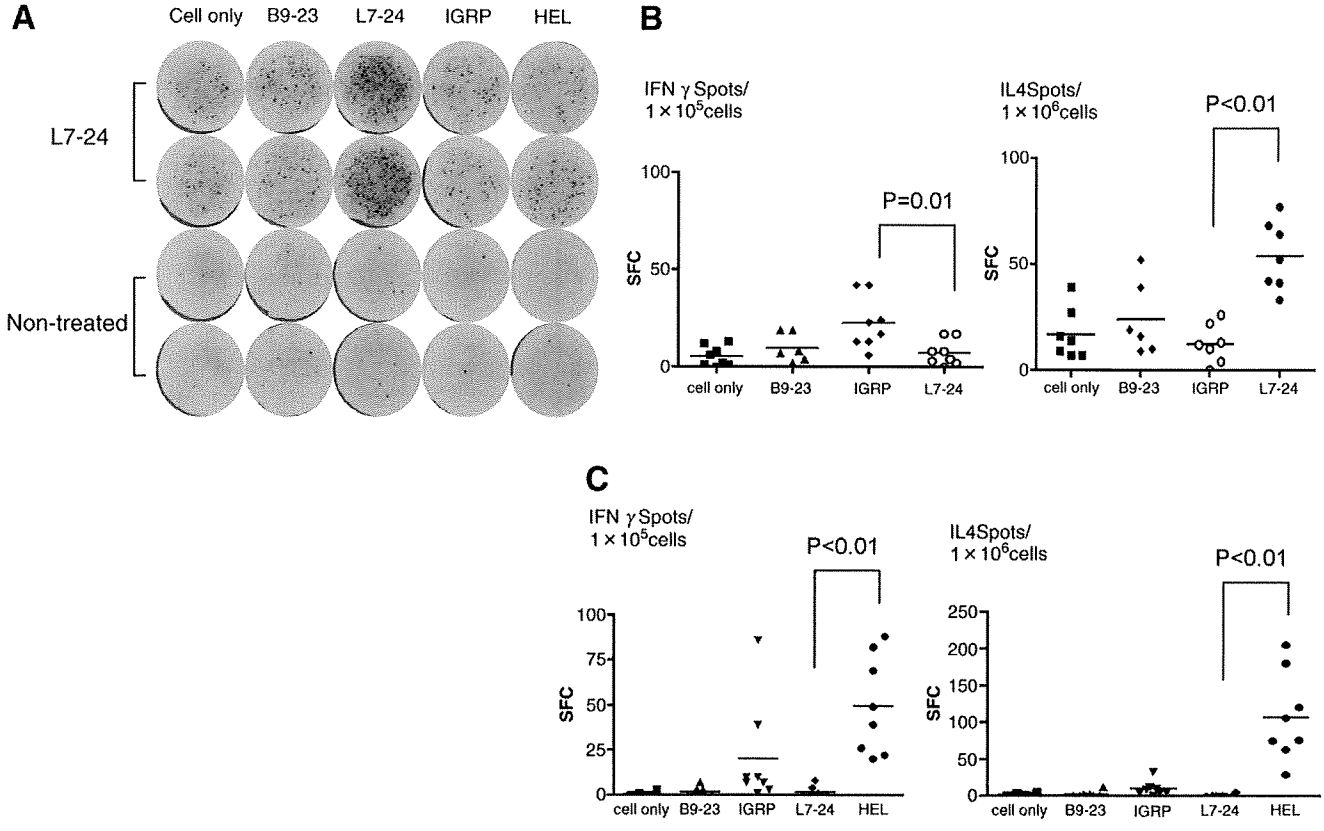

Figure 1 IFN-γ and IL-4 ELISPOT assay for splenocytes from L7–24 peptide-immunized mice (A and B) or HEL10–23 peptide-immunized mice (C). L7–24 or HEL10–23 peptide was injected subcutaneously (s.c.) into NOD mice twice weekly. Seven days after the last immunization, spleen cells were cultured with antigenic peptides (B9–23, IGRP206–214, and L7–24) in duplicates and analysed using an ELISPOT assay. A representative image of an IL-4 ELISPOT assay for splenocytes from L7–24 peptide-immunized mice is shown in (A). The antigen-stimulated spots were quantified as the mean number of IFN-γ spots/1 × 10^5 splenocytes or IL-4 spots/1 × 10^6 splenocytes (n=7), and individual IFN-γ or IL-4 spots of splenocytes from L7–24 peptide-immunized mice (B) or HEL10–23 peptide-immunized mice (C) are depicted as separate dots. Each bar indicates the mean of the spots. Spleen cells of L7–24 peptide-immunized NOD mice showed vigorous secretion of IL-4 and the lack of Th1 reaction, whereas spleen cells of HEL10–23 peptide-immunized mice show both Th1 and Th2 cells.

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follows: preproinsulin-1 L7–24 (FLPLLALLALWEPKPTQA), insulin-2 B9–23 (SHLVEALYLVCGERG), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) 206–214 (VYLKTNVFL), and hen egg lysozyme (HEL) 10–23 (AAMKRHGLDNYRGY).

Peptide administration

Each peptide (100 μg/mouse) in PBS was emulsified with Freund's incomplete adjuvant (IFA, Sigma-Aldrich Japan) and administered subcutaneously (s.c.) to posterior cervical region of the mice. Two subcutaneous administrations were conducted 1 week apart in all immunization. The blood glucose concentration was measured weekly using a FreeStyle® kit (Nipro, Osaka, Japan), and the mice were considered to be diabetic after two consecutive blood glucose (BG) values >300 mg/dL.

Islet-infiltrating cell isolation

Islets were isolated as described previously [13]. Briefly, collagenase (Wako Pure Chemical Industries, Osaka, Japan) was injected through the bile duct into the pancreas. After incubation at 37 °C for 10 to 16 min, islets were isolated by density gradient centrifugation with Histopaque®-1077 (Sigma-Aldrich Japan) and filtered through a cell strainer (BD Falcon, Franklin Lakes, NJ) to remove islets.

ELISPOT

A murine ELISPOT assay was performed using a modification of the human ELISPOT assay described previously [14,15]. In brief, splenocytes (1×10^5 for IFN-γ, 1×10^6 for IL-4) or islet-infiltrating cells (1×10^5) were cultured with antigenic peptides (50 μg/ml) in 96-well microtiter plates (Millititer, Millipore, Bedford, MA) in 200 μl of RPMI 1640 with 5% FCS for 48 h for IFN-γ and 70 h for IL-4. The ELISPOT assay was performed in duplicate well according to the manufacturer's instructions (Mabtech AB, Stockholm, Sweden). Spots were analyzed by the readers blinded to the nature of the sample with an ImmunoSpot Analyzer® (Cellular Technology, Cleveland, OH), and the spot size cutoff was determined at 0.0052 mm^2.

ELISA

The supernatant from splenocytes cultured with antigenic peptides was used for the ELISA. IL-10 concentration was measured using a Biotrak Easy ELISA® (GE Healthcare, Buckinghamshire, UK) and TGF-β concentration was measured using a Quantikine® immunoassay kit (R&D Systems,

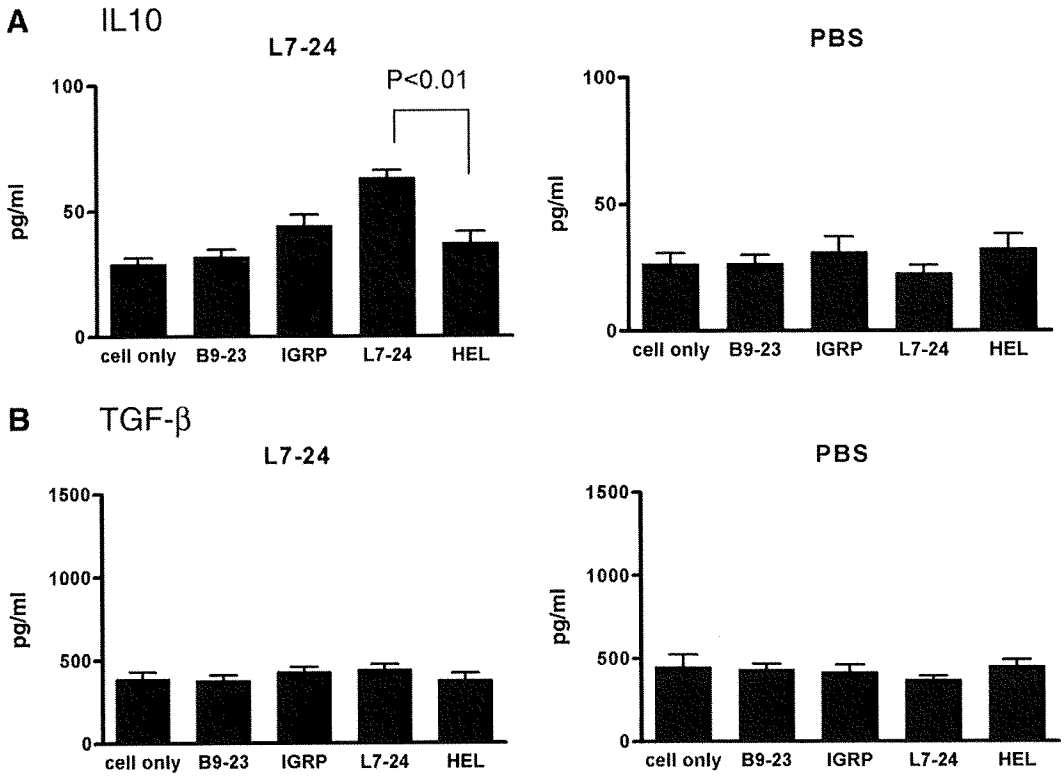

Figure 2 IL-10 and TGF-β ELISA assay for splenocytes from L7–24 peptide-immunized mice or unimmunized mice. Splenocytes (1×10^6) from immunized mice ($n=8$) and control mice ($n=8$) were cultured with antigenic peptides. The concentrations of IL-10 (A) and TGF-β (B) in the supernatant were measured by ELISA. Control mice were immunized with PBS emulsified in IFA. A significantly higher concentration of IL-10 was observed in the supernatant of splenocytes from mice treated with L7–24.

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Minneapolis, MN) according to the manufacturers' instructions.

Flow cytometry

Spleen cells or islet-infiltrating cells were stained with anti-CD4 and anti-CD25 monoclonal antibodies (BD Biosciences Pharmingen, San Diego, CA) for 30 min at 4 °C. Cells were permeabilized and stained using a Foxp3 staining buffer set according to the manufacturer's instructions (eBioscience, San Diego, CA).

Islet histology

The pancreas was fixed in 10% formalin solution, embedded in paraffin, and sectioned at a thickness of 5 μm. The sections were stained with hematoxylin and eosin. Immunostaining for insulin and glucagon was performed using guinea pig antibodies against insulin and rabbit antibodies against glucagon (Dako Japan, Kyoto, Japan). The immune complexes were detected with secondary antibodies conjugated with Cy3 and fluorescein isothiocyanate, respectively (Jackson ImmunoResearch Laboratories, West Grove, PA).

Statistical analysis

Student's *t* test was used to compare the means. A log-rank test was used to compare the incidence of diabetes between groups. GraphPad Prism 4® for Windows (GraphPad Software, San Diego, CA) was used for these analyses.

Results

Determinants of preproinsulin with high affinity to I-A^{g7}

Sequences of preproinsulin 1 and 2 were investigated to find a new immunological determinant with therapeutic effect against autoimmune diabetes. Four amino acids of the peptide are keys to determine the affinity to the binding pockets of MHC I-A^{g7} [12]. We found that the leader peptide of preproinsulin 1 contained three of these amino acids in their sequence (Table 1): 13 (leucine) matches to P4, 15 (alanine) to P6, and 18 (glutamic acid) to P9 of I-A^{g7} binding sites.

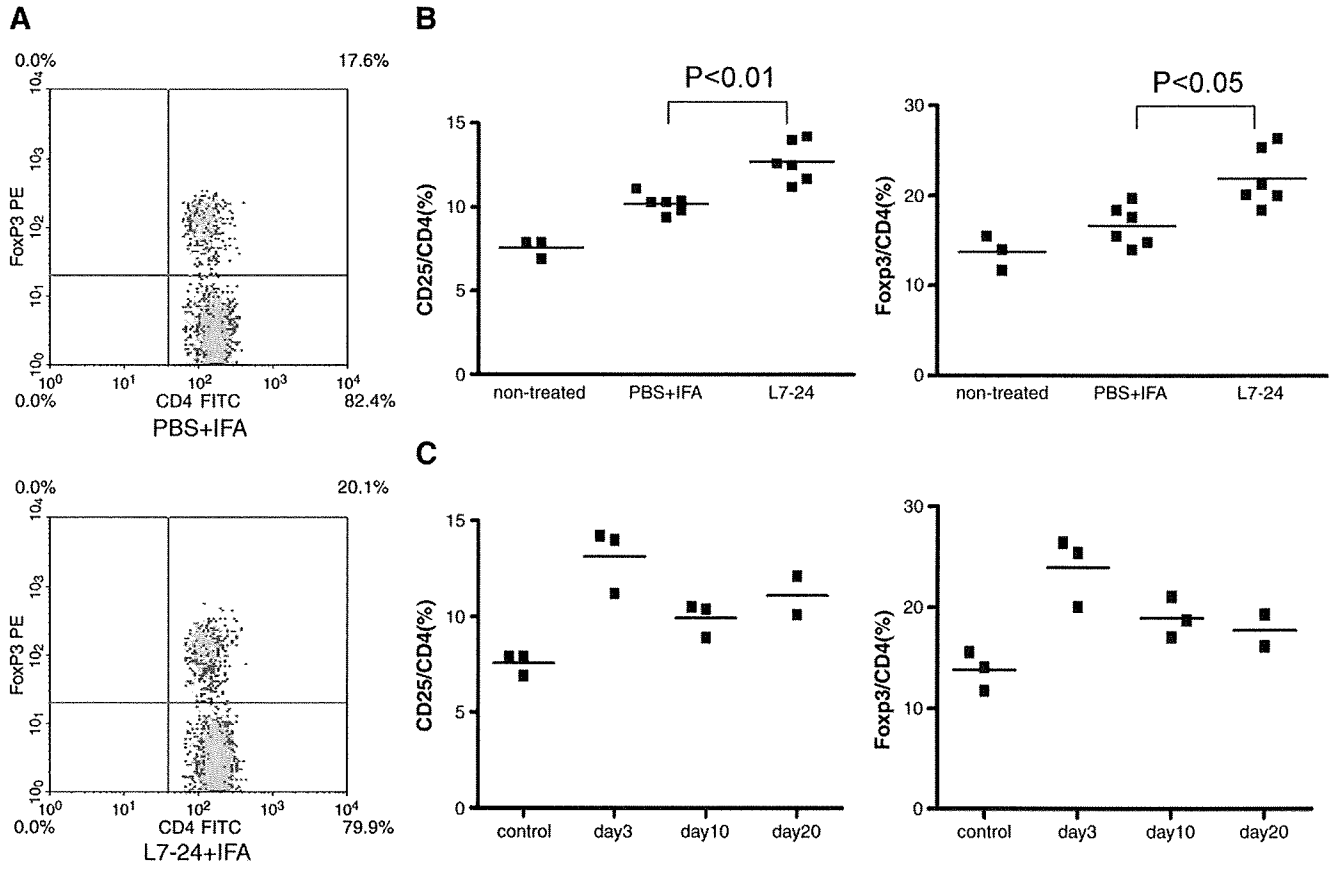

Figure 3 Regulatory T cells are induced by L7–24 peptide immunization. Ten- to 14-week-old NOD mice were immunized with 100 μg of L7–24 peptide twice weekly. The percentages of spleen and pancreatic lymph node cells were analysed by flow cytometry. The percentages of $CD25^+/CD4^+$ and $FoxP3^+/CD4^+$ T cells in spleen were analysed 3 days after the second administration (A and B). The change in the percentage of Tregs in the spleen from L7–24 peptide-immunized mice with time (C); the peak occurred on day 3.

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The leader sequence is a peptide that is required for the entry of preproinsulin into the endoplasmic reticulum. This peptide is synthesized in an equimolar ratio to proinsulin, but it is not released outside β cells and is cleaved by a signal peptidase between L24 and B1 at the endoplasmic reticulum and processed inside β cells after separation from proinsulin. An 18-mer of the peptide from preproinsulin 7 to 24 (L7–24) was selected as a candidate for the therapeutic peptide.

Administration of L7–24 peptide stimulates a Th2 response in NOD mice

To investigate the therapeutic potential of this peptide, 100 μg of this peptide was emulsified in IFA and administered subcutaneously into the neck of 10- to 14-week-old NOD mice. Seven days after the last immunization, spleen cells were cultured with several peptides and IFN-γ and IL-4 secretion were measured using an ELISPOT assay. ConA induced strong response in both IFN-γ and IL-4 secretion. Immunization with L7–24 did not induce IFN-γ secretion but it induced IL-4 secretion significantly in response to L7–24 peptide (Figs. 1A and B). Interestingly, immunization with HEL10–23 peptide, which also binds to I-A^{g7}, induced the secretion of both IL-4 and IFN-γ in response to HEL10–23 peptide (Fig. 1C). The different results for HEL10–23 peptide and L7–24 peptide might be explained by the fact that HEL is not an autoantigen but a foreign antigen that the immune system of the NOD mice had not been exposed to before immunization.

IL-10 and TGF-β are also critical immunoregulatory cytokines that suppress autoimmune processes in various diseases. Supernatants from splenocytes of L7–24-immunized mice were cultured with antigenic peptides, and the concentrations of cytokines in the supernatants were measured by ELISA. L7–24 peptide immunization also induced upregulation of IL-10 secretion in response to L7–24 peptide (Fig. 2A). The amount of TGF-β in supernatant was not different from that of control mice (Fig. 2B).

Administration of L7–24 peptide can induce regulatory T cells

$CD4^+CD25^+FoxP3^+$ regulatory T cells (Tregs) play a crucial role in suppressing autoimmune reactions. Tregs induced by a specific antigen have an antigen-specific immunoregulatory effect, which may be extended to include immunological responses to other antigens.

Three days after the second administration of L7–24 peptide, spleen cells were collected and $CD4^+CD25^+FoxP3^+$

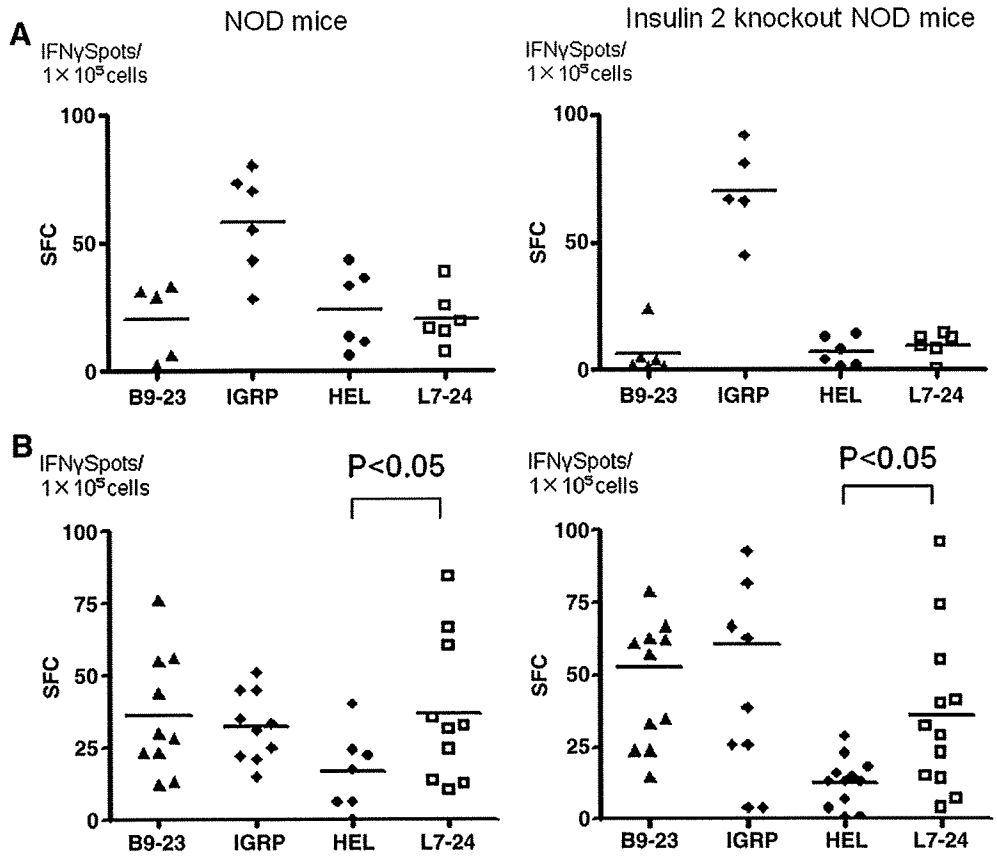

Figure 4 Spontaneous Th1 reaction to L7–24 peptide. Spleen cells and islet-infiltrating cells of 20- to 24-week-old NOD mice and 10–12-week-old insulin 2 knockout NOD mice were analysed by ELISPOT. (A) Ten thousand spleen cells were cultured for 48 h with each peptide. Secretion of IFN-γ in response to L7–24 was not observed. (B) IFN-γ spot forming colony (SFC) from islet-infiltrating cells was analysed. Islet-infiltrating cells of both NOD mice and insulin 2 knockout NOD mice showed vigorous production of IFN-γ in response to L7–24.

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cells were analysed by flow cytometry. The percentages of $CD25^+/CD4^+$ and $FoxP3^+/CD4^+$ cells were significantly higher in L7–24 peptide-immunized mice than in controls (Figs. 3A and B). The percentage of cells peaked on the third day after the second injection (Fig. 3C). Unexpectedly, the percentage of Tregs in the pancreatic lymph node did not differ between L7–24 peptide-immunized mice and unimmunized mice (data not shown).

Islet-infiltrating cells of late insulitis secrete IFN-γ in response to L7–24 peptide

To examine whether the L7–24 sequence is targeted by pathogenic T cells, we analysed splenocytes and islet-infiltrating cells of untreated NOD mice (20 weeks old) and insulin 2 knockout NOD mice (12 weeks old). IFN-γ secretion was not upregulated in response to L7–24 in splenocytes (Fig. 4A). However, islet-infiltrating cells of both NOD mice and insulin 2 knockout NOD mice showed vigorous production of IFN-γ in response to L7–24 (Fig. 4B).

Administration of L7–24 peptide prevents the development of diabetes

To explore the effect of L7–24 peptide in preventing diabetes, we injected 100 μg of the peptide in IFA subcutaneously twice weekly into 12-week-old NOD mice. Only three of 22 NOD mice treated with the peptide developed diabetes at 45 weeks of age. The protection was significant compared with the mice given IFA without peptide ($p=0.046$, Fig. 5A). The insulin 2 knockout NOD mouse model is an accelerated model of autoimmune diabetes that starts developing diabetes around 10 weeks of age, and the cumulative incidence of diabetes reaches almost 100% [7]. Unlike NOD mice, injection of L7–24 into 8-week-old insulin 2 knockout NOD mice did not significantly delay the development of diabetes (Fig. 5B).

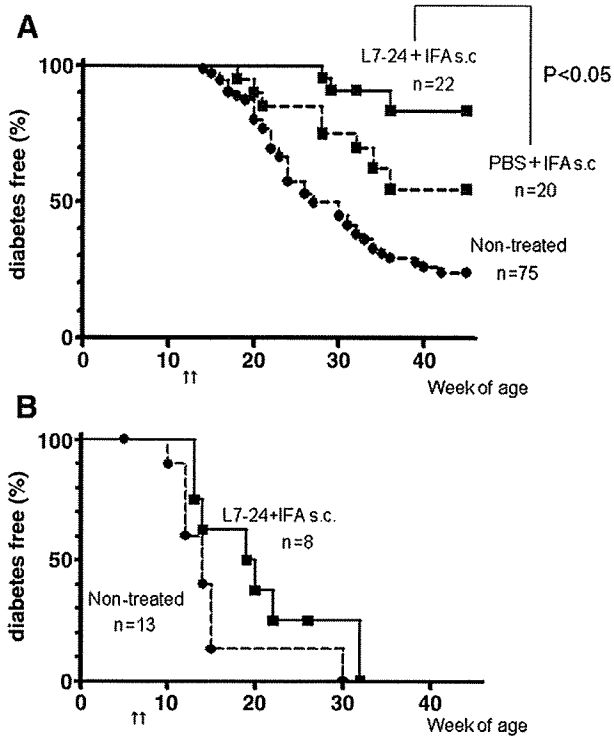

Figure 5 Administration of L7–24 peptide to 12-week-old NOD mice. (A) Twelve-week-old NOD mice were given two subcutaneous injections of L7–24 peptide. L7–24 peptide prevented diabetes compared with administration of IFA alone ($p=0.046$). (B) L7–24 peptide was administered similarly to insulin 2 knockout NOD mice. Only a slight delay in the onset of diabetes was observed. The little arrows indicate the timing of peptide immunization.

Administration of L7–24 peptide to diabetic NOD mice can restore normoglycemia

The therapeutic effect of L7–24 peptide on newly diabetic NOD mice was investigated next. Diabetic mice (BG >300 mg/dL) were assigned to receive injected L7–24, B9–23, or PBS only in IFA. After injections of the peptides twice a week, blood glucose concentration was monitored weekly. Five of 13 mice treated with L7–24 became normoglycemic 2–5 weeks after the last injection, whereas only one of 18 mice treated with PBS or B9–23 in IFA returned to normoglycemia (Figs. 6A–C). Histological examination of pancreata of the mice 16 weeks after restoration from hyperglycemia showed that some islets were preserved despite the remarkable infiltration of lymphocytes (Figs. 6D and E). Insulin and glucagon staining showed the retention of insulin-producing cells along with scattered glucagon-producing cells in the islets.

Discussion

The ultimate goal of treatment for autoimmune diabetes is antigen-specific suppression of the disease. There is increasing evidence that induction of antigen-specific Tregs is critical to halting the disease process [16,17]. Immunotherapy is currently applied in clinical practice to treat allergic diseases such as allergic rhinitis and asthma. However, the application of antigen-specific immunotherapy has not yet been successful in the treatment of autoimmune diseases.

There has been notable progress in immunotherapy for T1D. Subcutaneous injection of the GAD molecule with alum significantly reduces the progressive decline in β cell function without severe side effects [6,18,19]. Insulin is expected to be a promising autoantigen for the antigen-specific treatment of T1D. Several studies of NOD mice have shown the effectiveness of insulin administration in preventing the development of diabetes [20 22]. Although clinical studies of human T1D have been done, the clinical effectiveness of insulin administration has not been shown in humans, except for slight benefits in participants with a high titer of insulin autoantibody in the Diabetes Prevention Trial of T1D [10,11,23]. Nevertheless, insulin is undoubtedly the most abundant and specific molecule in the β cell, and there is increasing evidence that insulin is a primary antigen for T1D [24]. We hypothesized that additional epitopes besides those in the insulin A–B chain might be useful for providing tolerance in T1D, and we screened the sequence of the leader peptide that is removed from proinsulin in the rough

Please cite this article as: T. Arai, et al., Administration of a determinant of preproinsulin can induce regulatory T cells and suppress anti-islet autoimmunity in NOD mice, Clin. Immunol. (2010), doi:10.1016/j.clim.2010.02.016

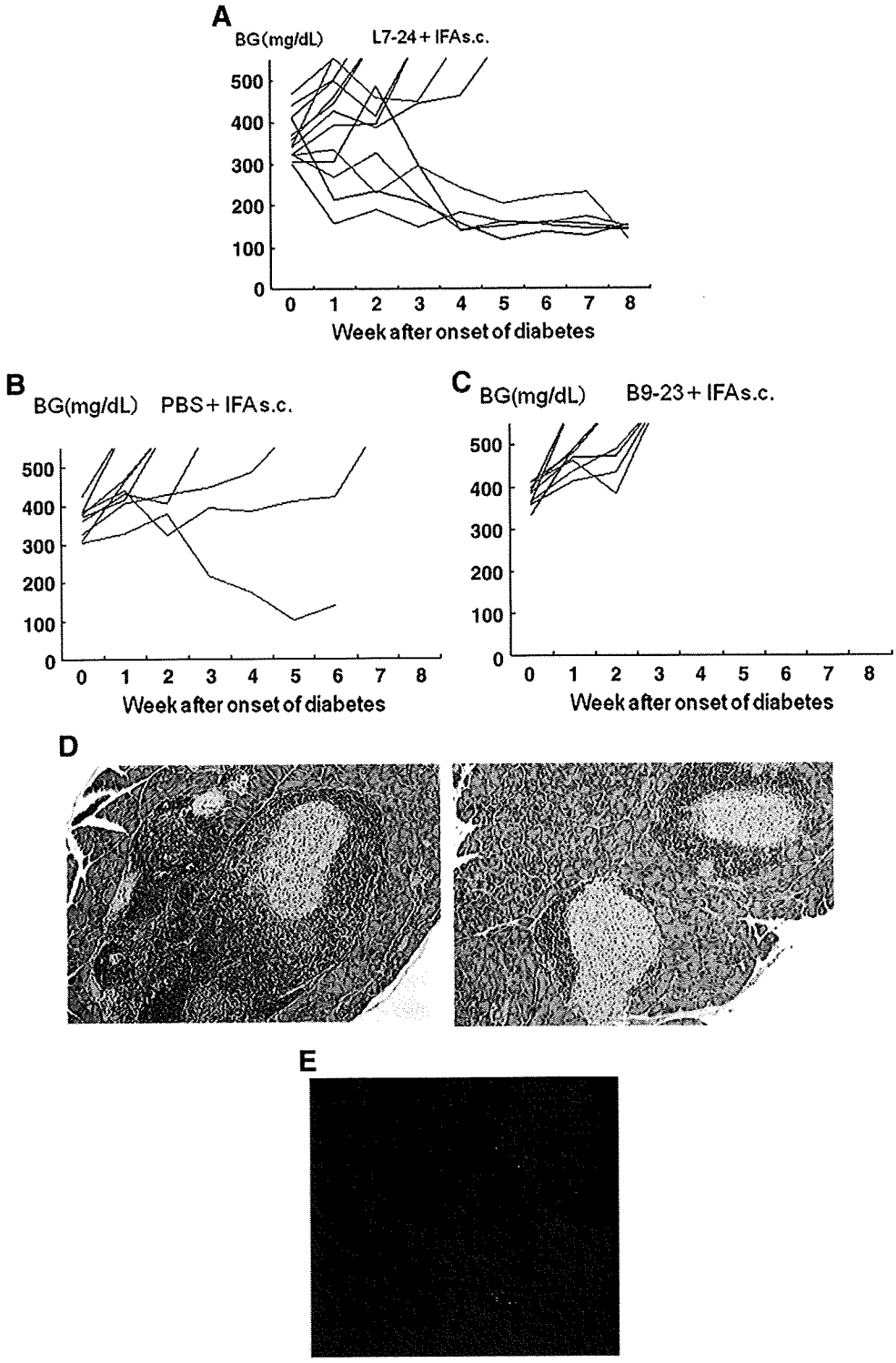

Figure 6 Administration of L7–24 to newly diabetic NOD mice. L7–24 peptide was administered to newly diabetic NOD mice (BG > 300 mg/dL). Five of 13 mice achieved remission of diabetes (A) whereas most mice did not by the administration of PBS in IFA (B) or B9–23 in IFA (C). Mean ± SD of blood glucose of each group on entry are as follows: L7–24, 362±53 mg/dl; PBS, 361±40 mg/dl; and B9–23, 380±26 mg/dl. Hematoxylin and eosin staining of islets of NOD mice that achieved remission show preservation of islets despite the marked lymphocyte infiltration (D). Insulin (red) and glucagon (green) staining showed the retention of insulin-producing cells along with scattered glucagon-producing cells in the islets (E).

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endoplasmic reticulum before the entry into the secretory granule.

We found that L7-24 peptide increased the frequency of $CD25^+Foxp3^+$ Tregs and the production of regulatory cytokines such as IL-4 and IL-10. Administration of this peptide to 12-week-old NOD mice significantly reduced the incidence of diabetes, probably by stimulating regulatory cytokine-producing cells. The milder suppression of diabetic development in groups that received PBS with IFA was an unexpected observation. The protective effect of Freund's complete adjuvant (CFA) but not IFA to the development of diabetes in NOD mice is widely recognized [25]. There was, however, a report that showed the milder protective effect of IFA similar to our observation [26].

Interestingly, analysis of islet-infiltrating cells in late-stage insulitis demonstrated vigorous IFN-γ secretion in response to L7-24 peptide. The result suggests that L7-24 is supposed to be naturally processed and presented in the course of autoimmune diabetes in NOD mice. A study using pools of preproinsulin peptides showed an IL-2 response after subcutaneous administration of L7-24 peptide emulsified in Freund's complete adjuvant but not in IFA, although the Th2 responses were not reported [27]. In our study, L7-24 peptide immunization produced a Th2 cytokine profile due to immunization in the presence of IFA. This peptide may have potency for both the Th1 and Th2 responses, although Th2 immunogenicity can be induced more predominantly in IFA. Unlike L7-24, HEL10-24 in IFA seems to induce equally both Th1 and Th2 response. Therefore, L7-24 can be administered more safely than HEL, which induce adverse side effect by Th1 response.

It was advocated recently that self-peptides with low affinity for MHC are important as autoantigenic epitopes [28 30]. The hypothesized reason for this phenomenon is that autoreactive T cells that recognize a weak MHC-binding peptide can escape negative selection in the thymus. In contrast, T cells that react with stronger MHC-binding peptides are anticipated to receive negative selection in the thymus and to survive as natural Tregs in the periphery [31]. L7-24 was selected based on an alignment algorithm described by E.R. Unanue et.al [13]. The peptide has three amino acids that have a high affinity for pockets of the MHC $I\text{-}A^{g7}$ molecule and is expected to be present frequently in the thymus. Tregs induced with L7-24 peptide may be explained by the high binding affinity of this peptide to the $I\text{-}A^{g7}$ molecule.

Although L7-24 peptide showed a therapeutic effect, the effect remains insufficient to protect fully against the disease. Administration of the peptide to 12-week-old NOD mice significantly prevented diabetes, but some mice became diabetic even after immunization. In addition, L7-24 peptide could not prevent diabetes in insulin 2 knockout NOD mice, which develop diabetes at a faster rate than normal NOD mice. As disease progresses, more epitopes are being targeted due to epitope spreading. At advanced stages of the disease, combination of more than one epitopes may prove more beneficial. Combined peptide therapy might be an effective immunotherapy in the future, and the combination of peptide therapy and CD3 antibody may be also a promising option [32].

Insulin-derived epitopes are among the autoantigens specific to β cell and the most abundant epitopes in β cell. The reason why whole insulin immunization have not succeeded in protecting from type 1 diabetes (T1D) in humans is probably due to some issues that remain to be resolved, such as the use of correct dosage, more efficient immunization schemes and possibility of critical epitopes other than A-B chain.

The leader peptide of insulin has not attracted the attention of researchers as an autoantigen relevant to T1D. However, a recent study using human HLA-A0201 showed that the CD8 epitope of the leader peptide is a candidate autoantigen in human T1D [33]. Taken together, these previous findings and our results suggest that the leader sequence contains some critical determinants for development of diabetes in both human T1D and NOD mice. Our study is the first to demonstrate a therapeutic effect of the peptide, which is contained in the leader sequence. The identification of the T cell response to the leader sequence in patients with T1D suggests that some of the leader sequence may also be applicable in the protection against and cure for humanT1D.

Acknowledgements

The authors thank Dr. George Eisenbarth (Barbara Davis Center for Childhood Diabetes, University of Colorado Health Science Center) for kindly providing insulin 2 knockout NOD mice. The authors also thank Ms. Atsumi Katsuta and Mr. Takeshi Hamada for the laboratory assistance.

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Please cite this article as: T. Arai, et al., Administration of a determinant of preproinsulin can induce regulatory T cells and suppress anti-islet autoimmunity in NOD mice, Clin. Immunol. (2010), doi:10.1016/j.clim.2010.02.016