Biochemical and Biophysical Research Communications 427 (2012) 513–517

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Daintain/AIF-1 (Allograft Inflammatory Factor-1) accelerates type 1 diabetes in NOD mice

Yan-Ying Zhao [a,*], Xin-Yuan Huang [b], Zheng-Wang Chen [c]

[a] College of Life Science and Technology, Southwest University for Nationalities, Chengdu 610041, China
[b] College of Life Science and Technology, Hubei Engineering University, Xiaogan 432000, China
[c] Key Laboratory of Molecular Biophysics of the Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

ARTICLE INFO

Article history:
Received 2 September 2012
Available online 26 September 2012

Keywords:
Daintain
AIF-1
Type 1 diabetes
Pancreatic β cell
Nitric oxide

ABSTRACT

A large body of experimental evidence suggests that cytokines trigger pancreatic β-cell death in type 1 diabetes mellitus. Daintain/AIF-1 (Allograft Inflammatory Factor-1), a specific marker for activated macrophages, is accumulated in the pancreatic islets of pre-diabetic BB rats. In the present study, we demonstrate that daintain/AIF-1 is released into blood and the levels of daintain/AIF-1 in the blood of type 1 diabetes-prone non-obese diabetic (NOD) mice suffering from insulitis are significantly higher than that in healthy NOD mice. When injected intravenously into NOD mice, daintain/AIF-1 stimulates white blood cell proliferation, increases the concentrations of blood glucose, impairs insulin expression, up-regulates nitric oxide (NO) production in pancreases and accelerates diabetes in NOD mice, while the antibody against daintain/AIF-1 delays or prevents insulitis in NOD mice. These results imply daintain/AIF-1 triggers type 1 diabetes probably via arousing immune cells activation and induction of NO production in pancreas of NOD mice.

1. Introduction

Type 1 diabetes is a multi-factorial autoimmune disease characterized by T cell mediated destruction of β cells in pancreas [1]. The early stages of the disease process are termed insulitis, an inflammatory reaction resulting from the infiltration of pancreas by mononuclear immune cells, including dendritic cells, macrophages, and T cells [2]. The death of β cells in this process is probably caused by direct contact with activated macrophages and T cells, and/or exposure to soluble mediators secreted by these cells, including cytokines, nitric oxide (NO), and oxygen free radicals [3]. Although the molecular pathways involved in the pathogenesis of type 1 diabetes are unknown, there is substantial evidence that suggests cytokines as key mediators [4,5].

Daintain, a 146 amino acid peptide, is produced by macrophage lineage first isolated from porcine intestines with effects on insulin release in the mid-1990s [6,7]. Contemporaneously, Allograft Inflammatory Factor-1 (AIF-1) was identified as a macrophage factor in rat and human cardiac allografts with chronic rejection [8,9]. Daintain and AIF-1 share the probable identical amino acid sequence, therefore, we call the polypeptide daintain/AIF-1 [10]. Although the mechanisms are still not very clear, studies from several diverse systems, demonstrate that the protein and its related proteins [11,12], are involved in inflammatory and immune responses such as survival and pro-inflammatory activity of macrophages [13], production of cytokines in a mouse macrophage cell line [14], neointimal hyperplasia, endothelial cell activation [15,16] and autoimmune diseases such as experimental autoimmune neuritis, encephalomyelitis, and uveitis [17].

Our previous work has revealed that a particularly dense accumulation of daintain/AIF-1 located in the pancreatic islets of pre-diabetic BB rats [7]. Later, we found that daintain/AIF-1 could decrease the cell viability and glucose-stimulated insulin secretion in INS-1 β cells [18]. In the present study, we aim to explore the role of daintain/AIF-1 in the pathology of type 1 diabetes using type 1 diabetes-prone non-obese diabetic (NOD) mice, a well established animal model for diabetes research [19].

2. Materials and methods

NOD mice were obtained from the standard animal center of China Medical College (Beijing, China). Animal studies were approved by the Huazhong University of Science and Technology Institutional Committee for the Care and Use of Animals.

2.1. Daintain/AIF-1 assay in blood

Blood samples were obtained from retro-orbital sinus of 8 NOD (female, 9 weeks) mice in heparin tubes. Plasma were collected by

* Corresponding author. Fax: +86 28 85522310.
E-mail address: biozyy@163.com (Y.-Y. Zhao).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.09.087

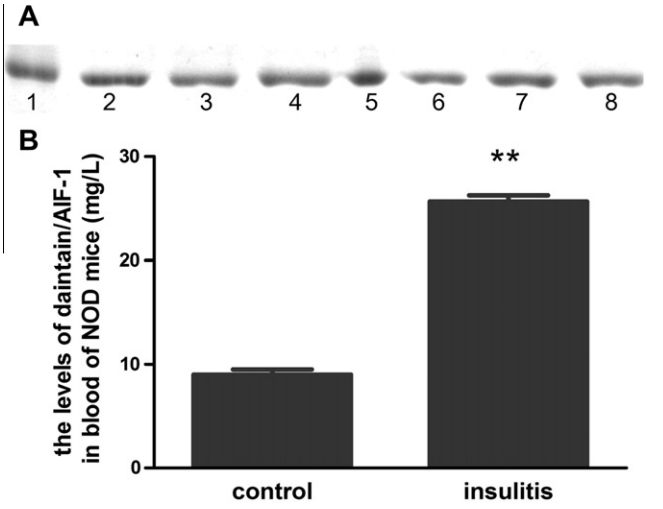

Fig. 1. Daintain/AIF-1 assay in blood. (A) Western blot analysis of daintain/AIF-1 in the blood of NOD mice. Proteins in the plasma of NOD mice were concentrated and applied to Western blot, daintain/AIF-1 was recognized with the mono-antibody against it. 1–8: Plasma from 8 NOD mice, respectively. (B) The levels of daintain/AIF-1 in the plasma of NOD mice suffering from insulitis (insulitis) and healthy NOD mice (control) were analyzed with competitive ELISA. Results are expressed as mean values ± SEM (**$p < 0.01$ vs. control).

centrifugation at 1500*g* for 10 min and concentrated by centrifugal filter devices (Amicon Ultra 3K device, Millipore, USA) according to the user guide. Western blot analysis was employed to determine whether daintain/AIF-1 could be released into blood. The monoclonal antibody against daintain/AIF-1[20] (dilution 1:1000) was used to recognize daintain/AIF-1 and then a goat anti-mouse IgG secondary antibody-conjugated horseradish peroxidase (1:3000, Santa Cruz, USA) was added. Reactions were visualized with CN/DAB detection kit (Thermo Scientific, USA).

The level of daintain/AIF-1 in blood was measured by competitive Enzyme-Linked Immunosorbent Assay (ELISA) as described previously [21]. Briefly, blood samples from 9 healthy NOD mice (female, aged 4 weeks) and 9 NOD mice suffering from insulitis (female, aged 9 weeks) were collected in heparin tubes from retro-orbital sinus, respectively. The samples were centrifuged at 1500*g* for 10 min and a 20 μL aliquot of each sample was diluted to 50 μL in saline for daintain/AIF-1 assay. 96 well polyvinylchloride plates were coated with 100 μL daintain/AIF-1 (purified from porcine intestine as described in Ref. [7], 5 mg/L in 50 mM bicarbonate buffer, pH 9.6) and incubated overnight at 4 °C. The wells were blocked with 120 μL of 1% gelatin at 37 °C for 40 min, and washed with phosphate buffered saline containing 0.05% Tween 20 (PBST, pH 7.4) for 5 times. An equal volume (50 μL) of anti-daintain/AIF-1 monoclonal antibody (dilution 1:1000), daintain/AIF-1 (20 mg/L was diluted to$1{:}2^{1-15}$) was mixed at room temperature for 30 min and then added to each well, incubated at 37 °C for 60 min and washed with PBST for 5 times, followed by addition of goat anti-mouse IgG-horseradish peroxidase (100 μL, 1:3000, Santa Cruz, USA) into each well at 37 °C for 60 min, washed again. Thereafter, 100 μL of substrate solution (5% H_2O_2, 0.2 M Na_2HPO_4, 0.1 M citric acid, pH 5.0) was added into each well and incubated for 30 min, finally 50 μL of 2 M H_2SO_4 was applied to terminate reactions. Results were measured photometrically at 492 nm. Standard curve was used to determine the concentrations of substance, plotting the concentration of daintain/AIF-1 on the *X* axis, and the absorbance at 492 nm on the *Y* axis. For daintain/AIF-1 assay in plasma, daintain/AIF-1 was substituted by the same volume (50 μL) of plasma from each NOD mouse to be mixed with the mono-antibody against it.

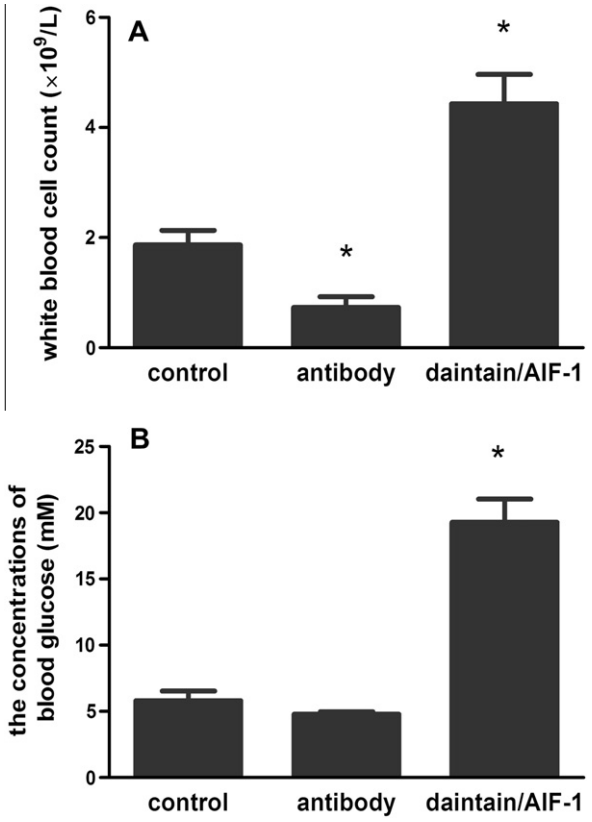

Fig. 2. White blood cell count and determination of blood glucose levels. Daintain/AIF-1, the antibody against daintain/AIF-1 or saline was intravenously injected into NOD mice for three times. (A) White blood cell count. (B) The concentrations of blood glucose were measured with an Accu-Chek Advantage blood glucose monitor. Data are the mean values ± SEM from 9 independent experiments (*$p < 0.05$ vs. the control group).

2.2. White blood cell count and determination of blood glucose levels

The NOD mice (female, aged 4 weeks) were randomly divided into three equal groups ($n = 9$) i.e. (a) control group, in which the mice received saline, (b) daintain/AIF-1 group, in which the mice received daintain/AIF-1 at 0.5 μg/g body weight (in saline), (c) antibody group, in which the mice received the mono-antibody against daintain/AIF-1 at 1 μg/g body weight (in saline) [20], intravenously on the first, 10th and 20th day, respectively. On the 35th day, white blood cell (WBC) count was performed using a Celltac α counter (MEK-6318K, Nihon Kohden, Japan). Then the mice were fasted for 8 h and the concentrations of blood glucose were measured with an Accu-Chek Advantage blood glucose monitor (Roche Diagnostics, Swiss).

2.3. Immunohistochemistry

The expression of insulin in pancreas of each mouse was detected by immunohistochemistry. Briefly, the mice were anesthetized and executed 35 days after the first injection. After fixation in 4% paraformaldehyde, tissue samples were incubated with the monoclonal antibody against insulin (1:200, Sigma, USA) and washed with PBS. Peroxidase-conjugated rabbit anti-mouse IgG (Santa Cruz, USA) diluted 1:500 in PBS was used as the second immune-reagent. Sections were counterstained with hematoxylin and reaction products were visualized with 3.3′-diaminobenzidine.

2.4. Detection of nitric oxide (NO) levels in pancreas

The pancreases of the NOD mice treated with saline, daintain/AIF-1 or antibody were washed with buffer (50 mM Tris–HCl, 0.15 M NaCl, pH 7.6) and cut into small pieces, then homogenized and centrifuged at 12000*g* for 10 min at 4 °C to remove the debris. Pancreatic NO production was measured with NO assay kit (Beyotime Biotechnology, Nanjing, China) using the Griess reagent method [22] according to the manufacturer's protocol. Briefly, 50 μL of tissue extract was collected and mixed with 50 μL of Griess reagent in a multi-well microtiter plate at room temperature for 5 min. Absorbance was measured at 540 nm in a microplate reader (Tecan Sunrise, Salzburg, Austria) and the amount of nitrite was calculated from a $NaNO_2$ standard curve. Proteins were quantified with bicinchoninic acid (BCA) protein assay Kit (Beyotime Biotechnology, Nanjing, China).

2.5. Statistical analysis

All data obtained were expressed as means ± SEM. The differences between the means were analyzed statistically with Student's *t*-test. Values of $p < 0.05$ were taken to imply statistical significance.

3. Results

3.1. Daintain/AIF-1 assay in blood

After concentrated, plasma from 8 NOD mice were applied to Western blot, daintain/AIF-1-immunoreactions were visible on the polyvinylidene difluoride (PVDF) membranes as shown in Fig. 1A. The result revealed that daintain/AIF-1 was released from its original cells into blood.

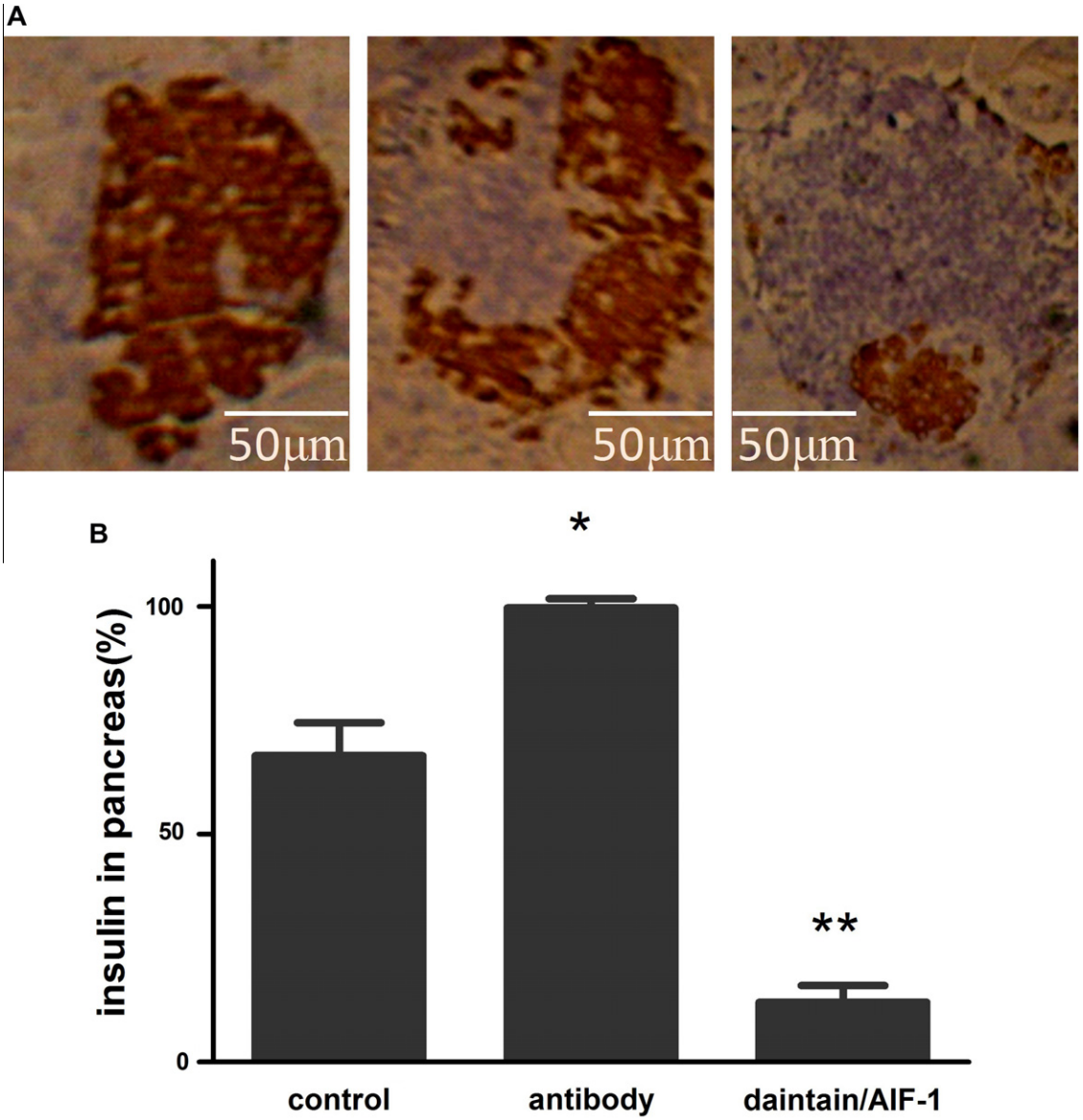

Fig. 3. Immunohistochemical localization of insulin in pancreas. (A) An insulin-stained image of cross sectional pancreatic β cells area in antibody group (left), the pancreatic islet was normal and positively stained. The control group (middle), the pancreatic islet was suffering from insulitis with infiltration of pancreas by mononuclear immune cells, part of pancreatic islet was stained. The daintain/AIF-1 group (right), the pancreatic islet was suffering from severe insulitis with infiltration of pancreas by large amounts of mononuclear immune cells, and only a little number of β cells remained. (B) The average mass of pancreatic β cells in the control group, the antibody group and daintain/AIF-1 group. Data are presented as mean ± SEM ($n = 9$) ($^{*}p < 0.05$, $^{**}p < 0.01$vs. the control group).

Competitive ELISA was carried out to examine the levels of daintain/AIF-1 in blood during diabetes progression in NOD mice. As show in Fig. 1B, the average level of daintain/AIF-1 in the blood of NOD mice suffering from insulitis was 25.7 ± 1.1 mg/L, significantly higher than that in the healthy NOD mice 9.0 ± 1.3 mg/L ($p < 0.01$). The results suggest that daintain/AIF-1 is a circulatory protein involved in type 1 diabetes.

3.2. White blood cell count and determination of blood glucose level

Report has shown that daintain/AIF-1 played an important role in the survival and pro-inflammatory activity of macrophages [13]. Activation of autoimmune cells is a critical issue in type 1 diabetes. To explore the role of daintain/AIF-1 in type 1 diabetes, daintain/AIF-1 or the antibody was intravenously injected into NOD mice. As shown in Fig. 2, white blood cell count and blood glucose levels in daintain/AIF-1 group were obviously higher than that in control group (white blood cell: $4.4 \pm 1.6 \times 10^9$/L vs. $1.9 \pm 0.8 \times 10^9$/L, $p < 0.05$; blood glucose: 19.3 ± 6.5 mM vs. 5.4 ± 1.1 mM, $p < 0.05$), white blood cell count in antibody group was significantly lower than that in control group ($0.7 \pm 0.4 \times 10^9$/L vs. $1.9 \pm 0.8 \times 10^9$/L, $p < 0.05$), but there were no statistically significant difference between the blood glucose levels in the antibody group and the control group (4.8 ± 0.6 mM vs. 5.4 ± 1.1 mM). Here, daintain/AIF-1 stimulated white blood cell proliferation, probably enhanced inflammatory activity in NOD mice. In addition, the NOD mice treated with daintain/AIF-1 exhibited the classic diabetes symptoms such as frequent urination, excessive thirst and unusual fatigue. The results imply that daintain/AIF-1 probably participates in the initiation of diabetes in NOD mice.

3.3. Immunohistochemistry

Type 1 diabetes results from the destruction of insulin-producing pancreatic β cells by a β cell-specific autoimmune process. Immunohistochemistry was carried out to observe insulin expression in pancreases of NOD mice. Pancreases were collected 35 days after the first injection and fixed in 4% paraformaldehyde. β cells were recognized by the mono-antibody against insulin and the reaction products were visualized with 3.3′-diaminobenzidine. As shown in Fig. 3, Fig 3A is an insulin-stained image of cross sectional pancreatic β cells area in antibody group (left), the pancreatic islet was normal and positively stained. The control group (middle), the pancreatic islet was suffering from insulitis with infiltration of pancreas by mononuclear immune cells, part of pancreatic islet was stained. The daintain/AIF-1 group (right), the pancreatic islet was suffering from severe insulitis with infiltration of pancreas by large amounts of mononuclear immune cells, and only a little number of β cells remained. In fact, pancreatic islets could not be found in some samples with daintain/AIF-1 treatment. Compared with the antibody group, β cell mass was reduced to about 67 ± 22% in control group ($p < 0.05$, β cell mass in antibody group was considered to be 100%) and 13 ± 11% ($p < 0.01$, compared with the control group) in daintain/AIF-1 group as shown in Fig. 3B. These results indicate daintain/AIF-1 impairs insulin expression and may cause β cell dysfunction. This is consistent with our previous study that daintain/AIF-1 causes β cell dysfunction in INS-1 β cells [18]. Interestingly, the antibody against daintain/AIF-1 delayed or prevented insulitis, could protect pancreatic islet cells from autoimmune attack.

3.4. Detection of NO levels in pancreas

Macrophages, ductal cells, and endothelial cells in pancreatic islets may mediate β cell damage by producing either NO or cytokines that then stimulate NO production by β cells [23]. To clarify the mechanism of daintain/AIF-1 causing β cell dysfunction, the concentrations of NO in the pancreases of NOD mice were detected by NO assay kit. As shown in Fig. 4, the average value of NO in the control pancreases was 31.2 ± 6.5 μmol/g protein, while the average level of NO in the pancreases of the mice treated with daintain/AIF-1 was 48.7 ± 8.2 μmol/g protein ($p < 0.05$ compared with the control group), and the average value of NO in the antibody group was 20.7 ± 3.2 μmol/g protein ($p < 0.05$ compared with the control group). The data demonstrated that daintain/AIF-1 enhanced NO production in the pancreases of NOD mice, while the antibody against daintain/AIF-1 decreased NO production probably via daintain/AIF-1 elimination.

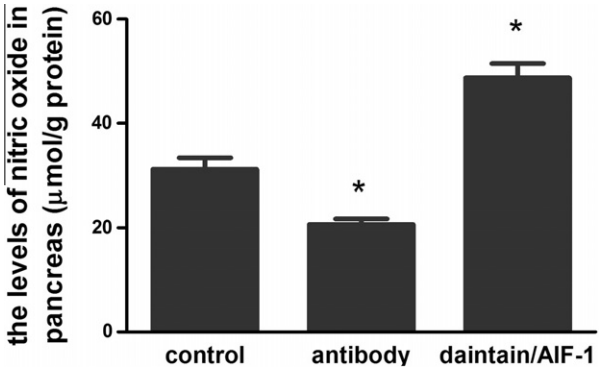

Fig. 4. Detection of nitric oxide (NO) levels in pancreas. The concentrations of pancreatic NO in control group, the antibody group and daintain/AIF-1 group were measured using the Griess reagent method. Data are presented as mean ± SEM ($n = 9$) (*$p < 0.05$ vs. the control group).

4. Discussion

In our original study, we reported that a particularly high concentration of daintain/AIF-1 located in the insulitis of prediabetic BB rats [7]. Using type 1 diabetes-prone NOD mice as model, we have further investigated the role of daintain/AIF-1 in the pathogenesis of type 1 diabetes. The result showed that this polypeptide could be released into blood and the level of the polypeptide in the blood of the NOD mice suffering from insulitis was significantly higher than that in healthy NOD mice. The data suggest that daintain/AIF-1 is involved in the pathogenesis of type 1 diabetes.

When intravenously injected, daintain/AIF-1 stimulated white blood cell proliferation. Several studies have demonstrated the immune regulation function of daintain/AIF-1 [13,14,24]. Our previous study also showed that daintain/AIF-1 increased the level of C-reactive protein and awoke fibrinogen in blood with higher blood viscosity and faster blood coagulation [25]. Therefore, daintain/AIF-1 could probably arouse inflammatory reactions in NOD mice. Moreover, daintain/AIF-1 directly increased high levels of blood glucose, and accelerated diabetes in NOD mice. The results suggest daintain/AIF-1 probably participated in the initiation of diabetes in NOD mice.

The pathophysiology of type 1 diabetes is basically a destruction of β cells in the pancreas, regardless of which risk factors or causative entities have been identified. In our experiment, pancreatic β cell mass decreased with daintain/AIF-1 administration. In addition, the levels of insulin in the blood of the NOD mice challenged with daintain/AIF-1 were too low to be detected (data not shown). Moreover, we found the concentrations of NO in the pancreases of NOD mice were higher than that in control pancreases. Excessive NO production from immune cells and/or cytokine-activated β cells has been implicated in β cell disruption in type 1 diabetes [23].

It is notable that the antibody against daintain/AIF-1 delayed or prevented insulitis, could protect pancreatic islet cells from autoimmune attack in NOD mice.

Collectively, these data indicate that daintain/AIF-1 may play important roles in the initiation and progress of type 1 diabetes. While the precise mechanism of daintain/AIF-1 triggering type 1 diabetes remains unclear. Further study is needed.

Acknowledgments

This work was supported by the Fundamental Research Funds for the Central Universities, Southwest University for Nationalities (11NZYQN36) and grant from the National Science Foundation of China (81172511).

References

[1] W. Gepts, Pathologic anatomy of the pancreas in juvenile diabetes, Diabetes 14 (1965) 619–633.
[2] W. Gepts, J. De Mey, Islet cell survival determined by morphology: an immunocytochemical study of the islets of Langerhans in juvenile diabetes mellitus, Diabetes 27 (1978) 251–261.
[3] D.L. Eizirik, T. Mandrup-Poulsen, A choice of death: the signal-transduction of immune-mediated β-cell apoptosis, Diabetologia 44 (2001) 2115–2133.
[4] I.L. Campbell, L.C. Harrison, Molecular pathology of type-I diabetes, Mol. Biol. Med. 7 (1990) 299–309.
[5] T. Mandrup-Poulsen, S. Helqvist, J. Molvig, Cytokines as immune effector molecules in autoimmune endocrine diseases with special reference to insulin dependent diabetes mellitus, Autoimmunity 4 (1989) 191–218.
[6] Z.W. Chen, C.G. Ostenson, T. Bergman, Isolation and structural analysis of a novel peptide daintain, Eur. J. Endocrinol. 130 (Suppl. 2) (1994) 32.
[7] Z.W. Chen, B. Ahen, C.G. Ösenson, A. Cintra, T. Bergman, C. Moller, K. Fuxe, V. Mutt, H. Jornvall, S. Efendic, Identification, isolation, and characterization of daintain (allograft inflammatory factor 1), a macrophage polypeptide with effects on insulin secretion and abundantly present in the pancreas of prediabetic BB rats, Proc. Natl. Acad. Sci. USA 94 (1997) 13879–13884.
[8] U. Utans, R.J. Arceci, Y. Yamashita, M.E. Russell, Cloning and characterization of allograft inflammatory factor-1: a novel macrophage factor identified in rat cardiac allografts with chronic rejection, J. Clin. Invest. 95 (1995) 2954–2962.
[9] U. Utans, W.C. Quist, B.M. McManus, J.E. Wilson, R.J. Arceci, A.F. Wallace, M.E. Russell, Allograft inflammatory factor-1. A cytokine-responsive macrophage molecule expressed in transplanted human hearts, Transplantation 61 (1996) 1387–1392.
[10] S. Liu, W.Y. Tan, Q.R. Chen, X.P. Chen, K. Fu, Y.Y. Zhao, Z.W. Chen, Daintain/AIF-1 promotes breast cancer proliferation via activation of the NF-kB/cyclin D1 pathway and facilitates tumor growth, Cancer Sci. 99 (2008) 952–957.
[11] Y. Imai, I. Ibata, D. Ito, K. Ohsawa, S. Kohsaka, A novel gene iba1 in the major histocompatibiliy complex class III region encoding an EF hand protein expressed in a monocytic lineage, Biochem. Biophys. Res. Commun. 224 (1996) 855–862.
[12] S. Tanaka, K. Suzuki, M. Watanabe, A. Matsuda, S. Tone, T. Koike, Up-regulation of a new microglial gene, mrf-1, in response to programmed neuronal cell death and degeneration, J. Neurosci. 18 (1998) 6358–6369.
[13] Z.F. Yang, D.W. Ho, C.K. Lau, Allograft inflammatory factor-1 (AIF-1) is crucial for the survival and pro-inflammatory activity of macrophages, Int. Immunol. 17 (2005) 1391–1397.
[14] K. Watano, K. Iwabuchi, S. Fujii, N. Ishimori, S. Mitsuhashi, M. Ato, A. Kitabatake, K. Onoé, Allograft inflammatory factor-1 augments production of interleukin-6, -10 and -12 by a mouse macrophage line, Immunology 104 (2001) 307–316.
[15] Laura J. Sommerville, C. Xing, S.E. Kelemen, S. Eguchi, M.V. Autieri, Inhibition of allograft inflammatory factor-1 expression reduces development of neointimal hyperplasia and p38 kinase activity, Cardiovasc. Res. 81 (2009) 206–215.
[16] Y. Tian, S. Jain, S.E. Kelemen, M.V. Autieri, AIF-1 expression regulates endothelial cell activation, signal transduction, and vasculogenesis, Am. J. Physiol. Cell Physiol. 296 (2009) C256–C266.
[17] H.J. Schluesener, K. Seid, R. Meyermann, Effects of autoantigen and dexamethasone treatment on expression of endothelial-monocyte activating polypeptide II and allograft-inflammatory factor-1 by activated macrophages and microglial cells in lesions of experimental autoimmune encephalomyelitis, neuritis, and uveitis, Acta Neuropathol. 97 (1999) 119–126.
[18] X.Y. Huang, Y.Y. Zhao, S.H. Jia, D.J. Yan, Z.W. Chen, Effects of daintain/AIF-1 on β cell dysfunction in INS-1 cells, Biosci. Biotechnol. Biochem. 75 (2011) 1842–1844.
[19] E.H. Leiter, The NOD mouse: a model for insulin-dependent diabetes mellitus, Curr. Protoc. Immunol. (2001) (Chapter 15, Unit 15.9).
[20] K. Fu, Y.Y. Zhao, W.X. Tang, Y. Zhong, T.B. Yang, J.H. Wang, Preparation and identification of monoclonal antibodies against daintain, Hybridoma (Larchmt) 25 (2006) 95–97.
[21] Y.Y. Zhao, W.X. Tang, Y. Wang, J. Lu, W. Wang, Z.W. Chen, Preparation of monoclonal antibodies against insulin and their applications, Hybridoma 26 (2007) 178–180.
[22] L.C. Green, D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnokand, S.R. Tannenbaum, Analysis of nitrate, nitrite and (15N) nitrate in biological fluids, Anal. Biochem. 126 (1982) 131–138.
[23] H.E. Thomas, R. Darwiche1, J.A. Corbett, W.H.K. Thomas, Interleukin-1 plus γ-interferon-induced pancreatic β-cell dysfunction is mediated by β-cell nitric oxide production, Diabetes 51 (2002) 311–316.
[24] G.W. Liu, H.X. Ma, L.L. Jiang, Y. Zhao, Allograft inflammatory factor-1 and its immune regulation, Autoimmunity 40 (2007) 95–102.
[25] Y.Y. Zhao, J.H. Wang, Z.W. Chen, Macrophage cytokine daintain stimulates inflammatory reaction in the blood, Nat. Prod. Res. Dev. 23 (2011) 425–427.