Effects of Daintain/AIF-1 on β Cell Dysfunction in INS-1 Cells

Xinyuan Huang,1,6 Yanying Zhao,2,6 Shaohui Jia,1 Dongjiing Yan,1 and Zhengwang Chen1,4

1Key Laboratory of Molecular Biophysics of the Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei, 430074, China
2School of Life Science and Technology, Southwest University for Nationalities, Chengdu, Sichuan, 610041, China

Received April 20, 2011; Accepted May 29, 2011; Online Publication, September 7, 2011
[doi:10.1271/bbb.110317]

We investigated the effects of daintain/AIF-1, a novel inflammatory cytokine, on INS-1β cells. Cells incubated with daintain/AIF-1 showed decreased cell viability and glucose-stimulated insulin secretion, as well as upregulated apoptosis and NO production. These deleterious effects of daintain/AIF-1 indicate that daintain/AIF-1 plays important roles in the dysfunction of pancreatic β cells in type-1 diabetes.

Key words: daintain/AIF-1; INS-1 cell; β cell dysfunction; type-1 diabetes; cytotoxicity

Allograft inflammatory factor-1 (AIF-1) is a 17-kDa IFN-γ-inducible cytokine originally cloned in the mid-1990s from activated macrophages of human and rat atherosclerotic allogenic heart grafts undergoing chronic transplant rejection.1–3 Contemporaneously, we isolated and characterized a polypeptide from porcine intestines and named it “daintain.”4-5 AIF-1 and daintain of rat, human, and pig origin are identical proteins with only species-specific amino acid differences.6 Hence, we call the polypeptide “daintain/AIF-1.”5,7 Daintain/AIF-1 is expressed mainly in macrophages and activated T cells and acts as an immune regulator in the activation and function of macrophage/microglial cells. Daintain/AIF-1 is regarded as a novel inflammatory factor that plays important roles in allograft rejection, vasculopathy, and autoimmune diseases such as experimental autoimmune neuritis, encephalomyelitis, and uveitis models,7,8 rheumatoid arthritis,9 and systemic sclerosis.10,11

Type-1 (insulin-dependent) diabetes (T1D) is characterized by a progressive autoimmune-mediated insulitis culminating in the death of pancreatic β cells. The β cell secretory dysfunction and apoptosis in the course of insulitis are perhaps caused by direct contact with activated macrophages and T-cells, and/or exposure to inflammatory mediators secreted by these cells, including cytokines (such as IL-1β, TNF-α, and IFN-γ) and NO.12,13 We have observed a particularly dense accumulation of daintain/AIF1-immunoreactive macrophages in insulitis affecting the pancreatic islets of prediabetic BB rats, suggesting that daintain/AIF-1 has a role in connection with the pathogenesis of T1D.13 In this study, we aimed to investigate further the impact of daintain/AIF-1 on β cell dysfunction in vitro using rat insulinoma INS-1 cells, a well-established insulin-secreting cell line for the study of diabetes.14

Recombinant human daintain/AIF-1 was prepared as described previously.15 The INS-1 cell line was obtained from CCTCC (China Center for Type Culture Collection, Wuhan, China) and cultured in growth medium composed of RPMI 1640 medium (Invitrogen, Carlsbad, CA), containing 11.1 mM glucose, 10% fetal bovine serum (Hyclone, Logan, UT), 10 nm HEPES, 2 nm l-glutamine, 1 mm sodium pyruvate, 50 μM β-mercaptoethanol, 100 units of penicillin/mL, and 100 μg of streptomycin/mL. Cultures were maintained at 37 ºC in a humidified 5% CO2 atmosphere.

Cells were seeded into 96-well culture plates at 2 × 104 cells/well. Twenty-four h after seeding, daintain/AIF-1 protein (1, 5, 10, 20 μM) was added to the cultures. For each culture condition, six identical samples were used. After incubation for 24 h, cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay.16 The medium was removed (or collected for NO assay as described in the following paragraph) and replaced with 0.5 mg/mL of MTT (Sigma-Aldrich, St. Louis, MO) dissolved in 100 μL PBS. Plates were incubated at 37 °C for 3 h. The resulting formazan crystals were solubilized in 100 μL of isopropanol with 40 mM HCl, and the optical density was read on a Microplate Reader (Tecan Sunrise, Salzburg, Austria) with a test wavelength of 570 nm and a reference wavelength of 630 nm. Cell viability was normalized to cells incubated in medium only (control), which were considered to be 100% viable.

The degree of apoptosis was detected by flow cytometry analysis using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) kit (Beyotime Biotechnology, Nanjing, China). Briefly, INS-1 cells in 6-well plates (3.5 × 105 cells/well) were incubated for 24 h without and with various concentrations of daintain/AIF-1 (1, 5, 10, and 20 μM). Fifty μM H2O2 was used as positive control to induce apoptosis.7 At the end of treatment, cells were harvested by trypsin-EDTA digestion and resuspended in binding buffer (2 × 105 cells/mL). Cells (195 μL) were incubated with 5 μL of Annexin V-FITC for 15 min at room temperature in the dark. After washing with PBS, they were co-stained with PI solution and analyzed by flow cytometer (Beckman Coulter FC500, La Brea, CA).

1 To whom correspondence should be addressed. Tel/Fax: +86-27-87792027; E-mail: zwchen@mail.hust.edu.cn
2 These authors contributed equally to this study.
NO production was measured in the culture medium as nitrite accumulation by the Griess reagent method\(^8\) according to the indication of the NO assay kit (Beyotime Biotechnology, Nanjing, China). Briefly, 50 μL of culture supernatant was collected and allowed to react with 50 μL of Griess reagent in a multiwell microtiter plate and incubated at room temperature for 5 min. The absorbance was measured at 540 nm using a Microplate Reader (Tecan Sunrise), and nitrite was calculated from a NaNO\(_2\) standard curve.

For the insulin secretion assay, INS-1 cells were cultured in the various treatment media (described above) in 12-well plates (2 × 10\(^5\) cells/well) for 24 h. The cells were then rinsed twice with HEPES-buffered Krebs Ringer Bicarbonate solution (KRBH buffer; 128.8 mM NaCl, 4.8 mM KCl, 1.2 mM KH\(_2\)PO\(_4\), 1.2 mM MgSO\(_4\), 2.5 mM CaCl\(_2\), 5 mM NaHCO\(_3\), and 10 mM HEPES, pH 7.4), followed by pre-incubation in KRBH buffer containing 3 mM glucose at 37°C for 30 min. After aspiration of the buffer, the INS-1 cells were subsequently incubated in fresh KRBH buffer supplemented with 3 mM or 20 mM glucose at 37°C for 60 min. The supernatants were collected and the insulin concentrations were determined with a commercial rat insulin immunoassay kit (R&D Systems, Minneapolis, MN) following the manufacturer’s instructions. The final insulin concentration per well was standardized to that of the total cellular protein content measured with a BCA kit (Beyotime Biotechnology, Nanjing, China).

All the experiments were repeated 3 times and the data in the text were expressed as mean ± SEM. Student’s t-test and One-way ANOVA were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA). The results were considered statistically significant at p < 0.05.

We found that daintain/AIF-1 had an inhibitory effect on INS-1 cell viability when administered at 10 μM and 20 μM (Fig. 1A). Compared with the untreated control, cell viability fell to 83.3% and 79.5% respectively. Cells incubated with 1 μM and 5 μM daintain/AIF-1 for 24 h showed a slight, but not significant, decrease in cell viability (approximately 90.7% and 92.6% survival). To confirm possible suppression of cell viability by daintain/AIF-1, INS-1 cells were double-stained with FITC and PI to examine apoptosis by flow cytometry. In the early stages of apoptosis, the INS-1 cells were stained by Annexin V-FITC, but excluded by PI (FITC+/PI\(^-\)), because the cell membranes remained intact. While in the late stages of apoptosis, plasma membrane integrity is broken, and this enables PI to penetrate and bind to DNA and makes the cells positive for both Annexin V and FITC (FITC+/PI\(^-\)). Hence we used flow cytometry to analyze the constitutions of the cells in early apoptosis (FITC+/PI\(^-\)) and late apoptosis (FITC+/PI\(^+\)). Figure 1B shows the quantitative results for changes in percentages of apoptotic cells. After culture with daintain/AIF-1 at various concentrations (1, 5, 10, and 20 μM) for 24 h, there was no significant increase in cell apoptosis in the late apoptosis groups, but the percentages of early apoptotic cells of daintain/AIF-1 treated cells and H\(_2\)O\(_2\) treated cells (as positive control) increased to 3.1, 4.2, 4.4, 6.6, and 9.0% respectively. These results indicate that daintain/AIF-1 is effective at inducing apoptosis in INS-1 cells, especially early apoptosis, suggesting that it plays stimulatory roles in the initial stages of apoptosis, leading to elimination of viable cells and subsequently a decrease in INS-1 cell viability.

It is well known that cytokines, such as IL-1β, IFN-γ, and TNF-α, induce the expression of NO within pancreatic β cells.\(^19\) This is perhaps a significant trigger for apoptotic or necrotic destruction of β cells in early TID.\(^20,21\) To determine whether NO is involved in daintain/AIF-1-induced apoptosis in INS-1 cells, NO generation was measured in culture medium using the Griess reaction.\(^19\) As shown in Fig. 2, NO production in the INS-1 cells increased significantly under daintain/AIF-1 treatment (1, 5, 10, and 20 μM) for 24 h as compared with control. NO levels were promoted 2.6-, 3.4-, 3.2-, and 4.6-fold respectively. This result suggests that an increase in NO level is one of the mechanisms of daintain/AIF-1-induced β cell toxicity.

We also examined the impact of daintain/AIF-1 on insulin secretion of INS-1 cells. Basal insulin secretion (BIS, 3 mM glucose) and glucose-stimulated insulin secretion (GSIS, 20 mM glucose) from INS-1 cells were measured with an ELISA kit. As shown in Fig. 3,
incubation with daintain/AIF-1 for 24 h caused no significant alteration in the BIS of the INS-1 cells, but reduced GSIS levels by 17–28%, as compared with control, implying an impairment role of daintain/AIF-1 on insulin secretion of INS-1 cells responding to high glucose levels. Besides the aforementioned suppression of cell viability, this inhibition of glucose-responsive-ness might be another toxic effect that daintain/AIF-1 exerts on β cell dysfunction in T1D.

The present report provides the first description of the cytotoxic effects of daintain/AIF-1 on INS-1β cells in vitro, as evidenced by decreased cell viability and GSIS as well as upregulated apoptosis and NO production. Collectively, these data indicate that daintain/AIF-1 plays important roles in the initiation and progress of T1D. But the precise mechanism of daintain/AIF-1 on β cell dysfunction remains unclear. Further study is needed.

Acknowledgments

This work was supported by the National Science Foundation of China (Grants 30370674 and 30470823).

References


Fig. 2. Effects of Daintain/AIF-1 on NO Production in INS-1 Cells. INS-1 cells were treated for 24 h with and without various concentrations of daintain/AIF-1 (1, 5, 10, or 20 μM), NO concentrations in the culture supernatants were measured by the Griess reagent method. Data are presented as mean ± SEM (n = 7). (***p < 0.001 vs. untreated control).

Fig. 3. Effects of Daintain/AIF-1 on Insulin Secretion from INS-1 Cells. INS-1 cells were treated with various concentrations of daintain/AIF-1 for 24 h. The insulin concentrations within the various media were assayed by ELISA, and were adjusted by intracellular protein content. The white column represents basal insulin secretion (BIS) induced by 3 mM glucose, and the black column represents glucose-stimulated insulin secretion (GSIS) induced by 20 mM glucose. Values are means ± SEM for three independent experiments (*p < 0.05 vs. control).