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Construction of a novel vaccine by conjugating a B-cell epitope of DPP4 to peptide IA2(5)-P2-1 to significantly control type 1 diabetes in NOD mice

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ABSTRACT

Type 1 diabetes is a chronic organ-specific autoimmune disease in which selective destruction of insulin-producing β cells leads to impaired glucose metabolism and its attendant complications. IA2(5)P2-1, a potent immunogenic carrier which designed by our laboratory, can induce high titer specific antibodies when carry a B cell epitope, such as B cell epitopes of DPP4, xanthine oxidase, and Urate transporter protein. In this report, we describe a novel multi-epitope vaccine composing a peptide of DPP4, an anti-diabetic B epitope of Insulinoma antigen-2(IA-2) and a Th2 epitope (P2:IPALDSLTPANED) of P277 peptide in human heat shock protein 60 (HSP60). Immunization with the multi-epitope vaccine in non-obese diabetic (NOD) mice successfully induced specific anti-DPP4 antibody, inhibited plasma DPP4 activity, and increased serum GLP-1 level. Moreover, this antibody titer was correlated with the dose of immunization (20μg, 100μg). Inoculation of this vaccine in NOD mice significantly control blood glucose level, improved glucose excursion and increased insulin level in vivo. Consistent with a lower diabetic and insulitis incidence, a induced splenic T cells proliferation and tolerance were observed. IFN-γ secretion reduced and IL-10 increased significantly in the D41-IA2(5)-P2-1 treated mice compared to P277 and control group due to the potential immunomodulatory effect of the epitope in the vaccine. Immunohistochemical analysis and cytometry showed a rebalance of Th1/Th2 in NOD mice. Our results demonstrate that this multi-epitope vaccine may serve as a promising therapeutic approach for type 1 diabetes.

1. Introduction

Type 1 diabetes mellitus (T1DM) is a disorder of glucose metabolism caused by a chronic autoimmune inflammation of the pancreatic islets of Langerhans. The ultimate outcome of type 1 diabetes is the loss of insulin-producing cells to numbers belows a threshold that is critically required to maintain physiological glucoregulation [1]. Many important factors in the pathogenesis of T1DM have been understood. Inflammation and autoimmunity to autoantigens are a part of the progression of the disease [2]. Given the fact that T1DM process is influenced by disease-associated autoantigen and determined by dendritic cells or T cells recognization of islet autoantigenic epitopes [3], the onset of insulitis can be postponed by endogenous β-cells autoantigens [4] such as insulin, glutamic acid decarboxylase65(GAD65), insulinoma antigen-2(IA-2) and heat shock protein 60(HSP60) [5]. Vaccination with diabetic autoantigens can inhibit islet-specific responses and induce regulatory responses [6]. However, the efficacy of vaccination with autoantigens is dependent on many factors including the route of administration, the dose and duration of treatment, and the composition of the vaccine. Autoantibodies to IA-2 are detected in the majority of patients at the time of diabetes onset and are being widely used as predictive markers to identify individuals at risk for developing T1DM [7,8]. P277 (437 VLGGGCALLRCIPALDSLTPANED460) is a peptide from the heat shock protein 60 (HSP60). An epitope (P1: VLGGGCALLRC), which locates on N-terminal of P277 peptide, contributes to anti-P277 antibodies and atherosclerosis; while the Th2 epitope (P2: IPALDSLTPANED) locates on the C-terminal of P277 [6]. To some extent, P277 can prevent type 1 diabetes in the early stage, but an effective therapy outcome is not observed and its clinical trial results were retracted.

Dipeptidyl peptidase 4 (DPP4) also known as CD26, is a 11kD cell surface type 2 transmembrane protein. DPP4 was initially identified as a therapeutic target for type 2 diabetes owing to its degradation of glucagon-like peptide-1(GLP-1) and glucose-

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dependent insulinotropic peptide(GIP). GLP-1 and GIP could stimulate insulin release, delaying gastrointestinal emptying, inducing satiety, decreasing glucagon release and preserving beta-cell mass thus regulating blood glucose levels after a meal [9,10]. DPP4 inhibitors, such as Sitagliptin, Vildagliptin and Saxagliptin are currently being used clinically in patients with type 2 diabetes [11–13]. In addition to their antidiabetic action, DPP4 inhibitors have also shown many other benefits, such as anti-inflammation [14] and cardiovascular protective effect [15]. In addition, serum DPP4 activity increased in type 1 diabetes children [16]. These information indicates that DPP4 inhibitors may have potential effects on type 1 diabetes such as regulating glucose by increasing serum GLP-1 level and exert immunomodulatory effect.

NOD mice, the most widely used preclinical model of T1DM, have shown to be responsive for DPP4 inhibitors. Sitagliptin was demonstrated to prolong islet graft survival in STZ-induced diabetic mice and NOD mice [17–19]. Tian et al. [20] have showed that a DPP4 inhibitor (NVP-DPP728, 30 mg/kg, twice daily) could reverse diabetes in new onset NOD mice, and provided explanations for the reversal in that the DPP4 inhibitor increased CD4+CD25 +FoxP3+regulatory T cells number, reduced islet inflammation, and promoted β-cell regeneration. Other studies also showed therapeutic effects for NOD mice by administering a DPP4 inhibitor in combination with a proton pump inhibitor or a TLR2 tolerisation [21–23]. It is noteworthy that GLP-1 treatment alone and a combination of GLP-1 and gastrin have been shown to reverse diabetes in NOD mice. These studies showed DPP4 inhibitors may reverse the diabetes in new onset diabetic mice, partially due to the increased circulating levels of GLP-1. However, the exact mechanisms through which DPP4 could affect the immune-mediated disease course are still unknown. Therefore, at present, applying DPP4 inhibitor treatment of type 1 diabetes patients is surely a challenge.

Based on the effect of DPP4 inhibitors and P277 in toxin-induced autoimmune diabetes, We initially constructed a new combinatorial peptide, D41-IA2(5)-P2-1, composes a B cell epitope within DPP4, a B cell epitope of insulinoma antigen-2 (IA-2), and a Th2 epitope of P277 peptide [24]. The peptide DiaPep277(437-460) is a peptide from the heat shock protein 60(HSP60), which acts as a control to further highlight the role of combinatorial B cell epitope D41. Meanwhile, physiological status and immune response were monitored. In our research, experimental group treated by D41-IA2(5)-P2-1 peptide maintained a lower blood glucose level and morbidity, compared with control group. Therefore, our study support that subcutaneous immunization of D41-IA2(5)-P2-1 can potently control blood glucose and ameliorate established diabetes in NOD mice.

2. Materials and methods

2.1. Vaccine design and synthesis

A combinatorial multi-epitope peptide D41-IA2(5)-P2-1, containing (i) B cell epitope of DPP4, (ii) B cell epitope of IA-2(626 FEYQD 630), and (iii) Th2 epitope of P277 peptide (448 IPALDSLTPANED 460) was constructed and synthesized through FMOC solid phase synthesis in Shanghai GL Biochem Co,. Ltd. (Shanghai, China). Three epitopes were conjugated together by flexible peptides (G,GGGG etc.). Diapep277 (437 VLGGGCALLRCIPALDSLTPANED 460) was synthesized by the same method above. Synthetic peptides were purified by reverse-phase high performance liquid chromatography (HPLC) (>92% purity).

2.2. Animal study and immunization

The studies were approved by the Animal Ethnics Committee of China Pharmaceutical University. Four-week-old female NOD/LtJ mice were purchased from the Hua fukang biotech (Beijing, China) and maintained under specific pathogen-free conditions. Animals received high quality care with free access to food and water. Mice were considered diabetic when two consecutive blood glucose values were at or above 11.1 mmol/L. Diabetic mice were randomized into the group of D41-IA2(5)-P2-1 (n = 8), P277 (n = 8) and placebo (n = 8). Each mouse in D41-IA2(5)-P2-1 treated group received 0.1 ml 20% Lipofundin (B Braun, Melsungen, Germany) with 20 μg or 100 μg D41-IA2(5)-P2-1 and 4mg mannitol (as a filler) dissolved in. In P277 treated group, each mouse received 0.1 ml Lipofundin with 100 μg P277 and 4mg mannitol dissolved in. Mice in control group were immunized with 0.1 ml Lipofundin and 4mg mannitol. Immunisation process is that mice in each group (n = 8) were injected s.c. at week 7, 19, 20, 21, 22, 23, 24, 26, 28, 30, 32, 34, 36, 38.

Body weights and 8-h fasting blood glucose levels were monitored using a glucometer (HMD Bio Medical, Taiwan, China) before every inoculation. Diabetes remission was defined as the absence of glycemia values <11.1 mmol/L on 2 consecutive measurements. Serum samples were collected before every inoculation and stored at −20 °C for use in assay.

2.3. ELISA assay

Serum parameters such as antibody subclass, GLP-1 levels (Meimian Biotech, Yancheng, China) and insulin (Aoqing, Nanjing, China) were measured via enzyme-linked immunosorbent assay (ELISA).

Circulating levels of IL-10 and interferon-gamma (IFN-γ) were measured in serum using ELISA kits purchased from (Aoqing, China, Nanjing) in accordance with the protocol of the manufacturer. Biosource recombinant mouse cytokines were used as standards for calibration curves. Cytokine levels are expressed as picograms per milliliter based on calibration curves. The lower limits of detection for the experiments described in this paper were 15 pg/ml for cytokines.

2.4. Measurement of DPP4 activity

The neutralizing ability of anti-DPP4 antibodies was measured using a colorimetric assay based on the liberation of *p*-nitroanilide (A405 nm) from the DPP4 substrate H-Gly-Pro-*p*NA (GL Biochem, Shanghai, China; purity >97%) at week 30 as previously described. Briefly, 10 μl of serum was mixed with assay buffer (100 mmol/L Tris/HCl, pH 8.0) and incubated at 37 °C for 30 min. Then, 10 μl of (0.5 U/L) recombinant DPP4 (ProSpec-Tany Technogene Ltd. USA) and 5 μl of (0.26 mmol/L) H-Gly-Pro-*p*NA was added to the wells. Each test well in the 96-well plate (total volume of 100 μl) contained 10 μl of serum, 10 μL of (0.5 U/L) DPP4, 5 μl of (0.26 mmol/L) H-Gly-Pro-*p*NA and 75 μl of assay buffer. In the negative-control wells, 10 μl of serum was replaced with the same volume of ddH_2O. Blank control wells contained no DPP4 or serum. Test blank control wells contained no DPP4. Plates were incubated at 37 °C for 60 min, and the absorbance was detected using a microplate reader (Thermo, USA). The neutralizing activity of anti-DPP4 antibodies was calculated by using the following formula:

$$\begin{aligned}\text{Inhibition } (\%) = [&(\Delta A_{405} \text{ of negative control} \\ &- \Delta A_{405} \text{ of blank control})/(\Delta A_{405} \text{ of test control} \\ &- \Delta A_{405} \text{ of test blank control})] \\ &/[(\Delta A_{405} \text{ of negative control} \\ &- \Delta A_{405} \text{ of blank control})] \times 100\%\end{aligned}$$

2.5. T Cell proliferation and cytokine assay

Spleens were harvested from 40-week-old NOD mice. T cell proliferative responses were assayed in vitro according to previous