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Original Research

Recombinant Interferon Alpha-2b is a High-Affinity Antigen for Type 1 Diabetes Autoantibodies

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ABSTRACT

Objectives: Type 1 diabetes results from T-cell-mediated destruction of the beta cells of the pancreas and is associated with several autoimmune phenomena. Many studies have suggested the involvement of interferon alpha (IFN α) in the development of type 1 diabetes, but the exact mechanism remains unclear. In this study, the binding of type 1 diabetes antibodies with recombinant interferon alpha-2b (hrIFN α-2b), their gene (cIFN α-2b gene) and commercially available interferon α-2b (IFN α-2b) were assessed. Furthermore, we also sought to use anti-hrIFN α-2b antibodies as a probe for the estimation of plasma IFN α in patients with type 1 diabetes.
Methods: The binding specificity of antibodies was analyzed by direct binding, inhibition ELISA and quantitative precipitin titration in 45 patients with type 1 diabetes and 30 control subjects. Competition ELISA was also used to estimate INF α in the serum of patients with type 1 diabetes.
Results: Antibodies from type 1 diabetes sera, purified in a protein A-agarose matrix, exhibited greater recognition of hrIFN α-2b than IFN α-2b ($p<0.05$) and cIFN α-2b gene ($p<0.001$). The relative affinity of type 1 diabetes antibodies for the hrIFN α-2b, IFN α-2b and cIFN α-2b genes was found to be 1.34×10^{-7}, 1.28×10^{-6} and 1.13×10^{-6}, respectively. The concentration of plasma INF α evaluated by induced antibodies was found to be significantly higher than in controls ($p<0.05$).
Conclusions: High binding of hrIFN α-2b with IgG from patients with type 1 diabetes might suggest involvement of hrIFN α-2b in type 1 diabetes, especially as an antigenic agent. Anti-hrIFN α-2b antibodies were shown to be good probes for estimation of plasma INF α in patients with type 1 diabetes.

Mots clés :
anticorps
dosage d'immunoabsorption par enzyme lié (ELISA)
interféron alpha–2b humain recombinant
diabète de type 1

RÉSUMÉ

Objectifs : Le diabète de type 1 résultant de la destruction médiée par les cellules T des cellules bêta du pancréas est associé à plusieurs phénomènes auto-immuns. De nombreuses études ont indiqué la participation de l'interféron alpha (IFNα) au développement du diabète de type 1, mais on ignore le mécanisme exact. Dans la présente étude, la liaison des anticorps du diabète de type 1 à l'interféron alpha–2b humain recombinant (IFNα–2b humain recombinant), leurs gènes (gène du cIFNα–2b) et l'interféron α–2b disponible commercialement (IFNα–2b) ont été évalués. De plus, nous avons cherché à utiliser les anticorps anti-IFNα–2b humain recombinant comme sonde pour estimer la concentration plasmatique de l'IFNα chez les patients atteints du diabète de type 1.
Méthodes : La spécificité de liaison des anticorps a été analysée par l'ÉLISA directe, l'ÉLISA par inhibition et le dosage quantitatif des précipitines chez 45 patients atteints du diabète de type 1 et 30 témoins. L'ÉLISA par compétition a également été utilisée pour estimer l'INFα dans le sérum des patients atteints du diabète de type 1.
Résultats : Les anticorps du sérum de diabétiques de type 1 purifiés dans une matrice d'agarose et de protéine A ont montré une plus grande reconnaissance du gène de l'IFNα–2b humain recombinant que des gènes de l'IFNα–2b ($p<0,05$) et du cIFNα–2b ($p<0,001$). Il a été constaté que l'affinité relative des anticorps du diabète de type 1 pour les gènes de l'IFNα–2b humain recombinant, de l'IFNα–2b et du cIFNα–2b était respectivement de $1,34\times10^{-7}$, de $1,28\times10^{-6}$ et de $1,13\times10^{-6}$. Il a été démontré que la concentration plasmatique de l'INFα évaluée par les anticorps induits était significativement plus élevée que les témoins ($p<0,05$).

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Conclusions : La forte liaison de l'IFNα–2b humain recombinant à l'IgG des patients atteints du diabète de type 1 suggérerait la participation de l'IFNα–2b humain recombinant dans le diabète de type 1, particulièrement comme un antigène. Il a été démontré que les anticorps anti-IFNα–2b humain recombinant étaient de bonnes sondes pour estimer les concentrations plasmatiques de l'INFα chez les patients atteints du diabète de type 1.

Introduction

Type 1 diabetes is an autoimmune disease in which insulin-producing beta cells of the pancreas are destroyed by autoreactive T lymphocytes. Although multiple factors have been suggested to be involved in causing this disease, many studies have suggested the association of interferon alpha (IFN α) in the development of type 1 diabetes (1,2). These studies have shown higher levels of IFN α mRNA and protein in the pancreases of patients with type 1 diabetes in comparison to patients without diabetes (1,2). In addition, elevated levels of IFN α were also found in plasma of 70% of patients with diabetes, and a positive detection of IFN α mRNA in the blood cells was also observed in 75% of these patients (3). IFN α treatment of patients with tumour or viral hepatitis is associated with increased incidence of type 1 diabetes (4,5). However, the role of IFN α in the pathogenesis of type 1 diabetes in nonobese diabetic (NOD) mice is controversial. Some studies have shown that oral treatment of prediabetic NOD mice with IFN α suppresses insulitis and diabetes (6,7), whereas other studies have shown a triggering role of IFN α in the process of type 1 diabetes in NOD mice (8). In addition, extensive blockade of IFN α action by a monoclonal antibody specific to the IFN α receptor 1 results in nearly complete prevention of type 1 diabetes in NOD mice. These studies have shown that plasmacytoid dendritic cell-derived IFN α is the prime initiator of the pathogenesis of type 1 diabetes in NOD mice (8).

IFN α is a cytokine that has an immunomodulatory function. It plays an important role not only in antiviral activity but also in several physiologic functions, such as activation of dendritic cells and accelerated expression of major histocompatibility complex I and II molecules that may cause increased antigen presentation (9). It also functions as a bridge between the innate and the adaptive immune systems and plays a role in immunologic self-tolerance. Interferon may also activate macrophages, cytotoxic T lymphocytes and natural killer cells, inducing islet cell injury and destruction (10). Overexpression of interferon in pancreatic islet cells caused insulin-dependent diabetes in transgenic mice. The inflammation and the diabetes could be prevented with a neutralizing antibody to IFN α in these mice (11). INF α is also linked to diabetes in that it may increase interleukin-1, and interleukin-1 is found to be cytotoxic to pancreatic islet cells (12). It may also stimulate glucagon, growth hormone and cortisol secretion, thus causing impaired glucose tolerance and insulin resistance (13). Furthermore, diabetes requiring insulin has been seen to develop following interferon therapy (14). Anti-islet autoantibodies were detected in 94.5% of the patients at the onset of diabetes (13). Thus, the expression of INF α by the beta cells could be causal in the development of type 1 diabetes, which suggests a therapeutic approach to this disease (11).

We have recently reported that recombinant interferon alpha-2b (hrINF α-2b) was highly recognized by circulating systemic lupus erythematosus (SLE) autoantibodies (15). In the present study, we report the preferential recognition of hrIFN α-2b by circulating antibodies in patients with diabetes. The gene encoding human interferon was cloned and expressed in *Escherichia coli*. Furthermore, experimentally induced anti-hrIFN α-2b antibodies have been used to estimate the concentration of IFN α in the serum of patients with type 1 diabetes. The role of hrIFN α-2b in type 1 diabetes etiopathogenesis is discussed and evaluated.

Methods

Patients and controls

Blood samples were obtained from 45 patients with type 1 diabetes and 30 normal individuals (90% Asian and 10% from Africa and the Middle East) who served as controls and who were without personal or family histories of autoimmune diabetes. The clinical and laboratory data concerning these subjects are given in Table 1 (glycated hemoglobin [A1C] levels, smoking history, age, duration of disease, sex, etc.). All patients with type 1 diabetes included in this study were treated with insulin. Type 1 diabetes was diagnosed according to the National Diabetes Data Group (16). All subjects gave prior informed consent for the analysis, and the protocol was approved by the Institutional Ethics Committee. Serum samples were isolated from all subjects and heated at 56°C for 30 minutes to deactivate complement protein and finally were stored at –20°C with sodium azide.

Cloning, transformation and expression of recombinant interferon

Cloning, transformation and expression of hrINF α-2b protein was done as described previously (15). The amplified cDNA segments were digested with restriction enzymes Xbal and Xhol. The vector pET 28a was then ligated with the digested fragments of the cDNA. The cloned recombinant DNA construct was transformed into *E. coli* BL21 (DE3)-competent cells. The transformants (colonies) were selected on LB agar plates supplemented with ampicillin (100 μg/mL)×gal (40 μg/mL). The recombinant protein was purified by using talon affinity chromatography (Clontech, Terra Bella, California, United States). Protein samples were loaded using SDS-PAGE to check the expression of hrINF α-2b (17).

Antibodies against hrIFN α-2b

Antibodies were induced in experimental animals (female rabbits) against hrIFN α-2b as describe elsewhere (18). Antibodies against IFN α-2b were also induced along with suitable controls.

Table 1
Clinical data and estimation of INF α by anti-hrIFN α-2b antibodies in the sera of patients with type 1 diabetes

No. of subjects	Type 1 diabetes	Normal
Number and gender (M/F)	32/13	21/9
Age (years)	38±12	37±11
Duration of disease (years)	12±4.8	—
Fasting blood glucose (mg/dL)	280±32.5	92±8.9
A1C (%)	7.8±0.6	5.4±0.5
Carbonyl content (nmoL/mg protein)	3.5±0.25	2.1±0.31
C-peptide (ng/mL)	0.45±0.1	0.54±0.21
Smoking duration (n=31)	16±3.8	—
Plasma INF α estimation by[a]		
Anti-hrIFN α-2b antibodies (U/mL)	12.5±4.3[b] (U/mL)	0.9±0.13
INF alpha 2 ELISA kit (U/mL)	12.15±5.9 (U/mL)	0.91±.21

INF, interferon.

[a] The amount of serum INF α was measured by competition ELISA, and values are presented in U/mL. For blood glucose estimations, blood was collected in oxalated fluoride containers, and the assays were performed immediately.

[b] Significantly higher than control ($p<0.05$).

Note: subjects with type 1 diabetes, n=10; control, n=15.