利用非肥胖性糖尿病老鼠為動物模型探討人類第二型去氧核糖核酸拓撲異構酶在胰島素依存性糖尿病之致病機轉中所扮演的角色

Study of the role of human DNA topoisomerase II in the pathogenesis of IDDM in NOD mice

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一、中文摘要

本研究計劃之主旨在於探討臺灣本土第一型糖尿病(type 1 diabetes mellitus)**病人血清中各種自體抗體的分佈及其臨床意義。雖然許多和第一型糖尿病有關之研究已逐漸使我們對此病有更深入的了解，但大多數是以白種人的第一型糖尿病病例為研究對象，所以有關臺灣地區第一型糖尿病病例的特性及臨床表現尚未有一系統且完整的研究。本研究主要在偵測臺灣本土之第一型糖尿病病人體內對抗麩氨酸脫羧酶、甲狀腺球蛋白**(thyroglobulin)**、甲狀腺微粒體**(thyromicrosome)**等自體抗體之分佈；同時我們也從統計學的觀點針對這些病例對抗麩氨酸脫羧酶、甲狀腺球蛋白、甲狀腺微粒體、人類第二型去氧核糖核酸拓撲異構酶等自體抗體之分佈及所代表之臨床意義加以分析。**

關鍵詞：第一型糖尿病、麩氨酸脫羧酶、甲狀腺球蛋白、甲狀腺微粒體、人類第二型去氧核糖核酸拓撲異構酶

Abstract

In a preliminary cross-sectional study, we discovered that DNA topoisomerase II autoantibodies (anti-topII) were detected in 49.2% of 195 Chinese type 1 diabetes mellitus (type 1 DM) patients with a mean age of 14.5yr and a mean duration of 4.6yr. For further characterizing the correlation between various autoantibodies and the clinical manifestations in Chinese type 1 DM patients, we collected the demographic and immunological data from our patients with regard to the frequency of antibodies to human DNA topoisomerase II (anti-topII), glutamic acid decarboxylase (anti-GAD), thyroglobulin (ATA) and thyroid microsome (AMiA) in this study.

Keywords：type 1 diabetes mellitus, human DNA topoisomerase II, glutamic acid decarboxylase, antithyroglobulin antibodies, antimicrosomal antibodies

二、緣由與目的

Glutamic acid decarboxylase (GAD) (1,2) occurs in islets as two isoforms: 65 kD (GAD65) or 67 kD (GAD67). Anti-GAD can be detected in most newly diagnosed patients with type 1 DM (3,4). Seropositivity to GAD in the asymptomatic prediabetes may be an early predictor of type 1 DM (5,6). The T-cell response to GAD (7) together with the early antibody response to GAD strongly suggests a central role for

autoimmunity to GAD in the development of type 1 DM.

In a previous cross-sectional study, we used purified overlapping human DNA topoisomerase II (topII) fragments as antigens to examine sera of 195 Chinese type 1 DM patients (8). The results showed that anti-topII were detected in 49.2% of our patients. Since most of the research referring to type 1 DM are focused on Caucasian subjects, the characteristics and clinical manifestations of Chinese type 1 DM patients have never been well studied. Therefore, we investigated the frequency of anti-GAD, anti-topII, ATA/AMiA and C-peptide concentrations in our patients for further demonstrating the appearance of anti-GAD and anti-topII with various clinical and immunological features. In addition, for evaluating our previous inference (8) that anti-topII might be originated from antigenic mimicry of other autoantigens or environmental agents, we compared the prevalence of anti-topII and anti-GAD to further testify our hypothesis.

一、結果與討論

Frequency and levels of autoantibodies.

Results from anti-topII, anti-GAD, ATA/AMiA and C-peptide concentration were available from 184 patients. Among them, 48 (26.1%) were negative for both anti-topII and anti-GAD. Overall, anti-GAD were detected in 49.5% (91/184), anti-topII in 52.2% (96/184), and ATA/AMiA in 14.1% (26/184) of patients. Although C-peptide was undetectable in the majority of our patients, 16 out of 184 (8.7%) remained C-peptide positive. The average level of anti-GAD in all patients and anti-GAD^+ patients were 18.7 ± 38.1 and 34.8 ± 46.6 units, respectively.

Associations with age and age at onset.

The pattern of anti-GAD and anti-topII positivity is similar in terms of age and the age at onset. Higher frequency of anti-GAD and anti-topII were found both in the adolescent age and age at onset. The average level of anti-GAD is also elevated in the adolescent age and age at onset but tends to gradually decrease with advancing age. The age of onset in anti-GAD^+ patients were statistically older (11.0 ± 6.5 vs. 8.2 ± 6.6 years, $p< 0.05$) compared to that in anti-GAD^- patients. Our findings of different frequencies of anti-GAD and anti-topII in relation to age and age at onset suggests that different autoantigens might be involved. The evidence that the frequency of anti-GAD and anti-topII is parallel while the temporal course of the prevalence of anti-topII and anti-GAD is somewhat different further supported out previous inference (8) that anti- topII might have been elicited secondarily and originated from antigenic mimicry to GAD (up to 35.0% identity and 55.6% homology), a putative initiator for this autoimmune response, after islet cell destruction. Therefore, anti-topII appears relatively late and will last longer than anti-GAD because of its complicated structure and stronger antigenicity or the molecular mimicry of relevant epitopes of topII by endogenous or exogenous antigen(s).

Association with sex.

We found a significantly higher female

to male ratio (2.25, 63/28), higher frequency (60% vs. 35.4%), and higher median levels (34.9 vs. 22.2 units) of anti-GAD, as well as a higher female to male ratio in ATA/AMiA (3.33, 20/6). This observation is in agreement with 3 other studies (9-11) and supports that organ-specific endocrine autoimmunity occurs more frequently in females regardless of racial difference (12).

Associations with disease duration.

The frequency of anti-GAD tends to gradually decline with longer disease duration (from 54.5% to 33.3%). The average duration of anti-GAD^+ patients were statistically shorter compared to the anti-GAD^- patients (3.8 ± 2.8 vs. 5.1 ± 2.8 years, $p< 0.05$). In a sequential observation, anti-GAD titers tended to decrease regardless of the coexistence of anti-topII, C-peptide and ATA/AMiA positivity. In addition, we also measured the serum C-peptide concentrations to monitor the residual -cell function. The average duration of 16 C-peptide positive patients was shorter than that of C-peptide negative patients (2.9 ± 2.3 vs. 4.6 ± 3.4 years, $p< 0.05$). On the contrary, the positivity of anti-topII is increased rather than decreased with longer duration (45.0 to 75%)

Associations of autoantibodies.

Among patients positive for anti-GAD, 24.2% (22/91) were positive for ATA/AMiA, compared with only 4.3% (4/92) among patients negative for anti-GAD ($p<0.05$). The ATA/AMiA positivity is also associated with the concurrent appearance of anti-topII and anti-GAD (30.8 % vs. 10.7%, $p< 0.05$). This phenomenon is obvious especially when the patients were anti-GAD^+ and titers between 10-50 units; in addition, among this subgroup of patients, the C-peptide positivity is significantly much lower (6.7% compared to 18.5% in other patients). This observation is in accordance with Martino *et al.* (9) and suggested that this may represent a subgroup associated with polyendocrine autoimmunity.

Comparison of childhood and adult type 1 DM.

The mean (±SEM) duration was longer in the adults (6.9 ± 4.6 vs.4.5 ± 3.3 years, $p< 0.05$) and the percentage with residual -cell mass and the mean serum C-peptide concentrations were higher (9.5% vs. 6.3% and 1.31 ± 0.90 vs.0.57 ± 0.32 nM, $p< 0.05$), suggesting that they had -cell reserve. Fifty-nine (41.5%), 67 (47.2%) and 17 (12.0%) out of 142 children (3.3 to 17.9 years old) were positive for anti-GAD, anti-topII and ATA/AMiA, respectively, as compared with 24 (57.1%), 30 (71.4%) and 9 (21.4%) of 42 adults (18.0 to 45 years old). According to the above results, we confirmed type 1 DM that begins in adulthood is characterized by preservation of residual -cell function and higher frequencies of anti-GAD, anti-topII and ATA/AMiA autoantibodies even though their duration of type 1 DM is longer than the childhood-onset counterpart. This observation indicated that the adult-onset type 1 DM might have the tendency of polyendocrine autoimmunity, this phenomenon sustained the notion that the association of persistence of antibody with a benign clinical course is usually interpreted as slowly progressive destruction

of -cell in this subgroup, and therefore, with a higher -cell function (13).

四、計劃成果自評

In summery, we examined that anti-GAD positivity and analyzed the putative associations between various autoantiboies and the clinical features among 184 Chinese type 1 DM patients from a statistical point of view. Several relationships between type 1 DM and the clinical characteristics along with polyendocrine autoimmunity were observed. We concluded that the pathogenesis of type 1 DM is not a simple process in Chinese patients suffered from this disease. Hopefully, we can provide further understanding to the clinical manifestations and immunological responses of Chinese type 1 DM patients through this study.

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