行政院國家科學委員會專題研究計畫 成果報告

第四型介白素對脂肪與葡萄糖代謝作用的調控(第 3 年)
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附件一

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第四型介白素對脂肪與葡萄糖代謝作用的調控

計畫類別：■個別型計畫 □整合型計畫
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目　　錄

1. 中英文摘要及關鍵詞

1.1. 中文摘要

第二型糖尿病(type 2 diabetes mellitus, T2DM)是常見的內分泌疾病，其致病機轉仍然未知。T2DM病患者雖然可自行分泌胰島素，但其細胞無法有效接收或傳遞胰島素的訊息而產生胰島素阻抗性(insulin resistance)；長期罹病患者的胰島素分泌能力也可能受損。許多因素可能會導致T2DM，其中以遺傳因子和環境因素最受矚目。研究可引起代謝異常的遺傳基因有助於了解糖尿病的致病因素及發展嶄新的治療與預防策略。許多研究已證實T2DM與慢性發炎反應有密切相關性，患者體內的急性發炎蛋白與細胞激素濃度都會增高。本文的主旨為探討細胞激素第四型介白素(interleukin-4, IL-4)在T2DM和新陳代謝中的角色。根據本研究之基因型分析結果，國人IL-4基因型與T2DM以及受檢者之高密度膽固醇發病有相關性；此發現表示IL-4可能與葡萄糖/脂肪之代謝作用有關而參與T2DM之致病。因此本實驗室進一步以細胞與動物模式檢測上述假說。實驗結果顯示IL-4可透過調控Akt之磷酸化，提升胰島素敏感性與葡萄糖耐受性；此外，IL-4也抑制脂肪聚集而調控脂肪代謝作用，減輕小鼠之體重與脂肪重量。簡而言之，本研究除發現IL-4基因型與T2DM之發病有關之外，亦證實IL-4透過提高胰島素敏感性與葡萄糖耐受性並抑制脂肪堆積而調節葡萄糖/脂肪之代謝作用。上述結果提供IL-4調控代謝作用之嶄新角色，也提出免疫作用、胰島素敏感性與新陳代謝三者交互作用之證據。

關鍵詞: 細胞激素、新陳代謝、介白素、糖尿病

1. 中英文摘要及關鍵詞

1.2. Abstract and Keywords

Type 2 diabetes mellitus (T2DM) is usually caused by insulin resistance, and often combined with progressive defect in insulin secretion. However, the etiology of T2DM remained unknown. Among various factors which lead to the onset of T2DM, host genetics and environmental factors are the focus of discussion. Identification and characterization of specific genes that can result in metabolic abnormalities are helpful for the effective prevention and therapeutic intervention of diabetes mellitus. Accumulating evidences have proved that T2DM is closely correlated with chronic inflammation, with increased levels of circulatory acute response proteins and cytokines in affected subjects. The aim of the present study is to investigate the roles of cytokines, mainly interleukin-4 (IL-4) in metabolism and T2DM. Significant association between IL-4 secreting genotypes and T2DM as well as that between the IL-4 genotypes and the lower circulatory HDL-C level was observed. It indicated that IL-4 levels might contribute to T2DM pathogenesis through participating in the regulation of lipid and glucose metabolism. We further elucidation the roles IL-4 in the diabetic pathogenesis as well as interactions between adipose tissues and immune responses. Our results reveal that IL-4 improves insulin sensitivity and glucose tolerance through up-regulating Akt phosphorylation while attenuating GSK-3β activities. IL-4 is also involved in lipid metabolism by inhibiting lipid accumulation in fat tissues which lead to decreased weight gain and fat mass. The above results suggest that IL-4 regulates glucose and lipid metabolism by promoting insulin sensitivity, glucose tolerance, and inhibiting lipid deposits. The present study uncovers the novel roles of IL-4 in metabolism and provides new insights in the interaction between cytokines/immune responses, insulin sensitivity and metabolism. Accomplishments of the immune-related genetic studies in a particular ethnic population can lead not only to the understanding of the interactions between immune responses and T2DM, but also potential clues for the designing of personalized type 2 diabetic treatment in the future.

Keywords: interleukin-4, glucose/lipid metabolism, insulin sensitivity, type 2 diabetes mellitus

2.1. INTRODUCTION

Insulin resistance, the major cause of type 2 diabetes mellitus (T2DM), is occurred when insulin-target cells can not effectively transduce insulin signaling and eventually become less sensitive to insulin stimuli. The consequences of impaired insulin signaling include reduced glucose uptake and disposal as well as increased hepatic gluconeogenesis.[1] In addition to T2DM, abundant evidence demonstrates that insulin resistance also plays an important role in other metabolic abnormalities, such as obesity, dyslipidemia, hypertension and cardiovascular diseases.[2,3]

T2DM is a chronic inflammatory state of elevated circulatory pro-inflammatory cytokines, such as interleukin-1β, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).[4,5] These increased cytokines act as negative regulators to modulate glucose homeostasis and contribute as a link between immune responses and T2DM. Excessive intake of glucose and macronutrients also induce oxidative stress and cytokine mediators, which subsequently block insulin signaling.[6-10] Triacylglycerols storage in adipose tissues is impaired by chronic inflammation, which leads to the elevation of circulating free fatty acids (FFA) and triacylglycerols, and eventually insulin resistance.[11] Accordingly, cytokines are associated with systemic insulin resistance, metabolic diseases and diabetes. Therefore, it is tempting to determine whether other cytokines are also involved in the pathogenesis of T2DM.

Interleukin-4 (IL-4) is a pleiotropic cytokine secreted by activated Th2 cells, FcεR1^{+} cells and eosinophils. IL-4 plays an important role in inflammatory reactions by modulating growth, differentiation and cytokine production.[12] While IL-4 signaling is known to trigger phosphorylation of insulin receptor substrate 2 (IRS2),[13,14] chronic insulin and glucose treatment attenuates IL-4-dependent IRS2 phosphorylation.[15] It suggests that IL-4 can positively regulate insulin signaling pathway. In addition, our recent study reveals a significant association between IL-4 genotypes and T2DM.[16] Nevertheless, the influence of IL-4 on metabolism has not been extensively addressed and deserves further investigation. In this context, the present study aimed at investigating *in vivo* effects of IL-4 on metabolism using mice T2DM model induced by single low-dose STZ administration and high-fat diet (HFD).[17-20] Our results show that IL-4 overexpression leads to better glucose utilization. In addition, body weights and fat mass of mice with IL-4 treatment are significantly lower than their counterparts. These observations suggest that IL-4 is involved in mediating glucose and lipid metabolism.

2.2. MATERIALS AND METHODS

Cell culture and adenovirus expansion

Human AD293 embryonic kidney cells were cultured in DMEM containing glucose (4.5 g/L) and 10% FBS. Recombinant adenoviruses expressing IL-4 (AdIL-4) and β-galactosidase (AdLacZ, as a negative control) were kindly provided by Dr. Nagayama.[21] AdIL-4 and AdLacZ were respectively propagated in AD293 cells and purified by cesium chloride density gradient ultracentrifugation, followed by dialysis in PBS with 10% glycerol. Number of viral particles was determined by measuring the absorbance at 260 nm.

Animal experiments

All mice were obtained from National Laboratory Animal Center and caged in groups of 5. For adenovirus experiments, 8-week-old male C57BL/6 mice were *i.p.* injected twice (once daily for 2 consecutive days) with 5 x 10^{11} particles of AdIL-4 or AdLacZ. The adenovirus-injected mice were *i.p.* administered with STZ (100 mg/kg; Sigma-Aldrich, St Louis, MO, USA) on the second day.[21] Intraperitoneal glucose tolerance test (IGTT), intraperitoneal insulin tolerance test (IITT) and circulatory biochemical parameters were assessed on the third day. For long-term IL-4 administration experiments, 4-week-old C57BL/6 mice were fed with HFD (60% kcal) or standard diet (SD), and *i.p.* administered with IL-4 (1,000 pg per mouse; BD Pharmingen) every other day for 8 weeks. Then the mice were sacrificed after IGTT, IITT and biochemical tests were performed. In IL-4 neutralization experiments, BALB/c mice with relatively high IL-4-secreting ability were used instead of low IL-4-secreting C57BL/6 mice.[22] Four-week-old male mice were first fed with SD or HFD for 10 weeks, then divided to 4 groups: (1) SI group received *i.p.* STZ-injection followed by IL-4 antibody administration on the second, third and fifth day (50 μg per mouse; Biosource, Camarillo, CA, USA); (2) IS group received IL-4 antibody administration on the first, second and fifth day, and STZ injection on the third day; (3) STZ group received only STZ treatment on the first day; and (4) Control group received citrate buffer (0.05 M, pH 4.5; USB, Cleveland, Ohio, USA) administration. Then the mice were sacrificed after IGTT, IITT and biochemical tests were performed. Animal protocols were reviewed and approved by the Chung Shan Medical University animal studies committee.

IGTT, IITT and blood parameters

IGTT was performed and blood glucose was measured using OneTouch monitoring system (LifeScan) before and after *i.p* glucose injection (2 g/kg; Sigma-Aldrich, Steinheim, Gremany) at the indicated time. IITT was conducted and blood glucose was monitored at the time points indicated after *i.p.* injection of

recombinant human insulin (1 U/kg; Eli Lily, Indianapolis, IN, USA). Serum levels of insulin (Mercodia, Uppsala, Sweden), IL-4 (R&D, Minneapolis, MN, USA), leptin (Millipore, Billerica, MA, USA), adiponectin (Millipore, Billerica, MA, USA), and FFA (BioVision, Mountain View, CA, USA) were measured after overnight fast using ELISA kits according to manufacturers' instruction. The epididymal fat pads were taken and weighted, and the adipocytic cross-sectional areas from staining images were calculated.

Western blot

Protein extracts from muscle and epididymal fat tissues were obtained after the tissues were homogenized using T-PER tissue protein extraction reagent (Pierce, Rockford, IL, USA) supplied with phosphatase and protease inhibitors (Roche, Indianapolis, IN, USA). Protein lysates were normalized using Bio-Rad protein reagent and resolved by SDS-PAGE, then electrotransferred to PVDF membrane. The membranes were blocked and incubated with mouse anti-actin monoclonal IgG (Abcam, Cambridge, UK), mouse anti-phospho-Akt serine 473, rabbit anti-Akt, rabbit anti-phospho-GSK3β serine 9 and rabbit anti-GSK3β, respectively. All primary antibodies were purchased from Cell Signaling (Danvers, MA, USA), except where otherwise indicated. Then, membranes were incubated with HRP-conjugated anti-mouse or anti-rabbit antibodies. Results were developed using ECL reagents and quantitated by densitometer. All secondary antibodies and ECL reagents were purchased from Millipore (Temecula, CA, USA).

Statistical Analysis

Results were presented as Mean ± SEM and the significant difference between groups was analyzed by one-way or two-way ANOVA using SPSS software. Statistical difference was defined as $p<0.05$ for all test.

2.3. RESULTS

Effects of in vivo IL-4 overexpression on glucose metabolism

To establish a mouse model with high IL-4 levels, 8-week-old C57BL/6 mice were first subjected to *i.p.* injection with AdIL-4, followed by STZ treatment (AdIL-4 mice). Mice with either citrate buffer (control mice) or AdLacZ administration and STZ treatment (AdLacZ mice) were served as controls. Serum IL-4 level of AdIL-4 mice (10.16±1.72 *p*M) was significantly higher than that of control (1.17±0.44 *p*M) and AdLacZ (1.64±0.65 *p*M) mice (Table 1). It indicated that the adenovirus administration can successfully turn the low-IL-4 secreting C57BL/6 mice into high-IL-4-expressing animal model. Biochemical parameters including adiponectin, leptin, insulin, and FFA were then analyzed to examine the effects of *in vivo* IL-4 overexpression on metabolism. As listed in Table 1, levels of adiponectin, leptin and insulin were all significantly increased in AdIL-4 mice. AdIL-4 mice had higher FFA levels, however, this difference did not reach statistical significance.

The effects of IL-4 overexpression on glucose homeostasis and insulin sensitivity were subsequently examined. The results showed that IGTT curves from control and AdLacZ mice were quire similar (Fig. 1A). Whereas, AdIL-4 mice demonstrated better glucose tolerance with a lower peak glucose level (approximately 13.89 mM) and a shorter time required for reaching glucose homeostasis (about 90 min). Consistent with the IGTT observations, IITT results showed that insulin sensitivity and glucose tolerance of AdIL-4 mice were significantly better than that of control and AdLacZ mice (Fig. 1B).

Effects of IL-4 neutralization on glucose metabolism

To verify the above observations, high IL-4 producing BABL/c mice were used to examine if the effects of IL-4 on metabolism could be reversed by depleting IL-4. BALB/c mice were first fed either with SD or HFD for 10 weeks, and their IL-4 bioactivities were neutralized before or after STZ treatment as described in Methods. IGTT results showed that blood glucose of all the mice groups with SD reached the peak levels at about 30~40 min, with high glucose levels sustained till 120 min in SI, IS and STZ mice (Fig. 1C). Interestingly, blood glucose levels in mice with IL-4 neutralization (SI and IS) were consistently and significantly higher than mice without IL-4 depletion (control and STZ; Fig. 1C). Similar phenomena were also observed in HFD-fed mice: glucose levels in mice with IL-4 neutralization remained high still 120 min after glucose injection, compared to control and STZ mice groups (Fig. 1D). These data suggested that mice with IL-4 overexpression had better glucose tolerance and metabolism.

Effects of in vivo IL-4 on Akt and GSK3β phosphorylation

To further examine the mechanism of IL-4 promoting glucose tolerance, influences of IL-4 to the

activity of the insulin downstream signaling mediators, Akt and GSK3β, were explored. Akt phosphorylation were significantly increased in muscle cells of AdIL-4 mice; whereas, no significant alterations of Akt phosphorylation were observed in epididymal fat (Table 2). It suggests that the IL-4-improved glucose tolerance might result from enhancing insulin action by up-regulating Akt activities in muscle cells. On the contrary, levels of phosphorylated GSK3β were decreased in AdIL-4 mice (Table 2).

Effects of long-term IL-4 treatment on lipid and glucose metabolism

The *in vivo* elevated IL-4 levels in mice with AdIL-4 administration could only last transiently for about 3 days. For creating mice model with long-term high IL-4 levels, mice were *i.p.* injected with recombinant IL-4 every other day for 8 weeks, and fed with either SD or HFD. Our results showed IL-4 levels in mice with IL-4 administration (IL-4, 12.02 ± 3.90 *p*M; HFD+IL-4, 11.34 ± 4.21 *p*M) were significantly higher than their counterparts (control, 3.14 ± 1.78 *p*M; HFD, 3.19 ± 1.33 *p*M; Table 1). It indicated that mice model with long-term high IL-4 levels can be achieved by this strategy. Similar trend were also observed in serum levels of adiponectin and FFA, with IL-4-treated mice having higher levels than control and HFD mice (Table 1). However, leptin levels were decreased in IL-4-treated groups (IL-4, 0.30 ± 0.12 *n*M; HFD+IL-4, 0.47 ± 0.10 *n*M), compared to mice without IL-4 treatment (control, 0.49 ± 0.09 *n*M; HFD, 0.82 ± 0.11 *n*M; Table 1).

Interestingly, body weights and fat mass of mice with IL-4 treatment (IL-4 and HFD+IL-4 mice) were significantly lower than the counterparts without IL-4 injection (Fig. 2A and Table 3; with the original data of Fig. 2A listed in Table S1). Weights of the epididymal fat pads were decreased in IL-4-treated mice (IL-4, 0.16 ± 0.02 g; HFD+IL-4, 0.92 ± 0.31 g), compared to the mice without IL-4 treatment (control, 0.28 ± 0.03 g; HFD, 1.08 ± 0.09 g; Table 3). The adipocytic cross-sectional areas in IL-4-treated mice were significantly smaller (with relative ratio to control mice: IL-4, 0.61 ± 0.03; HFD, 1.19 ± 0.05; HFD+IL-4, 1.01 ± 0.06; Table 3). The same phenomenon was also observed in epididymal adipocytes of AdIL-4 administered mice (with relative ratio to control mice: AdLacZ, 1.03 ± 0.11; AdIL-4, 0.66 ± 0.07; Table 3). Taking the results of decreased fat mass and the increased serum FFA levels (Table 1) in IL-4-treated mice together, it implied that overexpressed IL-4 may inhibit lipid accumulation in fat tissues and lead to the elevated levels of circulatory FFA.

IGTT and IITT were then conducted to analyze the effects of long-term IL-4 treatment on glucose and insulin tolerance. Consistent with the results from adenovirus administration experiments, IL-4-treated mice (IL-4 and HFD+IL-4) had better glucose tolerance than their control counterparts, with a shorter time required for reaching glucose homeostasis (IL-4, 60 min; HFD+IL-4, about 90 min) after glucose injection (Fig. 2B). Likewise, the IITT results showed that insulin sensitivity and glucose

tolerance of IL-4-treated mice were significantly better than that of the corresponding control mice (Fig. 2C).

Effects of long-term IL-4 treatment on Akt and GSK3β phosphorylation

The Akt phosphorylation was significantly decreased in muscle of HFD+IL-4 mice; nevertheless, it was dramatically increased in the epididymal fat of IL-4 and HFD+IL-4 mice (Table 2). It suggested that under long-term IL-4 treatment, the improved glucose tolerance and insulin sensitivities might result mainly from the enhanced insulin action by up-regulating Akt activities in fat tissues. The levels of phosphorylated GSK3β were significantly decreased both in the muscle and fat tissues of IL-4 and HFD+IL-4 mice, comparable to the results from adenovirus experiments, (Table 2).

2.4. DISCUSSION

T2DM is associated with chronic inflammation. Although the correlation between IL-6/TNF-α and insulin resistance has been extensively studied,[23-25] relatively few is known about the roles of other cytokines in diabetic pathogenesis. IL-4, another cytokine secreted by Th2 cells, participates in the regulation of inflammation by modulating expression of other pro-inflammatory cytokines and inflammation mediators.[26,27] Our present study uncovers the roles of IL-4 in glucose/lipid metabolism and insulin sensitivity using animal model.

In AdIL-4 experiments, our results suggest that transient IL-4 treatment improves glucose tolerance and insulin sensitivity (Fig. 1, A&B). This effect is also observed in the IL-4 neutralization experiments, in which glucose tolerance and insulin sensitivity are exacerbated in mice injected with IL-4 antibodies (Fig. 1, C&D). In mice with long-term exposure to high IL-4 levels, the capacity of IL-4 to promote glucose tolerance and insulin sensitivity is further confirmed (Fig. 2). Interestingly, while Akt phosphorylation is significantly induced in muscle of mice with transient IL-4 treatment, it is significantly induced in fat cells of mice with long-term IL-4 exposure (Table 2). It indicates that better glucose tolerance and insulin sensitivity under IL-4 treatment may be resulted from its capacity to promote insulin signaling.

In addition to the roles in promoting insulin signaling, the observation that insulin levels are significantly increased in AdIL-4 mice (Table 1) implies transient IL-4 treatment promotes insulin-secreting function of β-cells. The results that both transient and long-term IL-4 treatments significantly inhibit GSK3β phosphorylation in muscle and fat cells (Table 2) support the previous evidence that IL-4 mediates glucose metabolism by regulating glycolytic enzymes.[28,29] It is intriguing that IL-4 promotes glucose tolerance through activating Akt activities while suppressing GSK3β phosphorylation. There are 2 possible explanations to this contradictory observation. First of all, IL-4 might display differential regulatory function to different insulin-targeted organs for maintaining metabolic hemeostasis. The reduced GSK3β activities in muscle and fat tissues might not be applied to liver, the critical target organ of glycogen synthesis. Besides, a recent study reveals that fibroblast growth factor 19 facilitates postprandial hepatic protein and glycogen synthesis through a insulin-independent pathway.[30] Therefore, the downregulated GSK3β activities in fat and muscle cells may not represent that glycogen synthesis is suppressed in hepatocytes. Secondly, this contradictory regulatory effect of IL-4 might be in response to the increased energy needs from cells to maintain homeostasis for avoiding cellular apoptosis, as previous described.[31]

Production of the pro-inflammatory cytokine IL-6 is essential to induce glucose intolerance and

insulin resistance.[25] Lee et al. reveals that the genetic inactivation of PKCζ leads to a hyper-inflammatory state with increased synthesis of pro-inflammatory cytokine IL-6 in obese mice which are more glucose intolerant and insulin resistant.[32] Their study shows that PKCζ is a critical negative regulator of IL-6 in the control of obesity-induced inflammation because the glucose intolerance and insulin resistance phenotypes are corrected to normal by silencing IL-6 expression in PKCζ-deficient mice. The positive regulatory role of IL-4 to glucose tolerance and insulin sensitivity uncovered by this study is consistent to the conclusion from Lee et al. since PKCζ serves as a critical role in the anti-inflammatory effects of IL-4.[33,34] Accordingly, it supports our hypothesis that the positive regulatory role of IL-4 to glucose tolerance and insulin sensitivity may originate from its anti-inflammatory function by inhibiting the production of cytokines inducing insulin resistance, such as TNF-α and IL-6.[35]

Our results also suggest that IL-4 participates in lipid metabolism by regulating the circulatory levels of adiponectin and leptin. Intriguingly, adiponectin and leptin levels are both elevated under transient IL-4 treatment; whereas leptin levels are significantly decreased in HFD mice with long-term IL-4 administration. In addition, serum FFA levels are both increased after IL-4 treatments (Table 1). These data further support the capacity of IL-4 to promote insulin sensitivity and glucose metabolism because adiponectin has been shown to elevate insulin sensitivity and inhibit hepatic gluconeogenesis.[36] However, the inducing ability of IL-4 to β-cell insulin secretion is not observed in long-term IL-4 experiments, discrepancy in leptin levels is also observed in mice with different duration of IL-4 treatment (Table 1). Elevated levels of leptin and IL-4 in rats with acute pancreatitis are reported for increasing pancreatic resistance against inflammatory damages.[37] The increased levels of leptin in transient IL-4-treated mice support the suggestion of the above study, whereas, the food intake (e.g. HFD in this study) plays a dominant role in regulating leptin secretion. We suggest that the absence of induced insulin and leptin secretion under long-term IL-4 administration might be an adaption to the consistently elevated cytokine exposure for maintaining insulin levels and homeostasis.

The weekly weight gain in the IL-4-treated mice is lower (IL-4 and HFD+IL-4; Fig. 2 and Table S1), moreover, long-term IL-4 treatment leads to a reduction of fat mass and the cross-sectional areas of fat cells (Table 2). As evidence has revealed that leptin secretion is positively correlated with the amount of adipose tissues,[38] and adiponectin concentrations are reduced as adiposity increases,[39,40] we suggest that the significantly slower weight gain might result from the increased lipolysis in fat cells, which leads to the elevated circulatory FFA in IL-4 treated mice. Although the exact underlying mechanisms await further investigation, our results support the previous report that IL-4 transgenic mice contain less and smaller sized dermal fat tissues.[41] Our data also support another study in which documents that IL-4 secretion and Akt activity are both promoted in fatless A-ZIP/F1 diabetic mice.[42]

In summary, overexpression of IL-4 may promote glucose tolerance and insulin sensitivity through boosting insulin signaling by altering Akt and GSK3β activities. IL-4 is also involved in lipid metabolism by regulating adipokines and FFA levels. We suggest that IL-4 regulates metabolism by promoting insulin sensitivity, glucose tolerance, and inhibiting lipid deposits. The present study reveals evidence to uncover the novel roles of IL-4 in metabolism, and provide new insights in the interaction between cytokines/immune responses, insulin sensitivity and metabolism.

2.5. REFERENCES

1. Goldstein BJ. Insulin resistance as the core defect in type 2 diabetes mellitus. *Am J Cardiol* 2002; **90**: 3G-10G.
2. Sone H, Suzuki H, Takahashi A, Yamada N. Disease model: hyperinsulinemia and insulin resistance. Part A-targeted disruption of insulin signaling or glucose transport. *Trends Mol Med* 2001; **7**: 320–322.
3. Ginsberg HN. Insulin resistance and cardiovascular disease. *J Clin Invest* 2000; 106: 453–458.
4. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care* 2004; **27**: 813–823.
5. Crook M. Type 2 diabetes mellitus: a disease of the innate immune system? An update. *Diabet Med* 2004; **21**: 203–207.
6. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004; **25**: 4–7.
7. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM *et al.* IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 2005; **11**: 191–198.
8. Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* 1998; **41**: 1241–1248.
9. Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* 1997; **40**: 1286–1292.
10. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* 2003; **112**: 1785–1788.
11. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006; **444**: 860–867.
12. Wei LH, Jacobs AT, Morris SM Jr, Minor KD, Mazzocco VR, Freund GG. IL-4 and IL-13 upregulate arginase I expression by cAMP and JAK/STAT6 pathways in vascular smooth muscle cells. *Am J Physiol Cell Physiol* 2000; **279**: C248–C256.
13. Wurster AL, Withers DJ, Uchida T, Wither DJ, Grusby MJ. Stat6 and IRS-2 cooperate in interleukin 4 (IL-4)-induced proliferation and differentiation but are dispensable for IL-4-dependent rescue from apoptosis. *Mol Cell Biol* 2002; **22**: 117–126.
14. Sun XJ, Wang LM, Zhang Y, Yenush L, Myers MG Jr, Glasheen E *et al.* Role of IRS-2 in insulin and cytokine signaling. *Nature* 1995; **377**: 173–177.
15. Hartman ME, O'Connor JC, Godbout JP, Minor KD, Mazzocco VR, Freund GG. Insulin receptor substrate-2-dependent interleukin-4 signaling in macrophages is impaired in two models of type 2 diabetes mellitus. *J Biol Chem* 2004; **27**: 28045–28050.
16. Ho KT, Shiau MY, Chang YH, Chen CM, Yang SC, Huang CN. Association of IL-4 promoter

polymorphisms in Taiwanese patients with type 2 diabetes mellitus. *Metabolism* 2010; **59**: 1717-1722.

17. Luo J, Quan J, Tsai J, Hobensack CK, Sullivan C, Hector R *et al.* Nongenetic Mouse Models of Non-Insulin-Dependent Diabetes Mellitus. *Metabolism* 1998; **47**: 663–668.
18. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett PG, Gadbois TM *et al.* A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism* 2000; **49**: v1390–v1394.
19. Chen D, Wang MW. Development and application of rodent models for type 2 diabetes. *Diabetes Obes Metab* 2005; **7**: 307–317.
20. Nakamura T, Terajima T, Ogata T, Ueno K, Hashimoto N, Ono K *et al.* Establishment and pathophysiological characterization of type 2 diabetic mouse model produced by streptozotocin and nicotinamide. *Biol Pharm Bull* 2006; **29**: 1167–1174.
21. Nagayama Y, Mizuguchi H, Hayakawa T, Niwa M, McLachlan SM, Rapoport B. Prevention of autoantibody-mediated Graves'-like hyperthyroidism in mice with IL-4, a Th2 cytokine. *J Immunol* 2003; **170**: 3522–3527.
22. Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to Leishmania major in mice. *Nat Rev Immunol* 2002; **2**: 845–858.
23. Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* 2002; **51**: 3391–3399.
24. Klover PJ, Clementi AH, Mooney RA. Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* 2005; **146**: 3417–3427.
25. Sabio G, Das M, Mora A, Zhang Z, Jun JY, Ko HJ *et al.* A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* 2008; **322**: 1539–1543.
26. Lee YW, Eum SY, Chen KC, Hennig B, Toborek M. Gene expression profile in interleukin-4-stimulated human vascular endothelial cells. *Mol Med* 2004; **10**: 19–27.
27. Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. *J Clin Endocrinol Metab* 2005; **90**: 2282–2289.
28. Dufort FJ, Bleiman BF, Gumina MR, Blair D, Wagner DJ, Roberts MF *et al.* Cutting edge: IL-4-mediated protection of primary B lymphocytes from apoptosis via Stat6-dependent regulation of glycolytic metabolism. *J Immunol* 2007; **179**: 4953–4957.
29. Plas DR, Thompson CB. Cell metabolism in the regulation of programmed cell death. *Trends Endocrinol Metab* 2002; **13**: 75–78.
30. Kir S, Beddow SA, Samuel VT, Miller P, Previs SF, Suino-Powell K, Xu HE, Shulman GI, Kliewer SA, Mangelsdorf DJ. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. *Science* 2011; **331**: 1621-1624.
31. Vander Heiden MG, Plas DR, Rathmell JC, Fox CJ, Harris MH, Thompson CB. Growth factors can influence cell growth and survival through effects on glucose metabolism. *Mol Cell Biol* 2001; **21**:

5899–5912.

32. Lee SJ, Kim JY, Nogueiras R, Linares JF, Perez-Tilve D, Jung DY, Ko HJ, Hofmann SM, Drew A, Leitges M, Kim JK, Tschop MH, Diaz-Meco MT, Moscat J. PKCζ-regulated inflammation in the nonhematopoietic compartment is critical for obesity-induced glucose intolerance. *Cell Metab* 2010; **12**: 65–77.
33. Duran A, Rodriguez A, Martin P, Serrano M, Flores JM, Leitges M, Diaz-Meco MT, Moscat J. Crosstalk between PKCzeta and the IL4/Stat6 pathway during T-cell-mediated hepatitis. *EMBO J* 2004; **23**:4595-4605.
34. Martin P, Villares R, Rodriguez-Mascarenhas S, Zaballos A, Leitges M, Kovac J, Sizing I, Rennert P, Marquez G, Martinez-A C, Diaz-Meco MT, Moscat J. Control of T helper 2 cell function and allergic airway inflammation by PKCzeta. *Proc Natl Acad Sci USA* 2005; **102**: 9866-9871.
35. Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel CR, Goforth MH, Subramanian V, Mukundan L, Ferrante AW, Chawla A. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. *Cell Metab* 2008; **7**: 496-507.
36. Barnea M, Madar Z, Froy O. High-fat diet delays and fasting advances the circadian expression of adiponectin signaling components in mouse liver. *Endocrinology* 2009; **150**: 161–168.
37. Konturek PC, Jaworek J, Maniatoglou A, Bonior J, Meixner H, Konturek SJ *et al.* Leptin modulates the inflammatory response in acute pancreatitis. *Digestion* 2002; **65**: 149–160.
38. Bełtowski J. Adiponectin and resistin--new hormones of white adipose tissue. *Med Sci Monit* 2003; **9**: RA55–RA61.
39. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y *et al.* Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1595–1599.
40. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941–946.
41. Elbe-Bürger A, Egyed A, Olt S, Klubal R, Mann U, Rappersberger K *et al.* Overexpression of IL-4 alters the homeostasis in the skin. *J Invest Dermatol* 2002; **118**: 767–778.
42. Nunez NP, Oh WJ, Rozenberg J, Perella C, Anver M, Barrett JC *et al.* Accelerated tumor formation in a fatless mouse with type 2 diabetes and inflammation. *Cancer Res* 2006; **66**: 5469–5476.

2.6. TABLES

Table 1. Biochemical Profile of Mice Groups Divided by Treatment of Adenovirus Administration, IL-4 and Diet

	IL-4 (*p*M)	Adiponectin (*p*M)	Leptin (*n*M)	Insulin (*n*M)	Free fatty acid (*m*M)
Adenovirus treatment*					
Control	1.17±0.44	185.67±4.3	0.08±0.02	0.06±0.01	8.56±3.57
AdLacZ	1.64±0.65	187.67±15.67	0.41±0.16[a]	0.28±0.19	9.96±2.67
AdIL-4	10.16±1.72[a,b]	216.0±6.3[a,b]	0.58±0.10[a]	0.79±0.21[a,b]	14.7±3.14
IL-4/diet treatment**					
Control	3.14±1.78	185.67±6.0	0.49±0.09	0.08±0.01	7.39±0.88
IL-4	12.02±3.90[c]	206.33±14.67	0.30±0.12	0.10±0.002	8.25±1.52
HFD	3.19±1.33[d]	235.33±10.7[c]	0.82±0.11[c,d]	0.09±0.01	13.9±3.74
HFD+IL-4	11.34±4.21[c,e]	251.67±17.0[c,d]	0.47±0.10[e]	0.10±0.03	20.3±4.67[c,d]

*8-week-old mice fed with standard chow diet were subjected to *i.p.* adenovirus injection. All parameters were measured after 16 hours of overnight fast. Data were presented as Mean ± SEM; n=7. [a]$p<0.05$ versus control; [b]$p<0.05$ versus AdLacZ.

**4-week-old mice were subjected to *i.p.* IL-4 injection every 2 days and fed with either SD or HFD diet for 8 weeks. All parameters were measured as described. Data were presented as Mean ± SEM; n=5-10. [c]$p<0.05$ versus control; [d]$p<0.05$ versus IL-4; [e]$p<0.05$ versus HFD.

Table 2. Relative Levels of Phosphorylated Akt and GSK3β of Muscle and Epididymal Fat in Mice Groups Divided by Treatment of Adenovirus Administration, IL-4 and Diet

	Epididymal		Muscle	
	pAkt/Akt	**pGSK3β/GSK3β**	**pAkt/Akt**	**pGSK3β/GSK3β**
Adenovirus treatment				
Control	0.26±0.03	0.33±0.21	0.20±0.03	0.90±0.27
AdLacZ	0.30±0.04	0.13±0.04	0.22±0.01	1.07±0.09
AdIL-4	0.24±0.06	0.03±0.01[a]	0.35±0.02[a,b]	0.10±0.02[a,b]
IL-4/diet treatment				
Control	0.26±0.02	2.04±0.22	0.65±0.04	0.47±0.08
IL-4	1.97±0.39[c]	1.26±0.04[c]	0.85±0.08	0.24±0.08[c]
HFD	0.68±0.17[d]	1.55±0.27	1.24±0.65	0.48±0.03[d]
HFD+IL-4	1.76±0.29[c,e]	1.37±0.19[c]	0.57±0.05[e]	0.40±0.02[c,e]

*Data are presented as mean ± SEM which indicate the relative levels of phosphorylated protein using actin as represent an internal control. [a]$p<0.05$ versus control mice in adenovirus treatment; [b]$p<0.05$ versus AdLacZ; [c]$p<0.05$ versus control mice in IL-4/diet treatment; [d]$p<0.05$ versus IL-4; [e]$p<0.05$ versus HFD.

Table 3. Weights and Adipocytic Cross-sectional Areas of Epididymal Fat Pads in Mice Groups Divided by Treatment of Adenovirus Administration, IL-4 and Diet

	Cross-sectional area (AU)*	Epididymal fat weight (g)
Adenovirus treatment		
Control	1 ± 0.17	-
AdLacZ	1.03 ± 0.11	-
AdIL-4	0.66 ± 0.07[a,b]	-
IL-4/diet treatment		
Control	1 ± 0.05	0.28 ± 0.03
IL-4	0.61 ± 0.03[c]	0.16 ± 0.02
HFD	1.19 ± 0.05[d]	1.08 ± 0.09[c,d]
HFD+IL-4	1.01 ± 0.06[d]	0.92 ± 0.31[c,d]

*Data are presented as mean ± SEM which indicate the relative ratios of cross-sectional areas (AU: arbitrary units) using that from corresponding control mice as control. [a]$p<0.05$ versus control mice in adenovirus treatment; [b]$p<0.05$ versus AdLacZ; [c]$p<0.05$ versus control mice in IL-4/diet treatment; [d]$p<0.05$ versus IL-4.

2.7. FIGURES

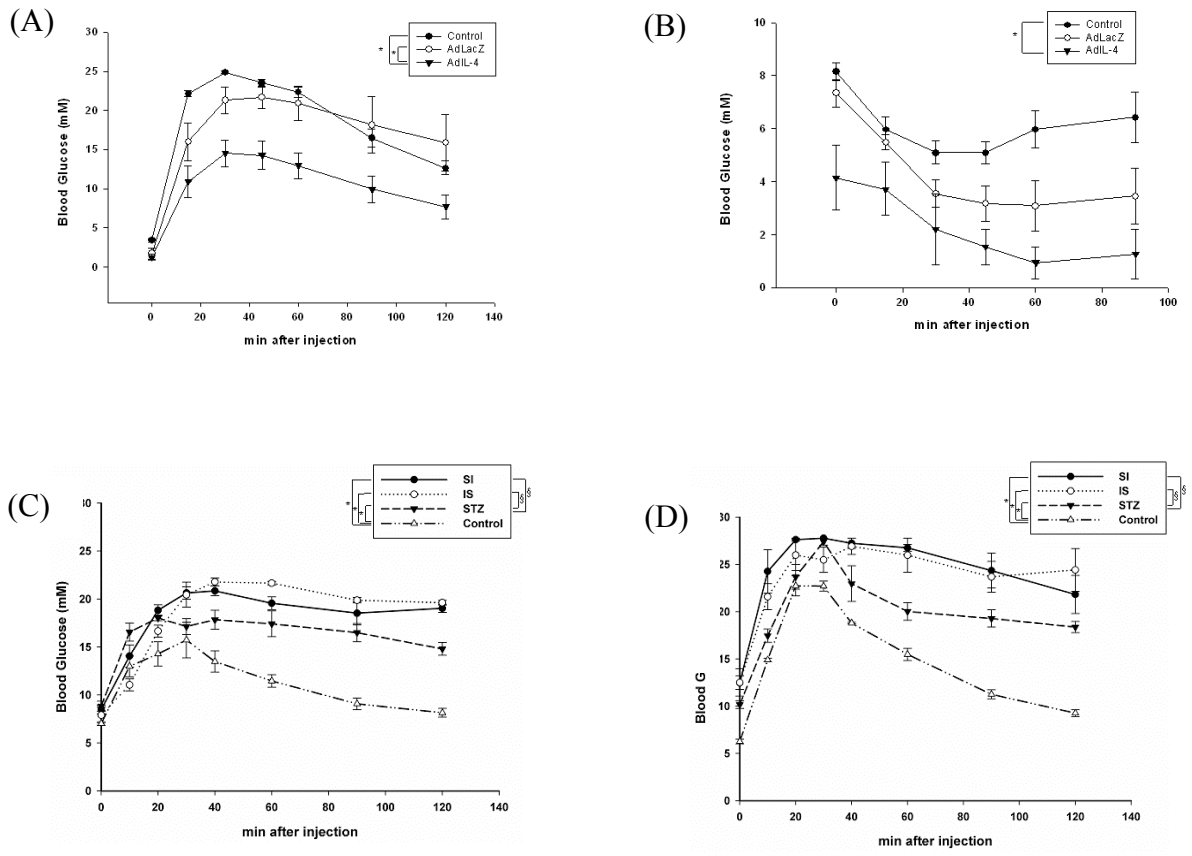

Figure 1. Glucose Tolerance and Insulin Sensitivity Are Improved by IL-4 Overexpression. (A-B) IGTT and IITT were conducted in overnight-fasting control (-●-), AdLacZ (-○-), and AdIL-4 (-▼-) mice after adenovirus and STZ treatments, respectively. Data were expressed as Mean ± SEM; n=7. $^{*}p<0.05$ v.s. AdIL-4. (C-D) IGTT was performed for overnight-fasting control (-Δ-), SI (-●-), IS (-○-), and STZ (-▼-) mice after 10 weeks of SD (B) or HFD (C) feeding and treatment with IL-4 antibodies and/or STZ. Data were expressed as Mean ± SEM; n=4. $^{*}p<0.05$ v.s. control; $^{\S}p<0.05$ v.s. STZ.

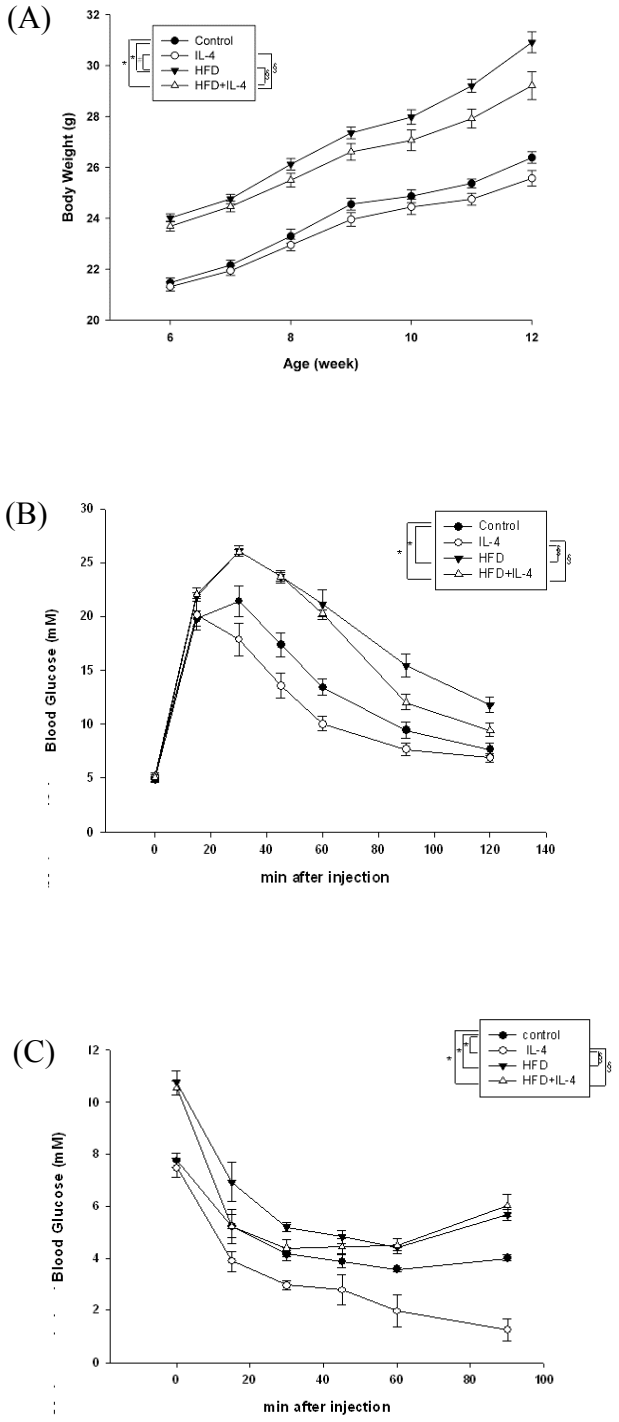

Figure 2. Effects of Long-term IL-4 Treatment on Body Weights and Glucose Metabolism. (A) Body weights of IL-4-treated mice groups (IL-4: -○-; HFD+IL-4: -Δ-) were significantly increased than their control counterpart without IL-4 administration (control: -●-; HFD: -▼-). $^{*}p<0.05$ v.s. control; $^{\#}p<0.05$ v.s. IL-4; $^{\S}p<0.05$ v.s. HFD; n=10. (B-C) Results of IGTT and IITT in mice with long-term IL-4 injection. Data were expressed as Mean ± SEM; n=5. $^{*}p<0.05$ v.s. control; $^{\S}p<0.05$ v.s. IL

附件二

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■ 達成目標

□ 未達成目標（請說明，以100字為限）

□ 實驗失敗

□ 因故實驗中斷

□ 其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 □無

技轉：□已技轉 □洽談中 □無

其他：（以100字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500字為限）

本文的主旨為探討細胞激素第四型介白素(interleukin-4, IL-4)在T2DM和新陳代謝中的角色。根據本研究之基因型分析結果，國人IL-4基因型與T2DM以及受檢者之高密度膽固醇發病有相關性；此發現表示IL-4可能與葡萄糖/脂肪之代謝作用有關而參與T2DM之致病。因此本實驗室進一步以細胞與動物模式檢測上述假說。實驗結果顯示IL-4可透過調控Akt之磷酸化，提升胰島素敏感性與葡萄糖耐受性；此外，IL-4也抑制脂肪聚集而調控脂肪代謝作用，減輕小鼠之體重與脂肪重量。簡而言之，本研究除發現IL-4基因型與T2DM之發病有關之外，亦證實IL-4透過提高胰島素敏感性與葡萄糖耐受性並抑制脂肪堆積而調節葡萄糖/脂肪之代謝作用。上述結果提供IL-4調控代謝作用之嶄新角色，也提出免疫作用、胰島素敏感性與新陳代謝三者交互作用之證據。

附件三

國科會補助專題研究計畫項下出席國際學術會議心得報告

計畫編號	NSC 97-2320-B-040-006-MY3
計畫名稱	第四型介白素對脂肪與葡萄糖代謝作用的調控
出國人員姓名 服務機關及職稱	張懿欣　中山醫學大學醫學檢驗暨生物技術學系　教授
會議時間地點	2009/04/25~2009/04/29 土耳其伊斯坦堡
會議名稱	第11屆歐洲內分泌大會 11th European Congress of Endocrinology
發表論文題目	台灣第二型糖尿病患者第四型介白素基因型之分析 Association of IL-4 Promoter Polymorphisms in Taiwanese Patients with Type 2 Diabetes Mellitus

一、 參加會議經過

本人於四月二十五日抵達土耳其伊斯坦堡，首先到住宿飯店辦理check in，稍作休息之後至會場辦裡報到手續，領取會議相關書面資料。隨後逕入會場聽取演講。接下來之議程中，除了聽講之外，也參加海報展覽。整個會議結束之後，搭乘飛機返台。

二、 與會心得

本次大會是由歐洲內分泌學會在土耳其主辦，每年舉辦之歐洲地區內分泌國際會議，主題除了討論糖尿病臨床與基礎知識背景與研究新知之外，也包括甲狀腺、生殖腺、腦下垂體等內分泌疾病之深入探討。本次會議之與會者計有美國、加拿大、國際糖尿病聯盟組織等單位之代表人物，共2,000餘位世界各地之內分泌相關領域的研究學者專家。在整個與會過程中，除可了解糖尿病等內分泌疾病與免疫研究之最新進展、資訊與技術之外，也可進一步其他內分泌疾病之相關研究，並有機會認識世界各地的學者專家。

三、建議

建議國科會應多多補助研究人員參與國際會議。除了可以幫助研究人員了解國際上研究之最新相關資訊之外，也可藉機認識國際友人與學者並提升學校知名度，不但拓展自身視野也可使台灣本土之學者立身於國際學術領域，增進學術交流，提升學校的學術地位。

四、攜回資料名稱及內容

會議議程、會議手冊與會議摘要CD。

Mini Review

Cyotkines, Metabolism, and Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus (T2DM) is usually caused by insulin resistance, and often combined with progressive defect in insulin secretion. However, the etiology of T2DM remained unknown. Among various factors which lead to the onset of T2DM, host genetics and environmental factors are the focus of discussion. Identification and characterization of specific genes that can result in metabolic abnormalities are helpful for the effective prevention and therapeutic intervention of diabetes mellitus. Accumulating evidences have proved that T2DM is closely correlated with chronic inflammation, with increased levels of circulatory acute response proteins and cytokines in affected subjects. The aim of this article is to provide a general overview on the epidemiology, classification, and roles of cytokines in metabolism and T2DM. In addition to focusing on the cytokines-related literatures of diabetic studies, a summary of our study results concerning the polymorphisms of cytokine genes among diabetic patients is also included. Accomplishments of the immune-related genetic studies in a particular ethnic population can lead not only to the understanding of the interactions between immune responses and T2DM, but also potential clues for the designing of personalized type 2 diabetic treatment in the future.

Key words: cytokines; metabolism; interleukins; diabetes

Epidemiology and Classification of Diabetes Mellitus

Diabetes mellitus (DM) is a syndrome of abnormal metabolism with inappropriate hyperglycemia due either to an absolute deficiency of insulin secretion or a reduction in the biologic effectiveness of insulin, or both. DM patients with long duration have the propensity to develop universal microangiopathy, neuropathy and atherosclerosis.

Traditionally, diabetes is classified according to the patients' age at onset of symptoms (juvenile-onset versus adult-onset). In 1997, the National Diabetes Data Group of American Diabetes Association recommended that DM be classified into one of two major types according to the correlation of diabetic onset and immune response: T1DM (T1DM; formerly designated as insulin-dependent DM, IDDM) and T2DM (T2DM; formerly designated as non-insulin dependent DM, NIDDM). T1DM is a severe form of DM and is associated with ketosis in the untreated state. It is a catabolic disorder in which circulating insulin is virtually absent, plasma glucagon is elevated, and the pancreatic β cells fail to respond to all known insulinogenic stimuli. T2DM is characterized by abnormally high blood glucose resulting from a relative deficiency of insulin [1]. Insulin secretion in T2DM patients is preserved, and such cases can be treated with dietary changes combined with oral antidiabetic drugs [2]. Therefore, patients are not necessarily dependent on exogenous insulin therapy to sustain life. Genetic factors are important in the aetiology of T2DM, and linkage studies have localized some of the genes that influence the development of this disorder [3, 4]. T2DM, in fact, comprises a heterogeneous group of the milder forms of diabetes, occurs predominantly in adults but may occa-

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sionally have its onset in childhood. Treatment of diabetes and its complications are an increasing health-care burden in our society [5].

Diabetic patients worldwide are estimated to be approximately 200 million, among which approximately 60~80% are obese [6]. The global prevalence of diabetes in year 2000 is about 2.8%, however, this prevalence is estimated to reach 4.4% in year 2030. Number of the diabetic patients is rapidly increasing globally. The population of global diabetic patients was 151 million in year 2000 and predicted to be 221 million in year 2010, with the increased rate of 46% within a single decade [5]. The prevalence of T2DM varies among different ethic populations, with the highest rate found in Pima Indians (as high as ~50%) [7]. Accompanied with the more and more westernized food-uptake habits, the living standard of people in Taiwan has been continuously elevated. Unfortunately, the prevalence of chronic metabolic diseases such as cardiovascular diseases and diabetes are also increasing. In Taiwan, more than 98% of DM patients are characterized as T2DM [8], affecting more than 1 million individuals. Type 2 diabetic prevalence in Taiwanese population is much lower than that of Caucasians (4~16%) [9]. The discrepancy indicates that unique genetic characteristics and possibly distinct etiological/environmental factors may be involved in the pathogenesis of T2DM in Taiwan.

T2DM, Acute-phase Response and Immune Responses

The etiology of T2DM is still an enigma. Though insulin resistance seems to be a central abnormality, the origin of the impaired insulin action and how it explains the many other abnormalities of T2DM await to be investigated. Pickup et al. first discovered that blood concentrations of acute-phase response markers, such as C-reactive protein and cortisol, as well as the cytokine mediators, such as interleukin-6 (IL-6), in circulation of type 2 diabetic patients are increased [8]. Since then, accumulating evidences have shown that T2DM is an acute-phase disease in which increased concentrations of cytokines are secreted from many cells under the influence of various stimuli such as overnutrition, increasing age, genetic or fetal metabolic preprogramming[10,11]. Their study implicated that acute inflammatory phenomena will result in glucose intolerance and diabetes, and many of the clinical biochemical features and the complications of T2DM may be explained by the augmented acute-phase response. Cytokines, mainly IL-1, IL-6 and tumor necrosis factor-alpha (TNF-α), act on the liver to produce the characteristic dyslipidaemia of T2DM (increased very low density lipoprotein [VLDL] and decreased high density lipoprotein [HDL]) and may contribute to obesity, hypertension and insulin resistance. Treating animals and humans with cytokine can induce hypertriglyceridaemia and insulin resistance [12,13]. For example, TNF-α is a potent inhibitor of the tyrosine kinase activity of the insulin receptor and has been implicated in the insulin resistance of T2DM and obesity [14]. Repeatedly giving IL-1β *in vivo* to normal rats would result in reduced glucose-stimulated first-phase insulin release from the isolated islets, without altering the islet insulin content or ultrastructure [15]. Moreover, many observations suggest that diabetes may be associated with enhanced cytokine production, raising the possibility that some of the metabolic abnormalities associated with diabetes may be due to or exacerbated by cytokine overproduction [16-19].

Polymorphisms of Cytokine Genes and T2DM in Taiwan

Accordingly, immune responses and inflammation are suggested to play certain roles in the development and complications of T2DM[10,11]. Among the elevated cytokines in T2DM subjects, IL-6 is one of the type 2 T helper cell (Th2) cytokines that contribute to the exquisite regulation of balance between Th1 and Th2 cells [reviewed in ref. 20]. In addition to IL-6, other cytokines that affect the Th1/Th2 balance include IL-4, IL-10, etc. Because proinflammatory cytokine production is increased in T2DM, it is intriguing to investigate if other Th2 cytokines are also involved in the pathogenesis of T2DM. Additionally, cytokine production ability is tightly controlled at the level of gene transcription [20], that is, the promoter activity. Therefore, it is tempting for us to identify whether the promoter polymorphisms that influence the transcription activity and the resulting cytokine secretion ability contribute to Taiwanese T2DM pathogenesis.

For verifying the above hypothesis that cytokines and immune response are involved in T2DM pathogenesis, genomic DNA was extracted from peripheral blood cells of T2DM patients and control subjects, with their information of body height, weight, body mass index (BMI), age, clinical biochemical parameters including fasting blood glucose level, renal function index (creatinine [CRE] and blood urea nitrogen [BUN]), and lipid profile filed and analyzed. Ten polymorphisms of 4

cytokine genes (*IL-4*: -34T>C, -81A>G, -285C>T and -589T>C [manuscript revised in submission]; *IL-6*: -174G>C [7]; *IL-10*: -592A>C and -819T>C [21]; and *TNF-α*: G-238A and G-308A [22]) and the α chain of IL-4 receptor (*IL-4Rα*: E400A [manuscript in submission]) were subsequently investigated by polymerase chain reaction and restriction fragment length polymorphism. Several significant associations between these cytokine genes and T2DM and/or the clinical biochemical parameters were found using multiple linear regression analysis with adjustment for subjects' age, sex and diabetic status (Table 1). First of all, polymorphisms in *IL-4* (-34T>C and -589T>C) and *IL-10* (-592A>C and -819T>C) were found to be associated with T2DM. Second, several polymorphisms, including *IL-4* -589T>C, *IL-4Rα* E400A and *TNF-α* -238G>A were associated with circulatory HDL-C levels. Third, *TNF-α* -308G>A polymorphisms was associated with fasting glucose concentrations. Fourth, significant correlations between *IL-4Rα* E400A genotypes with blood pressure, as well as with BUN, were also observed in lean control subjects.

Our observations suggested that while *TNF-α* polymorphisms are not associated with the prevalence of Taiwanese T2DM, its secretion levels might be linked to insulin resistance and diabetic complications. On the contrary, *IL-10* polymorphisms may play certain roles in determining susceptibility to diabetes, but do not seem to be important in the clinical manifestations of T2DM. Notably, significant associations between *IL-4* polymorphisms and HDL-C levels, as well as between polymorphisms in *IL-4Rα* and HDL-C levels, are identified. The correlation between IL-4Rα and the HDL-C levels is observed both in non-obese control individuals and the obese T2DM patients, which further implies that IL-4 might be involved in HDL-C and lipid metabolism. It might be premature to make solid conclusion regarding the role of cytokines in lipid metabolism, nevertheless, in addition to the external environmental factors such as food intake and lifestyle, we hypothesize that genotypes of cytokine genes might be one of the internal factors which affect lipid metabolism.

Possible role of IL-4 and IL-4α in Lipid Metabolism

It is intriguing to explain the correlation between IL-4 and lipid metabolism. In hypercholesterolemia, the accumulated low density lipoproteins (LDL) in the artery wall would be oxidized to release oxidation products that lead to activation of inflammatory responses. In mice model, severe hypercholesterolemia is associated with a switch to Th2 immune response, with increased IL-4 expression in the atherosclerotic lesions [23]. IL-4 mRNA can also be detected in atherosclerotic lesions in human body [24]. The microenvironmental IL-4 in the atherosclerotic lesions has multiple effects on atherogenesis, such as the augmentation of LDL cholesterol esterification by a concentration- and time- dependent manner [25]. In addition, IL-4 can regulate the expression of 15-lipoxygenase (15-LO), a key enzyme in LDL oxidation [26, 27]. Elbe-Burger et al. further demonstrated that the adipocyte layer in the dermis is reduced in IL-4 transgenic mice [28]. Accordingly, local microenvironmental expression of IL-4 is suggested to be involved in the atherogenic process.

Table 1. Association between cytokines polymorphisms and T2DM in Taiwan

Genotype	Association		Reference
	T2DM	Clinical parameter	
IL-4			[Manuscript revised in submission]
-34T>C	+	-	
-81A>G	-	-	
-285C>T	-	-	
-589T>C	+	HDL-C	
IL-4R			[Manuscript in submission]
E400A	-	blood pressure	
		BUN	
		HDL-C	
IL-6			
-174G>C	-	-	[7]
IL-10			
-592A>C	+	-	[21]
-819T>C	+	-	
TNF-a			
-238G>A	-	HDL-C	[22]
-308G>A	-	Fasting glucose	

IL-4Rα is a crucial component for binding and signal transduction of IL-4 [29]. It is reasonable that polymorphisms located in *IL-4Rα*, which alter the binding affinity to IL-4 or downstream signaling pathways and thus contribute to the fine tune of IL-4 responsive phenotypes, would also be linked to disease development. Several studies have reported that genetic polymorphisms of *IL-4* and *IL-4Rα* are associated with genetic predisposition to diseases, possibly through their influences on the activity of these genes or their products [30-33]. Georgea et al. studied the influence of IL-4 to fatty streak formation using IL-4 knockout mice and found that HDL and triglycerides in the IL-4-deficient mice were higher [34]. In support of the previous studies, our observations provide further evidence that IL-4 may be involved in lipid metabolism. We hypothesize that the contribution of IL-4Rα to HDL-C and lipid metabolism is likely due to influencing the strength of both IL-4:IL-4R interaction and the downstream IL-4 signaling, then eventually the lipid metabolism and the resulting diabetic incidence. Nevertheless, this speculation awaits further study.

Inter-ethnic Differences in T2DM

While more and more evidences regarding the investigation of cytokine genotypes in patients with T2DM are documented, some reported correlations are still controversial because discrepancies among different studies exist. Ethnic differences may play a role in these conflicting results, as the distribution of genetic polymorphisms in a certain gene is diverse among study subjects with different racial origins.

For example, the prevalence of *IL-6* -174 C allele is reported to be ranged from 4.45% in Afro Caribbean [35], 13.85% in Gujarati Indians [35], 40~50% in Caucasians [36] to 62% in Spanish Caucasians [37]. Studies demonstrated that individuals carrying *IL-6* -174C/C genotype have an increased insulin sensitivity index than carriers of the G allele with similar age and body composition [38, 39]. Study from Vozarova et al. [40] also demonstrated that the *IL-6* -174G/G genotypes are associated with T2DM in Spanish Caucasian subjects and American Indian subjects with non-Pima admixture. The above results demonstrated that individuals carrying *IL-6* -174 G allele would be more susceptible to develop insulin resistance and T2DM. However, contradictory results existed. Insulin sensitivity, glucose oxidation rates and nonoxidative glucose disposal are decreased in healthy Finnish normoglycemic subjects with *IL-6* -174 C/C genotype, compared with the subjects carrying the heterologous C/G and homologous G/G genotypes [41].

Our previous report showed that the *IL-6* -174G>C polymorphisms, which affects insulin sensitivity in Caucasians [40], is unlikely to play a role in development of T2DM in Taiwanese, because no polymorphism has been identified at this position in our population [7]. Taken the results of IL-6 in diabetic studies together, it reflects the unique genetic characteristics and possible distinct etiological/environmental factors may be involved in pathogenesis of Taiwanese T2DM.

Conclusions

Although the initiation and etiology of T2DM still await to be identified, accumulating evidences have proved the hypothesis that T2DM is a state of chronic inflammation, with increased acute phase proteins and various cytokines. Genetic studies regarding the exploration of susceptible or resistant genes for T2DM could provide clues for understanding the mystery of diabetic pathogenesis and for future designing of diabetic treatment. Particularly, the achievements of genetic studies in Taiwanese diabetic population should be able to echo the needs for the development of personalized medicine based on the contribution of distinct genetic heterogeneity to diabetic development and complications. Hopefully, this article can provide valuable references to this metabolic tragedy.

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References

1. Polonsky KS. Lilly Lecture 1994. The beta-cell in diabetes: from molecular genetics to clinical research. Diabetes 1995; 44: 705-17.
2. Fajans SS, Cloutier MC, Crother RL: Clinical and etiologic heterogeneity of idiopathic diabetes mellitus. Diabetes 1978; 27: 1112-5.
3. Hanis CL, Boerwinkle E, Chakraborty R, et al: A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. Nature Genet 1996; 13: 161-6.

4. Ahluwalia T, Khullar M, Ahuja M, et al: Common variants of inflammatory cytokine genes are associated with risk of nephropathy in type 2 diabetes among Asian Indians. PLoS ONE 2009; 4: e5168.
5. McCarty KS, Zimmet P. Diabetes 1994 to 2010: global estimation and projections (International Diabetes Institute, Melbourne, Australia, 1994)
6. Ahima R: Connecting obesity, aging and diabetes. Nat Med 2009; 15:996-7.
7. Chang YH, Huang CN, Lee YL, , et al: The C-174G Promoter polymorphism of the IL-6 gene that affects insulin sensitivity in Caucasians is not involved in the pathogenesis of taiwanese type 2 diabetes mellitus. Eur Cytokine Netw 2004; 15: 117-9.
8. Pickup JC, Mattock MB, Chuaney GD, et al: NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997; 40: 1286-92.
9. Shiau MY, Huang CN, Wu CY, et al: Association of IL-10 A-592C and T-819C polymorphisms with type 2 diabetes mellitus. Hum Immunol 2005; 66: 1258-63.
10. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 2004; 25: 4-7.
11. Lumeng C, Maillard I, Saltiel A: T-ing up inflammation in fat. Nat Med 2009; 15: 846-7.
12. Feingold KR, Grunfeld C: Role of cytokines in inducing hyperlipidemia. Diabetes 1992; 41[Suppl 2]: 97-101.
13. Vassilopoulou-Sellin R: Endocrine effects of cytokines. Oncology 1994; 8: 43-6.
14. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor a: a key component of the obesity-diabetes link. Diabetes 1994; 43: 1271-8.
15. Wang Y, Goodman M, Lumerman J, et al: In vivo administration of interleukin-1 inhibits glucose-stimulated insulin release. Diabetes Res Clin Pract 1989; 7: 205-11.
16. He J, Usui I, Ishizuka K, et al: Interleukin-1alpha inhibits insulin signaling with phosphorylating insulin receptor substrate-1 on serine residues in 3T3-L1 adipocytes. Mol Endocrinol 2006; 20: 114-24.
17. Somm E, Cettour-Rose P, Asensio C, et al: Interleukin-1 receptor antagonist is upregulated during diet-induced obesity and regulates insulin sensitivity in rodents. Diabetologia 2006; 49: 387-93.
18. Atsumi T, Cho YR, Leng L, et al: The proinflammatory cytokine macrophage migration inhibitory factor regulates glucose metabolism during systemic inflammation. J Immunol 2007; 179: 5399-406.
19. Kang K, Reilly SM, Karabacak V, et al: Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. Cell Metab 2008; 7: 485-95.
20. Paul WE, Seder RA: Lymphocyte responses and cytokines. Cell 1994; 76: 241-51.
21. Shiau MY, Huang CN, Wu CY, et al: Association of IL-10 A-592C and T-819C polymorphisms with type 2 diabetes mellitus. Hum Immunol 2005; 66: 1258-63.
22. Shiau MY, Wu CY, Huang CN, et al: Analysis of TNF-α G-238A and G-308A promoter polymorphism in Taiwanese patient with type 2 diabetes mellitus. Tissue Antigens 2003; 61: 393-7.
23. Zhou X, Paulsson G, Stemme S, et al: Hypercholesterolemia is associated with a T Helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E–knockout mice. J Clin Invest 1998; 101: 1717–25.
24. Stemme S, Faber B, Holm J, et al: T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci USA 1995; 2: 3893–7.
25. Cornicelli JA, Butteiger D, Rateri DL, et al: Interleukin-4 augments acetylated LDL-induced cholesterol esterification in macrophages. J Lipid Res 2000; 41: 376–83.
26. Conrad DJ, Kuhn H, Mulkins M, et al: Specific inflammatory cytokines regulate the expression of human monocyte 15-LO. Proc Natl Acad Sci USA 1992; 89: 217–21.
27. Cornicelli JA, Welch K, Auerbach B, et al: Mouse peritoneal macrophages contain abundant v-6 lipoxygenase activity that is independent of interleukin-4. Arterioscler Thromb Vasc Biol 1996; 16: 1488–94.
28. Elbe-Bürger A, Egyed A, Olt S, et al: Overexpression of IL-4 alters the homeostasis in the skin. J Invest Dermatol 2002; 118: 767-78.
29. Izuhara K, Shirakawa T. Signal transduction via the interleukin-4 receptor and its correlation with atopy. Int J Mol 1999; 3: 3–10.
30. Hershey GK, Friedrich MF, Esswein LA, et al: The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. N Eng J Med 1997; 337: 1720-5.
31. Marsh D, Neely J, Breazeale D, et al: Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. Science 1994; 254: 1152–6.
32. Song Z, Casolaro V, Chen R, et al: Polymorphic nucleotides within the human IL-4 promoter that mediate overexpression of the gene. J Immunol 1996; 156: 424–9.
33. Rosenwasser L, Klemm DJ, Dresback JK, et al: Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 1995; 25: 74–8.
34. George J, Mulkins M, Shais A, et al: Interleukin (IL)-4 deficiency does not influence fatty streak formation in C57BL:6 mice. Atherosclerosis 2000; 153: 403–11.
35. Fishman D, Faulds G, Jeffery R, et al: The effect of novel polymorphisms in the interleukin 6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998; 102: 1369-76.
36. Georges JL, Loukaci V, Poirier O, et al: IL-6 gene polymorphisms and susceptibility to myocardial infarction: the ECTIM study. Etude Cas-Temoin de l'Infarctus du Myocarde. J Mol Med 2001; 79: 300-5.
37. Villuendas G, San Millan JL, Sancho J, et al: The -597 G-->A and -174 G-->C polymorphisms in the promoter of

the IL-6 gene are associated with hyperandrogenism. J Clin Endocrinol Metab 2002; 87: 1134-41.
38. Fernández-Real JM, Broch M, Vendrell J, et al: IL-6 Gene polymorphism and insulin sensitivity. Diabetes 2000; 49: 517-20.
39. Shen J, Arnett D, Pérez-Martínez P, et al: The effect of IL6-174C/G polymorphism on postprandial triglyceride metabolism in the GOLDN study. J Lipid Res 2008; 49: 1839-45.
40. Vozarova B, Fernández-Real J-M, Knoler WC, et al: The interleukin-6 (-174) G/C promoter polymorphism is associated with type-2 diabetes mellitus in Native Americans and Caucasians. Hum Genet 2003; 112: 409-13.
41. Kubaszek A, Pihlajamäki J, Punnonen K, et al: The C-174G promoter polymorphism of the IL-6 gene affects energy expenditure and insulin sensitivity. Diabetes 2003; 52: 558-61.

細胞激素、新陳代謝與第二型糖尿病

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第二型糖尿病(type 2 diabetes mellitus, T2DM)是常見的內分泌疾病，其致病機轉仍然未知。T2DM病患者雖然可自行分泌胰島素，但其細胞無法有效接收或傳遞胰島素的訊息而產生胰島素阻抗性(insulin resistance)；長期罹病患者的胰島素分泌能力也可能受損。許多因素可能會導致T2DM，其中以遺傳因子和環境因素最受矚目。研究可引起代謝異常的遺傳基因有助於了解糖尿病的致病因素及發展嶄新的治療與預防策略。許多研究已證實T2DM與慢性發炎反應有密切相關性，患者體內的急性發炎蛋白與細胞激素濃度都會增高。本文的主旨爲討論糖尿病的流行病學與分類，以及細胞激素在T2DM和新陳代謝中的角色。此外，本文亦納入本實驗室近年來有關T2DM病患細胞激素基因型的研究結果，提供參考。特定族群的糖尿病免疫基因學研究結果不僅有助於釐清免疫反應與T2DM的相關性，也可供發展個人化醫療與疾病預防的參考資訊。

關鍵詞：細胞激素、新陳代謝、介白素、糖尿病

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Association of interleukin-4 promoter polymorphisms in Taiwanese patients with type 2 diabetes mellitus

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Abstract

Many factors have been implicated in the onset of type 2 diabetes mellitus (T2DM). Recently, immune response and inflammation were suggested to play certain roles in the development and complications of T2DM. The aim of this study is to investigate the putative correlation between the promoter polymorphisms of interleukin-4 (IL-4), one of the immune-regulatory type 2 helper T-cell cytokines, and T2DM. Genomic DNA from 425 Taiwanese T2DM patients and 148 nondiabetic control study subjects were extracted, and their IL-4 promoter polymorphisms were analyzed by polymerase chain reaction–restriction fragment length polymorphism. Both of the distribution of IL-4 C-589T (P = .013) and C-34T (P = .05) genotypes were significantly different between T2DM patients and control subjects. Significant association between IL-4 C-589T alleles (P = .002) and T2DM, as well as C-34T alleles and T2DM (P =.024), was also identified. In addition, a statistically significant association between homologous IL-4 −589 C/C genotype and lower circulatory high-density lipoprotein cholesterol levels was observed. Our results suggested that IL-4 promoter polymorphisms are associated with T2DM. A significant association between IL-4 −589 C/C genotype and lower circulatory high-density lipoprotein cholesterol level was observed as well. The above results suggested that IL-4 may participate in lipid metabolism and diabetic susceptibility.

1. Introduction

Type 2 diabetes mellitus (T2DM) is caused by insulin resistance and often combined with symptoms of progressive defect in insulin secretion. The number of the diabetic patients is rapidly increasing globally, with an estimated increased rate of 46% from year 2000 to 2010. The prevalence of T2DM varies among different ethic populations, with the highest rate found in Pima Indians (as high as ~50 [1]). Type 2 diabetes mellitus prevalence in the Taiwanese population (about 1.5%) is much lower than that in whites (4~16%) [2]. The discrepancy indicates that unique genetic characteristics and possibly distinct etiologic/environmental factors may be involved in the pathogenesis of T2DM in Taiwan.

Many factors can lead to T2DM onset; however, host genetic factors are the focus of discussion. Crook et al [3] and Pickup et al [4] first proved that T2DM is an inflammatory condition characterized by elevated concentrations of acute phase inflammatory reactants in the plasma. These acute phase proteins are synthesized in liver and stimulated by cytokines, mainly interleukin-1 (IL-1), IL-6, and tissue necrosis factor-α. The circulatory IL-6 levels in T2DM patients are also increased, despite the basal production of IL-6 in cultured diabetic blood cells being markedly depressed [4,5]. Consequently, T2DM is

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an acute phase disease in which increased concentrations of cytokines contributing to the regulation of balance between Th1 and Th2 cells are involved [6,7]. These studies implicate that acute inflammation will result in glucose intolerance and diabetes, and many of the clinical and biochemical T2DM features as well as its complications may be explained by the augmented acute phase response. Moreover, many observations suggest that diabetes may be associated with enhanced cytokine production, raising the possibility that some of the diabetes-associated metabolic abnormalities may be due to or exacerbated by cytokine overproduction [8].

Accordingly, immune response and inflammation are suggested to play certain roles in the development and complications of T2DM. Type 2 T helper cell (Th2) cytokine IL-6, which contributes to the exquisite regulation of Th1/Th2 balance, is one of the well-studied cytokines in diabetic research (reviewed by Paul and Seder [7]). In addition to IL-6, other cytokines that affect the Th1/Th2 balance might also participate in T2DM development. Therefore, it is intriguing to investigate if other Th2 cytokines are involved in the pathogenesis of T2DM. Nevertheless, studies regarding the correlation between Th2 cytokines other than IL-6 and T2DM are limited.

Interleukin-4, mainly secreted by activated T cells, $Fc\varepsilon R1^{+}$ cells, and eosinophils, is an important anti-inflammatory cytokine that can inhibit the secretion of the proinflammatory cytokines from macrophages [9,10]. The production of IL-4 is tightly controlled at the level of gene transcription [11]. Several single nucleotide polymorphisms (SNPs) have been identified in the promoter region of the IL-4 gene, such as the SNPs located at positions −589 (C to T), −285 (C to T), −81 (A to G), and −34 (C to T) from the transcription start site [12-14]. These SNPs have been identified to influence promoter strength and thus mediate transcription and expression of IL-4 gene. Several studies have examined the possible correlation of IL-4 genotypes and type 1 diabetes mellitus (T1DM) pathogenesis, with the association of IL-4 SNPs and T1DM reported in some [15,16] but not all studies [17,18]. This may be, in part, the result of different ethnic populations being studied.

The putative association between IL-4 and the more prevalent T2DM did not cause much attention until a recent study demonstrated that the genetic polymorphisms of IL-4 intron-3 could serve as susceptibility indicators for T2DM in the Indian population [19]. Therefore, it is tempting to identify whether the IL-4 promoter polymorphisms that influence the transcription activity and cytokine secretion ability would contribute to T2DM pathogenesis. The present study aimed at investigating if the IL-4 gene promoter SNPs are associated with Taiwanese T2DM. To inspect this hypothesis, the inheritance of the IL-4 promoter SNPs among patients with T2DM and the association of these polymorphisms with patients' biochemical features were examined.

2. Materials and methods

2.1. Study subjects

Fasting venous blood samples were taken from 425 T2DM patients attending the diabetic clinic in the Department of Internal Medicine, Chung Shan Medical University Hospital. Fasting blood samples form 148 nondiabetic control subjects were collected from the Physical Check Up Unit, Taichung Veterans General Hospital. Written consents were obtained from all the study subjects after the nature of the procedure was explained. The information on body height, weight, age, fasting blood glucose, and renal function index (creatinine [CRE], blood urea nitrogen [BUN]), etc; listed in Table 1) were collected for further statistical analysis.

2.2. Analysis of IL-4 promoter polymorphisms

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs). An aliquot of the genomic DNA (50-100 ng) was used for amplifying the promoter region containing each polymorphism by polymerase chain

Table 1
Demographic and biochemical data of study subjects in this study

	Control subjects n = 148 (n = 98)[b]	T2DM patients n = 425 (n = 328)[b]	P[c]
Male/female	75/73	198/187	NS
	(48/50)	(166/162)	NS
Age (y)	57.93±10.62	57.24±11.44	NS
	(58.16±10.91)	(57.07±11.56)	NS
BMI (kg/m^2)	24.52±3.40	25.36±3.27	.014
	(24.43±3.51)	(25.34±3.16)	.021
Fasting glucose	95.69±6.79	177.70±68.50	<.001
(70-110 mg/dL)[a]	(96.07±6.63)	(180.38±70.65)	<.001
Systolic pressure	125.42±19.18	134.76±18.56	<.001
(120-140 mm Hg)[a]	(123.45±17.19)	(135.22±18.19)	<.001
Diastolic pressure	79.20±10.38	80.30±10.80	NS
(70-90 mm Hg)[a]	(78.28±9.33)	(79.99±10.51)	NS
BUN	15.86±5.09	17.54±8.00	.025
	(15.60±4.39)	(17.58±8.07)	.023
CRE (0.6-1.4 mg/dL)[a]	1.11±0.42	1.04±0.44	NS
	(1.09±0.24)	(1.04±0.44)	NS
Cholesterol	201.18±37.51	198.2±42.22	NS
(125-240 mg/dL)[a]	(202.62±39.21)	(198.02±42.12)	NS
HDL-C (>35 mg/dL)[a]	58.35±13.88	46.49±13.38	<.001
	(59.24±14.58)	(46.52±13.43)	<.001
TC/HDL-C	3.59±0.90	4.50±1.31	<.001
	(3.56±0.89)	(4.51±1.31)	<.001
Triglycerides	142.54±125.52	184.00±153.24	.004
(20-200 mg/dL)[a]	(139.45±122.86)	(184.51±153.74)	.008
Uric acid	6.56±1.64	6.11±1.87	.021
(2.4-7.2 mg/dL)[a]	(6.79±1.67)	(6.12±1.88)	.003

NS indicates nonsignificant; BMI, body mass index.

[a] Numbers in parentheses indicate the reference range of each biochemical test.

[b] Numbers in parentheses indicate the information of each demographic and biochemical variables from the data of 98 control and 328 diabetic subjects with available IL-4 −34 genotypic results.

[c] Student t test.

reaction (PCR). Generally, DNA amplification was performed in a 20-μL volume containing 10 pmol of each primer, 4.5 mmol/L $MgCl_2$, 0.25 mmol/L of each dNTP, 1 unit Taq polymerase, and 1.5-mmol/L buffer with a 95°C initial incubation for 5 minutes, followed by 30 amplification cycles and a final extension. Promoter polymorphisms were examined by restriction fragment length polymorphism (RFLP). Specific primers and PCR conditions, and respective enzyme digestion and conditions in RFLP were as described previously [20] and listed in Table 2.

2.3. Analysis of IL-4 secretion

Peripheral blood mononuclear cells were isolated from whole blood using Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation. A total of 2×10^6 PBMCs were cultured in RPMI medium (Hyclone, South Logan, UT) containing 10% fetal bovine serum (GIBCO, Mexico). After 24 hours of 10-μg/mL concanavalin A (Con A, Sigma, Steinheim, Germany) treatment, secreted IL-4 by the activated PBMC was determined using IL-4 enzyme-linked immunosorbent assay kit (R&D, Minneapolis, MN).

2.4. Statistical analysis

Data analysis started with descriptive statistics, including mean and standard deviation for continuous variables and frequency for categorical variables. If necessary, natural logarithm transformation was used to enhance normality for blood biochemistry parameters with skewed distribution. Student *t* test was applied for comparisons of age, body mass index, and each of the blood biochemistry parameters between diabetic subjects and controls; and χ^2 test was used for comparing frequencies of different genotypes and sex between groups. Moreover, 1-way analysis of variance was applied to compare means of respective blood biochemistry parameters among subjects with different IL-4 genotypes. Finally, multiple linear regression analysis was used to assess the associations between IL-4 genotypes and the biochemical parameters, with adjustment for diabetes status, age, and sex. The statistical software of SAS version 8 (SAS Institute, Cary, NC) was applied for the analyses. An α level of 0.05 was used for all statistical tests.

Table 2
The primer sequences used in the analysis of IL-4 promoter polymorphisms and reverse transcriptase PCR

Primer designation	Primer sequence
IL4 −589F	5′-TGGGTAAGGACCTTATGGACC -3′
IL4 −589R	5′- GGTGGCATCTTGGAAACTGTC -3′
IL4 −285F	5′-TGGGTAAGGACCTTATGGACC-3′
IL4 −285R[b]	5′-GAAGCAGTTGGGA*C*GTGAGA-3′
IL4 −81F	5′-CCAGCAGCAGCCCCAAGCTGA-3′
IL4 −81R	5′-TGCAGTGAGAATGTGAGGCAA-3′
IL4 −34F[a]	5′-CTCATTTTCC*G*TCGGTTTCAGC-3′
IL4 −34R[b]	5′-GAAGCAGTTGGGA*C*GTGAGA-3′

[a] One base-exchange substitution from C to G position at position −50 destroyed the *Mnl*I restriction site.

[b] One base-exchange substitution from G to C position at position +9 destroyed the *Mnl*I restriction site.

3. Results

Our study aimed at investigating the distribution of the IL-4 promoter SNPs among control and T2DM subjects to test the possible correlation between IL-4 genetic polymorphisms and T2DM. Polymorphisms of IL-4 C-589T were successfully investigated in all study subjects, whereas the subject numbers of other IL-4 SNPs investigated were variable, probably because of the insufficient quantity and/or quality of genomic DNA from certain study subjects. Demographic data and clinical biochemical manifestations of the patients with available genotypic data are listed in Table 1.

3.1. Significant association of IL-4 promoter polymorphisms with T2DM

Results regarding the distribution of the IL-4 promoter SNPs among the control and diabetic subjects are summarized in Table 3. Significant difference in distribution of IL-4 C-589T genotypes between T2DM patients and control subjects was observed ($P = .013$), as well as between IL-4 −589 alleles and T2DM ($P = .002$). In addition, significant difference of IL-4 C-34T genotypic distribution between T2DM and control individuals was also observed ($P = .05$). Although this genotypic difference only reached marginal significance, a strong association of IL-4 −34 alleles and T2DM was discovered ($P = .024$). Interestingly, no polymorphisms at IL-4 −81 and −285 positions were found. The above observations demonstrated that the IL-4 polymorphisms were associated with T2DM.

Table 3
Comparison of IL-4 −34 C/T and −589 C/T polymorphisms between T2DM subjects and controls

Genotype/allele	Control subjects n (%)	T2DM patients n (%)	P[a]
−589 C/T	148	425	
T/T	96 (64.86%)	324 (76.24%)	.013
T/C	45 (30.41%)	93 (21.88%)	
C/C	7 (4.73%)	8 (1.88%)	
T allele	237 (80.07%)	741 (87.18%)	.002
C allele	59 (19.93%)	109 (12.82%)	
−34 C/T	98	328	
T/T	63 (64.29%)	250 (76.22%)	.05
T/C	32 (32.65%)	69 (21.04%)	
C/C	3 (3.06%)	9 (2.74%)	
T allele	158 (80.61%)	569 (86.74%)	.024
C allele	38 (19.39%)	87 (13.26%)	
−285 C/T	132	140	–
C/C	132 (100%)	140 (100%)	
−81 A/G	125	114	–
A/A	125 (100%)	114 (100%)	

[a] χ^2 test.

3.2. Significant association between high IL-4 secreting ability and T2DM

To further investigate the association of IL-4 and T2DM, IL-4 secreting levels of Con A–activated PBMC from study subjects were determined (Table 4). The results showed that, although the basal IL-4 levels were similar in control and T2DM groups (0~8 pg/mL, data not shown), IL-4 secreting level after Con A stimulation was significantly higher in T2DM patients (19.01 ± 1.27 pg/mL, n = 106) compared with that in control subjects (5.44 ± 1.42 pg/mL, n = 26, $P <$ .001). The results indicated that PBMC from T2DM patients had higher IL-4 secreting ability.

3.3. Association of homologous IL-4 −589 C/C genotype with high-density lipoprotein cholesterol levels and the ratio of total cholesterol to high-density lipoprotein cholesterol

In addition to the significant association between IL-4 C-589T polymorphisms with T2DM, we further investigated the correlation between this polymorphism and study subjects' biochemical data using multiple linear regression analysis adjusted for age, sex, and diabetes status (Table 5). When the biochemical data of individuals carrying C/C genotype were compared with those of individuals carrying T/T genotype, a significant difference was found in high-density lipoprotein cholesterol (HDL-C) ($P = <.01$) and the ratio of total cholesterol (TC) to HDL-C ($P < .01$). These findings indicated that individuals with IL-4 −589 C/C genotype tend to have lower HDL-C (parameter estimate [standard error; SE] = −0.42) and higher TC/HDL-C (parameter estimate [SE] = 0.41). No significant difference of the biochemical data between subjects with T/C genotype and T/T genotype was found. The results implied that IL-4 −589 C/C genotype might be associated with HDL-C metabolism and, therefore, contribute to the observed difference of TC/HDL-C.

4. Discussion

Cytokines, secreted by a variety of activated cells and acting as regulators of immune responses, are believed to be involved in immune response–induced destruction of islet β-cells in T1DM [21]. In animal models, transgenic expression of IL-4 in β-cells [22] and systemic IL-4 administration [23] can prevent NOD mice from insulitis and diabetes. Interleukin-4 is suggested to protect human islets from cytotoxic damage induced by proinflammatory and Th1 cytokines [24]. However, IL-4 expression is reported not to correlate with destructive and benign insulitis in T1DM patients [25]. Another study shows that long-term exposure of rat pancreatic islets to IL-4 results in an inhibitory action to some of the islet functions [26], probably due to an influence on the islet glucose metabolism.

Several studies have documented the correlations between the genotypes of IL-4/IL-4 receptor (IL-4R) and diabetic status. Mirel et al (n = 282) [27] and Bugawan et al (n = 90) [16] revealed that IL-4R genotypes were associated with protection from T1DM; however, no significance was observed between IL-4 SNP and T1DM. On the contrary, other studies suggested that IL-4R genotype were not associated with T1DM [18,28-30]. Possible factors that contribute to the above conflicting results include linkage disequilibrium of IL-4 and/or IL-4R gene to a nearby noncausative polymorphism, or confounding due to either ethnic admixture or the source(s) of population stratification. Besides, sample size is also suggested to be one of the major factors that affect the results and interpretation.

Despite the inconsistent conclusions concerning the association between IL-4 and T1DM, it is intriguing to examine if IL-4 is involved in the more prevalent T2DM that has been proven to be closely associated with inflammation. However, very little is known regarding this issue. The major finding in the present study was the significant difference in the IL-4 −589 genotypic distribution between healthy and T2DM individuals (P = .002, Table 3). It suggested that individuals with IL-4 high-secreting genotypes might be more predisposed to T2DM. Individuals carrying either heterozygous or homozygous IL-4 −589 T allele are reported to be associated with higher IL-4 secreting ability [31,32]. Gervaziev et al [33] demonstrated that higher serum IL-4 levels are associated with IL-4 −34 T and −589 T alleles in the Russian population. It is also reported [34] that coronary artery bypass grafting patients with IL-4 −589 T/T genotype had significantly higher circulating levels of IL-4 postoperatively compared with patients with C/C and C/T genotypes. The observation that T2DM patients have higher IL-4 secreting ability (Table 4) further supported our speculation.

Table 4
Interleukin-4 secreting levels of activated PBMC from control and T2DM subjects

	Control subjects n = 26	T2DM n = 106	P[a]
Mean concentrations (pg/mL)	5.44 ± 1.42	19.01 ± 1.27	<.001

[a] Wilcoxon test.

Table 5
Association of IL-4 −589 genotypes and blood chemistry parameters

Outcome	IL-4 genotypes (C/C vs T/T)		IL-4 genotypes (T/C vs T/T)	
	B (SE)[a]	P value	B (SE)[a]	P value
Fasting glucose	0.04	.68	0.02	.55
BUN	0.22	.15	0.05	.39
CRE	0.09	.30	<0.01	.93
Cholesterol	<0.01	.10	0.02	.49
HDL-C	−0.42	<.01	−0.02	.67
TC/HDL-C	0.41	<.01	0.08	.13
Triglyceride	−0.34	.24	0.14	.16
Uric acid	0.01	.96	0.01	.80

[a] Parameter estimate (standard error).

In addition, we suggested that the homologous IL-4 −589 C/C genotype was associated with HDL-C metabolism, according to the linear regression results adjusted by variables of age, sex, and diabetic status (Table 5). It is intriguing to explain the observation that subjects carrying lower IL-4 secreting −589 C/C genotype would have lower peripheral HDL-C levels, which is independent of diabetic status. As a matter of fact, IL-4 is known to be correlated with atherosclerosis [35-37]. In a mice model, severe hypercholesterolemia is associated with a switch to Th2 immune response, with increased IL-4 expression in the lesions [35]. Interleukin-4 messenger RNA can also be detected in atherosclerotic lesions in human body [36]. The microenvironmental IL-4 in the atherosclerotic lesions has multiple effects on atherogenesis, such as the augmentation of low-density lipoprotein cholesterol esterification by a concentration- and time- dependent manner [37]. In addition, IL-4 can regulate the expression of 15-lipoxygenase, a key enzyme in low-density lipoprotein oxidation [38,39]. Elbe-Bürger et al [40] further demonstrated that the adipocyte layer in the dermis is reduced in IL-4 transgenic mice. Moreover, by exploring the influence of IL-4 to fatty streak formation, George et al [41] found that HDL and triglycerides in IL-4–deficient mice were higher. The above studies indicate that local microenvironmental expression of IL-4 is involved in lipid metabolism and eventually the atherogenic process. Accordingly, we speculated that, although individuals with IL-4 −589 T/T or C/T genotypes were more susceptible to diabetic onset, diabetic individuals with IL-4 −589 C/C genotype might be more susceptible to diabetic complications such as atherosclerosis and cardiovascular diseases. However, whether the interindividual differences in the IL-4 secretion levels contribute to the HDL-C metabolism and predisposition of diabetic complications needs further investigation.

The IL-4 promoter SNPs at positions −285 (C/T), −81 (A/G), and −34 among our patients were also characterized. Interestingly, no polymorphisms at positions −285 and −81 were found in our population. The IL-4 −34 C/T polymorphisms are reported to be associated with −589 C/T SNPs [13]; that is, −34 C allele and T allele are always associated with −589 C allele and T allele, respectively. Therefore, the observed significance between IL-4 −34 SNPs and T2DM might be in part a result of the linkage disequilibrium between −34 and −589 SNPs.

In addition to SNPs, IL-4 gene expression is tightly regulated at the level of transcription by multiple transcription factors that bind to *cis*-elements in the upstream proximal promoter. The promoter activity of A to G transition at −81 will enhance 8-fold because this transition makes the promoter a higher affinity binding to AP-1–specific transcription factors [14]. To the best of our knowledge, no transcription factors are reported to bind to IL-4 −589 promoter region. Therefore, it is also intriguing to investigate if there is any transcription factor(s) that can bind to this region. Once the candidate transcription factors are found, the binding capacity of the proteins to different genotypes and the resultant transcription ability of IL-4 promoter, as well as the effect of this SNP to HDL-C metabolism, can subsequently be determined.

In summary, we have demonstrated that IL-4 promoter polymorphisms are significantly associated with T2DM. Moreover, an association between homologous IL-4 −589 C/C genotype and lower HDL-C level is characterized in our study. Our results suggest that polymorphisms of the IL-4 promoter may contribute to the HDL-C metabolism, T2DM predisposition, and diabetic complications in Taiwanese subjects.

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References

[1] Chang YH, Huang CN, Shiau MY. The C-174G promoter polymorphism of the IL-6 gene that affects insulin sensitivity in Caucasians is not involved in the pathogenesis of Taiwanese type 2 diabetes mellitus. Eur Cytokine Netw 2004;15:117-9.

[2] Shiau MY, Huang CN, Wu CY, et al. Association of IL-10 A-592C and T-819C polymorphisms with type 2 diabetes mellitus. Hum Immunol 2005;66:1258-63.

[3] Crook MA, Tutt P, Pickup JC. Elevated serum sialic acid concentration in NIDDM and its relationship to blood pressure and retinopathy. Diabetes Care 1993;16:57-60.

[4] Pickup JC, Mattock MB, Chuaney GD, et al. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997;40:1286-92.

[5] Tsiavou A, Hatziagelaki E, Chaidaroglou A, et al. TNF-alpha, TGF-beta1, IL-10, IL-6, gene polymorphisms in latent autoimmune diabetes of adults (LADA) and type 2 diabetes mellitus. J Clin Immunol 2004;24:591-9.

[6] Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 1998;41:1241-8.

[7] Paul WE, Seder RA. Lymphocyte responses and cytokines. Cell 1994;76:241-51.

[8] Feingold KR, Grunfeld C. Role of cytokines in inducing hyperlipidemia. Diabetes 1992;41(Suppl 2):97-101.

[9] Paul WE. Interleukin 4: signaling mechanisms and control of T cell differentiation. Ciba Found Symp 1997;204:208-16.

[10] Garcia-Zepeda EA, Combadiere C, Rothenberg ME, et al. Human monocyte chemoattractant protein-4 is a novel CC chemokine with activates on macrophage, eosinophils, and basophils induced in allergic and non-allergic inflammation that signals through the CC chemokine receptors (CCR)–2 and –3. J Immunol 1996;157:5613-26.

[11] Georas S, Cumberland J, Burke T, et al. Characterization of a novel negative regulatory element in the human interleukin 4 promoter. Leukemia 2000;14:629-35.

[12] Rosenwasser LJ, Klemm DJ, Dresback JK, et al. Promoter polymorphism in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 1995;25(Suppl 2):74-8.

[13] Song Z, Casolaro V, Chen R, et al. Polymorphic nucleotides within the human IL-4 promoter that mediate overexpression of the gene. J Immunol 1996;156:424-9.

[14] Takabayashi A, Ihara K, Sasaki Y, et al. Novel polymorphism in the 5′-untranslated region of the interleukin-4 gene. J Hum Genet 1999;44:352-3.

[15] Steck AK, Bugawan TL, Valdes AM, et al. Association of non-HLA genes with type 1 diabetes autoimmunity. Diabetes 2005;54:2482-6.
[16] Bugawan TL, Mirel DB, Valdes AM, et al. Association and interaction of the IL-4R, IL-4, and IL13 loci with type 1 diabetes among Filipinos. Am J Hum Genet 2003;72:1505-14.
[17] Nuñez C, Santiago JL, Varadé J, et al. IL-4 in the 5q31 context: association studies of type 1 diabetes and rheumatoid arthritis in the Spanish population. Immunogenetics 2008;60:19-23.
[18] Reimsnider SK, Eckenrode SE, Marron MP, et al. IL4 and IL4Ralpha genes are not linked or associated with type 1 diabetes. Pediatr Res 2000;47:246-9.
[19] Bid HK, Konwar R, Agrawal CG, et al. Association of IL-4 and IL-1RN (receptor antagonist) gene variants and the risk of type 2 diabetes mellitus: a study in the north Indian population. Indian J Med Sci 2008; 62:259-66.
[20] Shiau MY, Huang CN, Yang TP, et al. Cytokine promoter polymorphisms in Taiwanese patients with Graves' disease. Clin Biochem 2007;40:213-7.
[21] Rabinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet β-cell destruction and insulin-dependent diabetes mellitus. Biochem Pharmacol 1998;55:1139-49.
[22] Mueller R, Krahl T, Sarvetnick N. Pancreatic expression of interleukin-4 abrogates insulitis and autoimmune diabetes in nonobese diabetic (NOD) mice. J Exp Med 1996;184:1093-9.
[23] Rapoport MJ, Jaramillo A, Zipris D, et al. Interleukin 4 reverses T-cell proliferative unresponsiveness and prevents the onset of diabetes in NOD mice. J Exp Med 1993;178:87-99.
[24] Marselli L, Dotta F, Piro S, et al. Th2 cytokines have a partial, direct protective effect on the function and survival of isolated human islets exposed to combined proinflammatory and Th1 cytokines. J Clin Endocrinol Metab 2001;86:4974-8.
[25] Rabinovitch A. An update on cytokines in the pathogenesis of insulin dependent diabetes mellitus. Diabetes Metab Rev 1998;14:129-51.
[26] Sandler S, Sternesjö J. Interleukin 4 impairs rat pancreatic islet function in vitro by an action different to that of interleukin 1. Cytokine 1995;7:296-300.
[27] Mirel DB, Valdes AM, Lazzeroni LC, et al. Association of IL4R haplotypes with type 1 diabetes. Diabetes 2002;51:3336-41.
[28] Maier LM, Twells RC, Howson JM, et al. Testing the possible negative association of type 1 diabetes and atopic disease by analysis of the interleukin 4 receptor gene. Genes Immun 2003;4:469-75.
[29] Maier LM, Chapman J, Howson JM, et al. No evidence of association or interaction between the IL4RA, IL4, and IL13 genes in type 1 diabetes. Am J Hum Genet 2005;76:517-21.
[30] Erlich HA, Lohman K, Mack SJ, et al. Type I Diabetes Genetics Consortium. Association analysis of SNPs in the IL4R locus with type I diabetes. Genes Immun 2009;10(Suppl 1):S33-S41.
[31] Noguchi E, Shibasaki M, Arinami T, et al. Association of asthma and the interleukin-4 promoter gene in Japanese. Clin Exp Allergy 1998; 28:449-53.
[32] Noguchi E, Nukaga-Nishio Y, Jian Z, et al. Haplotypes of the 5′ region of the IL-4 gene and SNPs in the intergene sequence between the IL-4 and IL-13 genes are associated with atopic asthma. Hum Immunol 2001;62:1251-7.
[33] Gervaziev YV, Kaznacheev VA, Gervazieva VB. Allelic polymorphisms in the interleukin-4 promoter regions and their association with bronchial asthma among the Russian population. Int Arch Allergy Immunol 2006;141:257-64.
[34] Bittar MN, Khasati NH, Deiraniya AK, et al. Interleukin-4 C-590T polymorphism has no role in coronary artery bypass surgery. Asian Cardiovasc Thorac Ann 2007;15:214-7.
[35] Zhou X, Paulsson G, Stemme S, et al. Hypercholesterolemia is associated with a T helper (Th) 1/Th2 switch of the autoimmune response in atherosclerotic apo E-knockout mice. J Clin Invest 1998; 101:1717-25.
[36] Stemme S, Faber B, Holm J, et al. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci USA 1995;92:3893-7.
[37] Cornicelli JA, Butteiger D, Rateri DL, et al. Interleukin-4 augments acetylated LDL-induced cholesterol esterification in macrophages. J Lipid Res 2000;41:376-83.
[38] Conrad DJ, Kuhn H, Mulkins M. Specific inflammatory cytokines regulate the expression of human monocyte 15-LO. Proc Natl Acad Sci USA 1992;89:217-21.
[39] Cornicelli JA, Welch K, Auerbach B. Mouse peritoneal macrophages contain abundant v-6 lipoxygenase activity that is independent of interleukin-4. Arterioscler Thromb Vasc Biol 1996;16:1488-94.
[40] Elbe-Bürger A, Egyed A, Olt S, et al. Overexpression of IL-4 alters the homeostasis in the skin. J Invest Dermatol 2002;118:767-78.
[41] George J, Mulkins M, Shais A, et al. Interleukin (IL)-4 deficiency does not influence fatty streak formation in C57BL:6 mice. Atherosclerosis 2000;153:403-11.

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ORIGINAL ARTICLE

Regulation of glucose/lipid metabolism and insulin sensitivity by interleukin-4

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Objective: Abundant evidence has demonstrated that long-term cytokine-mediated inflammation is a risk factor for obesity and type 2 diabetes mellitus (T2DM). Our previous study reveals a significant association between promoter polymorphisms of Th2-derived cytokine interleukin-4 (IL-4) and T2DM, which suggests possible roles of IL-4 in metabolism. In this study, we focused on examining the putative regulation of glucose and lipid metabolism by IL-4.
Methods: C57BL/6 mice were intraperitoneally injected with either adenovirus containing full-length IL-4 encoding gene (AdIL-4) or recombinant IL-4 for mimicking the status of transient and long-term IL-4 overexpression, respectively, and the effects of the overexpressed IL-4 to glucose/lipid metabolism and insulin sensitivity were subsequently investigated.
Results: Our results reveal that IL-4 improves insulin sensitivity and glucose tolerance through upregulating Akt phosphorylation while attenuating GSK-3β activities. IL-4 is also involved in lipid metabolism by inhibiting lipid accumulation in fat tissues, which lead to decreased weight gain and fat mass.
Conclusions: Our results suggest that IL-4 regulates glucose and lipid metabolism by promoting insulin sensitivity, glucose tolerance and inhibiting lipid deposits. This study uncovers the novel roles of IL-4 in metabolism and provides new insights in the interaction between cytokines/immune responses, insulin sensitivity and metabolism.
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Keywords: interleukin-4; glucose/lipid metabolism; insulin sensitivity; type 2 diabetes mellitus

Introduction

Insulin resistance, the major cause of type 2 diabetes mellitus (T2DM), is occurred when insulin-target cells cannot effectively transduce insulin signaling and eventually become less sensitive to insulin stimuli. The consequences of impaired insulin signaling include reduced glucose uptake and disposal as well as increased hepatic gluconeogenesis.[1] In addition to T2DM, abundant evidence demonstrates that insulin resistance also has an important role in other metabolic abnormalities, such as obesity, dyslipidemia, hypertension and cardiovascular diseases.[2,3]

T2DM is a chronic inflammatory state of elevated circulatory pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α.[4,5] These increased cytokines act as negative regulators to modulate glucose homeostasis and contribute as a link between immune responses and T2DM. Excessive intake of glucose and macronutrients also induce oxidative stress and cytokine mediators, which subsequently block insulin signaling.[6–10] Triacylglycerols storage in adipose tissues is impaired by chronic inflammation, which leads to the elevation of circulating free fatty acids (FFAs) and triacylglycerols, and eventually insulin resistance.[11] Accordingly, cytokines are associated with systemic insulin resistance, metabolic diseases and diabetes. Therefore, it is tempting to determine whether other cytokines are also involved in the pathogenesis of T2DM.

IL-4 is a pleiotropic cytokine secreted by activated Th2 cells, FcεR1$^+$ cells and eosinophils. IL-4 has an important role in inflammatory reactions by modulating growth, differentiation and cytokine production.[12] Although IL-4 signaling is known to trigger phosphorylation of insulin receptor substrate 2,[13,14] chronic insulin and glucose treatment attenuates IL-4-dependent insulin receptor substrate 2 phosphorylation.[15] It suggests that IL-4 can positively regulate insulin signaling pathway. In addition, our recent study reveals a significant association between IL-4 genotypes and T2DM.[16] Nevertheless, the influence of IL-4 on

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metabolism has not been extensively addressed and deserves further investigation. In this context, this study aimed at investigating *in vivo* effects of IL-4 on metabolism using mice T2DM model induced by single low-dose STZ administration and high-fat diet (HFD).[17–20] Our results show that IL-4 overexpression leads to better glucose utilization. In addition, body weights and fat mass of mice with IL-4 treatment are significantly lower than their counterparts. These observations suggest that IL-4 is involved in mediating glucose and lipid metabolism.

Materials and methods

Cell culture and adenovirus expansion
Human AD293 embryonic kidney cells were cultured in Dulbecco's modified Eagle's medium containing glucose ($4.5\,g\,l^{-1}$) and 10% fetal bovine serum. Recombinant adenoviruses expressing IL-4 (AdIL-4) and β-galactosidase (AdLacZ, as a negative control) were kindly provided by Dr Nagayama.[21] AdIL-4 and AdLacZ were respectively propagated in AD293 cells and purified by cesium chloride density gradient ultracentrifugation, followed by dialysis in phosphate-buffered saline with 10% glycerol. Number of viral particles was determined by measuring the absorbance at 260 nm.

Animal experiments
All mice were obtained from National Laboratory Animal Center and caged in groups of five. For adenovirus experiments, 8-week-old male C57BL/6 mice were intraperitoneally (i.p.) injected twice (once daily for 2 consecutive days) with 5×10^{11} particles of AdIL-4 or AdLacZ. The adenovirus-injected mice were i.p. administered with STZ ($100\,mg\,kg^{-1}$; Sigma-Aldrich, St Louis, MO, USA) on the second day.[21] Intraperitoneal glucose tolerance test (IGTT), intraperitoneal insulin tolerance test (IITT) and circulatory biochemical parameters were assessed on the third day. For long-term IL-4 administration experiments, 4-week-old C57BL/6 mice were fed with HFD (60% kcal) or standard diet (SD), and i.p. administered with IL-4 (1000 pg per mouse; BD Pharmingen) every other day for 8 weeks. Then the mice were killed after IGTT, IITT and biochemical tests were performed. In IL-4 neutralization experiments, BALB/c mice with relatively high IL-4-secreting ability were used instead of low IL-4-secreting C57BL/6 mice.[22] Four-week-old male mice were first fed with SD or HFD for 10 weeks, then divided to four groups: (1) SI group received i.p. STZ-injection followed by IL-4 antibody administration on the second, third and fifth day (50 μg per mouse; Biosource, Camarillo, CA, USA); (2) IS group received IL-4 antibody administration on the first, second and fifth day, and STZ injection on the third day; (3) STZ group received only STZ treatment on the first day; and (4) Control group received citrate buffer (0.05 M, pH 4.5; USB, Cleveland, OH, USA) administration. Then the mice were killed after IGTT, IITT and biochemical tests were performed. Animal protocols were reviewed and approved by the Chung Shan Medical University animal studies committee.

IGTT, IITT and blood parameters
IGTT was performed and blood glucose was measured using OneTouch monitoring system (LifeScan) before and after i.p. glucose injection ($2\,g\,kg^{-1}$; Sigma-Aldrich, Steinheim, Germany) at the indicated time. IITT was conducted and blood glucose was monitored at the time points indicated after i.p. injection of recombinant human insulin ($1\,U\,kg^{-1}$; Eli Lily, Indianapolis, IN, USA). Serum levels of insulin (Mercodia, Uppsala, Sweden), IL-4 (R&D, Minneapolis, MN, USA), leptin (Millipore, Billerica, MA, USA), adiponectin (Millipore) and FFA (BioVision, Mountain View, CA, USA) were measured after overnight fast using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions. The epididymal fat pads were taken and weighted, and the adipocytic cross-sectional areas from staining images were calculated.

Western blot
Protein extracts from muscle and epididymal fat tissues were obtained after the tissues were homogenized using T-PER tissue protein extraction reagent (Pierce, Rockford, IL, USA) supplied with phosphatase and protease inhibitors (Roche, Indianapolis, IN, USA). Protein lysates were normalized using Bio-Rad protein reagent and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then electrotransferred to polyvinylidine fluoride membrane. The membranes were blocked and incubated with mouse anti-actin monoclonal immunoglobulin G (Abcam, Cambridge, UK), mouse anti-phospho-Akt serine 473, rabbit anti-Akt, rabbit anti-phospho-GSK3β serine 9 and rabbit anti-GSK3β, respectively. All primary antibodies were purchased from Cell Signaling (Danvers, MA, USA), except where otherwise indicated. Then, membranes were incubated with horseradish peroxidase-conjugated anti-mouse or anti-rabbit antibodies. Results were developed using ECL reagents and quantitated by densitometer. All secondary antibodies and ECL reagents were purchased from Millipore (Temecula, CA, USA).

Statistical analysis
Results were presented as mean ± s.e.m. and the significant difference between groups was analyzed by one-way or two-way analysis of variance using SPSS software. Statistical difference was defined as $P<0.05$ for all test.

Results

Effects of in vivo *IL-4 overexpression on glucose metabolism*
To establish a mouse model with high IL-4 levels, 8-week-old C57BL/6 mice were first subjected to i.p. injection with

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Table 1 Biochemical profile of mice groups divided by treatment of adenovirus administration, IL-4 and diet

	IL-4 (pM)	*Adiponectin (pM)*	*Leptin (nM)*	*Insulin (nM)*	*Free fatty acid (mM)*
*Adenovirus treatment**					
Control	1.17 ± 0.44	185.67 ± 4.3	0.08 ± 0.02	0.06 ± 0.01	8.56 ± 3.57
AdLacZ	1.64 ± 0.65	187.67 ± 15.67	0.41 ± 0.16[a]	0.28 ± 0.19	9.96 ± 2.67
AdIL-4	10.16 ± 1.72[a,b]	216.0 ± 6.3[a,b]	0.58 ± 0.10[a]	0.79 ± 0.21[a,b]	14.7 ± 3.14
*IL-4/diet treatment***					
Control	3.14 ± 1.78	185.67 ± 6.0	0.49 ± 0.09	0.08 ± 0.01	7.39 ± 0.88
IL-4	12.02 ± 3.90[c]	206.33 ± 14.67	0.30 ± 0.12	0.10 ± 0.002	8.25 ± 1.52
HFD	3.19 ± 1.33[d]	235.33 ± 10.7[c]	0.82 ± 0.11[c,d]	0.09 ± 0.01	13.9 ± 3.74
HFD+IL-4	11.34 ± 4.21[c,e]	251.67 ± 17.0[c,d]	0.47 ± 0.10[e]	0.10 ± 0.03	20.3 ± 4.67[c,d]

Abbreviations: HFD, high-fat diet; IL, interleukin; i.p., intraperitoneal; SD, standard diet. *Eight-week-old mice fed with standard chow diet were subjected to i.p. adenovirus injection. All parameters were measured after 16 h of overnight fast. Data were presented as mean ± s.e.m.; $n = 7$. [a]$P < 0.05$ vs control; [b]$P < 0.05$ vs AdLacZ. **Four-week-old mice were subjected to i.p. IL-4 injection every 2 days and fed with either SD or HFD diet for 8 weeks. All parameters were measured as described. Data were presented as mean ± s.e.m.; $n = 5$–10. [c]$P < 0.05$ vs control; [d]$P < 0.05$ vs IL-4; [e]$P < 0.05$ vs HFD.

AdIL-4, followed by STZ treatment (AdIL-4 mice). Mice with either citrate buffer (control mice) or AdLacZ administration and STZ treatment (AdLacZ mice) were served as controls. Serum IL-4 level of AdIL-4 mice (10.16 ± 1.72 pM) was significantly higher than that of control (1.17 ± 0.44 pM) and AdLacZ (1.64 ± 0.65 pM) mice (Table 1). It indicated that the adenovirus administration can successfully turn the low IL-4-secreting C57BL/6 mice into high IL-4-expressing animal model. Biochemical parameters including adiponectin, leptin, insulin and FFA were then analyzed to examine the effects of *in vivo* IL-4 overexpression on metabolism. As listed in Table 1, levels of adiponectin, leptin and insulin were all significantly increased in AdIL-4 mice. AdIL-4 mice had higher FFA levels, however, this difference did not reach statistical significance.

The effects of IL-4 overexpression on glucose homeostasis and insulin sensitivity were subsequently examined. The results showed that IGTT curves from control and AdLacZ mice were quite similar (Figure 1a). Whereas, AdIL-4 mice demonstrated better glucose tolerance with a lower peak glucose level (approximately 13.89 mM) and a shorter time required for reaching glucose homeostasis (about 90 min). Consistent with the IGTT observations, IITT results showed that insulin sensitivity and glucose tolerance of AdIL-4 mice were significantly better than that of control and AdLacZ mice (Figure 1b).

Effects of IL-4 neutralization on glucose metabolism

To verify the above observations, high IL-4-producing BABL/c mice were used to examine if the effects of IL-4 on metabolism could be reversed by depleting IL-4. BALB/c mice were first fed either with SD or HFD for 10 weeks, and their IL-4 bioactivities were neutralized before or after STZ treatment as described in Materials and methods section. IGTT results showed that blood glucose of all the mice groups with SD reached the peak levels at about 30 ~ 40 min, with high glucose levels sustained till 120 min in SI, IS and STZ mice (Figure 1c). Interestingly, blood glucose levels in mice with IL-4 neutralization (SI and IS) were consistently and significantly higher than mice without IL-4 depletion (control and STZ; Figure 1c). Similar phenomena were also observed in HFD-fed mice: glucose levels in mice with IL-4 neutralization remained high still 120 min after glucose injection, compared with control and STZ mice groups (Figure 1d). These data suggested that mice with IL-4 overexpression had better glucose tolerance and metabolism.

Effects of in vivo *IL-4 on Akt and GSK3β phosphorylation*

To further examine the mechanism of IL-4 promoting glucose tolerance, influences of IL-4 to the activity of the insulin downstream signaling mediators, Akt and GSK3β, were explored. Akt phosphorylation were significantly increased in muscle cells of AdIL-4 mice; whereas, no significant alterations of Akt phosphorylation were observed in epididymal fat (Table 2). It suggests that the IL-4-improved glucose tolerance might result from enhancing insulin action by upregulating Akt activities in muscle cells. On the contrary, levels of phosphorylated GSK3β were decreased in AdIL-4 mice (Table 2).

Effects of long-term IL-4 treatment on lipid and glucose metabolism

The *in vivo* elevated IL-4 levels in mice with AdIL-4 administration could only last transiently for about 3 days. For creating mice model with long-term high IL-4 levels, mice were i.p. injected with recombinant IL-4 every other day for 8 weeks, and fed with either SD or HFD. Our results showed IL-4 levels in mice with IL-4 administration (IL-4, 12.02 ± 3.90 pM; HFD + IL-4, 11.34 ± 4.21 pM) were significantly higher than their counterparts (control, 3.14 ± 1.78 pM; HFD, 3.19 ± 1.33 pM; Table 1). It indicated that mice model with long-term high IL-4 levels can be achieved by this strategy. Similar trend were also observed in serum levels of adiponectin and FFA, with IL-4-treated mice

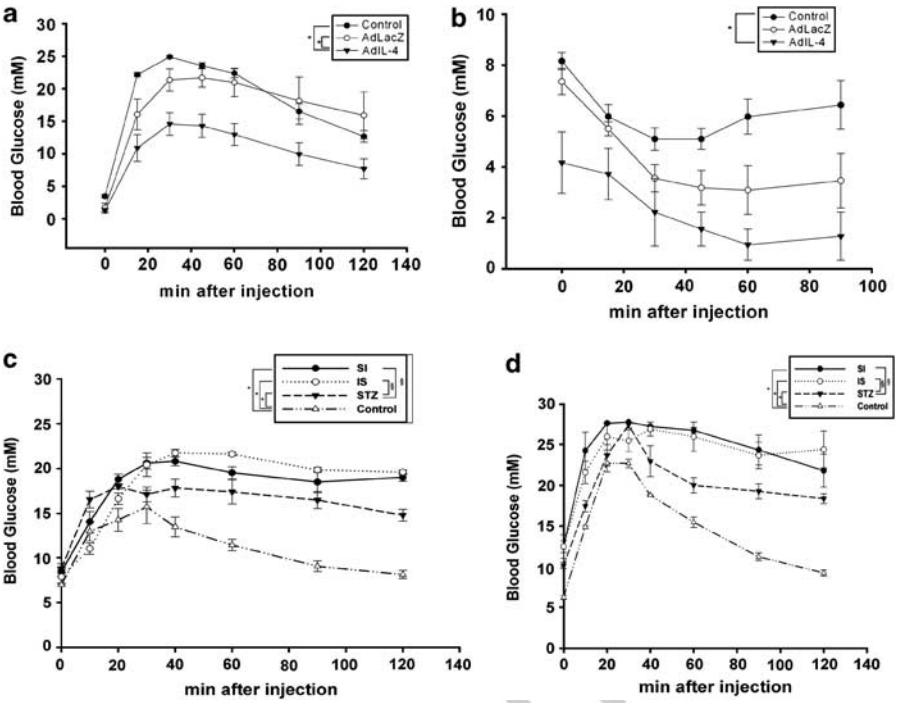

Figure 1 Glucose tolerance and insulin sensitivity are improved by IL-4 overexpression. (**a**, **b**) IGTT and IITT were conducted in overnight-fasting control (-●-), AdLacZ (-○-) and AdIL-4 (-▼-) mice after adenovirus and STZ treatments, respectively. Data were expressed as mean ± s.e.m.; $n=7$. *$P<0.05$ vs AdIL-4. (**c**, **d**) IGTT was performed for overnight-fasting control (-△-), SI (-●-), IS (-○-) and STZ (-▼-) mice after 10 weeks of SD (**b**) or HFD (**c**) feeding and treatment with IL-4 antibodies and/or STZ. Data were expressed as mean ± s.e.m.; $n=4$. *$P<0.05$ vs control; $^{\S}P<0.05$ vs STZ.

Table 2 Relative levels of phosphorylated Akt and GSK3β of muscle and epididymal fat in mice groups divided by treatment of adenovirus administration, IL-4 and diet

	Epididymal		*Muscle*	
	pAkt/Akt	*pGSK3β/GSK3β*	*pAkt/Akt*	*pGSK3β/GSK3β*
Adenovirus treatment				
Control	0.26 ± 0.03	0.33 ± 0.21	0.20 ± 0.03	0.90 ± 0.27
AdLacZ	0.30 ± 0.04	0.13 ± 0.04	0.22 ± 0.01	1.07 ± 0.09
AdIL-4	0.24 ± 0.06	0.03 ± 0.01[a]	0.35 ± 0.02[a,b]	0.10 ± 0.02[a,b]
IL-4/diet treatment				
Control	0.26 ± 0.02	2.04 ± 0.22	0.65 ± 0.04	0.47 ± 0.08
IL-4	1.97 ± 0.39[c]	1.26 ± 0.04[c]	0.85 ± 0.08	0.24 ± 0.08[c]
HFD	0.68 ± 0.17[d]	1.55 ± 0.27	1.24 ± 0.65	0.48 ± 0.03[d]
HFD+IL-4	1.76 ± 0.29[c,e]	1.37 ± 0.19[c]	0.57 ± 0.05[e]	0.40 ± 0.02[c,e]

Abbreviations: HFD, high-fat diet; IL, interleukin. *Data are presented as mean ± s.e.m., which indicate the relative levels of phosphorylated protein using actin as represent an internal control. [a]$P<0.05$ vs control mice in adenovirus treatment; [b]$P<0.05$ vs AdLacZ; [c]$P<0.05$ vs control mice in IL-4/diet treatment; [d]$P<0.05$ vs IL-4; [e]$P<0.05$ vs HFD.

having higher levels than control and HFD mice (Table 1). However, leptin levels were decreased in IL-4-treated groups (IL-4, 0.30 ± 0.12 nM; HFD + IL-4, 0.47 ± 0.10 nM), compared with mice without IL-4 treatment (control, 0.49 ± 0.09 nM; HFD, 0.82 ± 0.11 nM; Table 1).

Interestingly, body weights and fat mass of mice with IL-4 treatment (IL-4 and HFD + IL-4 mice) were significantly lower than the counterparts without IL-4 injection (Figure 2a and Table 3; with the original data of Figure 2a listed in Supplementary Table S1). Weights of the epididymal fat pads were decreased in IL-4-treated mice (IL-4, 0.16 ± 0.02 g; HFD + IL-4, 0.92 ± 0.31 g), compared with the mice without IL-4 treatment (control, 0.28 ± 0.03 g; HFD, 1.08 ± 0.09 g; Table 3). The adipocytic cross-sectional areas in IL-4-treated mice were significantly smaller (with relative ratio to control mice: IL-4, 0.61 ± 0.03; HFD, 1.19 ± 0.05; HFD + IL-4, 1.01 ± 0.06; Table 3). The same phenomenon was also observed in epididymal adipocytes of AdIL-4 administered mice (with relative ratio to control mice: AdLacZ, 1.03 ± 0.11; AdIL-4, 0.66 ± 0.07; Table 3). Taking the results of decreased fat mass and the increased serum FFA levels (Table 1) in IL-4-treated mice together, it implied that overexpressed IL-4 may inhibit lipid accumulation in fat tissues and lead to the elevated levels of circulatory FFA.

IGTT and IITT were then conducted to analyze the effects of long-term IL-4 treatment on glucose and insulin tolerance. Consistent with the results from adenovirus administration experiments, IL-4-treated mice (IL-4 and HFD + IL-4) had better glucose tolerance than their control counterparts, with a shorter time required for reaching

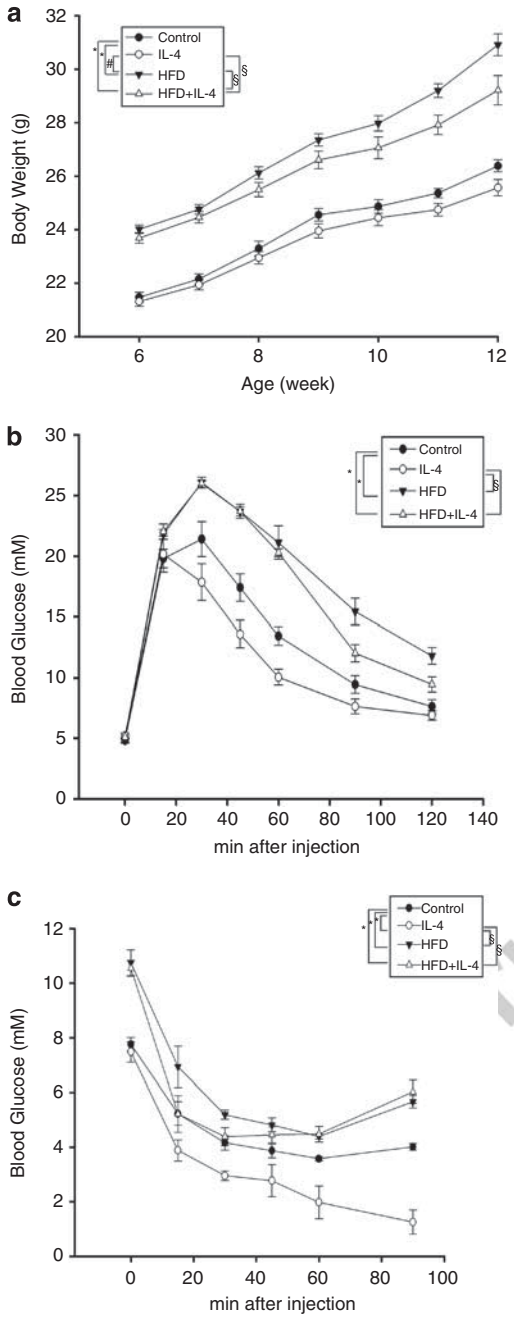

Figure 2 Effects of long-term IL-4 treatment on body weights and glucose metabolism. (**a**) Body weights of IL-4-treated mice groups (IL-4: -○-; HFD + IL-4: -△-) were significantly increased than their control counterpart without IL-4 administration (control: -●-; HFD: -▼-). *$P<0.05$ vs control; $^{\#}P<0.05$ vs IL-4; $^{\S}P<0.05$ vs HFD; $n=10$. (**b**, **c**) Results of IGTT and IITT in mice with long-term IL-4 injection. Data were expressed as mean ± s.e.m.; $n=5$. *$P<0.05$ vs control; $^{\S}P<0.05$ vs IL-4.

glucose homeostasis (IL-4, 60 min; HFD + IL-4, about 90 min) after glucose injection (Figure 2b). Similarly, the IITT results showed that insulin sensitivity and glucose tolerance of IL-4-treated mice were significantly better than that of the corresponding control mice (Figure 2c).

Table 3 Weights and adipocytic cross-sectional areas of epididymal fat pads in mice groups divided by treatment of adenovirus administration, IL-4 and diet

	*Cross-sectional area (AU)**	*Epididymal fat weight (g)*
Adenovirus treatment		
Control	1 ± 0.17	—
AdLacZ	1.03 ± 0.11	—
AdIL-4	0.66 ± 0.07[a,b]	—
IL-4/diet treatment		
Control	1 ± 0.05	0.28 ± 0.03
IL-4	0.61 ± 0.03[c]	0.16 ± 0.02
HFD	1.19 ± 0.05[d]	1.08 ± 0.09[c,d]
HFD+IL-4	1.01 ± 0.06[d]	0.92 ± 0.31[c,d]

Abbreviations: AU, arbitrary unit; HFD, high-fat diet; IL, interleukin. *Data are presented as mean ± s.e.m., which indicate the relative ratios of cross-sectional areas (AU) using that from corresponding control mice as control. [a]$P<0.05$ vs control mice in adenovirus treatment; [b]$P<0.05$ vs AdLacZ; [c]$P<0.05$ vs control mice in IL-4/diet treatment; [d]$P<0.05$ vs IL-4.

Effects of long-term IL-4 treatment on Akt and GSK3β phosphorylation

The Akt phosphorylation was significantly decreased in muscle of HFD + IL-4 mice; nevertheless, it was dramatically increased in the epididymal fat of IL-4 and HFD + IL-4 mice (Table 2). It suggested that under long-term IL-4 treatment, the improved glucose tolerance and insulin sensitivities might result mainly from the enhanced insulin action by upregulating Akt activities in fat tissues. The levels of phosphorylated GSK3β were significantly decreased both in the muscle and fat tissues of IL-4 and HFD + IL-4 mice, comparable to the results from adenovirus experiments, (Table 2).

Discussion

T2DM is associated with chronic inflammation. Although the correlation between IL-6/tumor necrosis factor-α and insulin resistance has been extensively studied,[23–25] relatively few are known about the roles of other cytokines in diabetic pathogenesis. IL-4, another cytokine secreted by Th2 cells, participates in the regulation of inflammation by modulating expression of other pro-inflammatory cytokines and inflammation mediators.[26,27] This study uncovers the roles of IL-4 in glucose/lipid metabolism and insulin sensitivity using animal model.

In AdIL-4 experiments, our results suggest that transient IL-4 treatment improves glucose tolerance and insulin sensitivity (Figures 1a and b). This effect is also observed in the IL-4 neutralization experiments, in which glucose tolerance and insulin sensitivity are exacerbated in mice injected with IL-4 antibodies (Figures 1c and d). In mice with long-term exposure to high IL-4 levels, the capacity of IL-4 to promote glucose tolerance and insulin sensitivity is further

confirmed (Figure 2). Interestingly, while Akt phosphorylation is significantly induced in muscle of mice with transient IL-4 treatment, it is significantly induced in fat cells of mice with long-term IL-4 exposure (Table 2). It indicates that better glucose tolerance and insulin sensitivity under IL-4 treatment may be resulted from its capacity to promote insulin signaling.

In addition to the roles in promoting insulin signaling, the observation that insulin levels are significantly increased in AdIL-4 mice (Table 1) implies transient IL-4 treatment promotes insulin-secreting function of β-cells. The results that both transient and long-term IL-4 treatments significantly inhibit GSK3β phosphorylation in muscle and fat cells (Table 2) support the previous evidence that IL-4 mediates glucose metabolism by regulating glycolytic enzymes.[28,29] It is intriguing that IL-4 promotes glucose tolerance through activating Akt activities while suppressing GSK3β phosphorylation. There are two possible explanations to this contradictory observation. First of all, IL-4 might display differential regulatory function to different insulin-targeted organs for maintaining metabolic hemeostasis. The reduced GSK3β activities in muscle and fat tissues might not be applied to liver, the critical target organ of glycogen synthesis. Besides, a recent study reveals that fibroblast growth factor 19 facilitates postprandial hepatic protein and glycogen synthesis through an insulin-independent pathway.[30] Therefore, the downregulated GSK3β activities in fat and muscle cells may not represent that glycogen synthesis is suppressed in hepatocytes. Second, this contradictory regulatory effect of IL-4 might be in response to the increased energy needs from cells to maintain homeostasis for avoiding cellular apoptosis, as previous described.[31]

Production of the pro-inflammatory cytokine IL-6 is essential to induce glucose intolerance and insulin resistance.[25] Lee *et al*.[32] reveals that the genetic inactivation of PKCζ leads to a hyper-inflammatory state with increased synthesis of pro-inflammatory cytokine IL-6 in obese mice, which are more glucose intolerant and insulin resistant. Their study shows that PKCζ is a critical negative regulator of IL-6 in the control of obesity-induced inflammation because the glucose intolerance and insulin resistance phenotypes are corrected to normal by silencing IL-6 expression in PKCζ-deficient mice. The positive regulatory role of IL-4 to glucose tolerance and insulin sensitivity uncovered by this study is consistent to the conclusion from Lee *et al*.[32] because PKCζ serves as a critical role in the anti-inflammatory effects of IL-4.[33,34] Accordingly, it supports our hypothesis that the positive regulatory role of IL-4 to glucose tolerance and insulin sensitivity may originate from its anti-inflammatory function by inhibiting the production of cytokines inducing insulin resistance, such as tumor necrosis factor-α and IL-6.[35]

Our results also suggest that IL-4 participates in lipid metabolism by regulating the circulatory levels of adiponectin and leptin. Intriguingly, adiponectin and leptin levels are both elevated under transient IL-4 treatment; whereas leptin levels are significantly decreased in HFD mice with long-term IL-4 administration. In addition, serum FFA levels are both increased after IL-4 treatments (Table 1). These data further support the capacity of IL-4 to promote insulin sensitivity and glucose metabolism because adiponectin has been shown to elevate insulin sensitivity and inhibit hepatic gluconeogenesis.[36] However, the inducing ability of IL-4 to β-cell insulin secretion is not observed in long-term IL-4 experiments, discrepancy in leptin levels is also observed in mice with different duration of IL-4 treatment (Table 1). Elevated levels of leptin and IL-4 in rats with acute pancreatitis are reported for increasing pancreatic resistance against inflammatory damages.[37] The increased levels of leptin in transient IL-4-treated mice support the suggestion of the above study, whereas, the food intake (for example, HFD in this study) has a dominant role in regulating leptin secretion. We suggest that the absence of induced insulin and leptin secretion under long-term IL-4 administration might be an adaption to the consistently elevated cytokine exposure for maintaining insulin levels and homeostasis.

The weekly weight gain in the IL-4-treated mice is lower (IL-4 and HFD + IL-4; Figure 2 and Supplementary Table S1), moreover, long-term IL-4 treatment leads to a reduction of fat mass and the cross-sectional areas of fat cells (Table 2). As evidence has revealed that leptin secretion is positively correlated with the amount of adipose tissues,[38] and adiponectin concentrations are reduced as adiposity increases,[39,40] we suggest that the significantly slower weight gain might result from the increased lipolysis in fat cells, which leads to the elevated circulatory FFA in IL-4-treated mice. Although the exact underlying mechanisms await further investigation, our results support the previous report that IL-4 transgenic mice contain less and smaller sized dermal fat tissues.[41] Our data also support another study in which documents that IL-4 secretion and Akt activity are both promoted in fatless A-ZIP/F1 diabetic mice.[42]

In summary, overexpression of IL-4 may promote glucose tolerance and insulin sensitivity through boosting insulin signaling by altering Akt and GSK3β activities. IL-4 is also involved in lipid metabolism by regulating adipokines and FFA levels. We suggest that IL-4 regulates metabolism by promoting insulin sensitivity, glucose tolerance and inhibiting lipid deposits. This study reveals evidence to uncover the novel roles of IL-4 in metabolism, and provide new insights in the interaction between cytokines/immune responses, insulin sensitivity and metabolism.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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References

1 Goldstein BJ. Insulin resistance as the core defect in type 2 diabetes mellitus. *Am J Cardiol* 2002; **90**: 3G–10G.
2 Sone H, Suzuki H, Takahashi A, Yamada N. Disease model: hyperinsulinemia and insulin resistance. Part A-targeted disruption of insulin signaling or glucose transport. *Trends Mol Med* 2001; **7**: 320–322.
3 Ginsberg HN. Insulin resistance and cardiovascular disease. *J Clin Invest* 2000; **106**: 453–458.
4 Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care* 2004; **27**: 813–823.
5 Crook M. Type 2 diabetes mellitus: a disease of the innate immune system? An update. *Diabet Med* 2004; **21**: 203–207.
6 Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004; **25**: 4–7.
7 Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM *et al*. IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 2005; **11**: 191–198.
8 Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia* 1998; **41**: 1241–1248.
9 Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* 1997; **40**: 1286–1292.
10 Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* 2003; **112**: 1785–1788.
11 Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006; **444**: 860–867.
12 Wei LH, Jacobs AT, Morris Jr SM, Minor KD, Mazzocco VR, Freund GG. IL-4 and IL-13 upregulate arginase I expression by cAMP and JAK/STAT6 pathways in vascular smooth muscle cells. *Am J Physiol Cell Physiol* 2000; **279**: C248–C256.
13 Wurster AL, Withers DJ, Uchida T, Wither DJ, Grusby MJ. Stat6 and IRS-2 cooperate in interleukin 4 (IL-4)-induced proliferation and differentiation but are dispensable for IL-4-dependent rescue from apoptosis. *Mol Cell Biol* 2002; **22**: 117–126.
14 Sun XJ, Wang LM, Zhang Y, Yenush L, Myers Jr MG, Glasheen E *et al*. Role of IRS-2 in insulin and cytokine signaling. *Nature* 1995; **377**: 173–177.
15 Hartman ME, O'Connor JC, Godbout JP, Minor KD, Mazzocco VR, Freund GG. Insulin receptor substrate-2-dependent interleukin-4 signaling in macrophages is impaired in two models of type 2 diabetes mellitus. *J Biol Chem* 2004; **27**: 28045–28050.
16 Ho KT, Shiau MY, Chang YH, Chen CM, Yang SC, Huang CN. Association of IL-4 promoter polymorphisms in Taiwanese patients with type 2 diabetes mellitus. *Metabolism* 2010; **59**: 1717–1722.
17 Luo J, Quan J, Tsai J, Hobensack CK, Sullivan C, Hector R *et al*. Nongenetic mouse models of non-insulin-dependent diabetes mellitus. *Metabolism* 1998; **47**: 663–668.
18 Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett PG, Gadbois TM *et al*. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism* 2000; **49**: v1390–v1394.
19 Chen D, Wang MW. Development and application of rodent models for type 2 diabetes. *Diabetes Obes Metab* 2005; **7**: 307–317.
20 Nakamura T, Terajima T, Ogata T, Ueno K, Hashimoto N, Ono K *et al*. Establishment and pathophysiological characterization of type 2 diabetic mouse model produced by streptozotocin and nicotinamide. *Biol Pharm Bull* 2006; **29**: 1167–1174.
21 Nagayama Y, Mizuguchi H, Hayakawa T, Niwa M, McLachlan SM, Rapoport B. Prevention of autoantibody-mediated Graves'-like hyperthyroidism in mice with IL-4, a Th2 cytokine. *J Immunol* 2003; **170**: 3522–3527.
22 Sacks D, Noben-Trauth N. The immunology of susceptibility and resistance to Leishmania major in mice. *Nat Rev Immunol* 2002; **2**: 845–858.
23 Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* 2002; **51**: 3391–3399.
24 Klover PJ, Clementi AH, Mooney RA. Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology* 2005; **146**: 3417–3427.
25 Sabio G, Das M, Mora A, Zhang Z, Jun JY, Ko HJ *et al*. A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. *Science* 2008; **322**: 1539–1543.
26 Lee YW, Eum SY, Chen KC, Hennig B, Toborek M. Gene expression profile in interleukin-4-stimulated human vascular endothelial cells. *Mol Med* 2004; **10**: 19–27.
27 Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. *J Clin Endocrinol Metab* 2005; **90**: 2282–2289.
28 Dufort FJ, Bleiman BF, Gumina MR, Blair D, Wagner DJ, Roberts MF *et al*. Cutting edge: IL-4-mediated protection of primary B lymphocytes from apoptosis via Stat6-dependent regulation of glycolytic metabolism. *J Immunol* 2007; **179**: 4953–4957.
29 Plas DR, Thompson CB. Cell metabolism in the regulation of programmed cell death. *Trends Endocrinol Metab* 2002; **13**: 75–78.
30 Kir S, Beddow SA, Samuel VT, Miller P, Previs SF, Suino-Powell K *et al*. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. *Science* 2011; **331**: 1621–1624.
31 Vander Heiden MG, Plas DR, Rathmell JC, Fox CJ, Harris MH, Thompson CB. Growth factors can influence cell growth and survival through effects on glucose metabolism. *Mol Cell Biol* 2001; **21**: 5899–5912.
32 Lee SJ, Kim JY, Nogueiras R, Linares JF, Perez-Tilve D, Jung DY *et al*. PKCζ-regulated inflammation in the nonhematopoietic compartment is critical for obesity-induced glucose intolerance. *Cell Metab* 2010; **12**: 65–77.
33 Duran A, Rodriguez A, Martin P, Serrano M, Flores JM, Leitges M *et al*. Crosstalk between PKCzeta and the IL4/Stat6 pathway during T-cell-mediated hepatitis. *EMBO J* 2004; **23**: 4595–4605.
34 Martin P, Villares R, Rodriguez-Mascarenhas S, Zaballos A, Leitges M, Kovac J *et al*. Control of T helper 2 cell function and allergic airway inflammation by PKCzeta. *Proc Natl Acad Sci USA* 2005; **102**: 9866–9871.
35 Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel CR, Goforth MH *et al*. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. *Cell Metab* 2008; **7**: 496–507.
36 Barnea M, Madar Z, Froy O. High-fat diet delays and fasting advances the circadian expression of adiponectin signaling components in mouse liver. *Endocrinology* 2009; **150**: 161–168.
37 Konturek PC, Jaworek J, Maniatoglou A, Bonior J, Meixner H, Konturek SJ *et al*. Leptin modulates the inflammatory response in acute pancreatitis. *Digestion* 2002; **65**: 149–160.
38 Bełtowski J. Adiponectin and resistin–new hormones of white adipose tissue. *Med Sci Monit* 2003; **9**: RA55–RA61.
39 Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y *et al*. Plasma concentrations of a novel,

adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1595–1599.
40 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K *et al*. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941–946.
41 Elbe-Bürger A, Egyed A, Olt S, Klubal R, Mann U, Rappersberger K *et al*. Overexpression of IL-4 alters the homeostasis in the skin. *J Invest Dermatol* 2002; **118**: 767–778.
42 Nunez NP, Oh WJ, Rozenberg J, Perella C, Anver M, Barrett JC *et al*. Accelerated tumor formation in a fatless mouse with type 2 diabetes and inflammation. *Cancer Res* 2006; **66**: 5469–5476.

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國科會補助專題研究計畫項下出席國際學術會議心得報告

計畫編號	NSC 97-2320-B-040-006-MY3
計畫名稱	第四型介白素對脂肪與葡萄糖代謝作用的調控
出國人員姓名 服務機關及職稱	張懿欣　　中山醫學大學醫學檢驗暨生物技術學系　教授
會議時間地點	2009/04/25~2009/04/29 土耳其伊斯坦堡
會議名稱	第11屆歐洲內分泌大會 11th European Congress of Endocrinology
發表論文題目	台灣第二型糖尿病患者第四型介白素基因型之分析 Association of IL-4 Promoter Polymorphisms in Taiwanese Patients with Type 2 Diabetes Mellitus

一、 參加會議經過

本人於四月二十五日抵達土耳其伊斯坦堡，首先到住宿飯店辦理check in，稍作休息之後至會場辦裡報到手續，領取會議相關書面資料。隨後逕入會場聽取演講。接下來之議程中，除了聽講之外，也參加海報展覽。整個會議結束之後，搭乘飛機返台。

二、 與會心得

本次大會是由歐洲內分泌學會在土耳其主辦，每年舉辦之歐洲地區內分泌國際會議，主題除了討論糖尿病臨床與基礎知識背景與研究新知之外，也包括甲狀腺、生殖腺、腦下垂體等內分泌疾病之深入探討。本次會議之與會者計有美國、加拿大、國際糖尿病聯盟組織等單位之代表人物，共2,000餘位世界各地之內分泌相關領域的研究學者專家。在整個與會過程中，除可了解糖尿病等內分泌疾病與免疫研究之最新進展、資訊與技術之外，也可進一步其他內分泌疾病之相關研究，並有機會認識世界各地的學者專家。

三、建議

建議國科會應多多補助研究人員參與國際會議。除了可以幫助研究人員了解國際上研究之最新相關資訊之外，也可藉機認識國際友人與學者並提升學校知名度，不但拓展自身視野也可使台灣本土之學者立身於國際學術領域，增進學術交流，提升學校的學術地位。

四、攜回資料名稱及內容

會議議程、會議手冊與會議摘要CD。

Dear colleague,

I am pleased to inform you that your abstract Association of IL-4 Promoter Polymorphisms in Taiwanese Patients with Type 2 Diabetes Mellitus, reference 0396 has been accepted for poster presentation at the 11th European Congress of Endocrinology.

Guidelines for presentations will shortly be posted on the conference website www.ece2009.com. Please do not forget to register for the conference immediately to confirm your participation. If you register before 16 February 2009 you can benefit from the lower early bird registration fee.
Registration must be done online through the following link:
http://www.ece2009.com/index.php?id=19

The format/size of the posters should be in portrait format and can not exceed 120 cm (height) x 90 cm (width).

We look forward to seeing you in Istanbul from April 25 - 29 2009.

With best wishes,
Conference Administrator

Association of IL-4 Promoter Polymorphisms in Taiwanese Patients with Type 2 Diabetes Mellitus

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a common endocrine disease. The aim of this study is to investigate the putative correlation between the promoter single nucleotide polymorphisms (SNPs) of interleukin-4 (IL-4) and T2DM. Genomic DNA from Taiwanese T2DM patients and control study subjects were extracted, and their IL-4 promoter SNPs were analyzed. Both of the distribution of IL-4 C-589T (p=0.013) and C-34T (p=0.05) genotypes were significantly different between T2DM patients and control subjects. Significant association between IL-4 C-589T alleles (p=0.002) and T2DM, as well as C-34T alleles and T2DM (p=0.024), was also identified. Additionally, we found a statistically significant association between homologous IL-4 -589 C/C genotypes and lower circulatory high density lipoprotein (HDL-C) levels using multiple linear regression analysis with adjustment for subjects' age, sex and diabetic status. Our results suggested that IL-4 promoter SNPs are associated with T2DM, as well as the significant correlation between IL-4 homologous C/C genotypes and the lower circulatory HDL-C level.

INTRODUCTION

T2DM is characterized by insulin resistance and abnormal insulin secretion. Previous reports suggested that Th2 cytokines play important roles in type 2 diabetic development. To investigate the role of IL-4 in T2DM, genomic DNA was extracted from peripheral blood and IL-4 promoter C-589T/C-34T SNPs were analyzed.

MATERIALS AND METHODS

Genomic DNA was extracted from peripheral blood of 425 Taiwanese T2DM patients and 148 unrelated healthy Taiwanese individuals, and IL-4 gene SNPs were analyzed by PCR-RFLP analysis. Student t test was applied for comparisons of each blood biochemistry parameter between T2DM subjects and controls. Others statistical significance of the difference was tested by Chi-squared analysis.

RESULT

TABLE 1 Demographic and biochemical data of study subjects.

	Control subjects n=148 (n=98)‡	Type 2 diabetic patients n=425 (n=328)‡	p †
BMI (kg/m²)	24.52±3.40 (24.43±3.51)	25.36±3.27 (25.34±3.16)	0.014 0.021
Fasting glucose (70-110 mg/dL)*	95.69±6.79 (96.07±6.63)	177.70±68.50 (180.38±70.65)	<0.001 <0.001
Systolic pressure (120-140 mmHg)*	125.42±19.18 (123.45±17.19)	134.76±18.56 (135.22±18.19)	<0.001 <0.001
Blood urea nitrogen	15.86±5.09 (15.60±4.39)	17.54±8.00 (17.58±8.07)	0.025 0.023
HDL-C (>35 mg/dL)*	58.35±13.88 (59.24±14.58)	46.49±13.38 (46.52±13.43)	<0.001 <0.001
TC/HDL-C	3.59±0.90 (3.56±0.89)	4.50±1.31 (4.51±1.31)	<0.001 <0.001
Triglycerides (20-200 mg/dL)*	142.54±125.52 (139.45±122.86)	184.00±153.24 (184.51±153.74)	0.004 0.008
Uric acid (2.4-7.2 mg/dL)*	6.56±1.64 (6.79±1.67)	6.11±1.87 (6.12±1.88)	0.021 0.003

*Numbers in parenthesis indicated the normal reference range of each biochemical test.
†Student t-test.
‡Numbers in the parentheses indicated the information of each demographic and biochemical variables from the data of 98 control and 328 diabetic subjects with available IL-4 -34 genotypic results.

TABLE 2 Comparison of IL-4 -34 C/T and-589 C/T SNPs between type 2 diabetic subjects and controls.

	Non-diabetic healthy control n (%)	Type 2 diabetic patients n (%)	p*
Genotype			
IL-4 -589 C/T	148	425	
T/T	96 (64.86%)	324 (76.24%)	
T/C	45 (30.41%)	93 (21.88%)	0.013
C/C	7 (4.73%)	8 (1.88%)	
IL-4 -34 C/T	98	328	
T/T	63 (64.29%)	250 (76.22%)	
T/C	32 (32.65%)	69 (21.04%)	0.05
C/C	3 (3.06%)	9 (2.74%)	
Allele			
IL-4 -589 C/T			
T	237 (80.07%)	741 (87.18%)	0.002
C	59 (19.93%)	109 (12.82%)	
IL-4 -34 C/T			
T	158 (80.61%)	569 (86.74%)	0.024
C	38 (19.39%)	87 (13.26%)	

* p<0.05 by Chi square test.

TABLE 3 Association of IL-4 genotypes and blood chemistry parameters, controlling for potential confounding factors*, the results of multiple linear regression analysis.

Outcome	IL-4 genotypes (C/C vs. T/T)		IL-4 genotypes (T/C vs. T/T)	
	b (se)†	p value	b (se)†	p value
Fasting glucose	0.03629	0.6816	0.02147	0.5467
BUN	0.21815	0.1476	0.04895	0.3905
Creatinine	0.08897	0.2964	0.00324	0.9281
Cholesterol	0.00027527	0.9973	0.01904	0.4936
HDL-C	-0.41838	0.0003	-0.01690	0.6730
TC/HDL-C	0.41418	0.0032	0.07508	0.1286
Triglyceride	-0.34156	0.2404	0.14315	0.1584
Uric acid	0.00510	0.9648	0.01134	0.7979

*Other factors included in the models are: case vs. control status, age and sex.
†Parameter estimate (standard error).

CONCLUSION

1. IL-4 promoter C-589T and C-34T polymorphism were associated with type 2 diabetes mellitus in Taiwan.

2. A significant association between IL-4 -589C/C genotypes and lower HDL-C levels was found. This result indicated that IL-4 -589C might be associated with HDL-C metabolism.

國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/18

國科會補助計畫	計畫名稱: 第四型介白素對脂肪與葡萄糖代謝作用的調控
	計畫主持人: 張懿欣
	計畫編號: 97-2320-B-040-006-MY3　　學門領域: 寄生蟲學、醫事技術及實驗診斷

無研發成果推廣資料

97 年度專題研究計畫研究成果彙整表

計畫主持人：張懿欣	計畫編號：97-2320-B-040-006-MY3
計畫名稱：第四型介白素對脂肪與葡萄糖代謝作用的調控	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	1	100%	篇	
		研究報告/技術報告	3	3	100%		
		研討會論文	0	3	100%		
		專書	0	1	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	1	100%	人次	
		博士生	1	1	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	2	2	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	3	5	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	2009/11/8 台灣醫事檢驗學會 98 年度學術論文獎 2009/11/8 台灣醫事檢驗學會 98 年度學術發表會/優秀壁報論文獎 2009/5/2~3 2009 亞太醫學檢驗科學國際研討會/(Asia-Pacifica Laboratory Science Forum 2009) 優秀壁報論文獎 2008/2/23~24 2008 亞太醫學檢驗科學國際研討會 (Asia-Pacifica Laboratory Science Forum 2008) 優秀壁報論文獎

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

本文的主旨為探討細胞激素第四型介白素(interleukin-4, IL-4)在 T2DM 和新陳代謝中的角色。根據本研究之基因型分析結果，國人 IL-4 基因型與 T2DM 以及受檢者之高密度膽固醇發病有相關性；此發現表示 IL-4 可能與葡萄糖/脂肪之代謝作用有關而參與 T2DM 之致病。因此本實驗室進一步以細胞與動物模式檢測上述假說。實驗結果顯示 IL-4 可透過調控 Akt 之磷酸化，提升胰島素敏感性與葡萄糖耐受性；此外，IL-4 也抑制脂肪聚集而調控脂肪代謝作用，減輕小鼠之體重與脂肪重量。簡而言之，本研究除發現 IL-4 基因型與 T2DM 之發病有關之外，亦證實 IL-4 透過提高胰島素敏感性與葡萄糖耐受性並抑制脂肪堆積而調節葡萄糖/脂肪之代謝作用。上述結果提供 IL-4 調控代謝作用之嶄新角色，也提出免疫作用、胰島素敏感性與新陳代謝三者交互作用之證據。