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ORIGINAL COMMUNICATION

Effect of obesity and insulin on immunity in non-insulin-dependent diabetes mellitus

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Objective: To understand the effect of obesity and insulin on immune functions in non-insulin-dependent diabetes mellitus (NIDDM).
Subject: Fourteen obese NIDDM (body mass index (BMI) = 30.6 ± 1.1), seven non-obese NIDDM (BMI = 24.2 ± 0.5) and five obese non-NIDDM (BMI = 28.3 ± 0.67).
Interventions: We first examined the influence of insulin on the proliferation of several human cell lines. Second, we compared several immune functions between obese and non-obese NIDDM, and obese non-NIDDM patients using peripheral blood mononuclear cells.
Result: Insulin decreased proliferation of T-cell lines but not that of other types of cell lines. Furthermore, obesity augmented the production of IL-1β which could have cytotoxity against islet β cells in NIDDM.
Conclusion: Our data suggested that the pathophysiology of NIDDM could be affected by the change of immunity due to obesity, and the treatment of obesity in NIDDM may be important from an immunological aspect.
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Keywords: obesity; immunity; insulin

Introduction

It has been reported that several immune functions are dysregulated in obesity (Tanaka *et al*, 1993; Nieman *et al*, 1999). In the studies of obese subjects and genetically obese animals, lymphocytes numbers and proliferative responses were lower than in controls (Tanaka *et al*, 1993, 1998; Nieman *et al*, 1999). We also reported that cytokine balance was changed in diet-induced obese mice (Mito *et al*, 2000). However, the mechanism and the factors affecting impaired immunity in obesity are not fully understood.

Although non-insulin-dependent diabetes mellitus (NIDDM) patients are often obese, the immune function in NIDDM with obesity has not been investigated. Many metabolic and endocrinological factors could be changed by obesity, such as insulin, leptin or corticosterone, which might affect immune functions (Hunt & Eardley, 1986; Brinkmann & Kristofic, 1995; Loffreda *et al*, 1998; Lord *et al*, 1998; Martin-Romero *et al*, 2000). Insulin is an important factor both in NIDDM and obesity, however, the role of insulin on immune system is not clear in these conditions.

Inter leukin (IL)-1β secreted by various immune cells is one of the important mediators regulating the host response to inflammation and immunity (Dinarello, 1984; Dinarello & Mier, 1987). As this cytokine is cytotoxic to insulin-producing β-cells (Mandrup-Poulsen *et al*, 1986), we investigated the change of IL-1β production in NIDDM with obesity.

The purpose of this study was to investigate the effect of insulin on immune cells *in vitro*, and the change of proliferative response and IL-1β production of PBMCs in obese NIDDM patients, in order to elucidate the effect of obesity on immunity in NIDDM. This is a novel study to deal with the immunological difference between obese and non-obese NIDDM patients.

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Materials and methods

Human cell lines study

Proliferative responses of cell lines. Human leukemic cell lines, HUT-102 and MOLT-4 (T-cell), RAJI (B-cell) and U937 (monocyte) were used in this study. Cells were maintained in RPMI-1640 medium (NISSUI, Tokyo, Japan) containing 10% heat-inactivated fetal calf serum (JRH, Lenaxa, Australia). Proliferation of these cells was assayed by a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) method after incubation for 72 h (Mito *et al*, 2000) with insulin (0, 0.01, 0.1, 1, 10, 100 or 1000 ng/ml). Following the addition of MTT solution (200 μg/ml), the cells were incubated for 3 h at 37°C in a 5% CO_2 atmosphere. The MTT–formazon product formation was dissolved by the addition of 10% SDS–0.01 N HCl. The optical density of each well was measured using test and reference wavelengths of 550 and 650 nm. The culture time of cells for maximum response and cell numbers were determined before the assay, and was not different between cell lines.

Human patients study

Subjects. Fourteen obese and seven non-obese NIDDM patients and five obese non-NIDDM subjects were included in the study. All patients were female. Duration of the disease was not different between obese and non-obese NIDDM. All but one NIDDM patients were treated with oral antidiabetic agents. Clinical profiles of the patients are summarized in Table 1. Informed consent was obtained from each patient.

Cell preparation. Blood samples were processed immediately for assay after collection. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by density gradient centrifugation with HISTOPAQUE-1077 (Sigma, St Louis, MO, USA).

PBMCs were adjusted to a density of 10^6 cells/ml with RPMI-1640 medium containing 5% heat-inactivated fetal calf serum, L-glutamine (2 mM, GIBCO, Grand Island, NY, USA), penicillin (100 U/ml, GIBCO), streptomycin (100 μg/ml, GIBCO), and cultured with or without phytohemagglutinin (PHA; 1 μg/ml, DIFCO, Detroit, MI, USA), for 48 or 72 h at 37°C in a 5% CO_2 atmosphere.

Proliferative responses of PBMCs. After 72 h of culture, the proliferative response of PBMCs was measured by MTT assay (Mito *et al*, 2000). Data are expressed as the ratio of the absorbency of mitogen-stimulated cultures to the absorbency of non-stimulated cultures (referred to as the stimulation index). The culture time of PBMCs for the maximum response was determined before the assay, and was not different between three groups.

Cytokine production and measurement. Supernatants of PBMCs were collected after 48 h of culture, centrifuged and stored at −30°C until analysis. For measurement of cytokine production, enzyme-linked immunosorbent assays (ELISA) were performed on culture supernatants. An ELISA kit was used for IL-1β measurement (Otsuka, Tokyo, Japan). The range of the ELISA assay used in this study was 15.6–500 pg/ml. The optimal incubation time of PBMCs for the cytokine production was decided before the assay.

Statistical analysis

Results are given as means ± s.e. Statistical comparisons were made between three groups of patients in parameters of clinical profiles, lymphocyte proliferative responses and cytokine production by PBMCs, using ANOVA, and each group was compared with the others by Fisher's protected least-significant test.

Results

Human cell lines study

In human cell lines, high concentrations of insulin (>10–100 ng/ml) suppressed proliferation of only T-cell lines, such as MOLT-4 and HUT-102. The suppression was dose-dependent over the concentration of 10 ng/ml. Proliferation of the B-cell line (Raji) or the monocyte cell line (U937) was not affected by any concentration of insulin (Figure 1). These findings may suggest the selective sensitivity of T-cell lines to insulin.

Table 1 Clinical profile of the patients

Characteristic	*Obese NIDDM (n=14)*	*Non-obese NIDDM (n=7)*	*Obese non-NIDDM (n=5)*
Age (y)	56±3.5	60±3.8	63±6.2
BMI (kg/m^2)	30.6±1.1**	24.2±0.5	28.3±0.67
Height (cm)	153.3±1.9	157.0±1.5	152±1.5
Weight (kg)	72.1±3.0**‡	59.5±1.5	65.6±2.9**
Blood pressure (systolic/diastolic, mmHg)	135±4.0/80±3.8	133±4.9/78±3.1	133±8.8/77±3.3
Serum insulin (pmol/l)	9.3±0.95*	20.3±6.95	13.4±6.4
HbA_{1c} (%)	7.9±0.5†	7.3±0.5	5.7±0.49
Duration of the disease (y)	10±1.6	14±6.6	—
Treatment of oral antidiabetic drugs	14/14	6/7	—

Values are mean ± s.e.
*$P<0.05$; **$PP<0.01$, *vs* non-obese NIDDM. †$P<0.05$; ‡$P<0.01$, *vs* obese non-NIDDM.

Human patients study

Clinical profile. The clinical profile of three groups (obese NIDDM, non-obese NIDDM and obese non-NIDDM patients) are shown in Table 1. Compared with non-obese NIDDM, obese NIDDM and obese non-NIDDM had significantly higher body mass index (BMI) and heavier weight. High levels of HbA_{1c} were found in both NIDDM groups. Serum fasting insulin level in non-obese NIDDM were significantly higher than those in obese NIDDM.

Proliferative responses of PBMCs. Proliferative responses of PBMCs with PHA stimulation was not different between obese and non-obese NIDDM patients. Proliferative response in obese non-NIDDM was lower than those in obese and non-obese NIDDM, although as significant difference was observed (Table 2).

Cytokine production from PBMCs. In cytokine production by PBMCs, IL-1β was significantly increased in obese NIDDM (216.4 ± 27.18 pg/ml) and obese non-NIDDM (279.6 ± 71.56 pg/ml) compared with non-obese NIDDM (98.0 ± 19.09 pg/ml; $P < 0.05$, $P < 0.01$, respectively, Table 2).

Discussion

NIDDM patients have an increased incidence of infections due to impaired immunity (Plouffe *et al*, 1978; Feng-Yee & Men-Fang, 1995; Reyzelman *et al*, 1999). Immune-competent cells can be regulated by multiple endocrine hormones including insulin through binding the surface-specific receptors (Kiess & Belohradsky, 1986). Therefore, it is hypothesized that change of immune system in NIDDM is associated with alterations of the endocrine homeostasis. Meanwhile, obesity is accompanied with impaired T-cell number and proliferation (Tanaka *et al*, 1993; Nieman *et al*, 1999). We already reported that cytokine balance were changed in diet-induced obese mice (Mito *et al*, 2000). As NIDDM and obesity have characteristic metabolic changes, such as alterations in insulin, leptin or corticosteron, it is possible that immune functions in NIDDM with obesity might be different from those in NIDDM with normal body weight.

In the study of human cell lines, exogenous insulin in high concentrations decreased proliferation of T-cell lines, such as MOLT-4 and HUT-102, but had no effect on B-cell and monocyte-cell lines. An effect of insulin on proliferation of other cell lines (HL-60 and K562) was also not observed (data not shown). This suggests that the effect of insulin may be different between cell types or various stages of differentiation of immune cells. T-cell lines may be particularly sensitive to insulin. In this respect, a previous study reported that super-physiological insulin concentration *in vitro* decreased the total number of lymphocytes, especially $CD45RO^+$, but did not change $CD45RA^+$, $CD4^+$ or $CD8^+$ (Kappel *et al*, 1998).

In human patients' study, a significant difference was not observed in proliferative responses of PBMCs (T-cells) between obese and non-obese NIDDM. It was previously reported that proliferation of T-lymphocytes was lower in obesity than in normal subjects (Tanaka *et al*, 1993; Nieman *et al*, 1999). Our observation in NIDDM patients was not necessarily consistent with those studies, although age- and sex-matched obese subjects, who did not have NIDDM, showed slightly decreased proliferation of PBMCs in this study. This might originate from the relatively suppressed proliferative response of T-lymphocytes in non-obese NIDDM due to elevated serum insulin level. We demonstrated that exogenous insulin suppressed the proliferation of T-cell line. Insulin receptors expressed on T-lymphocytes have a role in activating the glucose metabolism by binding with insulin (Helderman, 1981). Insulin receptors on T-lymphocytes could be changed by high concentrations of insulin (Helderman & Raskin, 1980) resulting in alteration of immune functions. In this study, non-obese NIDDM showed higher serum insulin levels compared with obese NIDDM,

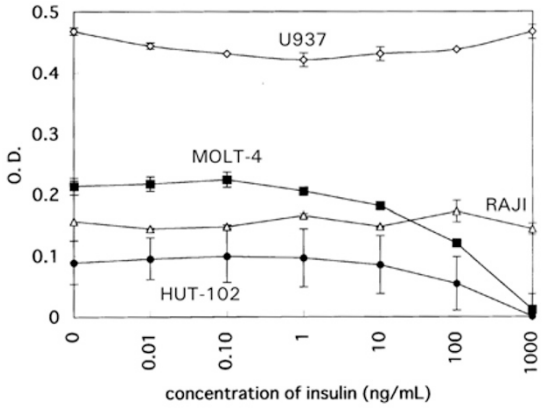

Figure 1 Effect of insulin on proliferative response of human cell lines, HUT-102, MOLT-4, U937 and RAJI.

Table 2 Proliferative response and cytokine production by PBMCs in three groups

Variable	*Obese NIDDM* (n = *14*)	*Non-obese NIDDM* (n = *7*)	*Obese non-NIDDM* (n = *5*)
Lymphocytes proliferation (SI)	2.15 ± 0.17	2.06 ± 0.17	1.82 ± 0.20
IL-1β production (pg/ml)	216.4 ± 27.18*	98.0 ± 19.09	279.6 ± 71.56**

Values are mean ± s.e.
*$P < 0.05$; **$P < 0.01$ *vs* non-obese NIDDM.

which might relate to the proliferation of T-cells in non-obese NIDDM. Since both diabetes and obesity are important causes of changes in serum insulin levels, it would be one of the modulating factors for T-lymphocyte functions in NIDDM and obesity. As exogenous insulin did not change the proliferation of PBMCs in this patient study (data not shown), further studies with separated T-cell populations investigating the effect of insulin on immune cells are necessary.

IL-1β secreted by activated monocytes and other various cells is an important mediators regulating the host response to inflammation and immunity (Dinarello, 1984; Dinarello & Mier, 1987). In previous reports, IL-1β production by PBMCs was higher in obesity and IDDM (Plouffe *et al*, 1978; Ciampolillo *et al*, 1993; Holden & Mooney, 1995; Bunout *et al*, 1996), although only a few papers have reported that NIDDM shows high levels of IL-1β (Davaraj & Jialal, 2000). Our investigation demonstrated that this cytokine was produced at significantly high levels in obese NIDDM and obese-non NIDDM compared with non-obese NIDDM. The IL-1β level was highest in obese non-NIDDM in the three groups. This suggested that obesity strongly affects the increment of IL-1β production from PBMCs. These findings require further investigation to clarify the mechanism of elevated IL-1β in obesity with NIDDM. Leptin, which has been reported to activate monocyte function or regulate cytokine balance (Mito *et al*, 2000; Loffreda *et al*, 1998; Santos-Alvarez *et al*, 1999), is increased in obesity (Frederichi *et al*, 1995; Considine *et al*, 1996). It has also been reported that leptin regulates inflammatory cytokine production by monocytes, such as TNF-α, IL-6 and IL-12 (Loffreda *et al*, 1998). In our study, obese groups showed high leptin levels compared with non-obese NIDDM, although a significant difference was not obtained because of the small sample size (data not shown). Therefore, increase of IL-1β production may be partly caused by hyperleptinemia in obesity. Several inflammatory cytokines including IL-1β have cytotoxity against insulin-producing islet β-cells (Mandrup-Poulsen *et al*, 1986) and microcirculation (Clausell *et al*, 1999). We reported that production of IFN-γ, pro-inflammatory cytokine, by splenocytes was increased in diet-induced obese mice (Mito *et al*, 2000). It has also been reported that T-cell function is impaired, and T-cell cytokine (IL-2) is decreased by obesity (Tanaka *et al*, 1993; Nieman *et al*, 1999; Mito *et al*, 2000). Other studies have produced evidence on the function of adipose tissue as a secreting organ with respect to cytokines, such as TNF-α or IL-6 (Mohamed-Ali *et al*, 1998). Accordingly, cytokines produced from T-cells, monocytes or adipose tissues in obesity may affect islet β-cell function or microcirculation in NIDDM. Further, obesity is often associated with hypertension, although the blood pressure of the patients in our study was not different from controls. Hypertensive patients have been reported to have higher levels of pro-inflammatory cytokines. Increased level of C-reactive protein, which indirectly reflects production of pro-inflammatory cytokine, was also found in obesity (Varner, 2000). These findings suggest that many factors in obesity may affect the inflammatory responses which influence pathophysiology of NIDDM. Consequently, it suggests that obesity may aggravate NIDDM through changing the production of pro-inflammatory cytokines. The treatment of obesity in NIDDM may be important from an immunological aspect. The factors affecting the immune system in NIDDM with obesity, such as leptin, corticosteron or other neuro-endocrine hormones in addition to insulin, should be further investigated.

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