

Different TNF α expression elicited by glucose in monocytes from type 2 diabetes mellitus patients

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Abstract

Increased plasma concentrations of tumor necrosis factor alpha (TNF α) system components appear in type 2 diabetes patients with poor glycemic control. We have analyzed the expression of TNF α , TNFR1 and TNFR2 when monocytes and lymphocytes isolated from a group of recent onset type 2 diabetic patients, with fasting glucose levels below 7.0 mM and glycated haemoglobin (Hb1Ac) in the normal range, were stimulated with high glucose or LPS endotoxin. We report, that cultured monocytes from these type 2 diabetic patients, in comparison to monocytes from non-diabetic individuals, had an enhanced response to LPS but did not respond to an acute glucose challenge ($p < 0.05$). No differences were observed in the cultured lymphocyte fractions. These results indicate the existence of differences, elicited by LPS or high glucose related stimulus, between monocytes isolated from non-diabetic subjects or from type 2 diabetes patients.

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1. Introduction

Diabetic patients have an increased susceptibility to atherosclerosis, defective wound healing and infection [1]. Hyperglycemia and low level chronic inflammation are important players in the pathogenesis of type 2 diabetes. Hyperglycemia has been implicated, through the production of superoxide by the mitochondrial electron transport chain, in the specific tissue damage associated with late diabetes complications: retinopathy, renal failure, cardiac and peripheral arterial disease, and neuropathy [1–4]. It is becoming clear that in type 2 diabetes, the best predictor of the severity of the underlying metabolic process is the degree of hyperglycemia [5,6].

On the other hand, it is becoming evident that a chronic low level inflammation is associated with obesity and type 2 diabetes conditions, and it is involved in all stages of the atherosclerotic process [7–9]. Increased plasma concentration of inflammatory mediators, such as members of the tumor necrosis factor alpha (TNF α) system and cytokines such IL-6, appear in type 2 diabetes and obesity [8,10–12].

The monocyte/macrophage system plays a prominent role on the onset of the atherosclerotic lesions when monocytes migrate and attach to the endothelium in the arterial wall to form foam cells [13]. Recent findings have also linked mononuclear cells to obesity in humans, where circulating monocytes/macrophages infiltrate adipose tissue [14–16]. Monocytes could also be associated with global insulin resistance in mice, where preventing the activation of the transcription nuclear factor κ B (NF- κ B), by deletion of its activator I κ B kinase β in myeloid cells, hampers the development of systemic insulin resistance [17]. Monocytes, endothelial cells, and other cell types in culture respond to

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high concentrations of glucose by releasing inflammatory mediators [8]. It has been found that the expression of proinflammatory cytokines, TNF α and IL-6, is regulated by glucose in human monocytes and monocytic cell lines [18–20]. Most of TNF α biological effects are mediated by means of two distinct membrane receptors, TNFR1 and TNFR2 [21].

Some metabolic alterations such as modified protein kinase C (PKC) activity [22,23], high TNF α response to LPS [24], decreased cell mediated immunity [25], increased expression of scavenger receptor CD36 [26], elevated oxidative stress [27], augmented response to interferon γ [28], and increased expression of TNFR2 receptor [29] have been observed in mononuclear cells from type 2 diabetes patients with plasma glucose levels above 8.0 mM. It is not known whether these inflammatory alterations in mononuclear cells are already present in the early stages of the disease. In this study, we have analyzed the *in vitro* response of the TNF α system to a challenge with high glucose or endotoxin, in mononuclear cells isolated from non-diabetic individuals and from a selected group of recent onset type 2 diabetic patients with fasting glucose levels below 7.0 mM and glycated haemoglobin (Hb1Ac) in the normal range. We have measured the mRNA concentration of TNF α and its receptors TNFR1 and TNFR2 and, in the cell culture medium, the secreted protein concentration of soluble TNF α and related cytokine IL-6. We have found differences in the scope of the responses to high glucose or endotoxin, between monocytes isolated from non-diabetic or type 2 diabetic patients, suggesting that monocytes from those type 2 diabetic patients with fasting glucose levels below 7.0 mM are already in a different activation status.

2. Materials and methods

2.1. Subject selection

Patients with recent onset of type 2 diabetes, according to American Diabetes Association criteria, were recruited from diabetes outpatient clinic on the basis of a stable metabolic control in the previous 6 months, as defined by stable HbA1c values. They were treated with diet alone and were selected according to their metabolic control; those with fasting glucose below 7.0 mM and Hb1Ac below 6.2 % (normal range 3.9–6.2%) were considered for this study. No medication was taken for hypertension or dislipidemia by any of the recruited patient. Control non-diabetic subjects were recruited from the hospital staff and all had fasting plasma glucose <7.0 mM and 2-h post-load plasma glucose <11.1 mM after a 75 g oral glucose tolerance test. Eleven healthy individuals (11 woman) and 9 patients with type 2 diabetes mellitus were included (7 women and 2 men). The age of type 2 diabetic patients were 51 ± 3 years and had a body mass index (BMI) of 31.5 ± 2.8 kg/m² and non-diabetic control subject's age was 39 ± 3 years with a BMI of 23.5 ± 0.8 kg/m². All analyses were adjusted for age, gender and BMI. Thereafter, all the

glucose or endotoxin mediated effects are found not to be influenced by age or BMI.

All included subjects were free of any infections in the previous month before the study. Liver and renal diseases were specifically excluded by biochemical work-up. Individuals who had infectious diseases, inflammatory conditions or cardiac, renal, or pulmonary diseases or who were under medications were excluded from the study. Local Ethical Committee approval for this study and written informed consent from the individuals participating was obtained to perform the study.

2.2. Analysis of plasma metabolites

Blood samples were drawn after an overnight fasting, between 8:00 and 9:00 a.m. Plasma samples were stored at -80°C until analytical measurements were performed, except for glucose that was determined immediately. Glucose was measured with a glucose oxidase method using a Hitachi autoanalyzer. Lipid profile (triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL)) were measured by usual enzymatic methods. HbA1c was measured by the high-performance liquid chromatography method (Bio-Rad, Muenchen, Germany, and autoanalyzer Jokoh HS-10, respectively). Intra-assay and inter-assay coefficients of variation were less than 4% for all these tests. The plasma levels of TNF α were measured with a high sensitive ELISA assay (Biotrak System, Amersham Biosciences, Buckinghamshire, UK). Plasma IL-6 levels were measured by ELISA assay Quantikine HS high sensitivity assay (R&D systems, Oxon UK). Soluble fractions of TNF α receptors TNFR1 and TNFR2 were determined by solid phase sandwich enzyme-immunoassay with amplified reactivity (Bio Source technique according to commercial kit instructions (EASIA, Biosource Europe, Nivelles, Belgium). Insulin was measured using a specific immunometric assay (Medgenix Diagnostics, Fleunes, Belgium).

2.3. Cell culture and treatments

Peripheral blood mononuclear cells were isolated from 40 ml of blood by density gradient centrifugation in Histopaque 1077 (Sigma–Aldrich, Sant Louis, MO, USA), following manufacturer's instructions. After centrifugation, mononucleated cells were recovered and washed twice with PBS. The yield was approximately 10^6 cells/ml of blood. Cells were cultured in 6 well plates (Corning Costar, Acton, MA, USA), at a density of 1×10^7 cells/well in 3 ml of glucose free RPMI-1640 culture medium (Gibco, Invitrogen Corporation, CA, USA) supplemented with 5.5 mM glucose (SIGMA, cell culture tested), 10% heat inactivated Foetal calf serum (Gibco), 50 $\mu\text{g/ml}$ gentamicin (Sigma), 20 mM HEPES (Sigma) and 3 mM glutamine. Cells were incubated for 1 h at 37°C in a humidified 5% CO₂ atmosphere to allow for monocyte attachment. The non-adherent fraction (lymphocytes), also used in the experiment, was reseeded in 6

well plates. The attached cells (monocytes) were washed four times with PBS. The purity of the monocytic-enriched fraction was assessed by measuring the enrichment of CD14 gene expression. More than 87% of the monocytes in both groups were viable at the end of the incubations as determined by Trypan Blue exclusion. The number of cells attached in the wells after the incubations was similar for both controls and diabetic groups: 0.85 ± 0.07 and 0.79 ± 0.07 millions of cells per well, for controls and diabetics, respectively. The human monocytic cell line THP-1 was purchased from the European Collection of Cell Culture (Wiltshire, UK). THP-1 cells were cultured in the same medium and were differentiated with 160 nM phorbol 12-myristate 13-acetate (Sigma) for 24 h prior its use. Cells (THP-1, monocytes and lymphocytes) were cultured under the following conditions: 1 $\mu\text{g}/\text{ml}$ LPS from *Escherichia coli* 026:B26 or 5.5, 10 and 20 mM glucose at 37 °C for 10 h in a 5% CO₂ atmosphere incubator. Mannitol was added up to 20 mM to discard any unspecific osmotic action on cytokine expression. Under this culture conditions an ineffective insulin concentration (10 times below its affinity constant, from the foetal bovine serum) was present.

2.4. Real time PCR analysis of gene expression

Total RNA was extracted with the RNeasy[®] Mini Kit (Qiagen Science, MD, USA) following manufacturer's instructions and was treated with RNase-free DNase (Qiagen). RNA integrity was verified electrophoretically and by the OD₂₆₀/OD_{280nm} absorption ratio. RNA was reverse transcribed (RT) using Promega reverse transcription system (Promega Corporation Madison, USA), 20 μl of RT mixture contained: 1 \times RT-buffer (10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton[®]X-100), 1 mM of each dNTP, 1 u/ μl Recombinant RNAsin[®] Ribonuclease inhibitor, 15 u of AMV Reverse Transcriptase, 0.5 μg of Random primers. The cDNA reaction was incubated for 10 min at 25 °C followed by 60 min at 42 °C and heated 5 min at 95 °C. For real time PCR, primer pairs for TNF α , TNFR1, TNFR2, CD14 and β -actin were selected to span exon-exon junctions with the assistance of Primer3 primer design on line software (http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi). The sequences of the primers pairs were: gagcactgaaagcatgatcc and gctggttatctctcagctcca for TNF α ; cgggtggaagtccaagctcta and tctaggctctgtggctgtg for TNFR1; ggaaactcaagctgcactc and ggatgaagtcgtgtggaga for TNFR2; tgccgctgtgtaggaaagaa and gctcacaaggtctggcg for CD14; ggacttcgagcaagatgag and agcactgtgtggcgtacag for β -actin.

Standard curve quantifications [30] were performed with a LightCycler (Roche Diagnostics, Basel, Switzerland). The reactions in a final volume of 20 μl contained MgCl₂ adjusted for each gene, 0.5 μM of oligonucleotide primers (except 0.2 μM for β -actin) and 2 μl of DNA LC-FastStart DNA Master SYBR green I (Roche) containing Taq DNA polymerase, reaction buffer, dNTP mix and the double stranded DNA (dsDNA)-specific fluorescent dye SYBR green I. LightCycler

mastermix (18 μl) was filled in the LightCycler glass capillaries and 2 μl sample cDNA was added as PCR template. The following LightCycler protocol was used: denaturation program at 95 °C for 10 min, 45 cycles of denaturation at 95 °C for 10", annealing for 5" at 60 °C for TNF α , CD14; 66 °C for TNFR1 and β -actin. The annealing temperature for TNFR2 was 63 °C. The purity of each amplified PCR product was confirmed by agarose gel electrophoresis and melting curve analysis. For the preparation of standard curves, the corresponding PCR products of TNF α , TNFR1, TNFR2, CD14 and β -actin cDNAs were cloned in the vector pCRII-TOPO (TOPO TA cloning kit; Invitrogen Corporation, CA, USA), sequenced and linearized with *Eco* RV or *Bam* H1. Concentrations were calculated spectrophotometrically and standard curve solutions were stored in small aliquots. The concentrations of the experimental samples were calculated extrapolating from the corresponding standard curves run in parallel, with the instrument software using the Crossing point value parameter (LightCycler, Roche).

2.5. Analysis of TNF α and IL-6 in cell culture supernatants

TNF α was measured with a high sensitivity enzyme-linked immunosorbent assay kit (Amersham Biosciences, UK) the sensitivity of the assay was below 1 pg/ml. IL-6 was determined with a commercial kit (QuantikineR high sensitivity, RD systems, Minneapolis, USA) with a sensitivity below 0.1 pg/ml. Cell culture media was concentrated with AmiconR Ultra-4 filters (Millipore USA) only prior cytokine TNF α quantification of glucose treated cells.

2.6. Protein determination

Total protein from monocytic cells was measured by the method of Bradford with the BIO-RAD protein assay reactive (BIO-RAD, Germany).

2.7. Statistical analysis

Analyses were performed using the Statistical package SPSS Version 11.5. Results are expressed as the mean \pm S.E., unless specified otherwise. Data were analyzed by Student's *t*-test, and Mann-Whitney or Kruskal-Wallis tests for non-parametric distributions. All analyses were adjusted for age, gender and BMI. Therefore, all the glucose or endotoxin mediated effects are found not to be influenced by age or BMI. All *p*-values were two-sided and *p*-values <0.05 were considered statistically significant.

3. Results

In the present study, we have carefully chosen a group of type 2 diabetic patients with low levels of fasting glucose, Hb1Ac and inflammatory markers. On the day of mononu-

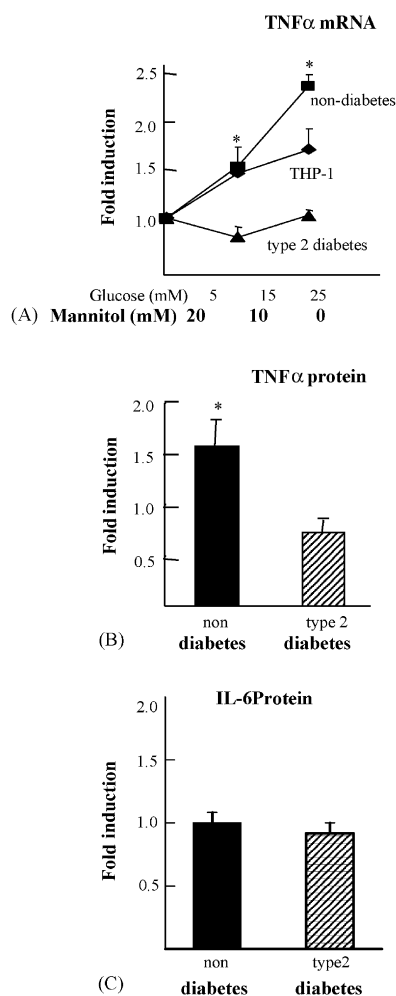


Fig. 1. Effect of high glucose on TNF α expression in monocytes of non-diabetic subjects and type 2 diabetes patients. (A) Fold induction with added glucose, relative to 5 mM glucose treatment. Symbols are: squares, monocytes from non-diabetic subjects; triangles, monocytes from type 2 diabetic patients and diamonds, monocytic cell line THP-1. The data are the ratios between TNF α and corresponding β -actin mRNAs. (B) Fold induction, relative to the incubation with 5 mM glucose, of the concentration of TNF α protein with 25 mM glucose in the cell culture media. (C) Fold induction, relative to the incubation with 5 mM glucose, of the concentration of IL-6 protein with 25 mM glucose in the cell culture media. * $p < 0.01$ compared to monocytes from type 2 diabetic patients.

clear cells isolation and culture, the average fasting glucose levels were 4.8 ± 0.2 mM for non-diabetic subjects group and 6.5 ± 0.1 mM for type 2 diabetic patients group (range 5.9–6.9 mM; $p < 0.001$), Hb1Ac for type 2 diabetic patients was $5.5 \pm 0.2\%$ (range 4.7–6.2). The plasma concentrations of TNF α , IL-6 and its receptors sTNFR1 and sTNFR2, leukocyte count, plasma levels of cholesterol, HDL-cholesterol and insulin were not significantly different from the corresponding ones in the non-diabetic group (data not shown). Triglycerides were significantly higher in the diabetic group (72 ± 9 mg/dl in non-diabetic versus 132 ± 15 mg/dl in type 2 diabetes group, $p < 0.003$).

3.1. High glucose increases TNF α expression only in primary cultures of monocytes from non-diabetic individuals, but not in primary cultures from type 2 diabetic patients

The effect of glucose treatment on TNF α gene expression was analyzed in primary cultures of monocytes and lymphocytes. The osmolarity of the different glucose incubations was kept constant with mannitol. Under these conditions, high glucose (25 mM) produced a small but significant upregulation of the TNF α mRNA expression and protein accumulation (Fig. 1A and B), in monocytes from non-diabetic individuals. Surprisingly, high glucose did not produce an upregulation of the TNF α mRNA or protein expression in primary cultures of monocytes isolated from type 2 diabetic patients (Fig. 1A and B). As a control, incubation with glucose of differentiated THP-1 cells produced an increased expression of TNF α mRNA, similar to what happened in the monocytic fraction of healthy subjects (Fig. 1A). No differences were observed in IL-6, a closely TNF α related cytokine, protein accumulation after high glucose treatment (Fig. 1C).

High glucose did not affect the mRNA expression of TNF α receptors TNFR1 and TNFR2 in monocytes isolated from non-diabetic or type 2 diabetic patients (Table 1). In isolated lymphocytes, the other mononuclear cell type analyzed, incubation with high glucose did not elicit any changes in the mRNA expression of TNF α or its receptors TNFR1 and TNFR2, in neither group (data not shown).

3.2. Activation of mononuclear cells with LPS endotoxin produced an enhanced response in monocytes isolated from type 2 diabetic patients

Contrary to the glucose treatment, which did not affect the levels of TNF α expression in type 2 diabetic monocytes, LPS significantly upregulated TNF α mRNA expression in primary cultures of monocytes isolated from non-diabetic subjects and patients with type 2 diabetic (Table 2). LPS did also significantly increase the mRNA expression of the TNF α receptor, TNFR2 in both groups (Table 2), but did not change the expression levels of TNFR1 (Table 2).

While LPS upregulated TNF α and TNFR2 expression in monocytes; in lymphocytes LPS did not affect the mRNA expression of TNF α and its receptors TNFR2 and TNFR1 in neither group (data not shown).

At protein level, LPS treatment of cultured monocytes from both non-diabetic subjects and type 2 diabetic patients produced an up regulation of TNF α protein over 100 times that of untreated controls (Fig. 2A). However, TNF α protein accumulation with LPS was significantly higher in primary cultures of type 2 diabetic monocytes, in comparison to the accumulation in primary cultures of non-diabetic subjects (Fig. 2A). The upregulation of related cytokine IL-6 protein by LPS treatment paralleled that of TNF α with an IL-6 increase over 100 times in both groups relative to untreated

Table 1

Glucose does not affect TNFR1 and TNFR2 mRNA expression in monocytes from non-diabetic subjects and type 2 diabetic patients

Treatment		Non-diabetic monocytes		Type 2 diabetic monocytes	
Glucose (Mm)	Mannitol (Mm)	TNFR2	TNFR1	TNFR2	TNFR1
5	20	1.1 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
15	10	0.9 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
25	0	1.1 ± 0.2	1.3 ± 0.2	0.9 ± 0.1	1.3 ± 0.1

Data are expressed as the logarithm of mRNA in femtograms.

Table 2

Similar effect of LPS treatment on mRNA expression of TNF α system components in monocytes from non-diabetic and type 2 diabetic subjects

Treatment, LPS (μ g/ml)	Non-diabetic monocytes			Type 2 diabetic monocytes		
	TNF α	TNFR2	TNFR1	TNF α	TNFR2	TNFR1
0	0.9 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.2 ± 0.2
1.0	1.9 ± 0.1 ^a	1.7 ± 0.2 ^a	1.1 ± 0.1	1.9 ± 0.2 ^a	1.6 ± 0.2 ^a	1.1 ± 0.1

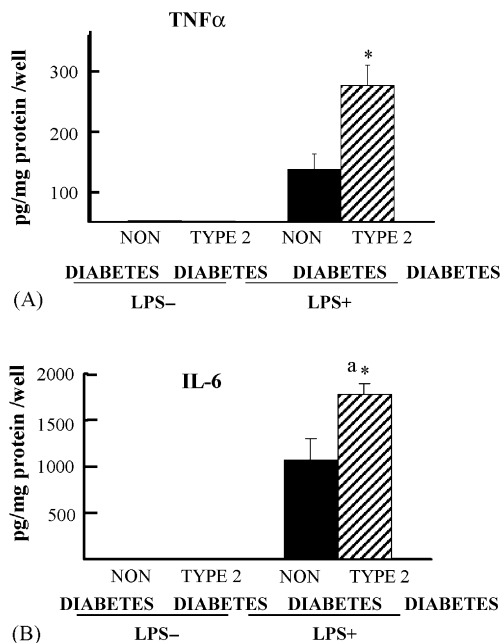
^a $p < 0.01$, LPS 1.0 μ g/ml vs. LPS 0 μ g/ml. Data are the logarithm of mRNA in femtograms.

Fig. 2. Effects of LPS incubation in the TNF α and IL-6 protein expression. (A) TNF α protein accumulation in primary cultures of monocytes from non-diabetic or patients with type 2 diabetes. TNF α protein concentrations without treatment with LPS were 0.66 ± 0.19 (range 0.1–1.5) and 0.08 ± 0.03 (range 0.01–0.25) pg/mg protein/well for non-diabetic and type 2 diabetes, respectively. (B) IL-6 protein accumulation. IL-6 protein values with out treatment with LPS were 2.5 ± 0.8 (range 0.3–7.7) and 1.6 ± 0.7 (range 0.2–6.1) pg/mg protein/well for non-diabetic and type 2 diabetes, respectively. * $p < 0.01$ and ^a $p < 0.031$ compared to LPS non-diabetic group.

controls (Fig. 2B), and with a significantly higher increase in the LPS treated type 2 monocytic culture when compared with the treated non-diabetic group (Fig. 2B).

4. Discussion

Although chronic inflammation in individuals with obesity and type 2 diabetic, occurs principally at metabolic

relevant sites such as liver, muscle and adipose tissue [31], mononuclear cells in a proinflammatory state have been found recently in obesity and in severe hyperglycaemic type 2 diabetic patients [32,33]. These findings are of great importance, as the proinflammatory status of circulating monocytes could impinge on the development of the atherosclerotic plaque when monocytes are recruited to the endothelial lesion. In addition to this proinflammatory status, several reports have described other alterations in monocytes from type 2 diabetic patients with poor glycemic control: reduced PKC activity and cell mediated immunity [23,25] and increased TNF α response to LPS, expression of scavenger receptor CD36, oxidative stress response to interferon γ and expression of TNFR2 [24,26–29]. In this study, we have found that, in comparison to non-diabetics, LPS endotoxin treatment produced a higher protein expression of TNF α and IL-6 proinflammatory cytokines in primary cultures of monocytes from recent onset type 2 diabetic patients with low fasting glucose levels (Fig. 2). Surprisingly, these supposedly activated type 2 monocytes did not increase TNF α mRNA and protein synthesis when acutely treated with high glucose during 10 h (Fig. 1). It is noteworthy that the lack of response to high glucose is taking place in the same set of conditions in which monocytes from non-diabetic individuals reacted as expected. Monocytes from non-diabetic subjects when treated with high glucose increased the expression of TNF α at mRNA and protein levels (Fig. 1) as it has previously been described in primary cultures of monocytes and THP-1 monocytic cells [18–20]. Despite differences in age and BMI between healthy and patients with type 2 diabetes, no effect of these variables on the above mentioned findings was observed. The possible mechanisms and implications of this lack of response of monocytes from type 2 diabetic patients will be discussed below.

Nevertheless, our observation of an increased in vitro accumulation of TNF α and IL-6 in monocytes from type 2 diabetic patients in comparison with non-diabetic individuals is in agreement with previous observations in more severe

type 2 diabetic patients and suggests that these monocytes are already in a proinflammatory status [24,33,34].

The response to LPS of the TNF α receptors TNFR1 and TNFR2 was identical in both monocytes from type 2 diabetic patients and the monocytes from the non-diabetic group. The non-diabetic group response to LPS was in agreement with what is already known, that LPS only increases the expression of TNFR2 [35].

Regarding the isolated lymphocyte fractions from both non-diabetic and type 2 diabetic patients, we have found that isolated lymphocytes are completely unresponsive to either LPS or glucose. In our monocytes depleted lymphocytes fractions, this observation agrees with LPS being a potent inducer of human T-lymphocyte proliferation and cytokine production only when direct cell-to-cell contact with accessory monocytes exists [36].

Before discussing the possible mechanisms and implications of this lack of response of monocytes from type 2 diabetic patients, it shall be noted that in our primary cultures of monocytes the response of TNF α to glucose in non-diabetic monocytes is well below the response to LPS (two-fold increase in TNF α protein accumulation with glucose versus over a 100-fold increase with the endotoxin) (Figs. 1 and 2). In a way, this observation mimics what happens in vivo in which the degree of inflammation among obesity and diabetic patients is well below the one produced by an acute infection [8]. What could be the mechanisms behind the relatively minor induction of TNF α expression by glucose in monocytes? In normal monocytes, both glucose and LPS act upon a similar overlapping set of molecular pathways. High glucose has been associated in monocytes with the activation of nuclear factor NF- κ B, PKC, mitogen activated protein kinase p38, extracellular regulated kinases (ERKs) and NADPH oxidase; in addition to the increased production of superoxide by the mitochondria and the metabolism of glucose to glucosamine [37–39,22,19]. A similar set of molecular pathways have been found to be activated by LPS and other endotoxins in human monocytes [40,41]. Nevertheless, the cellular mechanism of action of glucose and LPS could account for the difference in the degree of activation we have seen, since LPS cell actions are mediated by its binding to a specific membrane receptor that triggers an acute cell response [40]. Glucose, on the other hand, needs to be actively transported inside the cells by specific transporters to activate the different molecular pathways. The effects of high glucose in gene expression are at least in part mediated through the production of superoxide by the mitochondrial electron transport chain when glucose accumulates inside the cells in those tissues which are unable to keep a constant intracellular glucose concentration [2,3]. Part of the glucose effect in cell cultures of monocytes could be caused by an increased osmotic pressure [18]; to avoid this possibility we have kept the osmotic pressure constant in our primary cell cultures. In addition, different molecular pathways could be implicated. It is known that cells of monocyte-macrophage lineage can be induced to secrete

inflammatory cytokines with a variety of exogenous agents through several molecular pathways and at least two pathways induce TNF α in monocytic cells: one that is phorbol ester- or okadaic acid-dependent and one that is LPS-dependent [42,43].

On the other hand, how it can be explained that monocytes from patients with type 2 diabetes, in comparison with monocytes from non-diabetic individuals, showed an increased response to a LPS, suggesting an activated state, but had an impaired response to glucose? An impaired glucose uptake could be involved in the unresponsiveness towards glucose of the monocytes from type 2 diabetic patients. Defects in glucose uptake and metabolism (such as an overall impaired glucose removal from the circulatory system and an increased endogenous glucose production mostly in the liver) have been described in type 2 diabetes [44–46]. The step of glucose transport/phosphorylation is a likely candidate for the decrease in uptake [45]. At cellular level, an impaired glucose transport in type 2 diabetes have been found in muscle cells, with a reduction of around 40% in glucose uptake in the absence of insulin [47,48]. In monocytes from insulin dependent diabetes mellitus, a lower basal and insulin stimulated glucose transport rates have also been found [49]. Even if glucose transporters in human monocytes had been scarcely studied, the pattern of glucose transporters being expressed it is not fully resolved, and discrepancies still exist about insulin dependent GLUT 4 transporter expression [50–52], our finding of an impaired secretion of TNF α by glucose may point to a defective uptake or metabolism in these type 2 diabetic monocytes. Although an impaired glucose uptake could be partially responsible for the lack of response to glucose of the monocytes from type 2 diabetic patients, other possibilities may exist.

What could be the clinical significance of the differences in response to glucose of monocytes from control and type 2 diabetic individuals? A key role for activated monocytes in global insulin resistance has recently come out when it was found that deletion, only in myeloid cells, of the gene that codes for the transcription factor NF- κ B protected mice from high-fat-diet-induced insulin resistance in liver and muscle [17]. As many proinflammatory genes are induced by NF- κ B, this information suggests that the downregulation of inflammatory proteins in monocytes has a protective role in insulin resistance. A paracrine effect mediated through resident macrophages has been pointed out as the most likely mechanism for this protective effect because circulating inflammatory mediators were not altered [17]. Our observation that high glucose does not induce TNF α synthesis in monocytes from type 2 diabetic patients could be protective in these patients with fasting glucose levels below 7.0 mM and glycated haemoglobin (Hb1Ac) in the normal range. Glucose in comparison with LPS produces a very small change in TNF α production in monocytes, that will probably not be reflected in circulating levels of TNF α , as it happened after downregulating the production of inflammatory proteins in monocytes [17], and is in agreement with the absence of

differences in circulating levels of TNF α between diabetics and controls after a glucose load [53].

In conclusion, this study adds information to the growing body of reports about the differences between monocytes from normal and patients with type 2 diabetes, and does so in a population with a good metabolic control as close as possible to healthy individuals. The different reaction to endotoxin or glucose of monocytes from type 2 diabetic patients suggest a different role for immune system related LPS stimulus than for high glucose in the activation of monocytes in type 2 diabetic patients. Although the biological relevance of this phenomenon in the type 2 diabetes is not known at present (whatever the molecular mechanisms responsible for this kind of response), our observation could be important for the study of development of diabetic complications such as atherosclerosis.

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