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## Original Article

# Role of Peripheral Blood Mononuclear Cells in the Predisposition of Obese Individuals to Inflammation and Infection

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## Key Words

Obesity · Peripheral blood mononuclear cells · Cytokines · Inflammation · Infection

## Abstract

**Objective:** To compare the production of pro- and anti-inflammatory cytokines by peripheral blood mononuclear cells (PBMC) from obese but otherwise healthy individuals to that of normal-weight volunteers. **Methods:** 25 healthy normal-weight subjects and 41 obese individuals were enrolled. Weight and height were measured twice. PBMC were examined for their capacity to generate pro-inflammatory (TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-6, IL-2) and anti-inflammatory IL-10 and IL-1ra) cytokines. **Results:** PBMC from obese individuals, compared to those from subjects with normal weight showed an increased production of the pro-inflammatory cytokines IL-2 ( $6.7 \pm 0.4$  vs.  $4.9 \pm 0.3$  ng/ml;  $p = 0.003$ ), TNF- $\alpha$  ( $505 \pm 45$  vs.  $277 \pm 32$  pg/ml;  $p = 0.001$ ), and IFN- $\gamma$  ( $93.8 \pm 6.0$  vs.  $73.9 \pm 2.7$  ng/ml;  $p = 0.0016$ ). However, PBMC from obese individuals produced a lower amount of the anti-inflammatory cytokine IL-10 ( $651 \pm 72$  pg/ml) versus those from subjects with normal weight ( $951 \pm 133$  pg/ml;  $p = 0.039$ ). **Conclusions:** The findings imply that obese individuals are in a 'low-grade inflammatory state', presumed to be connected with metabolic and cardiovascular co morbidities. The surplus of pro-inflammatory cytokines produced by circulating mononuclear cells of obese individuals, together with those secreted by adipocytes and non-fat cells in the adipose tissue, may contribute to the predisposition of obese patients to inflammation and infections.

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## Introduction

Since the enduring spread of obesity and its co-morbidities is increasing at an alarming rate, its impact on human health presents a serious concern to health care providers [1]. The prevalence of obesity in adults of both genders in the USA in 2004 was 32.2% [2]. A cross-sectional survey carried out in Israel during 1999–2000 showed a similar incidence of 22.9% (19.9% for men and 25.8% for women, aged 25–64 years) [3]. The major complications of obesity include cardiovascular diseases and development of metabolic syndrome, including type 2 diabetes, malignancy, obstructive sleep apnea, and others [1, 4]. In addition, clinicians have paid attention to the increased susceptibility of obese individuals to infections compared to their normal-weight counterparts [5–8]. A similar trend has been observed in obese mice [9]. However, while the typical co-morbidities have drawn widespread attention, the increased risk of infections, as an obesity-associated complication, is still not completely elaborated. Karisson and Beck [5] have pointed out that obesity may affect negatively the immune system resulting in altered immune responses and susceptibility to infections. According to Bastard et al. [10] the innate immune system of obese subjects is persistently activated, a process that triggers generation of pro-inflammatory factors resulting in a low-grade inflammation of the white adipose tissue. Based on their T-cell source, cytokines are characterized as Th1 and Th2 types. Th1 cells produce IL-2, IFN- $\gamma$  and IL-12 capable to promote cellular immunity. Th2 cells are the source of IL-4 and IL-10, known to suppress cellular and to endorse humoral immunity. The pro-inflammatory cytokines, i.e. TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra), are produced mainly by macrophages. The linkage between obesity and chronic low-grade inflammation has been supported by others [11, 12]. Lumeng and Saltiel [12] have thoroughly reviewed the factors promoting obesity-induced inflammation, such as altered immune responses, leukocyte activation, and increase in acute phase proteins. In this review and in other studies, macrophages infiltrating the fatty tissues and producing pro-inflammatory molecules have been shown to be an important issue in the etiology of chronic inflammation observed in obese individuals [13–15]. While these studies focus on the function of adipocytes and tissue macrophages, the aim of the present study was to assess the role of peripheral blood mononuclear cells (PBMC) from obese but otherwise healthy individuals in production of pro- and anti-inflammatory cytokines and to compare it to that of normal-weight healthy volunteers.

## Subjects and Methods

The Rabin Medical Center-Human Studies Committee approved the study. 25 healthy normal-weight adult subjects, (21 females, and 4 males) and 41 obese individuals (32 females, 9 males) were included in the study. The participants from both groups were in a good general condition, they denied any complaints, and their family history was not contributory. Except for their everyday activities, the participants were not engaged in any additional physical exercises. Weight and height were measured twice without shoes and in light clothing. In the presence of a difference of 0.5 cm and 0.5 kg in height and weight, respectively, a third measurement was carried out. Table 1 shows that there was no noticeable difference in age, height, and blood pressure between individuals from both groups. On the other hand, their body weight, BMI value, and waist circumference differed significantly. The relevant serum biochemistry examinations are presented in table 2. Two obese individuals showed elevated fasting blood glucose (132 and 140 mg/dl) without any complaints suggestive for diabetes.

### *Cell Preparation and Culture Conditions*

PBMC were isolated from heparinized venous blood using lymphoprep gradient centrifugation. The cells were washed twice in phosphate buffered saline (PBS) and suspended in new-born calf serum (Biological Industries, Beith Haemek, Israel) supplemented with 10 % dimethyl sulphoxide (DMSO) Sigma-

**Table 1.** Demographic data of individuals from the two groups

	Non obese (n = 25)		Obese (n = 41)		p value
	range	mean ± SD	range	mean ± SD	
F/M ratio	21/4		32/9		
Age, years	20–53	33.1 ± 9.7	28–54	40.4 ± 7.1	0.001
Body weight, kg	45–78	55.1 ± 9.8	69–125	93.7 ± 12.8	<0.0001
Height, m	1.5–1.76	1.64 ± 0.07	1.5–1.84	1.63 ± 0.07	NS
BMI	17.8–25	20.95 ± 2.4	30.1–48.28	35.2 ± 4.0	<0.0001
Waist circumference, cm	63–92	74.8 ± 8.9	86–124	108.4 ± 9.0	<0.0001
SBP, mm Hg	93–152	113 ± 12.4	102–149	126.5 ± 11.3	<0.0001
DBP, mm Hg	53–95	69.7 ± 9.7	63–92	79.4 ± 8.0	<0.001

SBP = Systolic blood pressure; DPP = diastolic blood pressure; NS = not significant.

**Table 2.** Serum biochemistry of individuals from the two groups

	Non obese (n = 25)		Obese (n = 41)		p value
	range	mean ± SD	range	mean ± SD	
Glucose, mg/dl	72–112	90.1 ± 8.3	69–140	92.4 ± 17.2	NS
Cholesterol, mg/dl	154–252	190.3 ± 30.7	127–289	189.7 ± 31.4	NS
Triglycerides, mg/dl	41–430	91.2 ± 75.7	52.3 ± 46.0	142.0 ± 77.8	0.0128
HDL, mg/dl	32.2–94.3	63.6 ± 13.9	28.1–66.8	44.1 ± 9.3	<0.0001
LDL, mg/dl	63–174	108.5 ± 29	66–149	117.3 ± 22.6	NS

NS = Not significant.

Aldrich, Rehovot, Israel). Cell suspensions were frozen at  $-75^{\circ}\text{C}$  until assayed for cytokine secretion. On the day of assay, the cells were rapidly thawed and suspended in RPMI-1640 medium containing 1% penicillin, streptomycin and nystatin, and supplemented with 10% fetal calf serum, designated as complete medium (CM).

#### *Cytokine Production*

$2 \times 10^6$  PBMC suspended in 1 ml CM were incubated for 24 h with 20 ng/ml lipopolysaccharide (LPS, *E. coli*, Sigma) to determine the secretion of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-1ra and IL-10. To evaluate IL-2 and IFN- $\gamma$  production the cells were incubated for 48 h with 1  $\mu\text{g}/\text{ml}$  of phorbol myristate acetate (PMA) and 0.5  $\mu\text{g}/\text{ml}$  of ionomycin (both from Sigma-Aldrich). The plates were incubated at  $37^{\circ}\text{C}$  in a humidified atmosphere containing 5%  $\text{CO}_2$ . At the end of the incubation period, the culture media were collected, the cells were removed by centrifugation, and the supernatants were kept at  $-75^{\circ}\text{C}$  until assayed for cytokine content.

#### *Cytokine Content in the Supernatants*

The concentration of cytokines in the supernatants was tested using ELISA kits specific for human cytokines (Biosource International, Camarillo, CA, USA) as detailed in the guideline provided by the manufacturer. The detection level of all cytokines was 30 pg/ml.

#### *Statistical Analysis*

Data was analyzed using two tailed, independent Student's t-test. The results are expressed as mean  $\pm$  SEM. A p value  $< 0.05$  was considered as statistically significant.

**Table 3.** Cytokine production by PBMC from non-obese and obese individuals

Cytokines	Non-obese (n = 25)	Obese (n = 41)	p value
<i>Pro-inflammatory</i>			
TNF- $\alpha$ , pg/ml	277 $\pm$ 31	505 $\pm$ 45	0.0006
IFN- $\gamma$ , ng/ml	74.0 $\pm$ 2.65	93.1 $\pm$ 6.0	0.019
IL-1 $\beta$ , ng/ml	3.94 $\pm$ 0.34	3.78 $\pm$ 0.28	0.7299
IL-6, ng/ml	15.63 $\pm$ 2.32	13.53 $\pm$ 1.13	0.3648
IL-2, ng/ml	4.92 $\pm$ 0.34	6.66 $\pm$ 0.38	0.0029
<i>Anti-inflammatory</i>			
IL-10, pg/ml	952 $\pm$ 127	651 $\pm$ 73	0.04
IL-1ra, pg/ml	930 $\pm$ 99	895 $\pm$ 52	0.264

## Results

### *Pro-Inflammatory Cytokines (Table 3)*

The secretion of TNF- $\alpha$  by PBMC of the obese patients was significantly higher than that of the controls (505  $\pm$  45 pg/ml vs. 277  $\pm$  31 pg/ml; p = 0.0006). Similarly, IFN- $\gamma$  production by PBMC of obese patients was significantly higher than that produced by cells of control individuals (93.1  $\pm$  6.0 vs. 74.0  $\pm$  2.65 ng/ml; p = 0.019).

The secretion of IL-1 $\beta$  and that of IL-6 by PBMC of the obese individuals did not differ significantly from that of healthy subjects (3.67  $\pm$  0.29 vs. 3.94  $\pm$  0.32 ng/ml for IL-1 $\beta$ ; p = 0.7299, and 13.53  $\pm$  1.13 vs. 15.63  $\pm$  2.32 ng/ml for IL-6; p = 0.3648).

The production of IL-2 by PBMC from obese individuals was significantly higher than that of cells from the control group (6.66  $\pm$  0.38 vs. 4.92  $\pm$  0.34 ng/ml; p = 0.0029)

### *Anti-Inflammatory Cytokines (Table 3)*

The capacity of PBMC from obese subjects to produce IL-10 was significantly lower than that of cells from normal-weight individuals (651  $\pm$  73 vs. 952  $\pm$  127 pg/ml; p = 0.04). As for IL-1ra secretion by cells of obese subjects, it did not differ significantly from that of the controls (895  $\pm$  52 vs. 930  $\pm$  99 pg/ml; p = 0.264).

## Discussion

The results of the present study demonstrate that PBMC from obese individuals, compared to those from subjects with normal weight, show an increased secretion of the pro-inflammatory cytokines TNF- $\alpha$ , IFN- $\gamma$  and IL-2 as well as a lower production of the anti-inflammatory cytokine IL-10. These findings suggest that obese individuals are in a state of a concealed inflammation, designated as 'low-grade inflammatory state', a condition presumed to be connected with the metabolic and cardiovascular co-morbidities of obesity [11–15]. Moreover, this inflammatory process may predispose obese individuals to infections that might be promptly activated by triggers that would not affect the immune system of non-obese subjects. It is notable, that the secretion of the pro-inflammatory cytokines IL-1 $\beta$  and IL-6 and that of the anti-inflammatory cytokine IL-1ra by PBMC of the two groups did not differ significantly. It is conceivable that in the absence of a potent trigger, the PBMC from obese individuals will not produce the all array of pro- and anti-inflammatory cytokines. The factors accountable for the predisposition of obese individuals to infections are not understood completely. It has been reported that obese individuals, compared with normal-weight controls, have decreased NK and CD8+ cells, as well as altered CD4+ cell numbers with a

consequent increase in pro-inflammatory cytokine production [5]. In the present study, the Th1/Th2 balance in the group of obese individuals shifted from Th2 dominance towards a Th1 profile as indicated by a decrease in IL-10 production and an increased IL-2 and IFN- $\gamma$  secretion. It is noteworthy that the white adipose tissue itself may produce both pro- and anti-inflammatory cytokines with a consequent modification of the immune functions in obese patients [16]. A distinctive role in maintaining chronic inflammation in obese persons has been attributed to macrophages infiltrating the white adipose tissue [10, 14–17]. Moreover, studies have shown the existence of a correlation between an increased number of adipose-infiltrating macrophages and obesity. On the other hand, weight reduction has brought to a diminution of macrophage infiltration and improved immune competence [10, 18]. Studies in mice suggest that adipose-tissue macrophages originate in the bone marrow [17]. Canello and Clement [16] have reviewed in details the mechanisms by which these macrophages infiltrate adipose tissue and the way by which they contribute to the process of 'low-grade inflammation'. According to the authors, this process depends on paracrine, autocrine, and endocrine signals as well as on various cytokines, including leptin. In our study, the altered cytokine production detected in obese individuals, expressed by an increase of pro-inflammatory cytokines and a decrease of the anti-inflammatory IL-10, was observed while using PBMC. There are two ways to explain this observation – either the immune cells have been activated in the circulating blood by inflammatory molecules and metabolites in the vessels of obese individuals as it has been previously suggested [16], or their activation occurs in the adipose tissue resulting in an altered immune surveillance. It has been shown that the adipose tissue itself is a potent source of a considerable number of pro-inflammatory cytokines, such as TNF- $\alpha$ , IL-6, IL-8, IL-10, IL-17, contributing to the development of insulin resistance as well as to morbidity and mortality in obese patients [19–23]. Moreover, pro-inflammatory cytokines have been detected not only in the circulating blood but also in the follicular fluid of infertile obese women [24]. It is noteworthy that, although the adipose tissue itself is a resource for a large number of cytokines, many of them, excluding leptin and adiponectin, are produced by non-fat cells infiltrating the adipose tissue [25, 26].

In short, the present study demonstrates that PBMC from obese individuals produce more pro-inflammatory and less anti-inflammatory cytokines than those of normal-weight subjects. It is suggested that the alteration of the immune response of PBMC from obese persons is acquired either during the process of infiltration and stopover of the cells in the fat tissue or by products released from the adipose tissue into the circulation. The excess of pro-inflammatory cytokines produced by adipocytes and non-fat cells in the fatty tissue and those produced by circulating mononuclear cells may contribute to the predisposition of obese patients to inflammation and infections.

### Disclosure Statement

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