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On the impaired immune function of mononuclear cells in obesity-who is to be blamed?

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ABSTRACT

Background: Alterations of the immune system in obese individuals have been well documented. Studies have shown that peripheral blood mononuclear cells (PBMC) are closely associated with the tendency to inflammation and infections observed in obese, otherwise healthy persons, in part because of their increased capability to produce inflammatory cytokines. The aim of the study was to elucidate the question if this phenomenon is innate or it is acquired during their contact with the fatty tissue and/or abnormal substances in the serum of obese individuals. **Methods:** PBMC from normal-weight individuals were incubated for 24 hours with increasing concentrations of sera from obese subjects and from healthy normal weight individuals their capacity for inflammatory cytokines production was detected and compared. **Results:** While the effect of low concentrations of sera from obese and normal weight individuals on cytokine production by normal PBMC was similar, higher concentrations induced increased secretion of the pro-inflammatory cytokine IL-1β, and a decreased production of the ant-inflammatory cytokine IL-10. The secretion of the remaining cytokines examined was not affected. **Conclusions:** This observation suggests that the capability PBMC from obese patients to produce increased levels of pro-inflammatory cytokines is probably acquired.

KEYWORDS

PBMC;
Cytokines;
Obesity;
Inflammation.

INTRODUCTION

The rapidly increasing number of over-weight individuals, those with obesity without or with co morbidity conditions, such as type 2 diabetes, hypertension, cardiovascular diseases, and even malignancy[1] is of great concern for the medical care providers, since it presents a serious health financial burden[2,3]. Furthermore, the linkage between obesity, predisposition to inflammation and infections has been well documented[4] indicating the existence of an altered immune function in obese individuals. A sizeable number of studies have focused on various targets for explanation of its occurrence, such as the impact of the adipose tissue itself[5], release of pro-inflammatory molecules from the adipose tissue-mainly adipokines and cytokines[6], in-

creased production of TNFα by mononuclear cells[7] and even the effect of leptin, which, although being an anti-obesity hormone, may impair immune functions by activating monocytes for cytokine production[8]. The role of the mononuclear cells in the modification of inflammatory processes in obesity has been well established. It is expressed not only by the imbalance in their cytokine production, but also by their strongly correlation of their number with the BMI and body fat[4,9]. According to Womack et al.[10] individuals with morbid obesity showed the most expressed association with both total white blood and immune cell counts. On the contrary, a weight loss by 5% in obese women caused a significant improvement of the mononuclear cells pro-inflammatory state[11]. We have reported that PBMC from obese individuals, compared to those of normal weight subjects show an increased production of pro-inflammatory cytokines and reduced secretion of anti-inflammatory ones[12]. The question arises if the altered cytokine production by PBMC in obese individuals is due to a reprogramming of their immune function occurring during their passage through the obese tissue, or it is a result of a cellular innate defect. Therefore, it was the purpose of the present work to make an attempt to clarify the issue by examination of inflammatory cytokine production following incubation of PBMC from normal weight individuals with sera obtained from obese subjects as compared with sera from healthy normal weight individual.

SUBJECTS AND METHODS

The Rabin Medical Center-Human Studies Committee approved the study. Thirteen healthy normal weight adult subjects (BMI < 25, 9 females, and 4 males) and 19 obese, but otherwise healthy individuals (BMI > 30, 16 females, 3 males), were included in the study after signing an inform consent. The participants in both groups were in a good general health they denied any complaints and were not under any medication. Their family history was unremarkable. Weight and height were measured twice without shoes and in light clothing. All participants underwent the following biochemistry tests: liver and kidney function tests, electrolytes, glucose, lipid profile and blood counts.

Cell preparation and culture conditions

PBMC were separated from venous blood obtained from adult blood bank donors by gradient centrifugation using Lymphoprep-1077 (Axis-Shield PoC AS, Oslo, Norway). The cells were washed twice in phosphate buffered saline (PBS) and suspended in RPMI-1640 medium (Biological Industries, Beith Haemek, Israel) containing 1% penicillin, streptomycin and nystatin.

Serum collection

Serum was separated from whole blood of obese and non-obese individuals within 1 hr from blood withdrawal using serum separator tubes. The sera were stored at -75°C until assayed for their effect on cytokine secretion.

Cytokine production

$2x10^6$/ml of PBMC suspended in RPMI-1640 were incubated for 24 hrs with 10 ng/ml lipopolysaccharide (LPS, E. coli, Sigma) to determine the secretion of TNFα, IL-1β, IL-6, IL-1ra and IL-10. To evaluate IL-2 and IFNγ production the cells were incubated for 48 hrs with 0.5μg/ml of phorbol meristate acetate (PMA-Sigma, Israel) and 0.25 μg/ml of ionomycin (Sigma, Israel). The sera were added at the onset of the cultures at a final concentrations between 2.5% and 75%. Separate incubation was performed for each patient's serum. None of the sera were pooled. The plates were incubated at 37°C in a humidified atmosphere containing 5% 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°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) as detailed in the guide-line provided by the manufacturer. Each kit is specific for one individual cytokine. The detection level of all cytokines was 30 pg/ml. The percentage of the CV of the ELISA assay for the cytokines examined was 2% to the mean or less.

Statistical analysis

Data was analyzed using two tailed, independent Student's t-test. The results are expressed as mean ± SEM. One way ANOVA test was used to evaluate the effect of serum concentration on the on cytokine production. P value <0.05 was considered as statistically

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significant.

RESULTS

Demographic and biochemical data

There was no noticeable difference in height and blood pressure between individuals from both groups (TABLE1). The participants in the control group were younger (34.3±2.8 vs 42.9±1.5 years, respectively, p=0.006) and their body weight, BMI value and waist circumference were significantly lower from those of obese individuals (p<0.000). The relevant serum biochemistry examinations are presented in TABLE 1. One obese individual showed elevated fasting blood glucose (140 mg/dL) without any complaints suggestive for diabetes. Except for HDL values being 27.3% lower in the obese group (p=0.0004), there was no significant difference in blood biochemistry.

Pro-inflammatory cytokines at low serum concentration (TABLE 2)

Incubation of LPS stimulated PBMC with increasing concentrations of serum from individuals in both groups (between 2.5% and 10%) caused a dose dependent enhancement in the secretion of TNFα (p=0.028), the difference being not significant. However, increasing concentrations of sera from participants in both groups (2.5%-10%) had no effect on the pro-inflammatory cytokines IL-1β or IL-6 production (p=0.42, p=0.39, respectively) and the anti-inflammatory cytokines IL1-ra and IL-10 (p=0.12, p=0.89, respectively) by LPS stimulated PBMC. PBMC stimulated with PMA and ionomycin exposed to 2.5% and 10% serum showed a dose dependent reduction in IL-2 and IFNγ secretion (p<0.001) without any significant difference between the two groups.

TABLE 1 : Demographic data of individuals from the two groups

	Non obese (n=13)		Obese (n=19)		P value
	Range	Mean ± SD	Range	Mean ± SD	
Age, years	20-53	34.3±2.8	33-54	42.9±1.5	0.006
Body weight, kg	54-75	59.5±2.7	76-114	94.3±2.6	<0.001
Height, m	1.55-1.76	1.63±0.02	1.55-1.72	1.63±0.01	NS
BMI	20-24.6	22.1±0.7	30.1-48.3	35.7±1.1	<0.001
Waist circumf., cm	71-92	79.0±2.3	93-124	109.5±2.2	<0.001
SBP, mm Hg	105-152	118±3.6	105-147	125±2.8	NS
DBP, mm Hg	53-95	72±3.2	63-92	78.6±2.2	0.074
Glucose, mg/dL	78-112	91.8±2.6	72-140	94.6±3.9	NS
Cholesterol, mg/dL	155-246	206±8.4	127-153	186.7±7.0	0.083
Triglycerides,mg/dL	50-430	115.8±27.5	81-460	128.4±8.0	NS
HDL, mg/dL	62.2-89.9	59.4±3.9	31.5-62.7	43.2±2.1	0.0004
LDL, mg/dL	88-174	123.9±7.7	66-143	114.3±5.3	NS

P value- comparing between the two groups; BMI- Body mass index; SBP-Systolic blood pressure; DPP-Diastolic blood pressure

TABLE 2 : Cytokine production by PBMC treated with lower concentrations of sera from non-obese and obese individuals.

	Non-obese			Obese		
Serum concentration	2.5%	5%	10%	2.5%	5%	10%
TNFα, ng/ml	1.18±0.08	1.36±0.08	1.47±0.10	1.22±0.08	1.42±0.77	1.44±0.14
IFNγ, ng/ml	61.4±2.6	57.7±2.5	49.4±4.7	61.2±2.3	59.4±2.6	49.0±3.2
IL-1β, ng/ml	13.0±0.8	13.0±0.5	12.5±0.7	13.6±1.1	13.1±0.9	12.1±0.7
IL-6, ng/ml	56.0±4.0	55.3±4.1	50.3±4.4	58.7±5.0	53.6±2.8	53.1±4.6
IL-2, ng/ml	54.4±3.6	51.8±5.3	37.0±5.2	59.6±3.7	54.5±3.6	34.1±2.7
IL-1ra, ng/ml	2.1±0.24	1.98±0.04	1.77±0.07	1.83±0.08	1.80±0.07	1.74±0.08
IL-10, ng/ml	1.11±0.23	1.10±0.21	1.01±0.19	1.26±0.21	1.25±0.19	1.19±1.62

The results are expressed as Mean ± SEM. A comparison the effect of the same serum concentrations between the two groups did not show any statistical difference.

Anti-inflammatory cytokines at low serum concentrations

The secretion of IL-10 or IL-1ra by LPS-stimulated PBMC was not affected by addition of 2.5 and 10% serum (p=0.89, p=0.12, respectively). There was no significant difference in the secretion of the two anti-inflammatory cytokines when PBMC were exposed to sera from obese subjects or from normal weight individuals (TABLE 2).

Pro- and anti-inflammatory cytokines at high serum concentrations

The results are shown in TABLE 3. Among the pro-inflammatory cytokines tested, higher level of IL-1β production was found when LPS-stimulated PBMC were incubated with 75% serum from obese individuals as compared with that of non-obese persons (24% increase, p=0.026). The production of TNFα, and IL-6 was similar in the presence of high concentrations of serum derived from individuals from the two groups. On the other hand the secretion of the anti-inflammatroy cytokine IL-10 by LPS-stimulated PBMC was significantly lower by 43% when the cells were incubated with either 50% or 75% serum from obese persons as compared with non-obese ones (p=0.03, p=0.015 respectively).

NF-kappaB binding to DNA and transcription of pro-inflammatory genes regulated by NF-kappaB. On the other hand, the inhibitor of NF-kappaB-beta (IkappaB-beta) was decreased. Teran-Cabanillas et al.[14] have found elevated level of the pro-inflammatory cytokine IL-6 produced by PBMC from obese patients. Lacasa et al.[15] have treated preadipocytes with macrophage-conditioned medium and observed a decline in adipogenesis, as well as an increased production of pro-inflammatory cytokines. It is notable that the alteration in number and activation of CD14(++) monocytes has been detected in obese children aged 6-16 years[16], as well as those with dyslipidemia[17]. Tam et al.[18] reported that while at the age of eight there was no difference in serum cytokine levels between obese and normal weight children, in fifteen- year-old girls, the IL-6, IL-8 and IL-10 serum levels were higher compared with those of girls with normal weight. Conversely, studies with PBMC from obese young adults with a BMI of 28.3 did not reveal a significant difference in inflammatory cytokine production compared to lean subjects[19].

The results of the present study indicate that the capacity for cytokine production by PBMC from normal weight individuals was not affected differently following incubation with low concentrations of either normal, or obese individuals' sera. However, at higher concentrations, serum of obese individuals caused a significant increase of the pro-inflammatory cytokine IL-1β. This finding suggests the existence of noxious factor(s) in the sera of obese individuals whose capacity to affect inflammatory cytokines production becomes evident only at high concentrations. The fact that weight loss in obese women correlated with a significant improvement of the pro-inflammatory state of their mono-

TABLE 3 : Cytokine production by PBMC treated with higher concentrations of sera from non-obese and obese individuals.

	Non-obese	Obese	P value	Non-obese	Obese	P value
Serum conc.	**50%**			**75%**		
TNFα, ng/ml	1.43±0.11	1.35±0.06	NS	1.12±0.06	1.18±0.06	NS
IFNγ, ng/ml	57.3±6.4	59.2±0.11	NS	NE	NE	
IL-1β, ng/ml	10.5±0.6	10.5±0.4	NS	15.8±1.07	19.56±0.76	0.026
IL-6, ng/ml	41.4±0.9	43.0±1.4	NS	48.5±3.2	51.9±3.1	NS
IL-2, ng/ml	10.3±1.0	12.2±2.5	NS	NE	NE	
IL-1ra, ng/ml	1.05±0.09	1.07±0.07	NS	1.24±0.12	1.24±0.16	NS
IL-10, pg/ml	457±84	260±29	0.03	677±95	391±56	0.015

The results are expressed as Mean ± SEM; NS- not significant. NE-not examined. P values express the difference at the same serum concentrations between the two groups.76

DISCUSSION

The evidence for existence of a relationship between the immune function of PBMC and a pro-inflammatory state in obesity has been well documented. In a study reported by Ghanim et al.[13] it was shown that mononuclear cells from obese individuals express an increased

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nuclear cells[12] supports this assumption. However, since there is no way to examine in vivo the immune function of the PBMC from obese individuals before and after their contact with the serum, as well as before and after their passage through the fatty tissue, it is difficult to definitely conclude if the altered function of the PBMC in obese individuals is innate or acquired. It is noteworthy, that at the same concentrations of sera from obese subjects the production of the anti-inflammatory cytokine IL-10 was decreased. We have demonstrated that PBMC from obese, but otherwise healthy individuals, showed a marked increase in the production of pro-inflammatory cytokines, such as IL-2, TNFα and IFNγ, while the secretion of the anti-inflammatory cytokine IL-10 was lower[12]. The interpretation of these observations leads to the possibility that the immunological alterations observed in PBMC from obese individuals may be acquired during their passageway through the fatty tissue and their extensive contact with pathological components in the peripheral blood[20]. One may argue why in the present work only IL-1β was increased, while the production of the other pro-inflammatory cytokines examined, remained unaffected. This phenomenon may be explained by the fact that the control PBMC were incubated with sera from obese individuals for 24 hours, a time period not comparable with the conditions occurring in vivo. The increased IL-1β production by PBMC that occurred only after incubation with higher concentration of sera from obese subjects sustains this assumption. This concept is further supported by the report of O'Rourke et al.[21] who have found that there is not any correlation between the serum and PBMC cytokine levels in obese individuals and suggested that PBMC may not be the source of modified cytokine production in obesity. The question, what causes immunological PBMC activation in obese persons is intriguing. Studies have shown that in this sense, a number of factors may be involved. In a review on the subject Cancello and Clement[20] emphasize the role of close interaction between the macrophages infiltrating the adipose tissue as a reason for increased production of inflammatory cytokines. According to Weisberg et al.[22] adipose tissue macrophages are almost the main source for TNF-α and Il-6 expression, both being pro-inflammatory cytokines. Furthermore, in vitro studies have shown that mononuclear cells interacting with adipocytes stimulated IL-6 production by the later cells[23]. Exposure of immune cells and neutrophils to sex steroids, cholesterol and low density lipoproteins have been further implied in the up regulation of inflammatory cytokines in obesity[24,25]. In addition, the role of leptin, has been stressed as an important factor for modulation of monocyte function in obesity and particularly-generation of the pro-inflammatory cytokines IL-6 and TNF-α[26,27].

In short, the results of the study indicate that the capability of PBMC from obese patients to produce increased level of pro-inflammatory cytokines is probably acquired during their contact with both the fatty tissue and abnormal peripheral blood ingredients.

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