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Impaired Mononuclear Cell Immune Function in Extreme Obesity is Corrected by Weight Loss

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ABSTRACT

Background: Obesity is associated with an increased prevalence and severity of infections. The mechanism(s) responsible for the increased risk of infections is unclear. We evaluated the effects of excessive adiposity and weight loss on peripheral blood mononuclear cell (PBMC) chemokine (macrophage chemoattractant protein-1 [MCP-1] and cytokine (interferon- γ [IFN- γ]) production, which is an important component of the immune response to infectious pathogens. **Methods:** Lipopolysaccharide (LPS)- and phorbol 12-myristate 13-acetate plus ionomycin (PMA+I)-stimulated PBMC MCP-1 and IFN- γ production were determined in six extremely obese subjects (body mass index [BMI] = 62.4 ± 8.6 kg/m²) before and 1 year after gastric bypass surgery and in six age-matched lean subjects (BMI = 22.7 ± 1.4 kg/m²). **Results:** At baseline, LPS-stimulated MCP-1 production and PMA+I-stimulated IFN- γ production by PBMCs were $93.6\% \pm 4.9\%$ and $88.8\% \pm 9.6\%$ lower, respectively, in obese than in lean subjects ($p < 0.03$). Obese subjects lost $30.3\% \pm 10.6\%$ of their body weight at 1 year after gastric bypass surgery ($p < 0.001$). Weight loss completely restored LPS-stimulated MCP-1 production and PMA+I-stimulated IFN- γ production in obese subjects to normal. **Conclusions:** Agonist-stimulated production of IFN- γ and MCP-1 are markedly suppressed in subjects with extreme obesity. Weight loss completely normalizes the ability of stimulated PBMCs to produce MCP-1 and IFN- γ . These findings could have important implications in understanding the increased risk of infections associated with obesity, and demonstrate a unique beneficial effect of weight loss on immune function.

INTRODUCTION

OBESITY IS ASSOCIATED WITH an increased prevalence and severity of local and systemic infections. Obesity increases the risk of nosocomial systemic and wound infections after general,¹⁻⁵ gastrointestinal,⁶ cardiac,^{7,8} organ transplantation,⁹ and knee replacement surgery,¹⁰ and after serious burn injury.¹¹ The

mechanism(s) responsible for the increased risk of infections is not known, but likely involve alterations in immune system function, including impairment of polymorphonuclear and natural killer cells killing function, and lower capacity of lymphocytes to proliferate in response to mitogen activation.¹²⁻¹⁴

Cytokines and chemokines, produced by activated circulating mononuclear cells, help di-

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rect the immune response needed for optimal protection against foreign pathogens. Chemokines, such as macrophage chemoattractant protein-1 (MCP-1), are essential for accumulating and activating neutrophils and macrophages at infectious foci, whereas cytokines, such as interferon- γ (IFN- γ), increase the ability of macrophages and cytotoxic T cells to kill infectious pathogens.¹⁵ Mice deficient in MCP-1 or IFN- γ are killed by normally sublethal doses of bacteria and viruses.^{16,17}

The purpose of the present study was to evaluate the hypothesis that peripheral blood mononuclear cell (PBMC) immune function in extremely obese adults is impaired, but that weight loss can improve defective PBMC activity. Lipopolysaccharide (LPS)-stimulated and phorbol 12-myristate 13-acetate plus ionomycin (PMA+I)-stimulated PBMC cytokine and chemokine production were determined in lean subjects and in extremely obese subjects before and 1 year after marked weight loss induced by gastric bypass surgery.

MATERIALS AND METHODS

Study subjects

Six women with class III obesity (body mass index [BMI] ≥ 40 kg/m²) and six age-matched lean women participated in this study. Subjects completed a comprehensive medical evaluation, which included a history and physical examination, an electrocardiogram, and standard blood and urine tests. All subjects reported that they were weight stable ($\leq 2\%$ change in body weight) and sedentary (< 1 hour regular exercise per week) for at least 2 months before baseline studies were performed. Each subject provided written, informed consent before participating in this study, which was approved by the Human Studies Committee and the General Clinical Research Center (GCRC) Scientific Advisory Committee of Washington University School of Medicine in St. Louis, Missouri.

Experimental protocol

After subjects fasted overnight (12 hours), blood samples were obtained by venipuncture from an antecubital vein. Blood samples were

taken from the lean group on one occasion, and from the obese group before and 1 year after marked weight loss, induced by gastric bypass surgery. Peripheral blood was taken directly into sterile ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes. Mononuclear cells were isolated by using Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) density gradient centrifugation, washed two times in pyrogen-free saline, resuspended in RPMI 1640 (supplemented with 0.3 mg/mL L-glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin) with 10% sterile homologous human serum, and seeded in flat-bottom multiwell dishes (Costar, Cambridge, MA) at a concentration of 1×10^6 cells/mL. Cells were then incubated (humidified 5% CO₂, 37°C) for 24 hours without agonist, with LPS endotoxin (1 ng/mL), or with PMA (20 ng/mL) plus ionomycin (500ng/mL) (Sigma). We stimulated human PBMCs with PMA plus ionomycin (PMA+I) to preferentially activate T and natural killer (NK) cells, and with LPS to preferentially activate monocytes, even if these stimuli are not ideal, because LPS can activate lymphocytes and PMA can activate monocytes. Nonetheless, the stimuli we used still allowed us to distinguish between cell types, because MCP-1 is produced by monocytes, while IFN- γ is primarily produced by T_H1 CD4 T cells, CD8 T cells, and NK cells. Cell supernatants were obtained by centrifugation and stored at -70°C until subsequent analyses.

Sample analyses

Serum and supernatant MCP-1 and IFN- γ were measured by using Luminex™ technology (LINCO Research, Inc., St. Charles, MO). Serum insulin and leptin concentrations were determined by using radioimmunoassay (LINCO Research, Inc.).

Statistical analyses

The statistical significance of differences between measurements obtained in lean and obese subjects at baseline and in measurements obtained in lean subjects at baseline and in obese subjects 1 year after surgery were evaluated by using a Student's *t* test for unpaired samples. The statistical significance of differ-

ences between longitudinal measurements obtained at baseline and 1 year after surgery in obese subjects were assessed by using a Student's *t* test for paired samples. All reported *p* values are two-sided, and a value of ≤ 0.05 was considered to be statistically significant. All data were analyzed by using SPSS for Windows software, version 12.0 (SPSS Inc., Chicago, IL).

RESULTS

Body weight and metabolic characteristics

Body weight and BMI were much greater in obese than lean subjects, and decreased by $30.3\% \pm 10.6\%$ after bariatric surgery in the obese group ($p < 0.001$; Table 1). Serum insulin and leptin concentrations were much greater in obese than lean subjects at baseline, and decreased after bariatric surgery in the obese group ($p < 0.01$; Table 1). Serum MCP-1 and IFN- γ concentrations were not different between lean and obese subjects, and did not change after weight loss in the obese group. One year after gastric bypass surgery, plasma glucose concentration had decreased from 125 ± 32 to 81 ± 8 mg/dL ($p < 0.01$), plasma low-density lipoprotein (LDL)-cholesterol concentration from 115 ± 40 to 86 ± 23 mg/dL ($p < 0.05$), and plasma triglyceride concentration from 146 ± 24 to 94 ± 22 mg/dL ($p < 0.01$).

PBMC chemokine and cytokine production

The production of MCP-1 and IFN- γ by unstimulated PBMC were not different between lean and obese subjects, either at baseline or at 1 year after gastric bypass surgery; when PBMCs were incubated without agonist, MCP-1 concentrations were 73 ± 74 , 62 ± 59 , and 65 ± 18 pg/mL, and IFN- γ concentrations were 1.4 ± 0.8 , 1.0 ± 0.5 , and 1.4 ± 0.9 pg/mL for lean subjects, obese subjects at baseline, and obese subjects 1 year after gastric bypass surgery, respectively. However, LPS-stimulated MCP-1 production and PMA+I-stimulated IFN- γ production by PBMCs were $93.6\% \pm 4.9\%$ and $88.8\% \pm 9.6\%$ lower, respectively, in obese subjects than in lean subjects at baseline ($p < 0.03$; Fig. 1). Bariatric surgery-induced weight loss markedly increased LPS-stimulated MCP-1 and PMA+I-stimulated IFN- γ production to values that were not significantly different from baseline lean values.

DISCUSSION

Obesity is associated with an increased risk of infection,¹⁻¹¹ but the mechanism(s) responsible for this relationship is not clear. We hypothesized that obesity might have adverse effects on cytokine and chemokine production by PBMCs, which are important components of the immune system response to infectious or-

TABLE 1. BODY WEIGHT AND SELECTED SERUM HORMONE, CYTOKINE, AND CHEMOKINE CONCENTRATIONS

	Lean subjects	Obese subjects	
		Baseline	1 year after gastric bypass surgery
Weight (kg)	62.5 ± 6.9	166.4 ± 23^a	116.1 ± 25^b
BMI (kg/m ²)	22.7 ± 1.4	62.4 ± 8.6^a	43.7 ± 10^b
Insulin (μ U/mL)	3.8 ± 2.2	28.1 ± 23^a	7.3 ± 6.1^c
Leptin (ng/mL)	9 ± 5	63 ± 16^a	37 ± 11^c
MCP-1 (pg/mL)	365 ± 68	414 ± 241	469 ± 326
IFN- γ (pg/mL)	9.8 ± 0.7	11.1 ± 2.3	9.8 ± 0.4

Values are means $\pm$ 6 standard deviation (SD).

^aValue significantly different from corresponding lean group value, $p < 0.01$.

Value significantly different from corresponding obese group baseline values, ^b $p < 0.001$, ^c $p < 0.001$.

BMI, body mass index; MCP-1, macrophage chemoattractant protein-1; IFN- γ , interferon- γ .

FIG. 1. Lipopolysaccharide (LPS)-stimulated macrophage chemoattractant protein-1 (MCP-1) production (A) and phorbol 12-myristate 13-acetate plus ionomycin (PMA+I)-stimulated interferon- γ (IFN- γ) production (B) by peripheral blood mononuclear cells (PBMCs) in 6 lean and in 6 extremely obese subjects before and 1 year after marked weight loss induced by gastric bypass surgery. †Significantly different from lean value ($p < 0.03$); *Significantly different from baseline obese value ($p < 0.03$). Values are means $\pm$ standard error (SE).

ganisms. The results of the present study demonstrate that although basal PBMC cytokine (i.e., IFN- γ) and chemokine (i.e., MCP-1) production are the same in lean and extremely obese persons, PMA+I-stimulated production of IFN- γ and LPS-stimulated production of MCP-1 are markedly suppressed by obesity. However, weight loss completely restored the ability of stimulated PBMCs to produce MCP-1 and IFN- γ to normal. These data suggest that obesity is associated with considerable abnormalities in mononuclear cell immune function, but these abnormalities are completely reversible with weight loss.

Our data indicate that activated PBMC secretion of two key immune modulators, IFN- γ

and MCP-1, is almost completely suppressed in extremely obese patients. MCP-1 promotes monocyte migration into sites of inflammation, where monocytes differentiate into macrophages or dendritic cells.^{15,18} IFN- γ is involved in activating macrophage phagocytosis and generating bactericidal mediators, such as reactive oxygen species and nitric oxide.¹⁹ In addition, IFN- γ promotes antigen presentation by dendritic cells and activates polymorphonuclear (PMN) leukocytes.^{15,17,19} MCP-1 is also involved in regulating IFN- γ production; mice lacking the MCP-1 receptor have a profound defect in IFN- γ production.²⁰ Therefore, our data suggest that obesity is associated with severe defects in both innate and adaptive immune responses.

Several other abnormalities in immune cell function have been identified in obese persons. Obesity is associated with a decreased capacity of lymphocytes to proliferate in response to mitogen activation^{13,14} and impaired polymorphonuclear leukocyte bactericidal capacity.¹² These data, in conjunction with the results of the present study, demonstrate that obesity is associated with multiple defects in white blood cell functions, which can impair the host response to an attack from pathogenic organisms and likely contribute to the increased prevalence of infections observed in obese patients.

We found that weight loss normalized MCP-1 and IFN- γ production by activated PBMCs. Although our study cannot determine the mechanism responsible for this improvement, several factors could be involved. First, it is possible that weight loss decreases obesity-induced PBMC insulin and leptin resistance, and thereby normalize MCP-1 and IFN- γ production. This notion is supported by data demonstrating that insulin and leptin are involved in T-lymphocyte metabolism. Insulin receptor expression and signaling in T lymphocytes after *in vitro* stimulation are decreased in obese subjects,^{21–23} which could impair lymphocyte glucose uptake and cytokine production.²⁴ Leptin-deficient mice and humans have impaired T cell proliferation, phagocytosis, and cytokine production, which are normalized by leptin administration.^{25–27} Second, weight loss can decrease plasma IL1-Ra concentration, which could increase PBMC cytokine-induced pro-

duction of MCP-1 and IFN- γ .²⁸ Third, weight loss can decrease serum IL-10 concentrations, which would inhibit both monocyte and lymphocyte proinflammatory cytokine production.²⁹ Fourth, weight loss-induced changes in blood glucose and lipids could have improved phagocyte and lymphocyte function, which are impaired by high blood glucose and lipid concentrations.³⁰

The results from our study identify an apparent paradox in immune function in obese persons. Obesity is associated with increased inflammation, manifested by an upregulation of cytokine and chemokine production by adipose tissue, presumably from both adipocytes and infiltrated macrophages.^{31–33} At the same time, obesity is associated with a defective immune response to infection, manifested by a downregulation of stimulated cytokine and chemokine production by circulating mononuclear cells. Overactivity of the immune system during basal conditions is harmful because it is likely to contribute to insulin resistance, diabetes, and coronary heart disease,^{31,33} whereas underactivity of the immune response to invasive pathogens is also harmful because it is likely to increase host vulnerability to infectious agents. Weight loss normalizes both abnormalities by decreasing the production of cytokines and chemokines by adipose tissue^{34,35} and increasing their production by PBMCs when challenged by infectious agents. These findings underscore the complex relationship between obesity and the immune system. Additional studies are needed to identify the precise mechanisms responsible for the profound effects of adiposity on immune function.

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Luigi Fontana participated in the concept, design and implementation of the study, undertook plausibility testing, drafted the report. J. Christopher Eagon participated in the design, and in the implementation of the study. Marco Colonna participated in the con-

cept, design, implementation of the study, and drafting of the report. Samuel Klein participated in the concept, design, implementation of the study, and drafting of the report. All the authors declared that they participated in the study as mentioned above and that they reviewed and approved the manuscript in its final version.

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