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The Effect of Obesity on Neutrophil Fc Receptors and Adhesion Molecules (CD16, CD11b, CD62L)

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Background: There is a large body of epidemiological data associating obesity with a wide variety of clinical disease processes, including cancer and wound infections. However, defining the specific defects of neutrophils has proved difficult and often contradictory.

Methods: 27 patients having gastric bypass surgery for obesity (BMI>40) were compared with 10 normal controls (BMI<26). Relative neutrophil frequencies and expression of the activation antigens CD11b (integrin adhesion molecule), CD16 (Fc receptor), and CD62L (L- selectin), were evaluated by flow cytometry.

Results: The study control group had a mean age of 37 ± 7.6 yrs (range 30 to 57) with no significant health problems. Their mean BMI was 23 ± 2.5 kg/m² (range 21-26). The mean age of the sample group was 40.36 ± 13.7 yrs (range 18 to 60) with a mean BMI of 52 ± 8.2 kg/m2 (range 41 to 72). These patients had a large spectrum of diseases that afflict the morbidly obese, including hypertension (14), arthritis (10), exertional dyspnea (13), venous stasis (7), hypothyroidism (2), NIDDM (3), heart murmur (1), along with 8 smokers. The neutrophil frequency in the obese patients was comparable to the controls (control 49% vs obese 51%). Additionally, there was no apparent difference between obese and controls regarding CD11b or CD16 expression (424 vs 498 gmf) (267 vs 262 gmf). However, there was a significant reduction of CD62L (L-selectin) expression noted in the morbidly obese with respect to controls (102 vs 303 gmf, p<0.001). An increased percentage of eosinophils when compared to controls (6.7% vs 1.73%, p<0.001) was also observed.

Conclusion: Discordant CD11b/CD62L levels, depressed levels of CD62L, and elevated eosinophil percentages support the hypothesis that a chronic inflammatory state exists in morbid obesity. Decreased levels of CD62L in the morbidly obese neutrophil pool possibly affect the neutrophil's ability to activate and migrate to sites of inflammation.

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This may play a role in the higher incidence of infectious complications seen in morbidly obese individuals.

Key words: Morbid obesity, immune function, chronic inflammation, L-selectin, Fc receptor, CD62L, CD11b, CD16, neutrophil function, flow cytometry

Introduction

As early as 1934 Seifert attempted to document the increased morbidity and mortality associated with obesity.[1] Since that time progress has been made in outlining the epidemiological data associating obesity with a wide variety of clinical disease processes.[2-5] Defining the specific defects of the immune system, however, has proved difficult and often contradictory.

In the past, studies of neutrophil function have relied on such measures as total neutrophil counts, phagocytosis function in response to anti-body coated RBC, neutrophil proliferation in response to bacteria, maturation of neutrophils in culture media, adherence capability, chemotaxis ability, and opsonic capacity to judge neutrophil function.[6-10] Yet, despite these attempts at characterizing immune incompetence in neutrophils of the morbidly obese, we often have been left with contradictory data.

The purpose of this study was to further evaluate the neutrophil's immune function by looking at early activation cell surface markers and adherence ligands on peripheral blood leukocytes in morbidly obese patients, comparing them with non-obese controls.

Methods

The Institutional Review Board of Nassau University Medical Center, before beginning the study, reviewed and approved this protocol. All patients gave informed consent before their participation in the study.

Ten non-obese subjects with no comorbidities and BMI <26 kg/m² were recruited from the staff at University Hospital to function as normal controls. They were compared with 27 patients undergoing surgery for morbid obesity who had a BMI ≥40 kg/m². Exclusion criteria were steroid use or a recent history of cancer. All morbidly obese patients had a standardized history and physical done as part of their preoperative work-up.

Peripheral blood samples were obtained in sodium heparin tubes from all patients. A mixed leukocyte suspension was prepared by hypotonic lysis of the erythrocytes and subsequent centrifugation and washing with phosphate-buffered saline. The cells were then incubated on ice with antigen-specific monoclonal antibodies (CD11b, CD16, CD62L) conjugated with either fluoroisothiocyanate (FITC), phycoerythrin (PE), or allophycocyanin (APC) obtained from Becton-Dickinson Immunocytometry Systems (San Jose, CA). The cells were subsequently washed, and then fixed with 1% paraformaldehyde. The antigen panels were analyzed on a FACS Vantage flow cytometer (BDIS, San Jose, CA) after optimization with appropriate controls. Specific leukocyte subpopulations were gated using side scatter and CD14 plots. CD14 was used solely as a population discriminator, as this molecule is seen only on monocytes. From these gates, relative percentages of neutrophils were obtained. Further gating of the neutrophil subset by side scatter vs CD16 was used to separate neutrophil and eosinophil subsets.

The flow cytometry data was evaluated using FlowJo analytical software (Tree Star, Inc., Palo Alto, CA). Leukocyte antigen expression, measured as geometric mean fluorescence (gmf), of the bariatric group was then compared with the normal control group using Student's t-test with Sigma Stat (SPSS, Inc., Chicago, IL) software. Statistical significance was set at p≤0.05, and all values are expressed as mean ± standard deviation. Graphical representations in the figures are Tukey box plots with a mean line outlined by the 10th and 90th percentiles, and the 5th and 95th percentiles as error bars. Outliers are represented by dots.

Results

Our sample group was made up of 26 females and one male. Ethnically they consisted of 14 African Americans, seven Caucasians, and six Hispanics. The mean age of our sample group was 40 ± 13.8 yrs (range 18 to 60). These patients had a large spectrum of diseases that afflict the morbidly obese, including hypertension (14), arthritis (10), exertional dyspnea (13), venous stasis (7), hypothyroidism (2), type 2 diabetes (3), heart murmur (1), and smokers (8). The average BMI of our sample group was 52 ± 8.2 kg/m² (range 41 to 72).

The control group was comprised of seven females and three males. Ethnically they consisted of three Caucasian males, one Indian male, three Caucasian females, one Indian female, one Slavic female, and one African American female. Their mean age was 37 ± 7.6 yrs (range 30 to 57), and they had no comorbidities. Their mean BMI was 23 ± 2.5 kg/m² (range 21-26).

The following early leukocyte activation antigens were chosen for the study: CD11b, a monocyte and neutrophil complement receptor and adhesion integrin; the IgG receptor CD16 (FcRIII); and CD62L (L-selectin), a monocyte and neutrophil integrin.

Earlier work by Nieman et al[8-11] had shown that obesity elevates neutrophil counts. This disparity was not observed in this study where there was no apparent difference in the relative neutrophil percentages (control 49% vs 51% obese).

Gating for polymorphonuclear leukocytes (PMNLs) can be further delineated by examining the side scatter vs CD16 distribution of this subset (Figure 1). This plot yields two distinct groups of PMNLs – neutrophils and eosinophils. Eosinophils express CD16, but exhibit a distinctly weaker signal than neutrophils. While there was no apparent difference in the neutrophil percentages, the eosinophil subset was significantly greater in the obese group compared to controls (6.7% vs 1.73%,

Eosinophil Subset Percentages
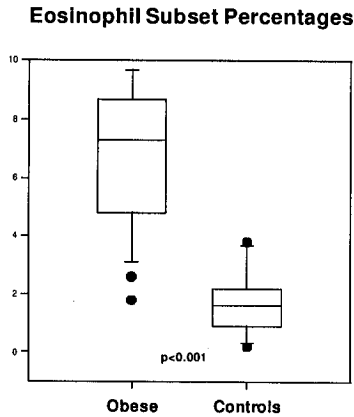
Figure 1. Expanded eosinophil subset percentages in morbid obesity.

p<0.001) (Figure 1). The expression of the FcRIII receptor, a low-affinity receptor for IgG, CD16, was not different on the obese patients' neutrophils in comparison with those of the normal group (267 vs 262 gmf).

We also chose to look at the adhesion antigens expressed on neutrophils, because there has been previous evidence of PMNL migration dysfunction in obesity.[6,9,12] The neutrophil activation and adherence antigen CD11b expression of the obese group showed no apparent difference from the normals (424 ± 282 vs 498 ± 295 gmf), but expression was highly variable. However, CD62L, a selectin that mediates the initial tethering and rolling to the endothelial surfaces, and is shed from the surface in the setting of acute stress,[14] was decreased significantly with respect to controls (102 vs 303 gmf, p<0.001) (Figure 2). This difference can be further demonstrated when looking at the ratio of CD11b

to CD62L expression. The ratio of CD11b to CD62L was 6.84 in the obese while that of the controls was only 1.6, p=0.013 (Figure 3)(Table 1).

Discussion

As early as 1975, Kjosen et al[10] demonstrated a decrease in glucose oxidation present in phagocytosing and non-phagocytosing PMNLs taken from obese patients when compared to controls and normals. Later, in 1977, Palmblad et al[9] showed that neutrophil bactericidal capacity was severely reduced in moderate obesity, with an increase in adherence. No differences were present in the mean neutrophil chemotaxic or opsonic abilities between obese and non-obese subjects. In addition none of these changes could be related to plasma lipid levels. In 1979, and again in 1980, Palmblad et al[6,13] demonstrated increased neutrophil adherence using the same model as in 1977, with a reduced bactericidal capacity. Also in 1980, Chandra and Kutty[7] showed that in obese children, there was decreased ability for intracellular killing while the ability to phagocytose was normal. McMurray et al[15] in 1990 demonstrated that serum from moderately obese individuals (BMI >25) decreased the ability of neutrophils for chemotaxis and intracellular microbial killing while the ability for phagocytosis was retained. In 1996 Plotkin,[16] using a rat model, showed that obese Zucker rats' PMNs were able to phagocytose yeast cells normally but their ability to kill them trailed signifi-

Neutrophil CD62L Expression
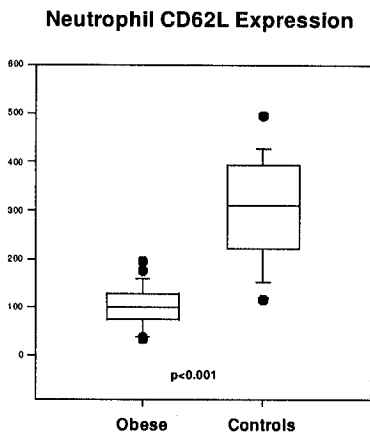
Figure 2. Graph illustrating the reduced expression of CD62L (L-selectin) in morbid obesity.

CDIIb/CD62 Ratio
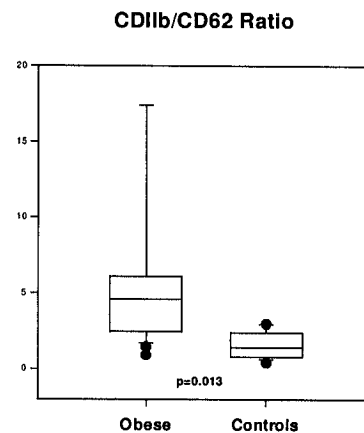
Figure 3. Graph illustrating increased ratio of CD11b/CD62L found in obesity.

Table 1. Neutrophil Function in Morbidly Obese and Control Subjects

	Obese±Mean	Controls±Mean	p-value
BMI	52±82	32±2.5	<0.001
Age	40±13.8	37±7.6	0.312
PMNL%	51±11.6%	49±15%	0.617
Eosinophil	6.7±2.7%	1.73±1.2%	<0.001
CD11b	424±282gmf	2362±106gmf	0.888
CD62L	303±109gmf	102±42gmf	<0.001
CD11b/ CD62L	6.8±7.7	1.6±0.86	0.013

cantly when compared with lean controls.[16] Nieman[12] in 1996 demonstrated that patients with a BMI >33 have higher numbers of neutrophils than similarly matched controls.[11] Similarly in 1999 Nieman[8] again showed higher numbers of neutrophils when compared with non-obese controls. Phagocytosis and oxidative burst activity also was elevated in obese patients when compared, and both these variables correlated with BMI.

The present study is unique because all but one of the above studies dealt with patients with moderate obesity (BMI <35). Our patients had a mean BMI of 52, placing them in the super-obese category. These patients have, on average, more medical problems and carry a higher morbidity and mortality than moderately obese patients.

Unlike the results obtained by Nieman,[12] we did not notice any significant differences in neutrophil percentages when comparing our controls with morbidly obese patients. The possible explanations include differing analytical techniques and the higher mean BMI of our patients. It also could suggest that despite the obvious chronic inflammatory condition that obesity produces, it does not down-regulate the production of neutrophils.

We chose to study several simultaneous variables of neutrophil adherence, as there has only been one model of *in vitro* adherence used in the past.[6,9,13] The first, CD11b, is a heterodimeric glycoprotein that is expressed on the surface of neutrophils. It is stored primarily in secondary and tertiary granules. Once a neutrophil becomes activated, the CD11b/CD18 complex rapidly translocates to the cell surface where it interacts with the split product of complement iC3b and the endothelial protein ICAM-1 to promote neutrophil adherence once rolling has been stopped by CD62L.[17-22] CD11b

also functions as an intestinal adhesive molecule during transepithelial migration of neutrophils to the gut lumen.[23] The CD11b complex is up-regulated in response to endotoxin, TNF, or IL-1.[24,25] CD11b expression was not elevated in our obese population.

The second adhesion molecule studied was CD-62 (L-selectin). L-selectin "mediates the first and essential step for extravasation of leukocytes, namely, leukocyte rolling."[17] It is also necessary for the subsequent homing of lymphocytes to lymph nodes and other immunologic organs.[26-30] In L-selectin deficient mice there was a dramatic decrease in the ability of neutrophils to migrate to sites of inflammation.[31] This finding of decreased migratory capacity has been confirmed *in vivo* using the clinical model of systemic inflammatory response syndrome.[32] In our study, morbidly obese patients were found to have severe depression in the levels of L-selectin while maintaining normal levels of CD11b. This dichotomy is unusual in acute inflammation, because both CD62L and CD11b are elevated in proportion to the insult.[33] However, in clinically relevant models of chronic inflammation such as chemotherapy and chronic renal failure, CD11b levels rise or remain unchanged, while L-selectin levels are depressed.[34] This alteration is evidence of chronic inflammation and is consistent with several large series that looked at the levels of C-reactive protein in the morbidly obese and found them to be elevated in proportion to BMI.[35-38] Thus, chronic inflammation results in lower levels of L-selectin expression, which could be a factor in the higher incidence of wound infection.

Fc receptors (FcR) are expressed on all cells of the immunologic system as well as epithelial and endothelial cells. There are three classes of these receptors: Fc RI (CD64), Fc RII (CD32), and Fc RIII (CD16). Fc RI is a high affinity receptor and binds IgG at normal physiologic concentrations. Fc RII and Fc RIII are low affinity receptors and as such only bind IgG when found in immune complexes. These molecules link the humoral and cellular immunity by binding the antibody to effector cells.[39] FcR receptors play a variety of roles in the human body that have been characterized using knock-out mice lacking FcR receptors. Among the most prominent of these roles are phagocytosis,

degranulation, release of inflammatory mediatory regulation of the production of oxygen radicals and regulation of antibody production in antibody-dependent cellular cytotoxicity. Our data found that the expression of this antigen was not apparently depressed in the morbidly obese when compared with normals.

Conclusion

Discordant CD11b/CD62L levels, depressed CD62L levels, and elevated eosinophil subset percentages confirm the hypothesis that a chronic inflammatory state exists in morbid obesity. Depression of CD62L suggests a functional decrease in the ability of neutrophils to migrate to sites of inflammation in obesity. This alteration may play a role in the higher incidence of infection seen in morbidly obese individuals. Further investigation is warranted and ongoing.

References

1. Seifert E. Zur Operationsprognose bei fettleibigen und bei mageren Kranken. Munch Med Wochenschr 1934; 81: 1917-21.
2. Lew, EA, Garfinkle L. Variations in mortality by weight among 750,000 men and women. Journal of Chronic Disease 1979; 32: 563-76.
3. Miller AB. Diet and cancer: a review. Acta Oncol 1990; 29: 87-95.
4. Norris SO, Provo, B, Scotts NA. Physiology of wound healing and risk factors that impede the healing process: clinical issues. Crit Care Nurs 1990; 1: 545-2
5. Fasol R., Schindler M, Schumaker B. The influence of obesity on perioperative morbidity: retrospective study of 502 aorto-coronary bypass operations. Thoracic Cardiovasc Surgeon 1992; 40: 126-9.
6. Palmblad J, Hallberg D, Engstedt L. Polymorph-onuclear function after small intestinal shunt operation for morbid obesity. Br J Haematol 1980; 44: 101-8.
7. Chandra RK, Kutty, KM. Immunocompetence in Obesity. Acta Paediatr Scand 1980; 69: 25-30.
8. Nieman DC, Henson DA. Influence of obesity on immune function. J Am Diet Assoc 1999; 99: 294-9.
9. Palmblad J, Hallberg D, Rossner S. Obesity, plasma lipids and polymorphonuclear granulocyte functions. Scand J Haematol 1977; 19, 293-303.
10. Kjosen B, Bassoe HH, Myking O. The glucose oxidation in isolated leukocytes from female patients suffering from overweight or anorexia nervosa. Scand J Clin Lab Invest 1975; 35: 447-54.
11. Wright SD, Ramos RA, Tobias PS et al. CD14, a receptor for complexes of LPS and LPS binding protein. Science 1990; 249(4975): 1431-3.
12. Nieman DC, Nehlsen-Cannarella SL, Henson DA. Immune response to obesity and moderate weight loss. Int J Obes 1996; 29: 353-60.
13. Palmblad J, Rossner S. Granulocyte functions during treatment of obesity. Int J Obes 1979; 3: 119-22.
14. Tedder TF, Steeler DA, Chen A et al. The selectins: vascular adhesion molecules. FASEB 1995; 9: 866-73.
15. McMurray RW, Bradsher RW, Russel WS. Effect of prolonged modified fasting in obese persons on In vitro markers of Immunity: lymphocyte function and serum effects on normal neutrophil. Am J Med Sci 1990; 299: 379-85.
16. Plotkin BJ, Paulson D, Chelich A. Immune responsiveness in a rat model for type II diabetes (Zucker rat, fa/fa): susceptibility to Candida albicans infection and leukocyte function. J Med Microbiol 1996; 44: 277-83.
17. Kawashima H, Watanabe N. Vascular and non-vascular ligands for L-selectin. Cell Adhesion and Communication 1998; 6: 135-9.
18. Lasky LA. Lectin cell adhesion molecules (LEC-CAMs): a new family of cell adhesion proteins involved with inflammation. J Cell Biochem 1991; 45: 139-46.
19. Stoolman LM. Selectins (LEC-CAMs): lectin-like receptors involved in lymphocyte recirculation and leukocyte recruitment. In: Fukuda, M ed. Cell Surface Carbohydrates and Cell Development. Boca Raton: CRC 1992: 71.
20. Bevilacqua MP, Wheeler ME, Pober JS et al. Endothelial-dependent mechanisms of leukocyte adhesion: regulation by interleukin-1 and tumor necrosis factor. In: Movat HZ, ed. Leukocyte Emigration and Its Sequelae. Basel: Karger 1987: 79.
21. Stoolman LM. Adhesion molecules controlling lym-

phocyte migration. Cell 1989; 56: 907-10.

22. Picker LJ, Warnock RA, Burns A et al. The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. Cell 1991; 66: 921-33.

23. Parkos CA. Cell adhesion and migration. I. Neutrophil adhesive interactions with intestinal epithelium. Am J Physiol 1997; 273(4 pt 1): G763-8.

24. Arnout MA. Structure and function of the leukocyte adhesion molecules CD11/CD18. Blood 1990; 75: 1037-50.

25. Springer TA. Adhesion receptors of the immune system. Nature 1990; 346: 425-34.

26. Lasky LA. Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science 1992; 258: 964-9.

27. Rosen SD, Bertozzi CR. The selectins and their ligands. Curr Opin Cell Biol 1994; 6: 663-73.

28. Smith CW. Endothelial adhesion molecules and their role in inflammation. Can J Physiol Pharmacol 1993; 71: 76-87.

29. McEver RP. Selectins: novel receptor that mediate leukocyte adhesion during inflammation. Thromb Haemost 1991; 65: 223-8.

30. Kanas GS. Structure and function of L-selectin. APMIS 1992; 100: 287-93.

31. Tedder TF, Steeber DA. L-selectin deficient mice have impaired leukocyte recruitment into inflammatory sites. J Exp Med 1995; 181: 2259-64.

32. Ahmed N, Christou N. Decreased neutrophil L selectin expression in patients with systemic inflam-

matory response syndrome. Clin Invest Med 1996; 19: 427-34.

33. Maekawa K, Futami S. Effects of trauma and sepsis on soluble L-selectin and cell surface expression of L-selectin and CD11b. J Trauma 1998; 44: 460-8.

34. Dou L, Brunet P. Effect of uremia and hemodialysis on soluble L-selectin and leukocyte surface CD11b and L-selectin. Am J Kidney Dis 1998; 31: 67-73.

35. Visser M, Bouter LM, McQuillan GM. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999; 282: 2121-35.

36. Ford ES. Body mass index, diabetes, and C-reactive protein among U.S. adults. Diab Care 1999; 22: 1971-7.

37. Yudkin JS, Stehouwer CDA, Emeis JJ. C-reactive protein in healthy subjects: sssociations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue. Arterioscler Thromb Vasc Biol 1999; 19: 972-8.

38. Barinas-Mithell E, Cushman M, Meilahn EN. Seum levels of C-reacive protein are associated with obesity, weight gain, and hormone replacement therapy in healthy postmenopausal women. Am J Epidemiol 2001; 153: 1094-101.

39. Hazenbos W, Gessner JE. Impaired Ig-G dependent anaphylaxis and arthus reaction in Fc RIII deficient mice. Immunity 1996; 5: 181-8.