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Context: Obesity-related chronic inflammation is implicated in the pathogenesis of type 2 diabetes (T2D).

Objective: The objective of the study was to determine the effects of weight loss on immune cells in T2D and prediabetes.

Design and Setting: Thirteen obese subjects with T2D or prediabetes underwent 24 wk dietary energy restriction with gastric banding surgery at 12 wk.

Main Outcome Measures: Measures included weight, waist, and insulin resistance; surface activation marker expression on circulating immune cells; T-helper cell polarization: type 1 (Th1), type 2 (Th2); adipose tissue macrophage number and activation in sc and visceral adipose tissue.

Results: Mean total weight loss was 13.5%. There were significant decreases in expression of proinflammatory activation markers: granulocyte CD11b, monocyte CD66b, and T cell CD69 and CD25. Proinflammatory Th1 cell numbers fell by greater than 80%, as did the Th1 to Th2 ratio. The fall in Th1 to Th2 ratio related to weight ($P < 0.05$) and waist loss ($P < 0.05$). Reduction in immune cell activation was more pronounced in subjects with prediabetes. Weight and abdominal fat loss were predicted by lower activation of adipose tissue macrophage in sc and visceral adipose tissue ($P < 0.05$).

Conclusions: Energy restriction before and after gastric banding attenuates activation of circulating immune cells of the innate and adaptive immune system in T2D and prediabetes. The role of immune cells in the chronic inflammation of obesity and T2D requires further investigation. **(*J Clin Endocrinol Metab* 95: 2845–2850, 2010)**

Obesity and its associated low-grade inflammation may contribute to the development of type 2 diabetes (T2D) (1). Bariatric surgery is an effective long term treatment of obesity (2), reverses T2D (3), reduces mortality at 10 yr (4), and is recommended in T2D with inadequate control where body mass index (BMI) exceeds 35 kg/m^2 (5).

Low-grade inflammation in obesity and T2D is considered the product of an activated innate immune system, with activated circulating (6) and tissue-based innate immune cells (7). The contribution of the adaptive immune system in obesity and T2D (particularly T lymphocytes) has not been interrogated. T helper lymphocytes can differentiate into either proinflammatory type 1 (Th1) or Th1-repressing type 2 (Th2) phenotypes; an appropriate balance is considered pivotal in immune regulation (8). Th1 predominance is present in autoimmune diseases (rheumatoid arthritis) (9), type 1 diabetes (8), and insulin-resistant obesity (10). Sparse data exist on T lymphocyte activation in obesity and T2D (11). The effects of weight

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Abbreviations: BMI, Body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; IGT, impaired glucose tolerance; MFI, mean fluorescence intensity; rMFI, relative MFI; SAT, sc adipose tissue; SVF, stromovascular fraction; T2D, type 2 diabetes; Th1, T helper type 1; Th2, T helper type 2; VAT, visceral adipose tissue.

loss on T lymphocyte activation or T helper phenotypes are unknown.

We investigated the effects of energy restriction with weight loss on the innate and adaptive immune system in obese people with T2D or prediabetes, with specific reference to T lymphocyte activation and Th1/Th2-polarization.

Subjects and Methods

Subjects

Thirteen morbidly obese Caucasians (seven males), aged 51 yr (35–65 yr) with T2D or impaired glucose tolerance (IGT) [American Diabetes Association criteria (5), examination of case records] were recruited from a city tertiary referral hospital. Inclusion criteria included age greater than 18 yr and BMI greater than 35 kg/m^2. Normal reference data for immune parameters were derived from 10 (five males) healthy, normal-weight-, age-, and sex-matched individuals [mean 45.3 yr (22–67 yr)]. Subjects gave written informed consent. The study protocol was approved by the institutional Research and Ethics Committee and registered at www.clinicaltrials.gov (NCT00592735).

Study design

A 24-wk protocol of energy restriction, with gastric banding surgery at 12 wk, was used. Individualized monthly counseling was also provided, and intake was limited to 3600–6000 kJ/d, depending on initial weight. Subjects were encouraged to replace one to two meals a day with a commercially available meal-replacement shake (560 kJ each: 18 g protein, 2 g fat, 14 g carbohydrate). Written instructions were provided for remaining meals [low fat (<10 g), modest carbohydrate (<80 g), and protein (<150–200 g)].

All subjects underwent laparoscopic gastric band placement using the Swedish Adjustable Gastric Band (Ethicon Inc., Johnson and Johnson Medical, Cincinnati, OH). A 1-cm^3 sample of abdominal sc (SAT) and visceral adipose tissue (VAT) was excised at surgery. A single band inflation to less than 50% capacity was permitted 6–10 wk postoperatively, with 3- to 6-ml inflations in 10 subjects.

Fasting blood samples (10 h overnight) were collected at baseline, 12 wk (preoperatively), and 24 wk (12 wk postoperatively). Overnight fasting was strictly enforced to avoid nutrient-induced cell activation (12).

Immune cell preparation and flow cytometry analysis

Fresh whole blood was stained with fluorochrome-conjugated antibodies to cell surface markers and analyzed on a BD FACSCalibur (all BD Biosciences, San Diego, CA). T helper lymphocytes were quantified after intracellular staining for interferon-γ (Th1) and IL-4 (Th2), following the manufacturer's protocol (BD Bioscience PharMingen, San Diego, CA). Data were analyzed with FlowJo software (version 7; TreeStar Inc., Ashland, OR). Activation marker mean fluorescence intensity (MFI) was divided by the MFI of the unstained control, giving the relative MFI (rMFI). The rMFI coefficient of variation was 17.0%.

Flow cytometry of adipose tissue stromovascular fraction

Adipose tissue samples were immediately digested with collagenase type IV to separate the stromovascular fraction (SVF) from mature adipocytes. SVF was analyzed by flow cytometry as described (13): preadipocytes/CD34+ progenitor cells identified by staining for stem cell marker CD34 and excluding CD31+ endothelial cells. Macrophages were stained for CD14. Results are expressed as percentage of total viable cells. Macrophage activation was determined by CD11b expression (expressed as rMFI).

Biochemical variables

Plasma glucose was measured by the glucose oxidase method (YSI glucose analyzer, model 2300 STAT PLUS 230V; YSI, Inc., Yellow Springs, OH), serum insulin by commercial RIA (Linco, St. Charles, MO), and insulin resistance by the homeostasis model assessment (HOMA-IR) (14).

Statistical analysis

Data are presented as mean ± SEM. Based on previous T-helper lymphocyte quantifications, 13 subjects provided greater than 90% power (α error 0.05) to detect a difference of 2%. Comparisons were performed by paired *t* test or sign test for skewed data; correlations expressed as Spearman's correlation coefficients (Statistica 6.0; StatSoft, Tulsa, OK). $P < 0.05$ was considered significant.

Results

Clinical parameters

Baseline data from 13 subjects, with mean BMI 42.8 ± 1.4 kg/m^2 (35.1–50.8), are shown in Table 1. T2D subjects (n = 9) received diet (n = 2), metformin (n = 2), metformin and sulfonylurea (n = 5), or lipid-lowering therapy (n = 4) and antihypertensives (n = 6). IGT subjects (n = 4) took no medications.

The protocol resulted in 5% weight loss at 12 wk (6.3 ± 1.3 kg, range +0.9 to −14.5, $P < 0.001$) and 13.5% weight loss at 24 wk (17.0 ± 2.4 kg, range +0.2 to −30.0 kg, $P < 0.0001$), with reductions in BMI and waist circumference. Fasting glucose and glycosylated hemoglobin fell significantly, insulin and HOMA-IR showed a downward trend. Antidiabetic medications were reduced in all T2D subjects after surgery: sulfonylureas ceased (three of five) or reduced (two of five); metformin ceased (four of seven).

Effects of energy restriction and weight loss on T helper cell phenotypes and immune cell numbers

Immune cell data are presented in Table 1. There was a dramatic reduction of Th1 cells at 12 wk (5.3 ± 1.6 to 0.8 ± 0.2%, $P = 0.03$), sustained at 24 wk (Table 1 and Fig. 1A). The Th1 to Th2 ratio decreased significantly at 12 wk (2.5 ± 0.6 to 0.6 ± 0.1, $P = 0.01$), sustained at 24

TABLE 1. Anthropometric, metabolic, and immune cell parameters in 13 subjects

Units	Baseline	12 wk	24 wk	Reference
Weight (kg)	125.9 ± 6.2	119.6 ± 5.5[a]	108.9 ± 5.4[b,c]	65.7 ± 2.5
BMI (kg/m^2)	42.8 ± 1.4	40.7 ± 1.3[a]	37.2 ± 1.3[b,c]	22.6 ± 0.7
Waist (cm)	132.2 ± 4.3	124.7 ± 3.9[a]	119.8 ± 4.3[b,c]	81.7 ± 2.9
Fasting glucose (mmol/liter)	5.9 ± 0.4	6.0 ± 0.4	5.2 ± 0.3[b,c]	4.7 ± 0.1
HbA1c (%)	7.1 ± 0.3	6.8 ± 0.3	6.5 ± 0.4[c]	N/A
Fasting insulin (μU/ml)	29.0 ± 6.1	27.8 ± 5.3	21.4 ± 2.7	8.5 ± 1.2
HOMA-IR	7.7 ± 1.7	7.2 ± 1.3	4.9 ± 0.6	1.8 ± 0.3
Immune cell numbers				
Granulocytes (percent of WBC)	39.4 ± 3.9	41.6 ± 4.5[a]	46.6 ± 2.1	42.4 ± 3.4
Monocytes (percent of WBC)	3.1 ± 0.6	3.2 ± 0.2	3.2 ± 0.3	4.6 ± 0.5
Lymphocytes (percent of WBC)	33.5 ± 2.1	34.5 ± 2.2	35.3 ± 2.4	28.7 ± 1.3
T cells (percent of WBC)	16.2 ± 2.5	19.3 ± 3.8	26.4 ± 1.9[c]	17.7 ± 1.7
CD4 (percent of lymphocytes)	45.8 ± 2.9	47.0 ± 3.6	46.0 ± 2.3	43.1 ± 4.7
CD8 (percent of lymphocytes)	15.0 ± 1.7	16.3 ± 1.7	13.5 ± 1.8	15.7 ± 1.8
Th1 (%)	5.3 ± 1.6	0.8 ± 0.2[a]	1.1 ± 0.2[c]	2.2 ± 0.9
Th2 (%)	2.6 ± 0.7	2.7 ± 1.1	4.7 ± 2.0	3.0 ± 0.9
Th1 to Th2 (ratio)	2.5 ± 0.6	0.6 ± 0.1[a]	0.5 ± 0.1[c]	1.0 ± 0.3
Immune cell activation				
G CD66b (rMFI)	9.7 ± 2.1	11.0 ± 2.1	11.4 ± 1.0	8.3 ± 1.6
G CD62L (rMFI)	42.6 ± 7.3	40.8 ± 9.3	55.0 ± 8.5	65.7 ± 16.9
G CD11b (rMFI)	19.7 ± 5.4	11.3 ± 4.0	6.2 ± 1.3[c]	2.3 ± 0.3
M CD66b (rMFI)	1.20 ± 0.09	1.01 ± 0.04	0.89 ± 0.05[c]	1.2 ± 0.2
M CD62L (rMFI)	32.3 ± 9.1	46.7 ± 11.2	64.2 ± 11.4	65.6 ± 15.3
M CD11b (rMFI)	40.7 ± 10.0	27.6 ± 8.3	21.7 ± 5.7	11.3 ± 2.2
T CD69 (rMFI)	1.11 ± 0.04	1.02 ± 0.02	0.97 ± 0.02[b,c]	1.1 ± 0.1
T CD62L (rMFI)	45.3 ± 4.9	45.6 ± 10.0	31.9 ± 3.7	60.1 ± 13.0
T CD25 (rMFI)	4.9 ± 1.1	2.3 ± 0.7[a]	1.3 ± 0.1[c]	1.5 ± 0.1

Summary of anthropometric and metabolic parameters as well as relative quantities of circulating immune cell subsets (percent) and expression of surface activation markers (rMFI) at baseline, after 12 wk of energy restriction preoperatively, and after 24 wk of energy restriction (12 wk after gastric banding surgery). Reference data were derived from 10 normal-weight subjects matched for age and sex. Data are presented as mean ± SEM. HbA1c, Glycosylated hemoglobin; WBC, white blood cells; N/A, not assessed.

[a] Baseline *vs.* 12 wk, $P < 0.05$.

[b] Twelve weeks *vs.* 24 wk, $P < 0.05$.

[c] Baseline *vs.* 24 wk, $P < 0.05$.

wk. Granulocytes increased at 12 wk and CD3$^+$ T lymphocytes at 24 wk. Monocytes, lymphocytes, and CD4$^+$/CD8$^+$ T lymphocyte numbers were unchanged.

Energy restriction and weight loss reduce immune cell activation

Energy restriction with weight loss significantly reduced the surface expression of activation markers on granulocytes (CD11b), monocytes (CD66b), and T lymphocytes (CD69 and CD25) (Table 1). The reduction in T lymphocyte CD69 expression at 24 wk related strongly to BMI reduction (r = 0.62, $P < 0.05$). Similarly, the fall in Th1/2 was associated with the magnitude of reduction in weight (r = 0.56, $P < 0.05$) and waist (r = 0.70, $P < 0.05$; Fig. 1B). No relationship was found between immune cell activation changes and changes in HOMA-IR and fasting glucose. Subjects with T2D and IGT had similar reductions in weight, waist, and glucose and insulin levels; however, the fall in immune activation in IGT was greater than that in T2D (Th1 −9.4 ± 3.9 *vs.* −2.0 ± 1.2%, $P = 0.03$; granulocyte CD11b −38.4 ± 2.7 *vs.* −2.5 ± 3.6 rMFI, $P < 0.001$; monocyte CD11b −49.4 ± 14.5 *vs.* −5.6 ± 8.9 rMFI, $P = 0.02$; T lymphocyte CD25 −7.9 ± 0.6 *vs.* −1.6 ± 0.9 rMFI, $P = 0.001$).

VAT and SAT macrophages and subsequent weight loss

There were greater numbers of macrophages and CD34+ precursor cells in SAT compared with VAT (macrophages: 6.7 ± 1.8 *vs.* 2.9 ± 1.0%, $P = 0.02$; precursor cells: 7.0 ± 2.7 *vs.* 1.8 ± 0.3%, $P = 0.04$; Fig. 1C). SAT and VAT had similar endothelial cell numbers and macrophage expression of activation marker CD11b. SAT and VAT macrophage number did not relate to measures of adiposity or insulin resistance at the time of surgery (data not shown). However, a greater weight loss after surgery was predicted by lower macrophage CD11b expression in SAT (r = 0.73, $P < 0.05$) and VAT (r = 0.82, $P < 0.05$). A greater waist reduction after surgery was predicted by lower VAT macrophage CD11b expression (r = 0.67, $P < 0.05$). SAT or VAT macrophage CD11b expression was

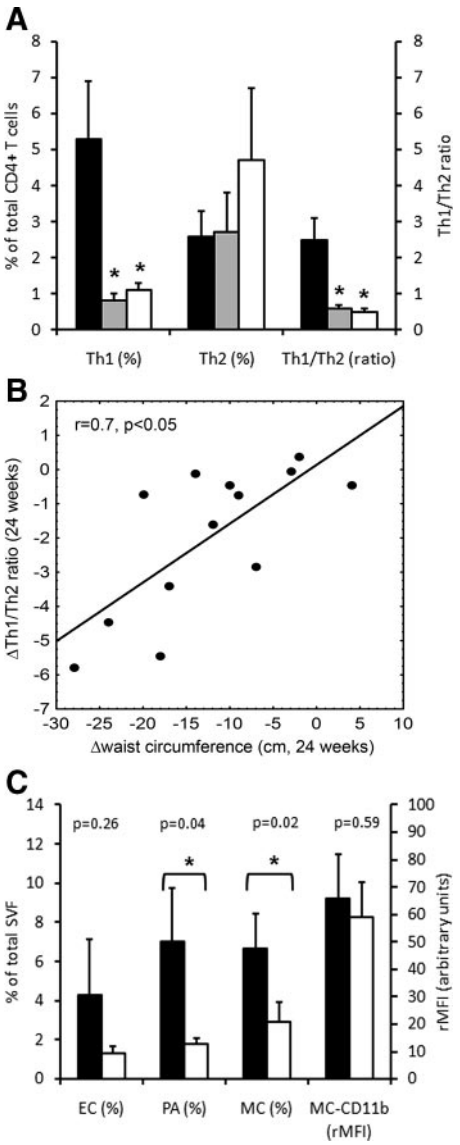

FIG. 1. A, Quantification of circulating T helper cells: Th1 and Th2 subsets at baseline (*black bars*), after 12 wk of energy restriction preoperatively (*gray bars*), and after 24 wk of energy restriction (12 wk after gastric banding surgery, *white bars*). Data are expressed as percent of total $CD4^+$ T cells and their ratio. *Error bars*, SEM. *, $P <$ 0.05. B, Association of change in Th1 to Th2 ratio and change in waist circumference (centimeters) during the 24-wk energy restriction ($r =$ 0.7, $P < 0.05$). C, SVF of SAT (*black bars*) and VAT (*white bars*), obtained during bariatric surgery: relative abundance (percent of total viable cells) of endothelial cells (EC), preadipocytes/$CD34^+$-precursor cells (PA), macrophages (MC), and expression of activation marker CD11b on MC (MC-CD11b), expressed as rMFI. *, $P < 0.05$.

not related to the weight lost in the 12 wk preceding gastric banding.

Discussion

This study of morbidly obese T2D and IGT subjects makes the novel observation that dietary energy restriction with a cumulative 13.5% weight loss reduced proinflammatory Th1 cell numbers by greater than 80%, shifting the Th1 to Th2 ratio toward an antiinflammatory Th2 phenotype. The intervention also reduced the activation status of T lymphocytes, monocytes, and neutrophils. A further novel finding was that lesser macrophage activation in SAT and VAT predicted a greater subsequent weight loss.

Our study is unique because all subjects had glucose disorders. We found that the reductions in immune cell activation and proinflammatory Th1 lymphocytes in response to energy restriction and weight loss were less in T2D compared with IGT, despite similar falls in weight and glucose and insulin levels. This suggests that glycemia or other factors specific to T2D, but not IGT, may influence immune cell phenotype and activation. We also examined the immune effects of energy restriction with weight loss before and after gastric banding, finding a continuous decline in immune cell activation over the whole 24 wk, suggesting that gastric banding did not have any additional effects on immune activation, apart from continued weight loss.

Data on the effects of weight loss after bariatric surgery on immune cells and inflammation are emerging, suggesting reduced circulating inflammatory markers (15). Gastric bypass in predominantly nondiabetic subjects decreased monocyte numbers and increased granulocyte CD62L expression, with no change in monocyte and neutrophil CD11b expression (16). Furthermore, gastric bypass decreased T lymphocyte CD69 and CD95 expression (17), indicative of reduced T lymphocyte activation, similar to our study. Our study builds on these data, indicating specific T lymphocyte phenotype shifts induced by energy restriction with weight loss.

The mechanisms by which energy restriction and weight loss attenuate proinflammatory activation of immune cells in obesity are unclear. Plausible intermediaries include functional changes in adipose tissue biology or distinct effects of energy restriction, independent of weight loss. Fasting in patients with rheumatoid arthritis (a Th1 mediated disease) reduced T lymphocyte numbers and activation status (CD69) and increased antiinflammatory Th2 lymphocytes (18), indicating acute energy restriction regulates immune cell activation. Improved insulin signaling may also contribute, promoting T lymphocyte differentiation toward a Th2 phenotype (19), consistent with our findings that the reduction in Th1/Th2 was associated with the reductions in weight and waist. Changes in adipose mass may also independently contribute.

Severe obesity is characterized by adipose tissue macrophage infiltration, considered to contribute to systemic insulin resistance (7). Our study found higher macrophage numbers in SAT compared with VAT. Controversy exists

as to whether any fat depot is more macrophage dense (20, 21). Our intriguing finding that lower SAT and VAT macrophage activation predicted a greater weight loss after surgery suggests adipose tissue-immune cells are involved in the regulation of weight loss after energy restriction and/or surgery. Two studies examined the interaction between adipose tissue macrophages and weight loss after energy restriction or surgery, observing weight loss is associated with decreased SAT macrophage density and SAT inflammatory gene expression (22, 23). Further studies are required to understand the effect of macrophage activation on adipose tissue biology during weight loss.

Limitations of this study include the lack of a control group undergoing dietary energy restriction without weight loss. Subjects were also in negative energy balance when studied at 12 and 24 wk. Therefore, this study cannot isolate the specific effects of weight loss, energy restriction, and gastric banding on the observed immune changes. Short-term refeeding studies would clarify this point. Another limitation is the use of HOMA-IR to measure insulin resistance in T2D; however, hyperinsulinemic-euglycemic clamps are technically difficult in morbid obesity. Insulin resistance is highly variable in obesity, perhaps contributing to this study not finding the expected reduction in insulin resistance.

In summary, this study found that dietary energy restriction with weight loss before and after gastric banding in obese subjects with T2D or IGT induces striking changes in immune cell phenotypes and activation status, resulting in a more antiinflammatory immune cell balance. Our findings indicate that both the innate and adaptive immune systems are worthy of further interrogation for their role in the regulation of chronic inflammation and metabolism in obesity-associated T2D.

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