Change of Cytokine Balance in Diet-Induced Obese Mice

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Although decreased T-cell function has been observed in obese human subjects and genetically obese animals, the precise role of immune functions in obesity is still unclear. To investigate immune functions in obesity, we examined the proliferative responses of splenic lymphocytes and their capacity to produce cytokines in the presence or absence of leptin, the protein produced by the *obese* gene, in diet-induced obese and control mice. For induction of obesity, C57BL/6J mice were fed a high-fat diet for 13 weeks. In mice fed the high-fat diet, body weight, fat pad weight, and tumor necrosis factor (TNF) α production by adipocytes were significantly increased relative to mice fed the normal diet. Lipopolysaccharide (LPS) stimulated proliferation of cultured splenocytes from diet-induced obese mice was also increased. However, production of interleukin (IL)-2 by splenic lymphocytes from obese mice was suppressed, whereas interferon (IFN)-γ and IL-4 production was increased. Exogenous lepitn regulated the cytokine production by cultured splenocytes from control and obese mice, respectively (upregulation of IFN-γ and downregulation of IL-2 in control mice, and downregulation of IL-4 in obese mice). These results suggest that changes in cytokine production by splenic lymphocytes in obesity are indicative of altered immune functions that might contribute to related complications, although the effect of difference in nutrient intake (macro and micro) may also have contributed to the changes.

OBESITY IS frequently associated with increased susceptibility to infections and several types of cancer.[1-3] These complications may be caused by changes in immune functions. Indeed, in recent studies of obese subjects and genetically obese animals, lymphocyte numbers and proliferative responses were lower than in controls.[4-9] Moreover, lymphocyte subsets have been shown to be altered in obesity.[8,9] However, most immune functions in obesity are not fully understood.

Recent studies have shown that leptin, the protein coded by the *obese* gene, is involved in some pathophysiological aspects of obesity.[10-12] Leptin is mainly derived from adipocytes and regulates appetite and energy expenditure.[13,14] Serum leptin concentration is increased in obesity and is strongly correlated with total body fat mass.[15,16] There are indications of a resistance to the effect of leptin in obesity.[17,18] The leptin receptor is expressed in several immune cell types,[19-21] and leptin can affect the proliferation and cytokine production in T cells and monocytes.[21,22] However, it is uncertain whether any role that leptin may play in obesity is linked to changes in the immune system. Genetically obese *ob/ob*[23] and *db/db* mice,[24] as well as Zucker fatty rats,[25] have mutations in the leptin or leptin receptor gene, but no equivalent mutations have been detected in the majority of humans with obesity.[26,27] It has been reported that C57BL/6J mice given a high-fat diet developed obesity and hyperinsulinemia.[28-30] Consequently, we have adopted this rodent model of diet-induced obesity as a means of examining the underlying causes of human obesity and have examined immune functions and the effect of leptin in this model by culturing splenocytes taken from normal or obese mice and then measuring proliferative responses and cytokine production after treatment with leptin and various mitogens.

MATERIALS AND METHODS

Animal and Diets

Twenty-four male C57BL/6J mice were obtained from Sankyo Laboratories (Tokyo, Japan) at 4 weeks of age. Animals were housed 4 per cage in a temperature-controlled room with a 12-hour light/dark cycle. They were allowed free access to water and diets. For induction of obesity, 12 mice were given a high-fat diet (AIN 93 supplemented with lard) and the other 12 mice were given a standard diet (AIN 93) as control. Diets were manufactured by Oriental Yeast (Tokyo, Japan). The composition of the diets is listed on Table 1.

Experimental Procedures

Both groups of mice were initially fed a standard diet for 1 week before the high-fat or control diets were started. All mice were maintained on their allotted diet for 13 weeks (weeks 2 to 14 of the study). At the end of every week in the entire study period, body weight and food consumption were measured. Mice were killed by cervical dislocation at the end of week 14.

Splenocyte Culture

After killing the mice, spleens were aseptically removed and placed in RPMI 1640 medium (NISSUI, Tokyo, Japan). Single cell suspensions were made by teasing spleens apart with a stainless steel mesh and filtering through a 250-μm nylon mesh. Cell suspensions were collected in sterile conical tubes and were washed 3 times in RPMI 1640 medium containing 5% heat-inactivated fetal calf serum (JRH, Lenaxa, Australia), L-glutamine (2 mmol/L, GIBCO, Grand Island, NY), penicillin (100 U/mL, GIBCO), and streptomycin (100 μg/mL, GIBCO), followed by centrifugation at 1,200 rpm for 10 minutes at 4°C. Cells were counted using a hemocytemeter and diluted in medium to a density of 4×10^6 cells/mL. Splenocytes were cultured in the presence of phytohemagglutinin (PHA; 1 μg/mL, DIFCO, Detroit, MI) or lipopolysaccharide (LPS; 10 μg/mL, Sigma, St Louis, MO) stimulation for 24 hours or 72 hours at 37°C in a 5% CO_2 atmosphere.

Proliferative Response of Splenocytes

After 72 hours of culture, the proliferative response of splenocytes was measured by an [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] (MTT) assay. After the addition of MTT solution (200 μg/mL), the cells were incubated for 3 hours at 37°C. The MTT-formazon product formation was dissolved by the addition of 10%

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Table 1. Composition of the Two Experimental Diets

Variable	Control Diet	High-fat Diet
Ingredients (g/100 g)		
Casein	14.0	14.0
L-cystine	0.2	0.2
Corn starch	46.6	17.6
α-corn starch	15.5	15.5
Sucrose	10.0	10.0
Soybean oil	4.0	4.0
Cellulose powder	5.0	5.0
AIN-93M mineral mix	3.5	3.5
AIN-93M vitamin mix	1.0	1.0
Heavy tartaric acid choline	0.3	0.3
Tertiary butyl hydroquinone	0.0008	0.0008
Lard	0	29.0
kcal/100 g	348.2	518.4
Caloric basis (as % of total calories)		
Fat	10.3	50.3
Carbohydrate	73.6	38.9
Protein	16.1	10.8
Fatty acid (g/100 g)		
Saturated fatty acid	0.6	12.1
Monounsaturated fatty acid	0.9	14.1
Polyunsaturated fatty acid	2.3	5.3

sodium dodecyl sulfate (SDS) 0.01N-HCl. The optical density of each well was measured using test and reference wavelengths of 550 nm and 650 nm. Data are expressed as the ratio of the absorbency of mitogen-stimulated cultures to the absorbency of nonstimulated cultures (referred to as the stimulation index). Culture time of splenocytes for the maximum response was determined before the assay and was not different between 2 groups.

Cytokine Production

For measurement of cytokine production, enzyme-linked immunosorbent assays (ELISA) were performed on supernatants of PHA or LPS-stimulated splenocytes in the presence or absence of recombinant leptin (Pepro Tech EC, London, England). Supernatants were collected after incubation for 24 hours, centrifuged, and stored at −30°C until analysis. Mouse-matched antibody pairs (Endogen, Cambridge, MA) for interleukin (IL)-2 and IL-5 measurement and enzyme-linked immunosorbent assay (ELISA) kit (Biosource International, Camarillo, CA) for interferon (IFN)-γ, IL-4, and tumor necrosis factor (TNF)-α measurement were used. Optimal incubation time of splenocytes for the cytokine production was decided before the assay.

Adipocyte Culture

Subcutaneous and visceral fat pads from each mouse were dissected and weighed separately. Adipocytes were isolated according to a previously published method.[31,32] Briefly, after washing by phosphate-buffered saline (PBS), visceral adipose tissue was cut into small pieces and digested with collagenase solution while shaking at 37°C. The cells were filtered through nylon mesh (250 μm). After centrifugation and washing with Hanks medium, the cells were counted using a haemocytometer. Cells were adjusted to a density of 2×10^5 cells/mL in Ham's F12 (NISSUI) and Dulbecco's Modified Eagle's (NISSUI) medium containing 10% heat-inactivated fetal calf serum (JRH), L-glutamine (2 mmol/L), penicillin (100 U/mL), and streptomycin (100 μg/mL). Adipocyte suspensions (0.5 mL) were placed in 24-well plates and cultured for 120 hours at 37°C in a 5% CO_2 atmosphere.

TNF-α Production by Adipocytes

TNF-α production by adipocytes was determined by ELISA using an ELISA kit (Biosource International). Supernatants of adipocyte cultures were collected after incubation for 120 hours, centrifuged, and stored at −30°C until analysis.

Statistical Analysis

Results are expressed as the mean ± SE. Statistical comparisons were made between obese and control mice for body weight, food and energy intakes, proliferative response, and cytokine production using the unpaired Student's *t* test. Differences between cultures of cells incubated with and without leptin were analyzed by the paired Student's *t* test.

RESULTS

Body Weights, Fat Pad Weights and Energy Intakes

Mice fed a high-fat diet (obese) had a significantly higher body weight in comparison to mice fed the control diet (control) from week 3 onwards until the end of the study. At the end of the study, the mean body weight of obese mice was 30.8 ± 1.24 g (n = 12), whereas in control mice, it was 23.5 ± 0.43 g (n = 12) (Fig 1A, and Table 2; $P < .001$, obese *v* control.).

The weights of both subcutaneous and visceral fat pads were heavier in obese mice. After feeding of experimental diets for 13 weeks, subcutaneous fat pad weights averaged 2.4 ± 0.35 g in obese mice and 0.6 ± 0.09 g in control mice (n = 12). Similarly, the mean visceral fat pad weight was 2.4 ± 0.32 g in obese mice and 0.5 ± 0.06 g in control mice (n = 12) (Table 2, $P < .0001$, obese *v* control.).

Although daily energy intakes in obese mice were elevated gradually and significantly higher than in control mice at week 14, the average of daily energy intakes in experimental periods showed no significant difference between the 2 groups (Fig 1B and Table 2).

TNF-α Production by Visceral Adipocytes

TNF-α concentrations in the culture supernatants of visceral adipocytes isolated from obese mice were higher than those from control mice (Fig 2; $P < .05$, obese *v* control.).

Proliferative Response of Splenocytes

Splenocytes isolated from obese mice had greater proliferative responses to LPS than those from control mice (Fig 3; $P < .001$), but there was no difference in their responses to PHA.

Cytokine Production by Splenocytes

IL-2 production by splenocytes isolated from obese mice was lower than that from control mice ($P < .05$). However, IL-4 ($P < .01$) and IFN-γ ($P < .05$) production by splenocytes from obese mice was higher than that from control mice. Although TNF-α and IL-5 production did not change significantly between the two groups of mice, obese mice tended to show a high level of cytokine production compared with control mice (Fig 4).

The Effect of Leptin on Cytokine Production by Splenocytes

To understand the effect of leptin on cytokine production by splenocytes from obese and control mice, we added leptin to the

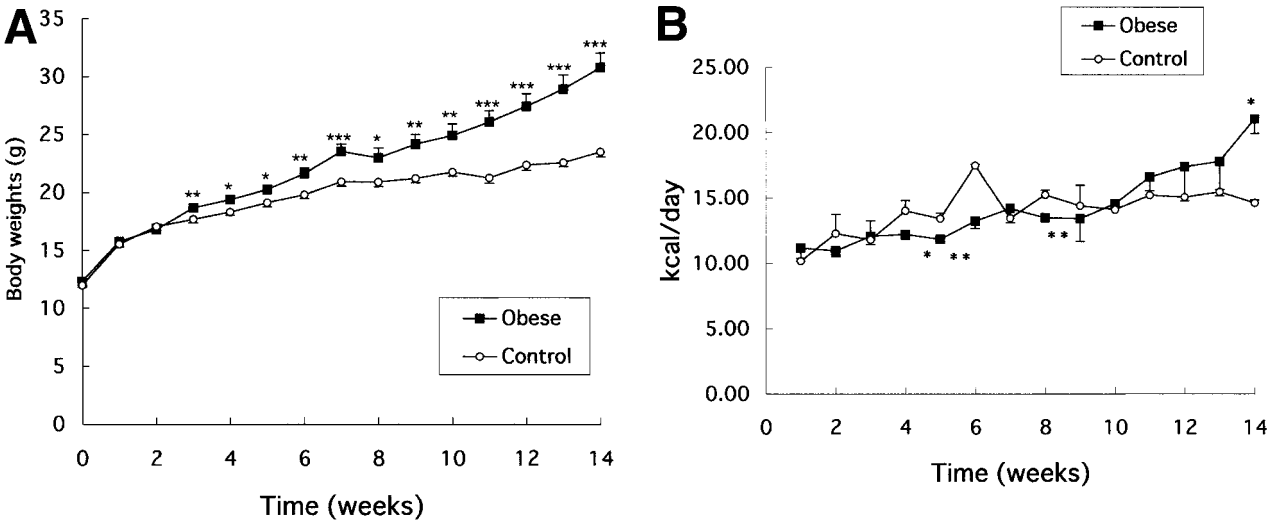

Fig 1. (A) Time course of changes in body weight of control mice (control) and mice with diet-induced obesity (obese). The difference between the 2 groups of mice was highly significant at all points from weeks 3 to 14 (obese *v* control, **P* < .05, ***P* < .01, ****P* < .001). Values are mean ± SE (n = 12 per group). (B) Time course of changes in energy intake of control mice (control) and mice with diet-induced obesity (obese) (obese *v* control, **P* < .05, ***P* < .01). Values are mean ± SE (n = 12 per group).

culture medium (500 ng/mL) and measured cytokine activities in supernatants. The optimal concentration of leptin was chosen from previous studies and confirmed before the assay (data not shown).

The addition of leptin to the culture medium suppressed IL-2 ($P < .01$) and increased IFN-γ ($P < .05$) production by splenocytes stimulated with PHA in control mice. In contrast, production of both cytokines was not changed by the addition of leptin in obese mice (Fig 5). IL-4 production by splenocytes stimulated with PHA was higher in obese mice than in control mice and was significantly suppressed by leptin (Fig 5; $P < .05$). In control mice, leptin slightly increased IL-4 production, although no statistically significant difference was observed. IL-5 and TNF-α production by splenocytes from both groups of mice was not altered by supplementation with leptin (data not shown).

DISCUSSION

As a means of understanding immune functions in human obesity, we have characterized immune functions and the influence of leptin on cytokine production in mice with and without diet-induced obesity.

Table 2. Energy Intake, Body Weight, and Fat Pad Weight

	Obese Group (n = 12)	Control Group (n = 12)	*P*
Energy intake (kcal/mouse/d)	14.28	14.08	.4
Final body weight (g)	30.8 ± 1.24	23.5 ± 0.43	<.001
Body weight gain (g)	18.5 ± 1.18	11.6 ± 0.43	<.0001
Subcutaneous fat pad weight (g)	2.4 ± 0.35	0.6 ± 0.06	<.0001
Visceral fat pad weight (g)	2.4 ± 0.32	0.5 ± 0.09	<.0001

NOTE. Obese *v* control. Values are means ± SE.

The C57BL/6J mice fed a high-fat diet in this study are a good model of human obesity and type II diabetes, developing weight gain and hyperinsulinemia.[28-30] It might be pointed out that not only was the fat intake different, but also the micronutrients and the protein intake of these obese mice, as compared with the control. Impaired immunity can be ascribed to the associated deficiency of protein or micronutrients intake in human and animals,[33-36] although selected nutrients in the diet used were completely removed or excessively reduced in those animal studies. Furthermore, there are a large number of reports on the effect of dietary lipids, both the amount and type of fat, on immune function.[33,37,38] Dietary lipids, particularly polyun-

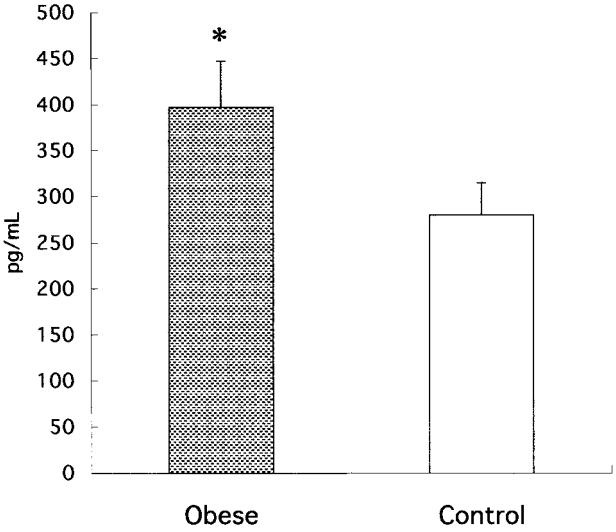

Fig 2. TNF-α production by cultured adipocytes of visceral fat cells from control mice (control) and mice with diet-induced obesity (obese) (obese *v* control, **P* < .05). Values are mean ± SE (n = 8 per group).

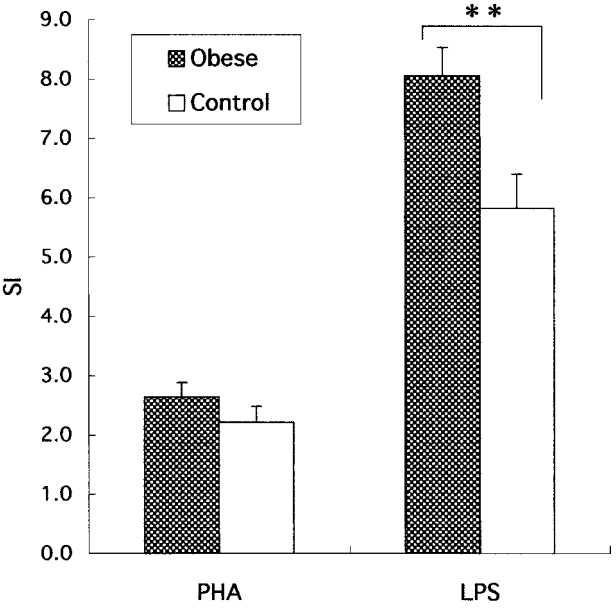

Fig 3. Proliferative responses of splenocytes isolated from control mice (control) and mice with diet-induced obesity (obese) after stimulation with PHA or LPS (obese *v* control, **$P < .01$). Values are mean ± SE (n = 8 per group). Unstimulated base line responses were not different between the 2 groups.

saturated fatty acids (PUFA), may influence immune functions by changing cell membrane fluidity or the production of eicosanoids and cytokines.[39-41] As we used lard for high-fat diets, because of a low level of PUFA in the present study, no significant difference was observed in PUFA intake between obese and control mice. Therefore, alteration of immunity in this study could be associated with induction of obesity itself. However, the effect of difference in nutrient intake (macro and micro) may also have contributed to the results observed.

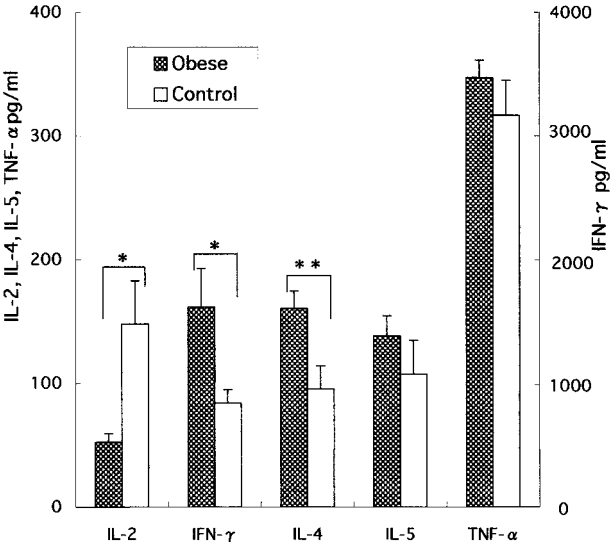

Fig 4. Cytokine production by splenocytes isolated from control mice (control) and mice with diet-induced obesity (obese) after stimulation with PHA (IL-2, IFN-γ, IL-4, IL-5), and LPS (TNF-α) (obese *v* control, *$P < .05$, **$P < .01$). Values are mean ± SE (n = 8 per group).

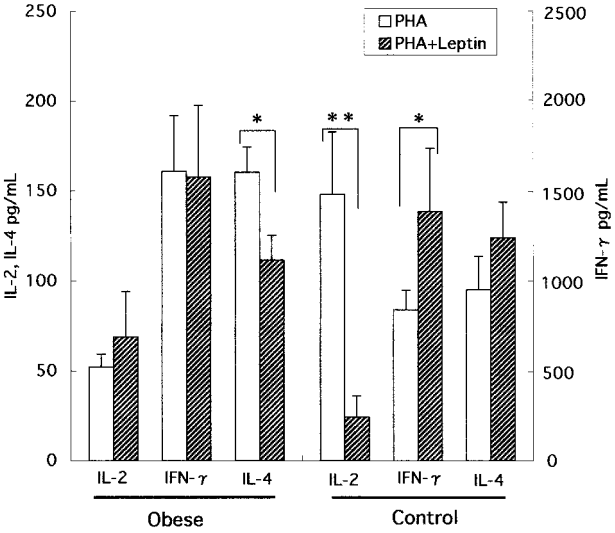

Fig 5. Effect of supplementation of medium with leptin (500 ng/mL) on the production of IL-2, IFN-γ, and IL-4 by splenocytes isolated from control mice (control) and mice with diet-induced obesity (obese) (*$P < .05$, **$P < .01$). Values are mean ± SE (n = 8 per group).

Recent studies have reported that TNF-α, an immunoregulatory cytokine, was produced by adipocytes.[42-44] In human obesity and animal obese models, expression of this cytokine in adipose tissue and its levels in serum are higher than in lean controls.[42-45] We have now also shown an increased TNF-α production by cultured adipocytes from mice with diet-induced obesity. Because TNF-α can inhibit the tyrosine kinase of the insulin receptor in muscle and fat,[46] it is possible that an increase in this cytokine may be one of the factors contributing to obesity-related insulin resistance.

Changes in cytokine balance within the immune system are correlated with many diseases. $CD4^+$ T cells (Th) are subcategorized into Th1 and Th2 cells, which have specific, but different functions. Th1 cells secrete cytokines, such as IL-2 and IFN-γ, and function in cellular immunity, whereas Th2 cells secrete cytokines, such as IL-4 and IL-5, and act to support antibody production.[47] A predominance of Th2 cells results in an altered cytokine balance that is frequently observed in allergic diseases.[48-50] Some surveys have found that obesity was more prevalent in children and adults with symptoms of asthma.[51,52] Recent reports have also shown that morbidity from atopy and rhinitis symptoms were higher in teen-age girls of high body mass index (BMI) than in girls of low BMI.[53] In this study, development of obesity in mice induced a change in cytokine profile as evidenced by a decreased IL-2 and increased IFN-γ, IL-4, and possibly IL-5 production, which supports the hypothesis that obesity is a risk factor for allergic diseases, as well as for infectious diseases.

Although both IL-2 and IFN-γ were produced by Th1 cells, we observed opposite results in cytokine production in these obese mice. IL-2 (Th1) production by splenocytes was suppressed in this obese mice. Conversely IFN-γ (Th1) and IL-4 (Th2) production was increased in obese mice. IFN-γ plays an important role, which protects against microbiological infection, however the high level of this cytokine may suppress

T-cell function after antigenic stimulation.[54] Alteration of cytokine production profile in obese mice in this study was also consistent with previous reports in aged animals. IL-2 production by lymphocytes was lower in old animals compared with those in control,[55-57] while an enhanced IFN-γ and Th2 cytokine production by the old group was reported.[55,56,58,59] Previous analysis showed that production of IFN-γ and IL-4 is predominantly, if not exclusively, restricted to the CD45RO high memory/effector T-cell subset, whereas IL-2 may be produced by both the CD45RA high and CD45RO low subsets.[60]

An impaired proliferative response of splenocyte from diet-induced obese mice was not obtained in this study, which is not consistent with previous reports using human obesity and genetically obese animals. This may be due to the differences of body weight, level of serum factors, and T-cell subset profiles between this obese model and genetically obese animals. Moreover, it is possible that lymphocyte proliferation can be regulated by not only IL-2, but other factors, which might be changed by obesity.

Leptin regulates appetite and energy expenditure through binding to leptin receptors in the hypothalamus. Recent studies reported that leptin receptors are expressed not only in the hypothalamus, but also in peripheral tissues,[61,62] and it has been suggested that leptin plays important roles in peripheral tissues, including immune cells. Indeed, leptin affects proliferation and differentiation of hematopoietic cells and T cells.[19,21,22] Leptin administration protected lymphoid atrophy in mice with low leptin level by starvation and increased lymphocyte cellularity in *ob/ob* mice, which could not produce normal leptin.[63] However, elevations in serum leptin and resistance to the effects of leptin in hypothalamus develop in human obesity and diet-induced obese mice.[15,16] No mutation in the genes encoding leptin or the leptin receptor have been detected in the majority of human obesity cases studied.[26,27] Therefore, it is possible that impaired immunity in obesity is related to the change of sensitivity of immune cells to leptin.

In the present study, we found that the effects of leptin on the response of splenocytes were altered in diet-induced obese mice. Exogenous leptin had different effects on cytokine production between obese and control mice. Leptin attenuated IL-4 production by splenocytes from obese mice, but not from control mice, and suppressed IL-2 and increased IFN-γ production by the cells from control mice, but not from obese mice. It was reported that leptin modulated IL-4 production only in memory T cells (CD45RO) producing a measurable amount of this cytokine from normal subjects, whereas IL-2 and IFN-γ production was affected in most T-cell subsets.[21] These observations suggest that more sensitive responsiveness of IL-4 to leptin might occur in obesity. Changes in cytokine balance in mice with diet-induced obesity in this study may be related to sensitivity to leptin. It was reported that fasting increased leptin receptor RNA expression in normal C57BL/6J mice brain.[64] Hence, it is possible that functional defects of leptin receptors may explain the change of sensitivity to leptin in obesity. In any case, the number and function of leptin receptors on immune cells during obesity should be further investigated.

In conclusion, this study has shown that obesity is associated with alterations in cytokine balance and sensitivity to leptin on immune cells. These results suggest that changes of cytokine production in obesity are indicative of altered immune functions that might contribute to related complications.

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REFERENCES

1. Marks HH: Influence of obesity on morbidity and mortality. Bull NY Acad Med 36:296-312, 1960

2. Tracey VV, Dew C, Harper JR: Obesity and respiratory infection in infants and young children. Br Med J 1:17-19, 1971

3. Garfinkel L: Overweight and cancer. Ann Intern Med 103:1034-1036, 1985

4. Tanaka S, Inoue S, Isoda F, et al: Impaired immunity in obesity: Suppressed but reversible lymphocyte responsiveness. Int J Obes 17:631-636, 1993

5. Nieman DC, Henson DA, Nehlsen-Cannarella SL, et al: Influence of obesity on immune function. J Am Diet Assoc 99:294-299, 1999

6. Candra RK: Cell-mediated immunity in genetically obese (C57BL/6J *ob/ob*) mice. Am J Clin Nutr 33:13-16, 1980

7. Fernandes G, Handwerger BS, Yunis EJ, et al: Immune response in the mutant diabetic C57BL/K*s-db*+ mouse. Discrepancies between in vitro and in vivo immunological assay. Nature 394:897-900, 1998

8. Tanaka S, Isoda F, Yamakawa T, et al: T lymphopenia in genetically obese rats. Clin Immunol Immunopathol 86:219-225, 1998

9. Sharon F, Elike E, James M, et al: T-lymphocyte subsets in patients with abnormal body weight: Longitudinal studies in anorexia nervosa and obesity. Int J Eat Disord 20:295-305, 1996

10. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. Science 274:1185-1188, 1996

11. Girard J: Is leptin the link between obesity and insulin resistance. Diabete Metab 23:16-24, 1997

12. Chehab FF, Mounzih K, Lu R, et al: Early onset of reproductive function in normal female mice treated with leptin. Science 275:88-90, 1997

13. Campfield LA, Smith FJ, Buisez Y, et al: Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546-549, 1995

14. Halaas JL, Gajiwala KS, Maffei M, et al: Weight-reducing effects of the plasma protein encoded by the *obese* gene. Science 269:544-546, 1995

15. Maffeei M, Halaas J, Ravussin E, et al: Leptin level in human and rodent: Measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. Nat Med 1:1155-1161, 1995

16. Considine RV, Sinbe MK, Heiman ML, et al: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292-295, 1996

17. Frederichi RC, Hamann A, Anderson S, et al: Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. Nat Med 1:1311-1314, 1995

18. Heek MV, Compton DS, France CF, et al: Diet-induced obese mice develop peripheral, but not central, resistance to leptin. J Clin Invest 99:385-390, 1996

19. Gainsford T, Willson T, Metcalf D, et al: Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc Natl Acad Sci USA 93:14564-14568, 1996

20. Nakao T, Hino N, Yamane T, et al: Expression of the leptin receptor in human leukaemic blast cells. Br J Haematol 102:740-745, 1998

21. Lord GM, Matares G, Howard JK, et al: Leptin modulates the T-cell immune response and reverses starvation-induced immunosupression. Nature 394:897-901, 1998

22. Loffreda S, Yang SQ, Lin HZ, et al: Leptin regulates proinflammatory immune responses. FASEB J 12:57-65, 1998

23. Zang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-432, 1994

24. Lee GH, Proenca R, Montez JM, et al: Abnormal splicing of the leptin receptor in diabetic mice. Nature 379:632-635, 1996

25. Chua SCJ, Chung WK, Wu-Peng XS, et al: Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271:994-996, 1996

26. Shigemoto M, Nishi S, Ogawa Y, et al: Molecular screening of both the promoter and the protein coding regions in the human *ob* gene in Japanese obese subjects with non-insulin-dependent diabetes mellitus. Eur J Endocrinol 137:511-513, 1997

27. Considene RV, Considine EL, Williams CJ, et al: The hypothalamic leptin receptor in humans. Diabetes 19:992-994, 1996

28. Ikemoto S, Takahashi M, Tunoda N, et al: High-fat diet-induced hyperglycemia and obesity in mice: Differential effect of dietary oils. Metabolism 45:1539-1546, 1996

29. Rebuffe-Scrive M, Surwit R, Feinglos M, et al: Regional fat distribution and metabolism in a new mouse model (C57BL/6J) of non-insulin-dependent diabetes mellitus. Metabolism 42:1405-1409, 1993

30. Surwit RS, Feinglos MN, Rodin J, et al: Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism 44:645-651, 1995

31. Rodbell M: Metabolism of isolated fat cells. J Biol Chem 239:375-381, 1963

32. Sugihara H, Yonemitsu N, Miyabara S, et al: Primary culture of unilocular fat cell: Characteristics of growth in vitro and changes in differentiation properties. Differentiation 31:42-49, 1986

33. William RB: Single nutrients and immunity. Am J Clin Nutr 35:417-468, 1982

34. Nikawa T, Odahara K, Koizumi H, et al: Vitamin A prevents the decline in immunogolobulin A and Th2 Cytokine levels in small intestinal mucosa of protein-malnourished mice. J Nutr 129:934-941, 1999

35. Dawson H, Li N, DeCicco K, et al: Chronic marginal vitamin A status reduces natural killer cell number and function in aging Lewis rats. J Nutr 129:1510-1517, 1999

36. Shin H, Scott M, Stevenson M, et al: Energy restriction and zinc deficiency impair the functions of murine T cells and antigen-presenting cells during gastrointestinal nematode infection. J Nutr 128:20-27, 1998

37. Lin B, Huang C, Chiang B, et al: Dietary fat influences Ia antigen expression, cytokines and prostaglandin E2 production of immune cells in autoimmune-prone NZB $\times$ NZW F1 mice. Br J Nutr 75:711-722, 1996

38. Pablo MD, Ortega E, Gallego A, et al: The effect of dietary fatty acid manipulation on phagocytic activity and cytokine production by peritoneal cells from Balb/c mice. J Nutr Sci Vitaminol (Tokyo) 44:57-67, 1998

39. Gallai V, Sarchielli P, Trequattrini A, et al: Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. J Neuroimmunol 56:143-153, 1995

40. Xi S, Cohen D, Chen L: Effects of fish oil on cytokines and immune functions of mice with murine AIDS. J Lipid Res 39:1677-1687, 1998

41. Jolly C, McMurray D, Chapkin R: Effect of dietary n-3 fatty acids on interleukin-2 and interleukin-2 receptor alpha expression in activated murine lymphocytes. Prostaglandins Leukot Essent Fatty Acids 58:289-293, 1998

42. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. Science 259:87-91, 1993

43. Hotamisligil GS, Arner P, Caro JF, et al: Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 95:2409-2415, 1995

44. Kern PA, Saghizadeh M, Ong JM, et al: The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 95:2111-2119, 1995

45. Dandona P, Weinstock R, Thusu K, et al: Tumor necrosis factor-α in sera of obese patients: Fall with weight loss. J Clin Invest 83:2907-2910, 1998

46. Hotamisligil GS, Budavarid A, Murray D, et al: Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-α. J Clin Invest 94:1543-1549, 1994

47. Abbas AK, Murphy KM, Sher A: Functional diversity of helper T lymphocyte. Nature 383:787-793, 1996

48. Paul WE: Interleukin-4: A protopic immunoregulatory lymphokine. Blood 77:1859-1870, 1991

49. Abehsira-Amer O, Gibert M, Joliy M, et al: IL-4 play a dominant role in the differential development of Th 0 into Th 1 and Th 2 cells. J Immunol 148:3820-3829, 1992

50. Hogan SP, Mould A, Kikutani H, et al: Aeroallergen-induced eosinophilic inflammation, lung damage, and airway hyperreactivity in mice can occur independently of IL-4 and allergen-specific immunoglobulins. J Clin Invest 99:1329-1339, 1997AU#2

51. Luder E, Melnik TA, DiMaio M: Association of being overweight with greater asthma symptoms in inner city black and Hispanic children. J Pediatr 132:699-703, 1998

52. Teran LM: Obesity and asthma: Cause for concern? Clin Exp Allergy 29:291-293, 1999

53. Huang SH, Shiao GM, Chou P: Association between body mass index and allergy in teenage girls in Taiwan. Clin Exp Allergy 29:323-329, 1999

54. Ozawa H, Aiba SN, Tagami H: Interferon-gamma and interleukin-10 inhibit antigen presentation by Langerhans cells for T helper type 1 cells by suppressing their CD80 (B7-1) expression. Eur J Immunol 26:648-652, 1999

55. Wakikawa A, Utsuyama M, Wakabayashi A, et al: Age-related alteration of cytokine production profile by T cell subsets in mice: A flow cytometric study. Exp Gerontol 34:231-242, 1998

56. Pioli C, Pucci S, Barile S, et al: Role of mRNA stability in the different patterns of cytokine production by $CD4^+$ cells from young and old mice. Immunology 94:380-387, 1998

57. Ginaldi L, De Martinis M, D'Ostilio, et al: The immune system in the elderly: II. Specific cellular immunity. Immunol Res 20:109-115, 1999

58. Engwerda CR, Fox BS, Handwerger BS: Cytokine production by T lymphocytes from young and aged mice. J Immunol 156:3621-3630, 1996

59. Paganelli R, Scala E, Rosso R, et al: A shift to Th0 cytokine production by $CD4^+$ cells in human longevity: Studies on two healthy centenarians. Eur J Immunol 26:2030-2034, 1996

60. Picker LJ, Singh MK, Zdraveski Z, et al: Direct demonstration of cytokine synthesis heterogeneity among human memory/effector T cells by flow cytometry. Blood 86:1408-1419, 1995

61. Fei H, Okano HJ, Li G, et al: Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. Proc Natl Acad Sci USA 94:7001-7005, 1997

62. Lollman B, Gruninger S, Stricker-Krongrad A, et al: Detection and quantification of the leptin receptor splice variants ob-Ra, b, and, e in different mouse tissues. Biochem Biophys Res Commun 238:648-652, 1997

63. Howard JK, Lord GM, Matarese G, et al: Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in *ob/ob* mice. J Clin Invest 104:1051-1059, 1999

64. Lin S, Huang XF: Fasting increases leptin receptor mRNA expression in lean but not obese (*ob/ob*) mouse brain. Neuroreport 10:3625-3629, 1997