Effect of Diet-Induced Obesity on Ovalbumin-Specific Immune Response in a Murine Asthma Model

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Some epidemiologic surveys have demonstrated that asthma is more prevalent in obese children and adults. However, the mechanism of association between obesity and asthma has not been fully clarified. This report investigates a murine model for antigen-induced asthma and diet-induced obesity from an immunologic perspective. For the induction of obesity, C57BL/6J mice were fed a high-fat diet supplemented with lard or soybean oil. Mice were then sensitized and challenged with ovalbumin (OVA) to induce allergic lung inflammation. OVA-specific serum immunoglobulin levels were lower in obese mice compared with non-obese control mice. The decline of OVA-specific IgE in the soybean oil group was found to be especially pronounced. However, obese mice with OVA-induced asthma showed a higher sensitivity of antigen-induced T-cell responses, and increased gamma interferon (IFN-γ) production of splenocytes with phytohemagglutinin (PHA) stimulation. Furthermore, mast cell numbers in the tracheal mucosa were increased in obese mice upon sensitization by OVA. These results suggest that obesity-induced changes in T-cell function may be partly involved in the pathophysiology of asthma in human obesity, rather than Ig E-mediated allergic responses.

OBESITY IS ASSOCIATED with an increased susceptibility to infection and several types of cancer, due to impaired immunity.[1,2] It is generally accepted that part of the cause of these complications with obesity is related to changes in immune function. Previous studies reported that T-cell function but not B-cell function was impaired in obesity. In obesity, T-cell numbers are decreased, and proliferation of T cells is lower than in controls.[3-5] Moreover T-cell subsets are altered in obesity.[4-6] However, many metabolic and endocrinologic factors that can affect immunity are involved in human obesity, and those factors are frequently different in each individual case. Accordingly, immune function in obesity, including antigen-specific allergic responses, is not fully understood. Recent epidemiologic studies showed that the prevalence of asthma in obesity has increased in children and adults.[6,7] It is also reported that morbidity due to atopy and rhinitis symptoms was higher in teenage girls of high body mass index (BMI) than in girls of low BMI.[8] However, there is no experimental evidence regarding the high prevalence of allergic disease in human obesity.

C57BL/6J mice fed a high-fat diet that develop weight gain and hyperinsulinemia are a good model of human obesity and type 2 diabetes.[9,10] We previously reported on changes of cytokine balance in obesity.[11] Production of certain cytokines by splenocytes was decreased, but levels of others were increased in diet-induced obese mice. These changes in obesity may be involved in allergic disease, such as allergic asthma, because cytokines play an important role in the pathophysiology. In this study, to elucidate the relationship between obesity and asthma, effects of obesity induced by different foods (high-fat diet supplemented with lard or soybean oil) on allergic immune responses were examined.

MATERIALS AND METHODS

Animals and Diets

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. Two groups (14 to 15 mice per group) of mice were induced to obesity by a high-fat diet supplemented with lard or soybean oil. Another group of mice was given a standard diet as a control. On a caloric basis, the high-fat diet consisted of 10.8% protein, 38.9% carbohydrate, 50.3% fat, whereas the standard diet consisted of 16.1% protein, 73.6% carbohydrate, 10.3% fat. Mice were allowed free access to water and food. Diets were manufactured by Oriental Yeast (Tokyo, Japan). Once every week for the study period of 16 weeks, body weight and food consumption of all mice were measured. Subcutaneous (inguinal) and visceral (retroperitoneal) adipose tissues were dissected from each animal according to defined anatomic landmarks, and weighed after the mice were killed.

Induction of Airway Hypersensitivity

After all mice were maintained on their allotted diets for 13 weeks, induction of airway hypersensitivity was started. Mice were sensitized to ovalbumin (OVA; Sigma, St Louis, MO) and challenged according to a modification of the method described in previous reports.[12] Briefly, 7 or 8 mice in each diet group (L [lard]-OVA, S [soybean oil] -OVA, C [standard]-OVA) were immunized by intraperitoneal (IP) injection with 50 μg OVA absorbed to 4 mg alum in 0.5 mL phosphate-buffered saline (PBS). Seven days later, the mice received an identical booster IP immunization. On day 7 and 14 after the second immunization, mice were challenged with aerosolized 1% OVA (wt/vol) in PBS inside a plastic chamber for 30 minutes, twice on each challenge day. The aerosols were generated using a nebulizer (NE-U07; Omron, Mie, Japan). Another 6 to 7 mice in each diet group (L [lard]-PBS, S [soybean oil]-PBS, C [standard]-PBS) were sensitized by IP injection of alum only in 0.5 mL PBS, and were challenged with aerosolized PBS only. Three days after the last aeroallergen challenge, mice were killed by cervical dislocation, and blood, spleen, and trachea were harvested.

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Table 1. Final Body Weight, Energy Intake, Fat Pad Weight, and Serum Leptin Concentration

	Lard (n = 15)	Soybean Oil (n = 14)	Control (n = 15)
Final boy weight (g)	26.8 ± 0.96*	27.7 ± 0.94*	20.8 ± 0.20
Energy intake (kcal/d)	17.1 ± 1.17*	17.3 ± 0.85*	9.1 ± 0.21
Subcutaneouse fat pad weight (g)	1.3 ± 0.13*	1.3 ± 0.14*	0.4 ± 0.03
Visceral fat pad weight (g)	1.1 ± 0.13*	1.2 ± 0.15*	0.3 ± 0.03
Serum leptin concentration (pg/mL)	511.4 ± 86.45*	553.1 ± 78.97*	97 ± 18.02

NOTE. Values represent mean ± SE.
*$P < .01$ *v* control.

Measurement of Serum Concentration of Antibodies and Leptin

Blood was drawn and serum was obtained by centrifugation. Serum OVA-specific antibodies (IgG, IgG1, IgG2a, and IgE), nonspecific total IgG, and leptin were measured by enzyme-linked immunosorbant assay (ELISA) using the standard protocol. For measurement of OVA-specific antibodies, the ELISA plate was coated with OVA (20 μg/mL in $NaHCO_3$ buffer, pH 9.6). After incubating the sera in the coated plate, biotinylated rabbit anti-mouse IgG, IgG1, IgG2a monoclonal antibody (mAb) (Zymed Laboratories, San Francisco, CA) was used for detection. In the measurement of IgE, biotinylated rat anti-mouse IgE mAb (LO-ME-3, Biosource International, Camarillo, CA) was used. For the measurement of total IgG, the plate was coated with rabbit anti-mouse IgG mAb (Zymed Laboratories). All antibodies were detected by peroxidase-conjugated streptavidin (DAKO, Kyoto, Japan), and *O*-phenylenediamine (Sigma). The ELISA for serum leptin concentration was conducted using antibody pairs and recombinant mouse leptin (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions.

Preparation of Splenocytes

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 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 hemocytometer and diluted in medium to a density of 4×10^6 cells/mL. Splenocytes were cultured in the presence of OVA (100 μg/mL), phytohemagglutinin (PHA, 2 μg/mL; DIFCO, Detroit, MI), or lipopolysaccharide (LPS, 10 μg/mL; Sigma) stimulation for 48, 72, or 120 hours at 37°C in a 5% CO_2 atmosphere.

Proliferative Response of Splenocytes

After 72 hours (PHA, LPS) or 120 hours (OVA) of culture, the proliferative responses of splenocytes were measured by [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] (MTT) assay. Following 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% sodium dodecyl sulfate (SDS) in 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 relative proliferative index calculated with optical density (OD). Culture time of splenocytes for the maximum response was determined before the assay, and was the same for all groups.

Interleukin-2 and Gamma Interferon Production

For measurement of cytokine production, ELISAs were performed on culture supernatants of OVA- or PHA-stimulated splenocytes. Supernatants were collected after incubation for 48 hours, centrifuged, and stored at -30°C until analysis. Mouse-matched antibody pairs (interleukin-2 [IL-2]) and ELISA Mini Kits (gamma interferon [IFN-γ], Endogen, Cambridge, MA) were used for this assay. Optimal incubation time of splenocytes for cytokine production was decided before the assay.

Histologic Analysis

The tracheas were embedded in OCT compound (Tissue-Tec, Tokyo, Japan), snap-frozen in liquid nitrogen, and sectioned at 8-μm thickness. The sections were stained with toluidine blue at pH 4. Numbers of mast cells were counted in each section using a light microscope.

The slides were evaluated for any abnormalities by a pathologist blinded to the treatment groups.

Statistical Analysis

Data are shown as the mean ± SE. Statistical comparisons of all data except for mast cell numbers were made by analysis of variance (ANOVA) including all possible 2-way interactions, and each group was compared with the others by Fisher's protected least-significant difference test. Unpaired Student's *t* test was used to determine differences in the means of mast cell numbers in tracheal mucosa. The relationship between serum leptin and body weight in mice was determined by Pearson linear regression 2-tailed analysis.

RESULTS

Body Weight, Energy Intake, Fat Pad Weight, and Serum Leptin Concentration

Mice fed high-fat diets supplemented with lard or soybean oil showed a significant increase in body weight, energy intake, subcutaneous and visceral fat pad weight, and serum leptin concentration compared with control groups (Table 1). Serum leptin concentration was strongly correlated with body weight or fat pad weight of mice in all groups (data not shown). In all diet groups, no significant differences were observed in these parameters between the groups with or without immunization and challenge by OVA.

Proliferation and IFN-γ Production of Splenocytes by Mitogen Stimulation

In the L-PBS and S-PBS groups without sensitization by OVA, the proliferative response of splenocytes stimulated with PHA was significantly lower than in the C-PBS group (Fig 1A). Responses to LPS stimulation also were low in L-PBS mice compared with C-PBS mice (Fig 1B). On the other hand, no significant differences were observed in these proliferative responses between diet groups sensitized with immunization and challenge by OVA (Fig 1A and B).

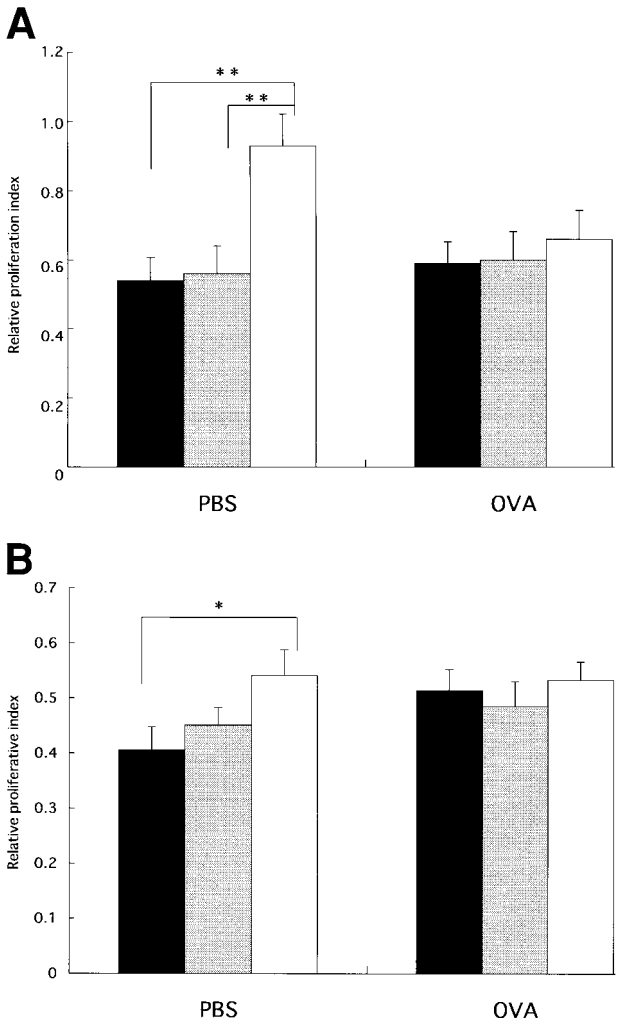

Fig 1. The effect of obesity and diet on proliferative responses of splenocytes stimulated with (A) PHA and (B) LPS. ■, Lard; ▨, soybean oil; □, control. Values are mean ± SE (*$P < .05$, **$P < .01$). n = 6 for L-PBS, S-PBS, C-PBS, and S-OVA; n = 7 for L-OVA, C-OVA.

Increased levels of IFN-γ production by splenocytes with PHA stimulation were shown in the L-OVA and S-OVA groups, although they were not statistically significant (data not shown).

Proliferation and IL-2 Production of Splenocytes in Response to OVA

Proliferation of splenocytes in response to OVA in the L-OVA group was significantly higher than in the C-OVA group. S-OVA mice also showed high sensitivity to OVA, compared with C-OVA mice, although this result was not found to be statistically significant. Both L-OVA and S-OVA groups showed a significantly higher induction of IL-2 production from splenocytes in response to OVA (Table 2). L-OVA mice showed the highest response to OVA among all groups in these 2 parameters.

OVA-Specific IgG, IgG1, IgG2a, and IgE, and Total IgG Concentration

All PBS groups (L-PBS, S-PBS, C-PBS) without immunization and challenge by OVA had low levels of OVA-specific antibodies in the sera. All OVA groups (L-OVA, S-OVA, C-OVA) showed significantly higher antibody titers for OVA in the sera, compared with the PBS groups. In the L-OVA and S-OVA diet groups, OVA-specific IgG, IgG1, IgG2a, and IgE titers were rather suppressed relative to those in the C-OVA group (Fig 2A through D). S-OVA mice, especially, showed low IgE concentration. On the other hand, serum nonspecific total IgG levels did not differ between the diet groups (data not shown).

Histologic Analysis

The PBS groups had fewer mast cells in the tracheal mucosa compared with the OVA groups (data not shown). In obese mice (L-OVA, S-OVA), mast cell numbers were higher than those in C-OVA mice, especially in S-OVA mice (Table 3).

DISCUSSION

It is well known that some immune functions are impaired in obesity. In obese subjects and genetically obese animals, lymphocyte numbers and proliferative responses are altered.[3-5] Immunologic dysfunctions in obesity are mainly in cell-mediated immunity, and B-cell functions remain unchanged.[5] However, the changes in antigen-specific immune responses in obesity have not been clarified. Although some epidemiologic studies have shown a high prevalence of asthma or allergic disease in human obesity,[6-8] the immunologic mechanisms are not understood. Previously, we reported that cytokine production by splenocyte was altered in diet-induced obese mice.[11] Accordingly, to analyze the immunologic effects of obesity in allergic reactions, we investigated antigen-induced allergic asthma in diet-induced obese mice. In order to identify the effects of diet on asthma in obesity, obesity was induced by 2 different high-fat diets, supplementation with lard or soybean oil, since fatty acids composition is different between the two. Lard is composed of a high percentage of saturated fatty acids and a low percentage of unsaturated fatty acids, in contrast to soybean oil. It was reported that these high-fat diets could induce obesity equally in C57BL/6J mice.[9]

Table 2. The Effects of Obesity and Diets on Proliferative Response and IL-2 Production of Splenocytes Stimulated With OVA

	Ratio of the Response (OVA group/PBS group)	
	Proliferation	IL-2
Lard	2.62 ± 0.55*	1.75 ± 0.15‡
Soybean oil	1.81 ± 0.18	1.66 ± 0.19†
Control	1.20 ± 0.25	0.83 ± 0.02

NOTE. Data are expressed as the ratio of the absorbency (proliferation) or concentration (IL-2) per mouse of OVA groups to the mean of absorbency or concentration of PBS groups in each diet group. Values are mean ratio ± SE. n = 6 for L-PBS, S-PBS, C-PBS, and S-OVA; n = 7 for L-OVA and C-OVA.

*$P < .05$, †$P < .01$, ‡$P < .001$ *v* control groups.

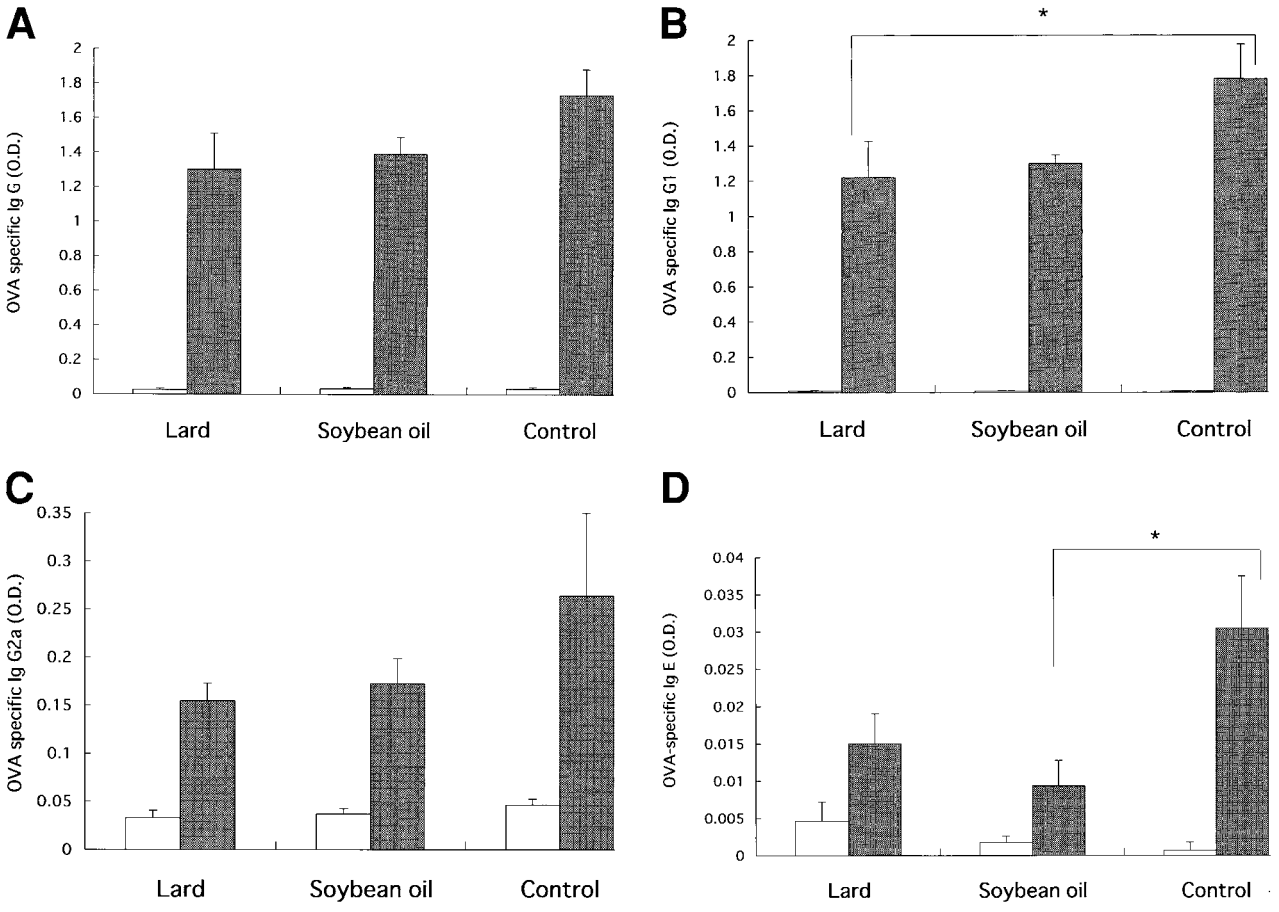

Fig 2. The effect of obesity and diets on serum OVA-specific (A) IgG, (B) IgG1, (C) IgG2a, and (D) IgE. □, PBS; ▨, OVA. Values are mean ± SE (*$P < .05$). n = 6 for L-PBS, S-PBS, C-PBS, and S-OVA; n = 7 for L-OVA, C-OVA.

In this study, final body weight and fat pad weight were significantly increased in the lard and soybean diets groups. Nonspecific T-cell proliferative responses stimulated with PHA were suppressed in all obese groups without sensitization by OVA. B-cell proliferation by LPS stimulation also showed a slightly lowered response in the lard group compared with the control, although that change was not as pronounced as the T-cell effect. There was no significant difference in total IgG concentrations in serum between all groups. The findings of nonspecific T-cell and B-cell responses in obese mice without OVA sensitization are consistent with previous reports.[3-5] Meanwhile, in the groups with OVA sensitization, no significant differences in proliferative responses were observed in PHA or LPS stimulation between all diet groups. In contrast, the obese groups showed high antigen-specific T-cell responses to OVA stimulation in vitro (proliferation and IL-2 production) from the low baseline response. These findings may suggest an increased antigen-specific T-cell sensitivity in obesity.

Table 3. Histologic Analysis of Mast Cell Numbers in the Tracheal Mucosa

	L-OVA (n = 8)	S-OVA (n = 5)	C-OVA (n = 7)
Mast cell number in trachea (no./mm length of trachea)	9.38 ± 1.22	10.85 ± 2.05*	6.15 ± 1.28

NOTE. Values represent mean ± SE.
*$P < .05$ *v* C-OVA.

Most allergic diseases, including asthma, are frequently linked to atopy in connection with elevated serum antigen-specific IgE antibodies. However, OVA-specific IgE, one of the important factors in allergic diseases, was lower in the obese groups in this study, especially in the soybean oil group. This result seems to be inconsistent with the evidence of increased IgE in human asthma. Although IgE and Th2-type cytokine predominance are important in immediate-type allergy, recent studies provided evidence of Th1-type immune response involvement in late-phase allergic reactions.[13,14] In the pathophysiology of asthma, chronic allergic inflammation in the late-phase response is important.[14] Some investigators found an increase in IFN-γ production in asthmatic subjects, and high numbers of IFN-γ–producing T cells in the affected tissues, during the late-phase response.[13-15] Also, a positive correlation between soluble intercellular adhesion molecule-1 (sICAM-1) and IFN-γ in sera from patients with infantile wheeze was shown.[15] These findings suggest that elevated IFN-γ may con-

tribute to allergic inflammation of asthma through its proinflammatory role. We previously reported changes in cytokine balance in diet-induced obese mice.[11] In that study, we found that IFN-γ production by splenocytes stimulated with PHA was significantly increased in obese groups compared with control groups. Similarly, in the present study, obese groups with OVA sensitization showed elevated levels of this cytokine production stimulated with PHA, although it was not changed with OVA stimulation. Therefore, the high prevalence of asthmatic symptoms in human obesity may be associated with functional changes in T-cell, especially in Th1-type immune response, by obesity. IL-4 (Th2-type cytokine) production stimulated with OVA was not changed by obesity in asthma groups (data not shown). However, in nonsensitized control groups, obese mice showed high IL-4 levels compared with control mice, which was consistent with our previous data.[11] Therefore, it is suggested that cytokine changed by original obese condition may be variously modified by OVA sensitization. Thus, it is possible that changes of cytokine balance in obesity may increase the risk of allergy or inflammation, and can be modified after the onset.

It is known that activated mast cells produce chemical mediators through binding of specific antigens to IgE in immediate-type allergic responses.[16] On the other hand, mast cells can mediate local inflammation without the IgE system.[17] In this study, high numbers of mast cells in the tracheal mucosa in obese groups were observed, but OVA-specific IgE antibody levels were lower in these groups. It has been previously reported that B-cell function might not be changed by obesity.[5] However, as antigen-specific antibody production of B cells is regulated by T cells, a change of T-cell function in obesity may contribute to the decline of antigen-specific IgE level. These findings may also explain the possible association of obesity and asthma based on T-cell–mediated inflammatory responses, rather than on IgE-mediated immediate allergic responses.

Adipose tissues have been shown to possess biologic activities. Adipocytes produce several physiologic mediators such as leptin, IL-6, or tumor necrosis factor-alpha (TNF-α).[18] These mediators, increased by obesity, could regulate inflammation.[19,20] Serum leptin was significantly increased in obese mice in the present study, consistent with previous reports. One recent study reported that leptin activated nuclear factor (NF)-κB.[21] Other investigators showed a central role of NF-κB in the pathogenesis of asthma because it is a transcription factor for most proinflammatory molecules, including adhesion molecules, enzymes, cytokines, and chemokines.[22,23] Furthermore, leptin increases monocyte chemotactic protein-1 (MCP-1) mRNA expression in human umbilical vein endothelial cells.[21] This chemokine directly induces mast cell degranulation in vitro.[17] As a direct link between leptin and asthma was not shown in this study, further studies are needed.

As for the difference of fatty acid composition between diets, body weight and fat pad weight did not differ significantly between the lard and soybean oil groups. The mast cell numbers in tracheal mucosa did not differ between these groups. However, a decline in serum OVA-specific IgE was observed, especially in the soybean oil group, while the highest levels of T-cell responsiveness to OVA were shown in the lard group. The significance of the difference is not clear at present. Other investigators have reported that fat cell volumes were larger in mice fed high-fat diets supplemented with soybean oil than in those with lard, although adipose tissue weight remaining unchanged.[25] Therefore, it is possible that the condition of adipose tissue may affect the production of mediators in allergic immune responses. Further investigation is needed to clarify the interrelationship of nutritional state, adipose tissue, and allergy.

In conclusion, we found that OVA-specific antibodies in diet-induced obese mice were lower, while sensitivity of T cells to OVA in the mice was higher than that in control mice. Accordingly, our study suggests that changes in T-cell function, rather than IgE-mediated allergic responses, by obesity may be partly involved in the pathophysiology of asthma in human obesity.

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