T Lymphopenia in Genetically Obese-Diabetic Wistar Fatty Rats: Effects of Body Weight Reduction on T Cells

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Patients with long-standing diabetes may have a propensity for infection-related mortality. In this study, lymphocyte subsets, the proliferative response of splenocytes to mitogens, and circulating levels of tumor necrosis factor alpha (TNF-α) in genetically obese-diabetic Wistar fatty (fa/fa) rats (WF) were longitudinally compared versus lean (+/?) litters (WL). Moreover, the effects of weight reduction with voglibose treatment on immunity were evaluated (WFV and WLV). Body weight was significantly increased in WF compared with WL. Hyperglycemia and hyperlipidemia developed, respectively, 11 weeks and 5 weeks thereafter throughout the observation periods. Circulating T cells and T-cell subsets of WF were significantly reduced after 22 weeks. There were also significant decreases in $CD4^+$ and $CD8^+$ thymocytes and the proliferative response of splenocytes. Circulating levels of TNF-α were significantly increased in WF. Treatment with voglibose resulted in significantly reduced blood glucose, insulin, cholesterol, triglyceride, and body weight in WFV. After weight reduction, circulating T cells and T-cell subsets were increased and TNF-α was decreased significantly in WFV. Our results suggest that the number and function of T cells in WF may be reduced, which may be related at least in part to elevated TNF-α levels, although the role of the other factors such as glucose, insulin, cholesterol, and triglycerides on T-cell immunity should be further investigated.

TYPE 2 DIABETES MELLITUS is a common and serious metabolic disorder characterized by obesity, insulin resistance, and hyperglycemia. Patients with long-standing diabetes may have a high incidence of infection[1] and infection-related mortality.[2,3] The same phenomenon was also shown in rat models of diabetes.[4] This has led to the view that the immune system may be impaired in diabetes.[3,5,6] To date, we have reported decreased lymphocyte responsiveness to mitogens in human obese subjects,[7] and T lymphopenia in genetically obese Zucker rats[8] and obese-diabetic db/db mice.[9] However, the details and mechanisms of this altered immunity are still poorly understood. Recently, we have reported enhanced tumor necrosis factor alpha (TNF-α) production in genetically obese-diabetic db/db mice[10] and genetically obese Zucker rats.[11] TNF-α is recognized as a multifunctional cytokine[12,13] that plays a central role in inflammation and immunity[13-15] and is relevant to the pathogenesis of insulin resistance.[10,16,17] TNF-α plays an important role in obese and diabetic animals and humans.[17-19]

In 1981, Ikeda et al[20] developed a new model of obesity and diabetes, the Wistar fatty rat (WF). This strain was derived from crosses between obese Zucker (13M strain, fa/fa) and Wistar-Kyoto rats. Male Wistar fatty (fa/fa) rats (WF), characterized by severe hyperglycemia, glycosuria, polyuria, and delayed-onset diabetes, are used as an animal model of human type 2 diabetes mellitus.[21,22] Voglibose is an inhibitor of α-glucosidase and is used for the treatment of diabetes mellitus.[23] Administration of voglibose has been shown to reduce blood glucose, plasma insulin, cholesterol, triglyceride, food intake, and body weight gain in Zucker fatty rats.[24]

In this study, we longitudinally measured lymphocyte subsets in the peripheral blood, thymus, and spleen in genetically obese-diabetic WF. The proliferative response of splenocytes to various mitogens and the circulating level of TNF-α were also quantified. Moreover, the effects of weight reduction on immunity were investigated.

MATERIALS AND METHODS

Animals and Treatment

All rats used in these experiments were males. WF and Wistar lean (fa/+ or +/+) rats (WL) were maintained at the Laboratory Animal Facility, Yokohama City University School of Medicine. All had free access to water and standard rat chow pellets and were housed under controlled temperature (24° ± 1°C) and humidity (50% to 60%) with a photoperiod from 7AM to 7 PM. WF cannot be distinguished from WL until the 4th week of life. However, by 5 weeks of age, there is a visible difference in body shape. At age 5 weeks, WF and WL were divided into 4 groups based on body weight, and the rats in 2 groups received chow supplemented with 0.0025% (wt/wt) voglibose throughout life (WFV and WLV) and the control group received standard rat chow pellets. Dr Takao Matsuo kindly provided the voglibose. Blood samples were obtained by puncture of the subclavian vein under ether anesthesia.

Cell Preparation and Immunologic Examination

Peripheral blood, splenocytes, and thymocytes were prepared as previously described.[8] The monoclonal antibodies (mAbs) used for analysis, OX19 (a mouse IgG_1 mAb that reacts with CD5 on rat thymocytes and T cells), OX8 (a mouse IgG1 mAb that reacts with CD8 on rat suppressor/cytotoxic T cells and NK cells), and OX33 (a mouse IgG1 mAb that reacts with CD45 on rat B cells), were purchased from Pharmingen (San Diego, CA). W3/25 (a mouse IgG1 mAb that reacts with CD4 on rat helper T cells) was purchased from Serotec (Kidlington, Oxford, UK). All antibodies were conjugated with fluorescein isothiocyanate or phycoerythrin. Splenocytes, thymocytes, and peripheral blood cells were stained with mAbs and analyzed with an EPICS ELITE cell sorter (Coulter, Hialeah, FL) as previously described.[8]

Viable splenocytes (2×10^6/mL) were cultured with or without phytohemagglutinin ([PHA-P] ×200; DIFCO Laboratories, Detroit,

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MI) or concanavalin A ([Con A] 1 μg/mL; Sigma, St Louis, MO) as previously described.[8]

Blood Analysis

Plasma glucose levels were determined by the glucose oxidase method with an APEC glucose analyzer (APEC, Danvers, MA). Serum triglyceride and total cholesterol concentrations were measured by an enzymatic assay with a Hitachi 736 Autoanalyzer (Hitachi, Tokyo, Japan). Serum rat insulin levels were determined by enzyme-linked immunosorbent assay ([ELISA] Morinaga, Yokohama, Japan) with rat insulin as a standard. White blood cell counts were obtained with an automatic counter (E-4000; Toa Medical Electronics, Tokyo, Japan), and differential percentages by microscopic examination of Wright-Giemsa–stained smears. TNF-α levels were measured using commercially available ELISA kits (Biosource International, Camarillo, CA) according to the supplier's protocol. Total plasma corticosterone was determined by radioimmunoassay using a scintillation proximity method. Briefly, plasma samples were denatured by incubation in borate buffer (133 mmol/L boric acid and 68 mmol/L NaOH, pH 7.4) containing bovine serum albumin (0.5%) in a 96-well microtiter plate (Falcon) at 80°C for 30 minutes. Then, the samples and a range of standards were incubated with ^{3}H-corticosterone (Amersham Life Sciences) and anti-corticosterone antibody in a total volume of 70 μL for 1 hour at room temperature. Scintillation proximity assay reagent (anti-rabbit, 50 μL; Amersham Life Sciences)AU#2 which holds antibody-bound radioactivity in close proximity to scintillant, was added and incubated for a further 24 hours at room temperature before counting in a β-scintillation counter.

Statistics

Data are expressed as the mean ± SD. Statistical analyses of differences were performed using the unpaired *t* test (2-tailed). The applicability of the *t* test to the data was verified by the F test correction. A χ^2 test was performed for comparison of serum levels of TNF-α at 5 weeks. Differences were considered significant at a level of *P* less than .05.

RESULTS

Characterization of WF

Body weight in WF increased throughout life. After the fifth week, WF showed a more rapid accumulation of body weight than WL. Beginning at 11 weeks and continuing throughout the observation period of the study (41 weeks), there were significant decreases in body weight in WFV and WLV (Fig 1). WF had remarkable hyperlipidemia throughout life (Table 1). Significant hyperglycemia and hyperinsulinemia were observed in WF after 11 weeks. With increasing age, serum insulin, blood glucose, and triglyceride of WF showed an early increase and a late decrease. However, serum cholesterol showed a gradual elevation. Significant reductions in cholesterol and blood glucose were present in WFV from 11 weeks onward and in WLV at 11 weeks but not at 22 weeks, and then again at 29 weeks and 41 weeks, compared with nontreated rats of the same genotypes. There were significant differences in triglyceride values at 11, 22, and 29 weeks of age and in serum insulin at 11 and 41 weeks in WFV compared with WF. Furthermore, there were significant differences in triglycerides at 11, 22, 29, and 41 weeks of age and in serum insulin at 11 and 41 weeks in WLV compared with WL. Voglibose did not influence corticosterone levels (data not shown).

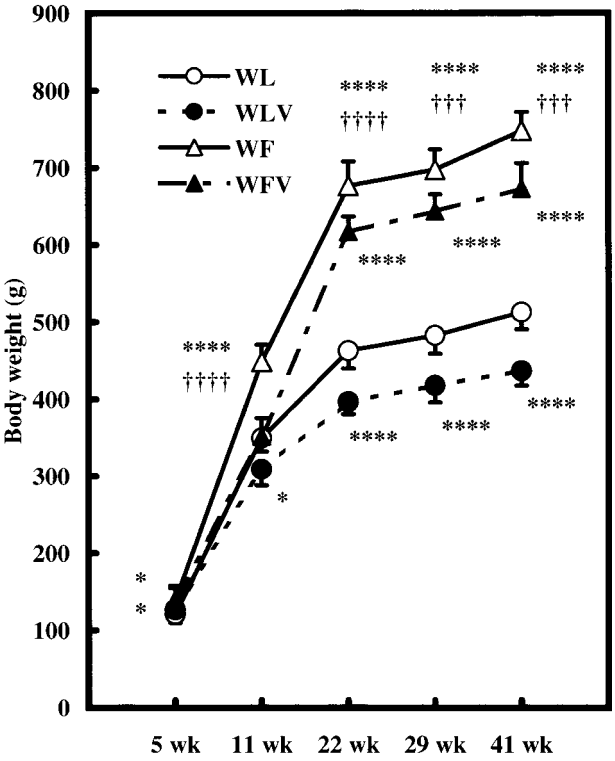

Fig 1. Body weight in WL, WLV, WF, and WFV. *$P < .05$, ****$P < .0001$ *v* WL; ††††$P < .001$, ††††$P < .0001$ *v* WF.

T-Cell and T-Cell Subset Counts

In peripheral blood, white blood cell, red blood cell, and platelet counts did not differ significantly in WF versus WL throughout the observation period (data not shown). However, after 22 weeks of age, in peripheral blood, significant and progressive T lymphopenia developed in WF (Table 2). Mean T-cell counts in WF were 46.3% of WL counts at 41 weeks of age. Until 5 weeks of age, no significant differences in the number of each T-cell subset was observed in peripheral blood. However, a nonselective reduction in all T-cell subsets developed after 22 weeks of age in peripheral blood, although $CD5^+CD8^+$ cells were already decreased after 11 weeks of age in WF compared with WL. There were significant increases in circulating T cells after 22 weeks of age throughout the experimental period and in the T-cell subsets, both $CD5^+CD4^+$ and $CD5^+CD8^+$ T cells, at 41 weeks of age in WFV. In thymocytes, significant differences in the number of $CD5^+$, $CD4^+$, and $CD8^+$ T-cell subsets at 5 weeks of age were observed. At 41 weeks of age, there were significant increases in $CD5^+$ and $CD8^+$ thymocytes after treatment with voglibose in WFV compared with WLV (Table 3). In addition, both $CD5^+CD4^+$ helper and $CD5^+CD8^+$ suppressor/cytotoxic T cells[25] decreased more rapidly in WF versus WL with increasing age. On the other hand, phenotypic NK cells, defined by $CD5^-CD8^+$ in rats,[26] phenotypic B cells (data not shown), and each splenic T-cell subset (Table 3) did not change significantly compared with those of WL. Each circulating T-cell subset in WLV also did not change significantly when compared with WL (Table 2).

Table 1. Blood Analysis

Age	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	Glucose (mg/dL)	Insulin (pmol/L)
5 weeks				
WL	2.28 ± 0.18	1.16 ± 0.11	9.4 ± 1.2	323 ± 188
WLV	2.12 ± 0.16	1.24 ± 0.14	8.8 ± 0.8	380 ± 234
WF	2.97 ± 0.13‡	1.46 ± 0.09*	10.0 ± 0.6	997 ± 478
WFV	3.13 ± 0.21‡	1.48 ± 0.12*	9.7 ± 1.0	956 ± 496
11 weeks				
WL	1.99 ± 0.13	1.30 ± 0.17	6.9 ± 0.3	729 ± 190
WLV	2.02 ± 0.16	1.00 ± 0.12†	6.4 ± 0.4*	366 ± 113‡
WF	2.66 ± 0.18§	3.22 ± 0.32§	18.1 ± 2.6§	4,661 ± 693§
WFV	2.40 ± 0.28*‖	1.41 ± 0.40**	7.4 ± 0.9**	3,634 ± 808§‖
22 weeks				
WL	2.28 ± 0.23	0.99 ± 0.18	6.3 ± 0.3	727 ± 208
WLV	2.33 ± 0.10	0.86 ± 0.16†	6.7 ± 0.6	393 ± 196†
WF	4.03 ± 0.41§	4.02 ± 0.54§	20.6 ± 1.6§	4,971 ± 648§
WFV	3.31 ± 0.23§#	2.94 ± 0.50§¶	11.3 ± 2.7†**	3,113 ± 349§
29 weeks				
WL	2.80 ± 0.30	1.67 ± 0.12	6.0 ± 0.4	756 ± 251
WLV	2.51 ± 0.14*	1.40 ± 0.07‡	5.4 ± 0.5*	572 ± 145
WF	5.50 ± 1.05§	3.77 ± 0.50§	19.2 ± 3.1§	4,084 ± 297§
WFV	4.14 ± 0.29§¶	2.80 ± 0.65†¶	15.0 ± 2.4§‖	4,113 ± 349§
41 weeks				
WL	3.15 ± 0.31	0.77 ± 0.12	6.9 ± 0.4	1,012 ± 172
WLV	2.46 ± 0.23‡	0.68 ± 0.12†	6.4 ± 0.3*	567 ± 84§
WF	6.59 ± 1.06§	2.50 ± 0.50§	13.2 ± 4.9†	3,736 ± 762§
WFV	4.40 ± 0.65†#	2.04 ± 0.51‡	8.0 ± 0.9*‖	2,470 ± 335§¶

NOTE. Values are the mean ± SD. WL and WF groups consist of 8 rats and WLV and WFV groups consist of 7 rats.

*$P < .05$, †$P < .01$, ‡$P < .001$, §$P < .0001$ *v* WL.

‖$P < .05$, ¶$P < .01$, #$P < .001$, **$P < .0001$ *v* WF.

Table 2. Circulating T-Cell Subsets

Age	$CD5^+$ ($10^3/mm^3$)	$CD5^+CD4^+$ ($10^3/mm^3$)	$CD5^+CD8^+$ ($10^3/mm^3$)
5 weeks			
WL (3)	1.79 ± 0.09	1.00 ± 0.21	0.60 ± 0.18
WLV (5)	1.67 ± 0.15	1.20 ± 0.33	0.61 ± 0.15
WF (3)	1.38 ± 0.17	0.81 ± 0.19	0.47 ± 0.10
WFV (3)	1.41 ± 0.15	0.82 ± 0.14	0.48 ± 0.13
11 weeks			
WL (8)	3.13 ± 0.09	2.03 ± 0.10	1.12 ± 0.08
WLV (7)	3.06 ± 0.09	2.03 ± 0.10	1.02 ± 0.10
WF (8)	3.00 ± 0.17	2.00 ± 0.12	0.94 ± 0.08*
WFV (7)	3.05 ± 0.25	2.06 ± 0.22	0.97 ± 0.03†
22 weeks			
WL (15)	3.30 ± 0.30	2.36 ± 0.21	1.10 ± 0.15
WLV (7)	3.10 ± 0.13	2.23 ± 0.16	1.09 ± 0.06
WF (12)	2.73 ± 0.54*	1.96 ± 0.42*	0.89 ± 0.06‡
WFV (7)	3.40 ± 0.20‖	2.59 ± 0.10¶	0.99 ± 0.07†
29 weeks			
WL (8)	2.82 ± 0.21	1.91 ± 0.20	0.92 ± 0.17
WLV (7)	2.80 ± 0.12	1.94 ± 0.10	0.85 ± 0.05
WF (8)	1.98 ± 0.27‡	1.44 ± 0.19†	0.55 ± 0.12†
WFV (7)	2.78 ± 0.25‖	2.04 ± 0.18¶	0.74 ± 0.07†
41 weeks			
WL (8)	2.87 ± 0.48	2.00 ± 0.33	0.81 ± 0.11
WLV (7)	3.37 ± 0.42	2.50 ± 0.29	0.84 ± 0.09
WF (8)	1.33 ± 0.32§	0.99 ± 0.27§	0.38 ± 0.15‡
WFV (7)	2.47 ± 0.14†¶	1.56 ± 0.22†‖	0.90 ± 0.05#

NOTE. Values are the mean ± SD. The number of rats examined is shown in parentheses.

*$P < .05$, †$P < .01$, ‡$P < .001$, §$P < .0001$ *v* WL.

‖$P < .05$, ¶$P < .01$, #$P < .001$ *v* WF.

Blastogenic Response to T-Cell Mitogens

The proliferative response of splenocytes to various T-cell mitogens, the mean (mean ± SD) intracellular incorporation of [^{3}H]-thymidine upon stimulation with PHA or Con A, was not significantly different in WF at 5 weeks of age versus WL. However, the response of splenocytes was significantly diminished in WF compared with WL at later time points (PHA, not significant [NS] at 22 weeks, $P < .05$ at 41 weeks; Con A, $P < .05$ at 22 weeks, $P < .01$ at 41 weeks). At 41 weeks of age, the proliferative response of splenocytes to various T-cell mitogens was not significantly different in WFV and WLV versus WF WL, respectively (Fig 2).

Serum Concentration of TNF-α

The serum concentration of TNF-α was measured using ELISA. Serum TNF-α was detectable in 7 of 7 (100%) WF and WFV at 5 weeks of age, with concentrations of 1.8 to 78.4 pg/mL. Only 3 of 7 (42.9%) WL had detectable levels of TNF-α (3.4 to 18.0 pg/mL). The difference in the fraction of WL and WF with detectable TNF-α was statistically significant ($P < .05$, χ^2 test). Serum TNF-α levels were significantly increased in WF at 41 weeks of age compared with WL (139.5 ± 41.5 *v* 36.7 ± 20.1 pg/mL, $P < .001$, n = 6 each). Furthermore, serum TNF-α levels were significantly decreased in WFV (88.2 ± 37.2 pg/mL, $P < .05$, n = 6) compared with WF. There were no significant differences in WLV compared with WL (Fig 3).

DISCUSSION

We designed this study to investigate the immune system in type 2 diabetes mellitus using genetically obese-diabetic WF. Quantitative impairment was evidenced by flow cytometric analysis that showed T lymphopenia in both helper and suppressor/cytotoxic T cells. Functional impairments in obese/diabetic rats were indicated by the response of splenocytes to mitogens, which was significantly reduced in WF. Both impairments appeared as age increased. Furthermore, the serum

Table 3. Subsets of Thymocytes and Splenocytes (10^7)

	Thymocytes		Splenocytes	
Age	$CD5^+$	$CD4^+$	$CD8^+$	$CD5^+$
5 weeks				
WL	86.8 ± 3.33 (4)	82.5 ± 3.71	80.0 ± 6.94	3.67 ± 0.39 (5)
WF	68.9 ± 2.32 (5)	64.3 ± 2.84*	63.7 ± 1.65*	3.04 ± 0.45 (5)
22 weeks				
WL	28.7 ± 0.18 (8)	27.2 ± 0.45	25.3 ± 2.40	3.16 ± 0.63 (6)
WF	21.5 ± 0.69* (6)	20.4 ± 0.35*	19.2 ± 1.15*	2.72 ± 0.56 (6)
41 weeks				
WL	13.3 ± 0.07 (4)	12.6 ± 0.30	12.1 ± 0.73	2.69 ± 0.46 (12)
WLV	13.4 ± 0.09 (7)	12.8 ± 0.48	12.6 ± 0.82	2.71 ± 0.82 (7)
WF	11.2 ± 0.30* (5)	10.5 ± 0.72*	10.4 ± 0.37*	2.38 ± 0.25 (13)
WFV	12.5 ± 0.34*‡ (7)	11.8 ± 0.81	11.6 ± 0.42†	2.42 ± 0.55 (7)

NOTE. Values are the mean ± SD. The number of rats examined is shown in parentheses.

*$P < .0001$ *v* WL.

†$P < .01$, ‡$P < .0001$ *v* WF.

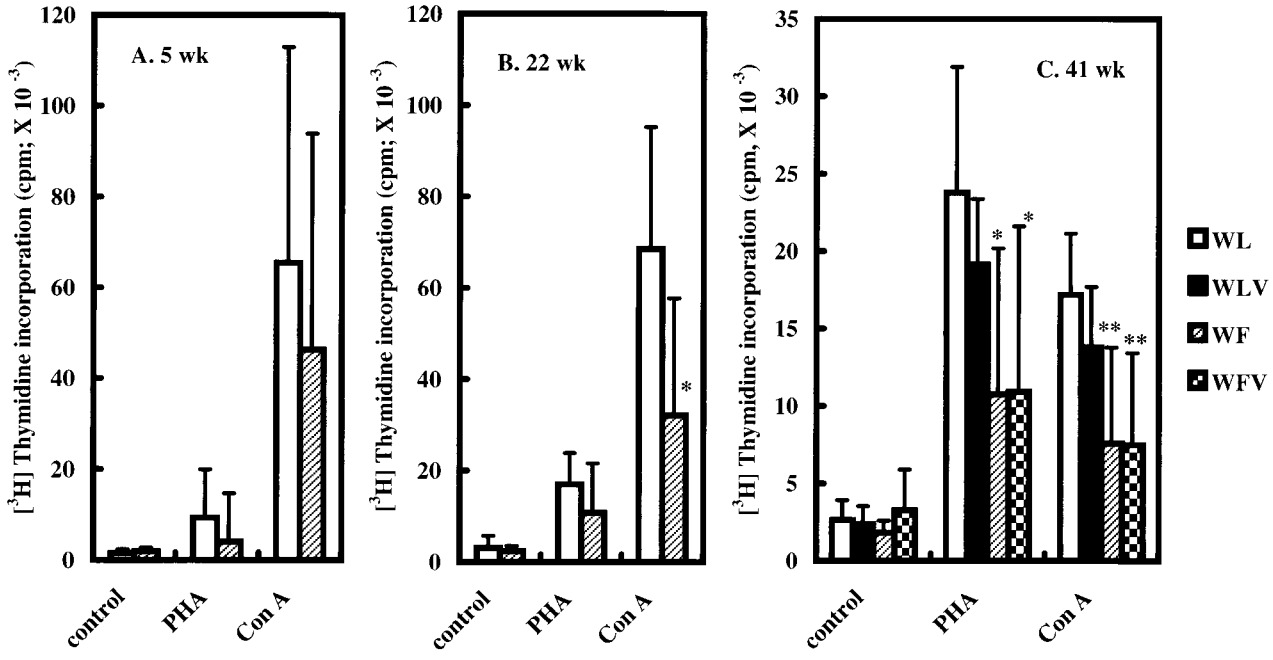

Fig 2. In vitro blastogenic response of splenocytes (2 × 10⁶/mL) in WL, WLV, WF, and WFV to T-cell mitogens, PHA (×200) and Con A (1 g/mL), at 5 weeks (A), 22 weeks (B), and 41 weeks (C) of age. The cells examined were not purified T cells, but total splenocytes. Each group consists of 5 rats. *$P < .05$, **$P < .01$ *v* WL.

concentration of TNF-α was significantly increased in WF compared with WL. T-cell subsets and TNF-α production were reversible with weight reduction, but not the response of splenocytes to mitogens.

WF are used as an animal model of human type 2 diabetes mellitus, as described in the introduction.[21,22] Recently, the concept of latent autoimmune diabetes in adults (LADA) has been developed.[27] WF might not be classified as a model of LADA for several reasons. For example, obese Zucker rats are homozygous for a mutation in the leptin receptor (OB-R) gene and are therefore leptin-insensitive. Thus, obesity and obesity-related abnormalities including diabetes mellitus in WF are thought to be due to leptin insensitivity. In addition, there have been no reports of ICA-positive or other autoimmune abnormalities in WF.

There are conflicting reports on the immune system in patients with diabetes. Bouter et al[5] reported that the number of $CD4^+$ and $CD8^+$ cells increased, but Chang et al[6] reported that the percentages of $CD3^+$, $CD4^+$, and $CD8^+$ were not significantly different between diabetic patients and healthy subjects. There are potential difficulties in studying the effects of type 2 diabetes mellitus on the immune system in humans. Patients with type 2 diabetes are not homogeneous in their individual dietary regimen, stress level, body weight, or social environment. Thus, genetically obese-diabetic WF are a useful model for elucidating the effects of type 2 diabetes on the immune system.

In the present study, obesity/diabetes influenced different parameters (including body weight, serum insulin, blood glucose, triglyceride, or cholesterol) at different rates. Serum levels of insulin, blood glucose, and triglyceride reached a peak at about 22 weeks of age, respectively, and thereafter declined. Although T-cell subsets started to decrease at 11 weeks, T-cell counts decreased after 22 weeks and persisted throughout the observation period. In contrast, since each splenic T-cell subset did not change significantly, there is a possibility that the hyporesponsiveness of splenocytes in WF may relate to the reduced responsiveness of the T-cell itself. However, serum cholesterol levels showed a gradual elevation. An effect of hypercholesterolemia on immune function was suggested by reports that a low-fat, low-cholesterol diet diminished the CD3, CD4, and CD8 lymphocyte subset counts that are elevated in children with hypercholesterolemia.[28] Taken together, it seems unlikely that impaired T-cell immunity in the diabetic rat is closely related to these metabolic characteristics. This study suggests that chronic diabetes may affect the immune system both quantitatively and functionally in WF.

Blood glucose levels were lower in WF and WFV at 41 weeks versus WF at 22 and 29 weeks. Although it is not clear why blood glucose levels in WF and WFV were reduced at 41 weeks compared with 22 and 29 weeks, there are 2 possibilities. First, animals with extremely high blood glucose might have died during the studies. However, in the present studies, no rats died after 22 weeks. Second, WF showed increased urinary protein excretion with age. Therefore, insulin inactivation due to renal dysfunction might improve blood glucose levels at 41 weeks. The same phenomenon was frequently shown in severe diabetic nephropathy in human diabetes mellitus. The basis for this phenomenon remains to be determined.

TNF-α plays an important role in obesity and diabetes in animals and humans.[17-19] Previous studies have reported that type 2 diabetes mellitus is associated with higher TNF-α

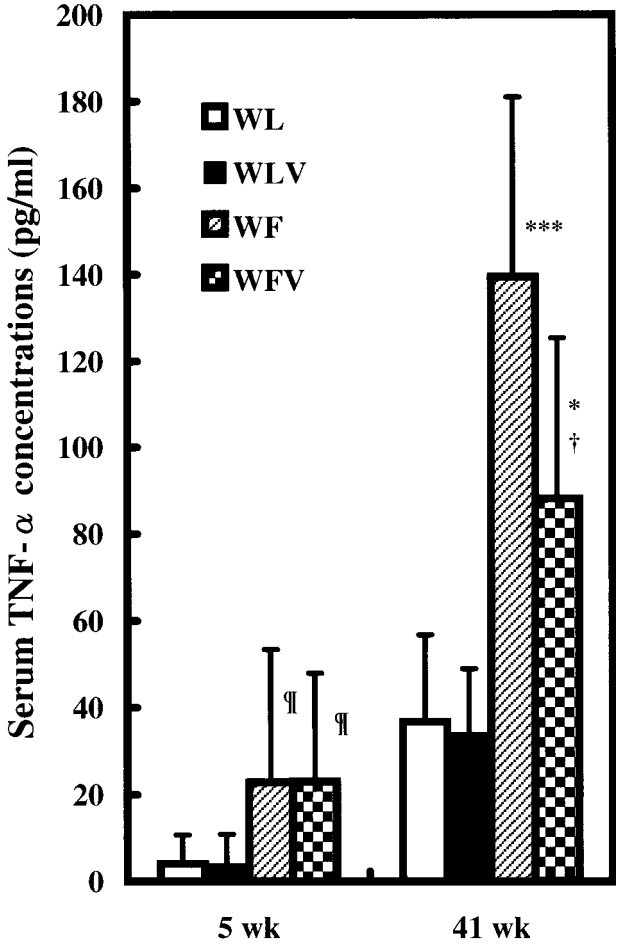

Fig 3. Serum concentration of TNF-α in WL, WLV, WF, and WFV at 5 weeks and 41 weeks of age. *$P < .05$, ***$P < .001$ *v* WL; †$P < .05$ *v* WF evaluated by *t* test; ¶$P < .05$ *v* WL evaluated by χ^2 test.

levels.[6,10,11,29,30] Murase et al[30] observed an increase in circulating levels of TNF-α in genetically obese-diabetic WF. We have also shown increased serum levels of TNF-α in genetically obese-diabetic WF after 5 weeks throughout the observation period. On the other hand, long-term TNF-α administration reduced splenic T- and B-cell counts and inhibited splenic lymphocyte proliferation to T-cell mitogens and NK activity in normal mice[30] and non-obese diabetic mice.[31] Rabinovitch et al[32] observed that TNF-α decreased type 1 cytokine gene expression. These inhibitory effects of TNF-α on type 1 cytokine gene expression may explain the selective inhibitory effects of TNF-α on cell-mediated, but not humoral, immunity in mice. In our study, after weight reduction with voglibose treatment, serum TNF-α levels were significantly decreased, but there were significant increases in circulating T cells, T-cell subsets, and $CD5^+$ or $CD8^+$ thymocytes. After weight reduction, WFV had higher T-cell counts than WF, but the number of T cells was similar in WLV and WL. Thus, the increase of T-cells in WFV was specific to obese rats and thus presumably not due only to voglibose. According to our results, we hypothesized that weight reduction can restore immunity in WF, partially mediated through a decrease of serum TNF-α. However, since the proliferative responses of splenocytes to various T-cell mitogens in WFV were not different, the weight reductions in WFV were not adequate. Although increased levels of circulating TNF-α may not fully explain the selective decrease in T cells and T-cell subsets, T lymphopenia and the reduced responsiveness of splenocytes in obese-diabetic rats may suggest possible inhibitory effects of increased TNF-α on cell-mediated immunity.

It is possible that the improvement of immune function in WFV may be the result of glucose and/or decreased lipid levels. However, several studies showed that decreased lipid levels were not linked to the improvement of immunity. For instance, relative to a high-cholesterol group, hypocholesterolemic men had significantly fewer circulating lymphocytes, fewer total T cells, and fewer $CD8^+$ cells (P values $<.05$).[33] In the case of glucose, it has been controversial as to whether a change in blood glucose may influence T-cell immunity.[5,34] On the other hand, TNF-α levels were regarded as closely related to the quantity and quality of T-cell immunity.[31,32] Thus, it is reasonable to state that the improvement of immune function in WFV may be caused by the decrease of TNF-α but not by the decrease of lipid, although we cannot deny the possibility that the change in glucose also may affect T-cell immunity. We need to further investigate these mechanisms.

As shown in the present study, impaired T-cell immunities were improved by treatment with voglibose. TNF-α production was also decreased with voglibose in WF. Although this effect of voglibose may be mediated through weight reduction, it is necessary to consider the possibility that voglibose is a direct immune modulator. We examined the effect of voglibose on [^{3}H]thymidine uptake and cytokine production of cultured splenocytes. Our preliminary results showed that in vitro culture of splenocytes with voglibose (1 to 1,000 μg/mL) produced no effect on [^{3}H]thymidine uptake or interleukin-12 or interferon gamma production. In addition, treatment with voglibose had no effect on T-cell subsets of peripheral blood, thymocytes, and splenocytes and TNF-α production in WLV (Tables 2 and 3), suggesting that voglibose had no immunomodulator activity. However, we are not able to deny the possibility that TNF-α production was directly suppressed with voglibose. Thus, we need to examine the direct effect of voglibose on TNF-α production.

In conclusion, we have shown that chronic type 2 diabetes may impair T-cell immunity in genetically obese-diabetic WF and that this impairment may be reversible with adequate weight reduction. This impairment may play a role in the increased susceptibility to infection in patients with diabetes.

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