Title: Immune alterations in male and female mice after 2-deoxy-D-glucose administration

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Running title: 2-DG-mediated immunomodulation

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Abbreviations:

2-DG, 2-deoxy-D-glucose; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; FSH, follicle stimulating hormone; IFN, interferon; IL, interleukin; LH, luteinizing hormone; PE, R-Phycoerythrin; TCR, T-cell receptor; WBC, white blood cell.

Abstract:

DRÉAU, D., D.S. MORTON, M. FOSTER, J.P. SWIGGETT AND G. SONNENFELD. *Immune alterations in male and female mice after 2-deoxy-D-glucose administration.* PHYSIOL BEHAV 000 000-000, 1997. Administration of 2-deoxy-D-glucose (2-DG) induces acute cellular glucoprivation. In the current study, we examined differences in immune parameters after 2-DG administration in both sexes. Male or female BDF_1 mice were injected three times, 48 hours apart, with either a saline solution (control group) or with 2-DG in saline (500 mg/kg). Two hours after the last injection, blood and spleens were collected. Plasma levels of Interleukin-1β, and interferon-γ levels were measured. Additionally, the levels of the following specific leukocyte antigens: CD3, CD4, CD8, TCR αβ, I-A^d, and H-$2L^d$/H-$2D^b$ were evaluated by flow-cytometry on both blood and spleen cells. The blastogenic response of leukocytes from both tissues to mitogens was assessed. Levels of glucose, corticosterone, testosterone, progesterone, 17β estradiol, follicle-stimulating hormone, and luteinizing hormone were also determined. Increases in the percentage of cells bearing TCR αβ and I-A^d in the blood and H-$2L^d$/H-$2D^b$ in the spleen were observed in the 2-DG-treated group for both sexes. In contrast, higher corticosterone and IL-1β plasma concentrations, as well as higher percentages of splenocytes bearing TCR αβ and I-A^d, and lower mitogen-induced proliferation of mature T splenocytes (79%) were observed in female but not in male mice injected with 2-DG compared with those injected with saline ($P<0.05$). Taken together, these results suggest that female are more sensitive than male mice to immune alterations induced by 2-DG administration.

Keywords: 2-deoxy-D-glucose; Corticosterone; Interleukin; Lymphocyte; Metabolism; Sex

Introduction

Administration of 2-deoxy-D-glucose (2-DG), an analog of glucose which inhibits glycolysis by competitive antagonism for phosphohexose isomerase (6,40), results in acute periods of intracellular glucoprivation and hyperglycemia resulting in hyperphagia (24,26). In addition to these changes in the carbohydrate metabolism, injection of 2-DG results in alterations of both the endocrine and neurological systems as suggested by modifications in oxytocin and glucocorticoid levels (7,39) and norepinephrine production (26). Moreover, alterations of the immune response, such as a decrease in the *in vitro* proliferation of splenocytes after mitogen-stimulation, were observed in mice injected with 2-DG (26). Sex, genotype and environment are among the factors that may modulate effects of catecholamines and hypothalamo-pituitary-adrenal axis on these immune changes (22).

Sexual dimorphism in immune function resulting from the effects of sex hormones on immune effector cells has been shown in both animals and humans (18,19). These observations have important implications, especially with regard to higher incidence of many autoimmune diseases in females (1). Evidence exists that reproductive hormones influence the immune system and increase the risk of immunologically related disorders in both animals and humans (29). Indeed, immunological responses in stressful situations may also be confounded by fluctuations of sex hormones especially in females (21).

Lymphocyte distribution, cytokine production, and the ability of lymphocyte to proliferate *in vitro* were analyzed in male and female mice to determine if sex influenced 2-DG immunomodulation. In addition, the influence of hormones, especially sex hormones, on these changes were evaluated.

Materials and methods

Animals

Specific pathogen-free female or male BDF_1 (C57BL/6 x DBA/2) mice each weighing 15 to 17g (4 to 5 weeks old) were purchased from Charles River Laboratories and were housed at two per cage in the Carolinas Medical Center AAALAC-accredited animal care facilities, under the supervision of a veterinarian and in accordance with animal care and use guidelines of the Public Health Service and the Carolinas Medical Center Institutional Animal Care and Use Committee. Mice were isolated in a colony room where a 12 hour day:night cycle (0700:1900h) was maintained using artificial illumination. Mice were allowed access to food (Tek Lad 8604 Laboratory Rodent Chow, Tek Lad, Madison, WI) and acidified water was given *ad libitum* except when noted.

Experimental injections

Experimental injections began after a 1-week acclimatization period. Eight mice were assigned randomly to control or experimental groups. Each mouse from the control and experimental groups was handled so that groups differed only by the content of the innoculum (0.2 ml/mouse/injection). Mice were injected intraperitoneally three times with sterile saline (Sigma Chemical Co., St. Louis, MO) for the control group and with 2-deoxy-D-glucose (2-DG; Sigma Chemical Co., St. Louis, MO) diluted in sterile saline (500 mg/kg) for the experimental group. Injections, administered 1.5 hours into the light phase of the day cycle, were performed once every 48 hours as previously described (26). All mice were deprived of food for 2 hours following the injection. Mice were rapidly euthanized with CO_2 two hours following the final injection of saline or 2-DG. Control and

experimental animals were killed at the same time, and assays on all groups were performed simultaneously.

Blood was collected in heparin-coated syringes (Elkins Sinn Inc., Cherry Hill, NJ) by cardiac puncture. After a 5 minute-centrifugation (1500 g), blood plasma was collected and stored at -70°C until used. The cell pellet was then resuspended and incubated in lysing buffer (15.5 mM NH_4Cl, 1 mM $KHCO_3$, 1 mM EDTA, pH 8.0) to lyse the red blood cells. After centrifugation, white blood cells (WBCs) were resupended in RPMI-1640 medium (Gibco, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Biowhittaker, Walkersville, MD). Spleens were removed aseptically and placed in RPMI-1640 medium with 10% fetal bovine serum and pressed through a sterile Falcon cell strainer (Becton-Dickinson, Lincoln Park, NJ; one spleen per strainer). Cell viability was recorded after trypan blue staining (Sigma, St. Louis, MO), and was greater than 95%. After enumeration using a cell counter (Coulter T890, Coulter Electronics, Hialeah, FL) and centrifugation, WBCs and splenocytes were resuspended at 2 x 10^6 cells/ml in complete RPMI-1640 medium [RPMI-1640 medium (Gibco, Gaithersburg, MD), 10% fetal bovine serum (Biowhittaker), 1 mM essential amino acids (Gibco), 1mM non-essential amino acids (Gibco), 2 mM L-glutamine (Gibco), 25 mM HEPES buffer (Sigma), 2 µg/ml fungizone (Gibco), 80 µg/ml gentamycin (Gibco), and 50 µM βmercaptoethanol (Sigma)]. WBCs and splenocytes were used in the cell staining and T cell proliferation procedures.

Plasma glucose, lactate, and hormone concentrations

Glucose and lactate levels in plasma were measured 2 hours after the third injection of either saline or 2-DG using a D-glucose and L-lactate analyzer (2300STET, YSI Bioanalytical Products, Yellow Spring, OH).

Levels of corticosterone, testosterone, progesterone, 17β estradiol, follicle-stimulating-hormone (FSH), and luteinizing hormone (LH) were determined in plasma

obtained 2 hours after the third injection using radioimmunoassay kits (ICN Pharmaceuticals Inc., Costa Mesa, CA) following the manufacturer's recommendations. Corticosterone, testosterone, progesterone, and 17β estradiol were measured using competitive radioimmunoassays using ^{125}I labeled corticosterone, testosterone, progesterone, and 17β estradiol, respectively. LH and FSH were detected by sandwich RIA using ^{125}I labeled anti-LH and anti-FSH antibodies, respectively. Levels of ^{125}I were counted using a gamma counter (Packard Instrument Company, Downers Grove, IL). Hormone levels, expressed in ng/ml for corticosterone, testosterone, progesterone, and 17β estradiol and in mIU/ml for FSH and LH, were then derived by comparison with standards assayed along with the samples.

Plasma cytokine concentrations

Innate concentrations of interleukin-1β (IL-1 β) and interferon-γ (IFN-γ) were determined in plasma collected 2 hours after the third injection using ELISA kits (Endogen Inc., Cambridge, MA) following the manufacturer's recommendations. Briefly, plasma samples were distributed on 96-well plates along with the standard dilutions. After a 2h incubation with the plasma samples at room temperature, the 96-well plates were washed. The 96-well plates were then incubated with anti-IL-1β and anti-IFN-γ conjugated antibodies, respectively. After a 1 h incubation and washings, the reaction was developed by adding the substrate solution. After 15 minutes, the reaction was stopped, and plates were read at 450 nm using an ELISA plate reader (Dynatech, Chantilly, VA). IL-1β levels expressed in pg/ml and interferon-γ expressed in ng/ml were derived by comparison with standards determined along with the samples.

Antibodies

Monoclonal antibodies, conjugated to either fluorescein isothiocyanate (FITC) or R-Phycoerythrin (PE), were used in the immunostaining procedure. Only the antibody for the major histocompatibility class I antigen H-2L^d/H-2D^b was used unconjugated. Rat (IgG2a) monoclonal anti-mouse CD4-FITC recognizing L3T4 antigen present on T helper cells, anti-mouse CD8-PE recognizing a 65kD antigen present on cytotoxic T cells, and hamster (IgG) anti-mouse-TCR-αβ-PE clone H57-597 against a 90-110kD antigen associated with the CD3 complex were obtained from Sigma ImmunoChemical (St. Louis, MO). Hamster (IgG) anti-mouse CD3-PE recognizing the ε chain of the CD3 complex, mouse (IgG2b) anti-mouse I-A^d-FITC with react with I-A^d major histocompatibility class II molecules, and rat (IgG2a) anti-mouse H-2L^d/H-2D^b which react with major histocompatibility class I molecules were purchased from Pharmingen (San Diego, CA). For H-2L^d/H-2D^b, a second step using rat anti-mouse IgG2a-FITC was performed. This last antibody alone was also used as control for this reaction. For one-step-staining reactions, the following control isotypes were used in similar conditions: rat IgG2a-FITC, rat IgG2a-PE, and rat IgG1-PE (Pharmingen) and hamster IgG-PE (Sigma ImmunoChemical).

Cell immunostaining and flow cytometric analysis

Cells isolated from blood and spleen 2 hours after the third injection of 2-DG or saline were distributed at the concentration of 2 x 10^5 cells per well in a 96-well round-bottom plate (Corning, New York, NY), and spun for 4 minutes, 1000 g at room temperature. The supernatant fluids were discarded. For one-step-staining reaction, cells were resupended with 50 µl of the antibody diluted according to the manufacturer's recommendations in RPMI-1640 medium (Gibco) plus 10% fetal bovine serum (Biowhittaker). After a 20-minute incubation with constant shaking on ice and protected

from light, red cells were lysed by an additional 5-minute incubation with 100 µl of lysing buffer (15.5 mM NH_4Cl, 1 mM $KHCO_3$, 1 mM EDTA, pH 8.0). After two additional washings with RPMI-1640 medium plus 10% fetal bovine serum, labeled cells were fixed with a 1% formaldehyde (Sigma Chemical, St. Louis, MO) solution, protected from light and stored at 4°C until analysis on the FACStar Plus (Becton Dickinson, San Jose, CA). For double staining, cells were first incubated with an unconjugated antibody. Following the first incubation and two washings with RPMI-1640 medium plus 10% fetal bovine serum, the procedure followed the directions for one-step staining outlined above. For each marker, the percentage presented in this study is the marker percentage minus the background (i.e., the percentage observed with the control isotype).

Leukocyte blastogenesis

In addition to the WBCs and splenocytes obtained 2 hours after the last injection, partially purified splenocytes were also used in the blastogenesis assay. Partially purified splenocytes were obtained as described previously (26). Briefly, after a 1 hour-incubation (37°C, 5% CO_2) in a tissue culture flask (Becton-Dickinson, Lincoln Park, NJ), non-adherent splenocytes were harvested. These cells were then incubated (37°C, 5% CO_2) in cell culture dishes precoated with J11d2 antibodies for 1 hour. This antibody has been shown to negatively select mature T cells (28). After the incubation, the non-adherent cells were washed once with RPMI-1640 medium plus 10% fetal bovine serum, counted and resuspended at 2×10^6 cells/ml. Cells were distributed in 96-well flat-bottom plates (Falcon; Becton-Dickinson, Lincoln Park, NJ) at 2×10^5 cells/well and cultivated in the presence of complete RPMI-1640 medium with or without Concanavalin-A 5 µg/ml (Sigma). After a 30 hour incubation (37°C, 5% CO_2), 1 µCi of tritiated thymidine (Amersham, Arlington Hts, IL) was added to each well. Following an additional 18 h incubation, cells were harvested onto glass filter paper using a semi-automated cell

harvester (Cambridge Technology Inc., Whatertown, MA). Uptake of thymidine was measured using a liquid scintillation counter (Packard, Meridien, CA).

Statistical treatment

All the results are expressed as mean±SEM. An analysis of variance (ANOVA, Staview 4.02 program; Abacus Concept, San Francisco, CA) was used to compare sex (male versus female), and treatment group (saline versus 2-DG). Significant differences between treatment means were identified using the Bonferroni's *t*-test. The critical level for significance was set at $p<0.05$ for all comparisons.

Results

Glucose and lactate levels in plasma

Glucose levels differed by both gender ($F(1,28)=5.6$, $p=0.02$) and treatment group ($F(1,28)=8.0$, $p=0.002$) effects. Differences in glucose levels between male and female related to gender were observed in the saline group (p=0.002) but not in the 2-DG group. Glucose levels were 35% higher in female mice treated with 2-DG than in control mice (168.2 and 120.2 mg/dL, $p=0.0015$, Fig. 1). No difference between saline and 2-DG groups was observed in males (163.5 and 179.2 mg/dL).

Lactate plasma levels were influenced by gender ($F(1,28)=10.7$, $p=0.003$) but not by the treatment group effects. Within treatment groups, lactate levels were higher in male than in female mice (5.4±0.3 versus 4.4±0.3 mmol/L, $p=0.05$) in the saline group and (4.8±0.5 versus 3.7±0.8 mmol/L, $p=0.03$) in the 2-DG group.

Corticosterone and sex hormone levels in plasma

Corticosterone levels varied by both gender ($F(1,24)=8.6$, $p=0.007$) and treatment group ($F(1,24)=4.5$, $p=0.04$) effects. Corticosterone levels were significantly higher in female mice injected with 2-DG compared to those injected with saline (163.0 and 91.3 ng/ml, $p=0.016$, Fig. 2). In contrast, corticosterone levels in male mice did not differ between treatment groups (79.1 and 85.9 ng/ml, Fig. 2).

To test whether 2-DG also affected levels of hormones produced preferentially by male or female mice, 17β estradiol , testosterone, and progesterone were measured in the bloodstream of mice injected with saline or 2-DG. In female or male BDF_1 mice, blood levels of 17β estradiol (11.4±0.6 and 7.6±1.1 pg/ml), testosterone (0.1±0 and 1.9±0.7 ng/ml), and progesterone (4.5±2.0 and 4.7±0.7 ng/ml) were not affected by treatments. Only testosterone levels differed by gender ($F(1,36)=4.7$, $p=0.036$). In addition, LH and FSH plasma levels measured in females were highly variable (range: 0 to 129.7 mIU/ml and 0 to 17.35 mIU/ml for FSH and LH, respectively).

In vitro leukocyte proliferation

After a 48h incubation in media alone, no difference was found in the proliferation levels of WBC, unpurified or partially purified mature T splenocytes as measured by thymidine uptake (0.6±0.2 x 10^3 cpm regardless of treatment group or gender). In the presence of concanavalin-A, proliferation of WBCs and unpurified splenocytes remained unchanged (0.8±0.2 and 85.3±8.9 x 10^3 cpm, respectively).

To test whether T lymphocytes rather than B cells were affected by 2-DG treatment, partially purified mature T splenocytes were used in similar conditions. Thymidine uptake of mature T splenocytes from female mice only was 79% lower in the 2-DG group compared to saline-injected mice (6.7±2.0 versus 31.9±10.6 x 10^3 cpm, $p<0.05$, Fig. 2).

Cytokine levels in plasma

Intrinsic levels of IL-1β, and IFN-γ were measured directly in the plasma. No difference between treatment groups was found in levels of IFN-γ (1.75±0.3 and 2.12±0.6 ng/ml) in female or male mice. IL-1β levels were higher in plasma from females injected with 2-DG (324.7±49.0 versus 679.9±106.9 pg/ml, p=0.007, Fig 5).

Distribution of the immune cell markers on WBCs and splenocytes

White blood cells expressing T cell markers CD3, CD4, or CD8 and the major histocompatibility complex class I molecules H-2L^d/H-2D^b were not affected by treatment or gender (Table 1). By contrast, percentages of TCR αβ, and the major histocompatibility complex class II molecules I-A^d borne by WBCs were significantly higher in both genders following 2-DG injections compared to saline controls ($F(1,25)$=17.9 p=0.0003 and $F(1,23)$=10.9 p<0.003, respectively, Table 1).

As observed in blood, splenocytes expressing T cell markers CD3, CD4, and CD8 were not affected by treatment or gender (Table 2). However, in both genders, percentage of cells bearing major histocompatibility complex class I molecules H-2L^d/H-2D^b was higher in the 2-DG group compared to the saline controls ($F(1,24)$=9.9, p=0.005, Table 2). Percentages of splenocytes bearing TCR αβ or I-A^d were affected by 2-DG treatment ($F(1,23)$=18.3, p=0.0003 and$F(1,27)$=5.4, p=0.03, respectively) and higher only in females injected with 2-DG compared to females injected with saline (p<0.005, Table 2).

Discussion

This study was designed to explore changes on the immune system of male and female mice after glucoprivation. Results demonstrate a greater sensitivity to 2-DG administration of female mice with regard to corticosterone levels and immune responses. These immune modifications included a decreased proliferation of splenocytes *in vitro*, increased intrinsic IL-1ß concentration in the bloodstream, and increased percentage of cells bearing TCR αβ or $I\text{-}A^d$ in the spleen.

Administration of 2-deoxy-D-glucose has been studied in many species including humans (5,16), rats (8,24) and mice (26,27). In contrast to studies using other nutrient deprivation (30), 2-DG administration (500 mg/kg) did not induce variation in general weight in female mice (26). The amount of 2-DG injected (500 mg/kg) was previously used in rats (24) and mice (26,27). In earlier studies, the protocol used here gave the greatest depression in T cell proliferation (26) and the greatest increase in *in vitro* mitogen-induced IL-1β, IL-3, and IL-6 production (27). As suggested before (26), administration route, number and time of injections, and dose of 2-DG potentially may have affected the present results. However, since experiments were conducted simultaneously and similarly in male and female mice, the observed differences may more likely be attributed to differences between male and female BDF_1 mice.

The increase of corticosterone concentration in the blood of female mice was consistent with previous values observed after 2-DG administration (24,26).Moreover, in female mice, acute starvation resulted in a marked reduction of the T cellularity which may be related to the corticosteroid production (46). The sympathetic nervous system could also contribute to 2-DG action on the immune system (43). Indeed, after 2-DG administration, higher levels of several metabolites released by the hypothalamo-pituitary-adrenal axis were observed the blood of female but not male mice (41), and of rat (36). Moreover, chemical sympathectomy (35) but not vagotomy (17,34) suppressed 2-DG effects.

Immunological alterations in female mice after 2-DG administration included a strong decrease of the *in vitro* proliferation of splenocytes in the presence of concanavalin-A, changes in the lymphocyte distribution and an increase of the interleukin-1ß concentration in the blood. These changes were not recorded in male mice, even with higher doses of 2-DG (up to 1500 mg/kg, data not shown). The decrease in proliferation of splenocytes isolated from females in the 2-DG treated group, also noted 24 hours after the last injection (26), was comparable to results described previously in 2-DG injected animals (24,26). Similar decreases were observed in sedentary compared to treadmill runners male rats (42), male rats following both social and foot-shock stress (4). By contrast, an increase in the cellular immune function was observed in mice during cold adaptation (47). 2-DG administration in similar conditions that resulted in the decrease in proliferation ability here, resulted in a previously observed increase in resistance to macrophage-dependent pathogens *in vitro* (31) and *in vivo* (Miller et al., unpublished) 2-DG induced stress experiments using female mice. This may be due to enhancement of macrophage activity by 2-DG (31). Such increases also have been described in viral infections (15) and after transplantation (12) and may lead to an enhanced protection. Therefore, in some cases, this acute cellular glucoprivation may benefit the individual (37). However, the proliferation decrease observed in female but not in male mice after 2-DG administration remains unique and may be related to a higher sensitivity in female as suggested by clinical studies (1).

The lymphocyte subpopulations (I-A^d+ and TCR αβ+) in the spleen and in the blood were drastically affected by the 2-DG injections in female mice. Increase in TCR αβ + cells was associated with an increase in CD3+ cells in both compartments in 2-DG injected female mice. These changes in lymphocytes subpopulations may also be related to the circadian rhythm (11) . In male rats, foot-shock resulted in a specific increase of the CD4/CD8 ration in mesenteric lymph nodes which was also related to the stressor administration route (13). However, changes in splenocyte subpopulations associated with

in vitro proliferation decreases suggest that these decreases may be induced higher concentrations of differentiated splenocytes (i.e., CD3+, TCR αβ+ and IA^d+), which have been previously shown to have lower levels of mitosis (20,33).

The increase in blood levels of interleukin-1β may partially explain other changes in female mice injected with 2-DG. Indeed, IL-1 β enhanced corticosterone secretion by acting directly on the rat adrenal gland (2) and affected T cell mitogenesis, natural killer activity, and IL-2 production (45). Recently, administration of IL-1 has been shown to induce long lasting production of corticosterone through interactions of neuro-endocrine-immune pathways (10).

Sexual dimorphism in immune function resulting from the effects of sex hormones on immune effector cells has been observed in both animals and humans (18,19). Previous work (21) has suggested an influence of sex hormones on the immune response and, therefore, on susceptibility to disease, especially with regard to higher incidence of many autoimmune diseases in females (1). In many species, females had more potent immune response than males (32). Variations in susceptibility to autoimmune disease (3,38), or stress effects on immune responses (9,14) based on sex have been studied to a limited extent (18). Moreover, sex hormones and some other steroids have been shown to exert immunoregulatory effects (32,44). In the present study, levels of several major sex hormones were also measured in the bloodstream of the 2-DG and saline injected mice. In contrast to previous work (44), no significant variation in levels of estradiol or testosterone was observed. Levels of luteinizing hormone, follicle stimulating hormone and progesterone, which are mainly related to the ovarian cycle, have been shown to affect the immune response (21,23), were not different between saline and 2-DG injected mice. By contrast, in rat, 2-DG induced feeding was suppressed during estrus or in ovarectomized female injected with progesterone (25). Finally, sexual dimorphism in basal hypothalamo-pituitary-adrenal axis activity, with higher levels in female, was observed in mice and the activity could modulated by sex steroid injection on gonadectomized animals (41).

Taken together, these observations suggest a sexual dimorphism in immune responses to glucoprivation with higher sensitivity of female mice. They also suggest that the decrease in lymphoproliferation induced by 2-DG administration is associated with changes in lymphocyte subpopulations.

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Table 1. Percentages of cells bearing markers (mean±SEM) after immunostaining of whole blood from male and female mice (n=8 per gender per group) injected three times with saline or 2-DG.

	Female		Male	
Marker	Saline	2-DG	Saline	2-DG
CD3+	13.3±3.8	17.6±4.5	10.9±1.4	15.9±3.0
TCR-α/β+	3.7±0.9	9.5[b]±1.5	5.7±1.0	10.7[a]±1.5
CD4+	3.7±1.3	4.9±2.8	1.7±0.3	2.9±1.0
CD8+	6.0±0.7	9.3±1.9	4.6±0.5	5.7±0.3
H-2L^d/H-2D^b+	30.9±6.4	33.7±3.6	48.9±5.9	47.1±1.8
I-A^d+	16.0±2.9	35.0[a]±7.4	15.1±2.6	23.2[a]±2.6

Probability that values obtained in saline and 2-DG injected mice within gender differ were determined by Bonferroni's *t*-test: [a]: $p<0.05$, [b]: $p<0.001$.

Table 2. Percentages of cells bearing markers (mean ± SEM) after immunostaining of splenocyte suspensions from male or female mice (n=8 per gender per group) injected three times with saline or 2-DG.

	Female		Male	
Marker	Saline	2-DG	Saline	2-DG
CD3+	12.0±3.4	20.7±5.7	12.9±0.6	14.0±0.6
TCR-α/β+	6.2±1.8	19.2[b]±2.8	11.8±0.5	12.5±0.9
CD4+	6.4±0.8	8.5±1.2	5.8±0.6	6.6±0.9
CD8+	8.0±1.1	6.4±1.1	6.9±0.5	7.9±0.3
H-2L^d/H-2D^b+	43.6±6.4	66.6[a]±8.3	63.4±3.1	72.4[a]±2.6
I-A^d+	37.5±3.4	53.9[b]±2.9	37.8±3.2	36.4±3.4

Probability that values obtained in saline and 2-DG injected mice within gender differ were determined by Bonferroni's *t*-test: [a]: $p<0.05$, [b]: $p<0.001$.

Figure legends:

Fig.1. Blood glucose levels in male and female mice (n=8 per group/sex) injected intraperitoneally three times with saline or 2-DG.

*P=0.0015, probability that values obtained in saline- (□) and 2-DG-injected (■) mice differ as determined by Bonferroni's *t*-test.

Fig.2. Blood corticosterone levels in male or female mice (n=8 per group/sex) injected intraperitoneally three times with saline or 2-DG.

*P=0.016, probability that values obtained in saline- (□) and 2-DG-injected (■) mice differ as determined by Bonferroni's *t*-test.

Fig.3. Proliferation of partially purified mature T splenocytes from male or female mice (n=8 per group/sex) injected intraperitoneally three times with saline or 2-DG in presence of ConA (5 mg/ml).

*P=0.042, probability that values obtained in saline- (□) and 2-DG-injected (■) mice differ as determined by Bonferroni's *t*-test.

Fig.4. Blood interleukin-1β levels in male or female mice (n=8 per group/sex) injected intraperitoneally three times with saline or 2-DG.

*P=0.007, probability that values obtained in saline- (□) and 2-DG-injected (■) mice differ as determined by Bonferroni's *t*-test.

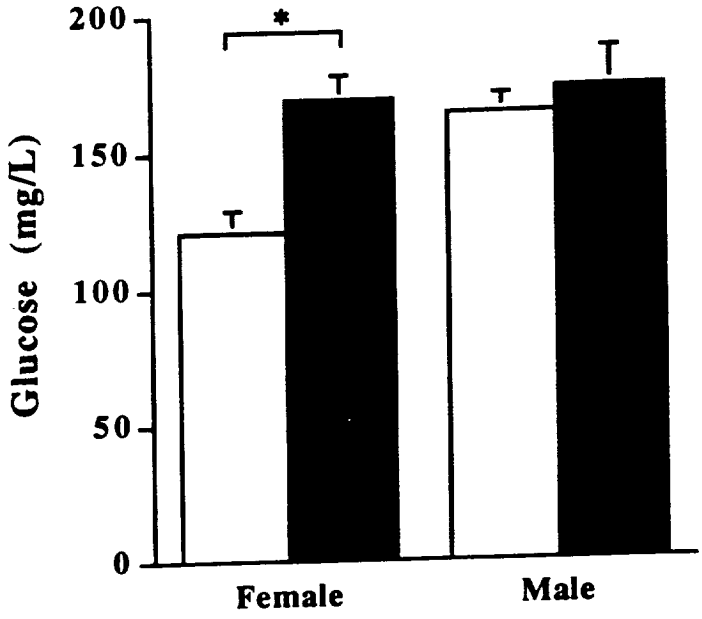
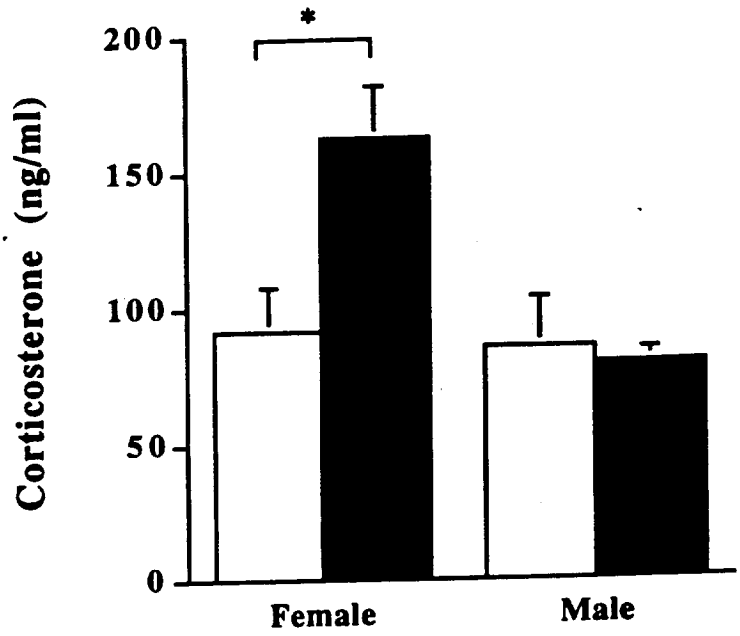


Figure 1.



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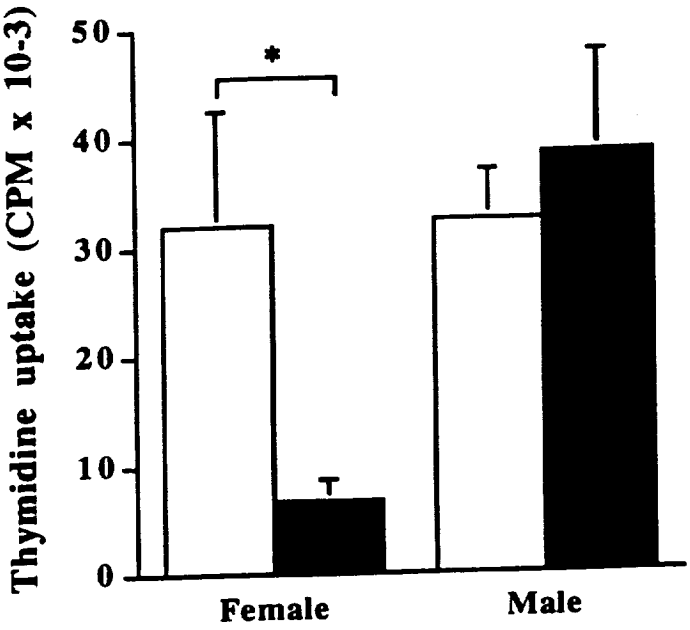

Figure 3.

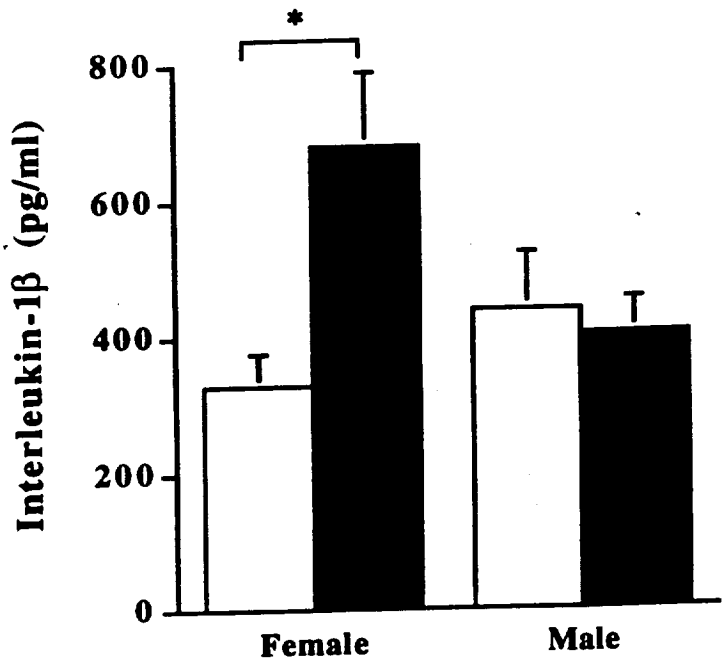

Figure 4.