Immune Response in the Mutant Diabetic C57BL/Ks-db+ Mouse

DISCREPANCIES BETWEEN IN VITRO AND IN VIVO IMMUNOLOGICAL ASSAYS

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ABSTRACT Cell-mediated and humoral immune responses of mutant diabetic db+/db+ mice were evaluated using in vivo and in vitro immunological assays. When compared to lean, nondiabetic db+/m+ or m+/m+ mice, db+/db+ mice demonstrated markedly altered in vivo immune responses characterized by a significantly diminished ability to reject allogeneic skin grafts, a markedly diminished capacity to generate cytotoxic cells after sensitization with allogeneic EL-4 lymphoma cells and a significantly enhanced plaque-forming cell response to sheep erythrocytes. In contrast, spleen cells from db+/db+ mice demonstrated only minimal alterations in in vitro responses to mitogens and allogeneic cells and no alteration in their capacity to generate an in vitro plaque-forming cell response. The spleens and thymuses of db+/db+ mice weighed significantly less than organs from db+/m+ mice. In addition, thymuses from db+/db+ mice demonstrated a marked deficiency in in vivo [^{125}I]UdR uptake. These data suggest that the altered metabolic status of the diabetic host influences immune function in vivo possibly due to abnormal function of lymphocyte subpopulations.

INTRODUCTION

It has been previously suggested that cell-mediated immunologic responses in patients with diabetes mellitus (1–5), in mutant diabetic C57BL/Ks-db+ mice (6, 7), chemically induced diabetic mice (7, 8), guinea pigs (9) and rats (10, 11) are impaired. The homozygous db+/db+ mutant mice of strain C57BL/KsJ develop spontaneous obesity and diabetes which is associated with insulin resistance, hyperglycemia, hyperinsulinemia (12), and a decreased number of insulin binding sites on several types of cells including lymphocytes (13). This report examines splenic lymphocyte function in C57BL/KsJ-db+ mice in response to mitogens and to both in vivo and in vitro stimulation with allogeneic cells and sheep erythrocytes. The results demonstrate that young db+/db+ mice have abnormalities in immune function detected by in vivo but not in vitro assays of cellular and humoral immunity.

METHODS

Animals. Nonmutant, nondiabetic C57BL/Ks (H-2^d) mice were obtained from the University of Minnesota Mouse Colony. Diabetic mice used in these experiments were obtained from a breeding colony of C57BL/KsJ-db+/m+ mice (The Jackson Laboratory, Bar Harbor, Maine). Because of the close linkage of the autosomal recessive diabetic (db) gene and misty (m) coat color gene (both are located on chromosome 4, linkage group VIII) and that in db+/m+ mice the misty allele is in repulsion to the db locus (i.e., on the other chromosome), mice of three distinct genotypes were produced by this mating procedure (14): (*a*) obese black diabetic mice homozygous for db (db+/db+); (*b*) lean, black, phenotypically nondiabetic mice heterozygous for db and m (db+/m+); and (*c*) lean, misty, nondiabetic mice homozygous for m and lacking the db gene (m+/m+). The origin, genetic background, and the breeding procedure are described in greater detail by Chick and Like (14). The db+/db+ mice used in the present studies weighed between 32 and 70 g, whereas db+/m+ mice weighed from 19 to 30 g. By 3 mo of age blood

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levels of glucose, as determined by the glucose oxidase method (15) in db+/db+ mice ranged from 315 to 656 mg/dl as compared with 130–190 mg/dl for db+/m+ and m+/m+ mice. CBA/H ($H\text{-}2^k$), and C57BL/6 ($H\text{-}2^b$) mice were obtained from the University of Minnesota Mouse Colony. Mice were kept in plastic cages with Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) and water ad lib. and maintained under standard conditions with 12 h of light (6 a.m.–6 p.m.) and 12 h of darkness (6 p.m.–6 a.m.). To minimize any circadian influence on immune response (16) both immunization and killing of animals was carried out strictly between 9 and 10 a.m.

Tumor cells. EL-4 lymphoma cells ($H\text{-}2^b$) (17) were maintained by weekly serial intraperitoneal injection of EL-4 cells into syngeneic C57BL/6 hosts. LSTRA lymphoma cells ($H\text{-}2^d$) (17) were maintained by serial intraperitoneal injection of LSTRA cells into syngeneic BALB/c hosts.

Mitogen stimulation. Details of this assay have been described previously (18). Nucleated spleen cells (5×10^5) from individual mice were cultured in 0.2 ml of RPMI-1640 medium containing 2% heat-inactivated (56°C, 45 min) fetal calf serum (FCS),[1] penicillin, streptomycin, and glutamine (Grant Island Biological Co., Grand Island, N. Y.) in triplicate wells of 3040 microtest II tissue plates (Falcon Plastics, Division of BioQuest, Oxnard, Calif.). Spleen cells were cultured with 0.25, 1.0, and 5.0 μg of phytohemagglutinin (PHA) per ml (HA17, Burroughs-Wellcome Co., Research Triangle Park, N. C.) 0.8, 2.5, and 5.0 μg of concanavalin A (Con-A) per ml (Sigma Chemical Co., St. Louis, Mo.), and 100 μg of *E. coli* lipopolysaccharide (LPS) per ml (Difco Laboratories, Detroit, Mich.) for 40, 64, 68, and 112 h. 16 h before harvest, 0.5 μCi of [*methyl*-^{3}H]thymidine (NET-027, New England Nuclear, Boston, Mass.) was added to each culture well. The plates were harvested on glass fiber filter papers, placed in scintillation fluid, and then counted.

Mixed lymphocyte reaction. Mixed lymphocyte reactions were performed by slight modification of the procedure described by Fathman et al. (19). Briefly, spleen cell cultures were prepared in RPMI-1640 medium containing 5% FCS, streptomycin, penicillin, and glutamine. Stimulator cells were treated with 25 μg/ml mitomycin C (Sigma Chemical Co.) for 30 min at 37°C. Triplicate cultures containing 5×10^5 responder cells and 5×10^5 stimulator cells were established in 3040 microtest plates. Cultures were incubated at 37°C in an atmosphere of 5% CO_2-95% air for 88 h. [*methyl*-^{3}H]Thymidine (0.5 μCi) was added during the final 16 h of culture.

Response to in vivo immunization with sheep erythrocytes (SRBC). The details of this assay were recently described (16). Each mouse was injected intraperitoneally with 0.2 ml of a 20% suspension of SRBC (Wilfer Farms, Minneapolis, Minn.) and 96 h later spleens were removed and the viable cell concentration adjusted to 10^7 cells/ml. A modified Jerne plaque-forming cell (PFC) assay (20) was used to measure the PFC response. The immunizing dose of SRBC was shown in preliminary studies to be optimal for both db+/db+ and db+/m+ mice.

Response to in vitro immunization with SRBC. Spleen cells free of erythrocytes (21) were prepared and cultured at a concentration of 1.2×10^7 cells/ml with SRBC (Grand Island Biological Co.) in RPMI medium with 10% FCS in plastic Petri dishes (model 3001, Falcon Plastics) by the method of McCarthy and Dutton (22). The cultures were fed daily and harvested on day 5 for measurement of PFC.

Cytotoxic response to immunization with allogeneic antigens. For in vivo immunization, db+/db+, db+/m+, and m+/m+ mice were injected intraperitoneally with 5×10^6 or 10×10^6 EL-4 lymphoma cells. 10–20 days after immunization the mice were sacrificed and the cytotoxicity activity of their spleen cells determined in a microplate ^{51}Cr-release assay system (23).

In vitro allogeneic sensitization was performed in plastic multiwell dishes (FB16-24TC, Linbro Chemical Company, Hamden, Conn.) using a modification of the method of Hodes et al. (24). Spleen cells (4×10^6) from db+/db+ or db+/m+ mice were admixed with 0.5×10^6 mitomycin C-treated (25 μg/ml, 37°C, 30 min) C57BL/6 spleen cells in 2 ml of RPMI medium with 10% FCS and 50 μM 2-mercaptoethanol, and incubated at 37°C in an atmosphere of 5% CO_2–95% air for 5 days. Replicate wells were then pooled and the cytotoxic activity of the viable cell population determined (23).

Allogeneic skin grafts. Grafting of full thickness skin grafts was performed as previously described (25). The grafts were examined daily and the interpretation of rejection was based upon the gross observation of skin hemorrhage in 50% of the grafted skin.

In vivo assay of DNA synthesis in spleen and thymus. 6- to 8-wk-old mice were injected intraperitoneally with 2 μCi/mouse of 5-[^{125}I]iodo-2′-deoxyuridine ([^{125}I]UdR) (sp act 102 μCi/μg, Amersham/Searle Corp., Arlington Heights, Ill.) and 18 h later the uptake of isotope both in the spleen and thymus was determined according to the method of Zatz and Goldstein (26).

Statistics. Statistical analyses were performed using the Student's *t* test.

RESULTS

Stimulation of lymphocytes by mitogens. No significant differences were observed in the mean response of spleen cells from db+/db+, db+/m+, or m+/m+ mice to stimulation with PHA, Con A or LPS after 40, 64, 88, or 112 h of culture. Fig. 1 illustrates the response to optimal concentrations of PHA (1 μg/ml), Con A (2.5 μg/ml), and LPS (100 μg/ml) after 64 h of culture (time of maximal response). The range of responses of diabetic mice of PHA and Con A was somewhat greater than the range of responses of nondiabetic mice.

Mixed lymphocyte reaction. Table I shows the mean proliferative response (as measured by stimulation index) of db+/db+, db+/m+, and m+/m+ (all $H\text{-}2^d$) spleen cells to allogeneic stimulation by C57BL/6 ($H\text{-}2^b$) and CBA/H ($H\text{-}2^k$) spleen cells. The mean stimulation indices of db+/db+ spleen cells to stimulation by C57BL/6 and CBA/H alloantigens was diminished when compared to the mean responses of db+/m+ or m+/m+ mice but the differences were not statistically significant ($P > 0.05$).

Response to immunization with SRBC. The PFC response of spleen cells from 2- to 6-mo-old db+/db+ mice was significantly increased (1.4- to 3.0-fold, $P < 0.05$) compared with the response of spleen cells

[1] *Abbreviations used in this paper*: Con A, concanavalin A; FCS, fetal calf serum; LPS, lipopolysaccharide; PFC, plaque-forming cell; PHA, phytohemagglutinin; SRBC, sheep erythrocytes.

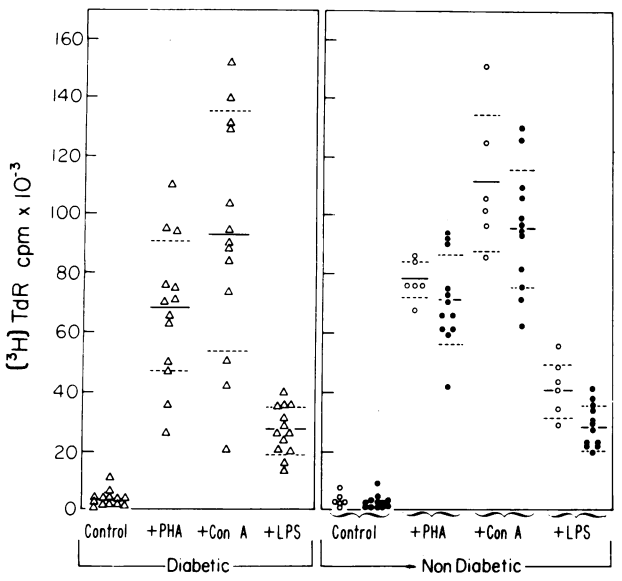

FIGURE 1 Mitogenic response of spleen cells to PHA (1 μg/ml), Con A (2.5 μg/ml), and *E. coli* LPS, 100 μg/ml) from 3-mo-old diabetic (*db+/db+*, Δ) and nondiabetic (db+/m+, ●, and m+/m+, ○) mice. 0.5 × 10^6 spleen cells from individual mice were cultured in triplicate in the presence of mitogen for 64 h. The results represent the mean ±1 SD.

from age-matched db+/m+ and m+/m+ mice after in vivo immunization with SRBC (Table II). The PFC response of spleen cells from db+/m+ and m+/m+ mice were comparable (Table II, exp. III).

In contrast to the in vivo responses, no significant differences were noted between the PFC response of spleen cells of db+/db+ and db+/m+ mice after 5 days of in vitro sensitization to SRBC (Table III).

TABLE I
Mixed Lymphocyte Reactivity of db+/db+, db+/m+, and m+/m+ to Allogeneic Stimulator Cells

Responder* cell	Stimulator cell	Stimulation indices‡	Mean stimulation index±SEM
db+/db+	C57BL/6	2.3, 2.1, 1.4, 2.9, 3.6	2.46±0.37
db+/m+	C57BL/6	2.4, 2.3, 4.1, 2.4	2.80±0.43§
m+/m+	C57BL/6	2.2, 1.9, 4.2	2.77±0.74§
db+/db+	CBA/H	3.2, 2.3, 2.2, 2.4, 2.6	2.54±0.18
db+/m+	CBA/H	2.3, 3.1, 4.2, 3.0	3.15±0.39§
m+/m+	CBA/H	2.8, 2.7, 3.8	3.10±0.36§

* All responder mice were 3-mo old.
‡ Stimulation index: mean counts per minute of allogeneic mixed lymphocyte reactivity divided by mean counts per minute of syngeneic mixed lymphocyte reactivity. Each value represents the stimulation index of an individual experiment.
§ The *P* values for comparisons of mean stimulation indices of db+/m+ and m+/m+ to mean stimulation indices of db+/db+ mice were >0.5.

Cell-mediated cytotoxicity of spleen cells after immunization with allogeneic cells. The cytotoxic response of spleen cells from db+/db+ mice after in vivo immunization with allogeneic EL-4 ($H\text{-}2^b$) lymphoma cells was significantly depressed ($P < 0.05$) when compared with the responses of spleen cells from db+/m+ or m+/m+ mice. Table IV presents the results after immunization with 5 × 10^6 EL-4 lymphoma cells. Mice were sacrificed 10, 12, 15, and 20 days after immunization and the cytotoxic activity of their spleen cells against ^{51}Cr-labeled EL-4 cells determined in a 6-h cytotoxicity assay. At each of the time

TABLE II
PFC Response by Spleen Cells after In Vivo Immunization with SRBC

Exp. no.	Mice	Age	No. of mice	Sex	PFC/1 × 10^6 cells	PFC/spleen
		mo			*mean±SEM*	
I	db+/db+	2	2	M	918±25*	138,168±20,426*
	db+/m+	2	2	M	374±66	62,424±17,064
II	db+/db+	3	4	M	1,005±55*	145,235±25,500*
	db+/m+	3	3	M	385±34	67,765±66,42
III	db+/db+	3	3	F	1,414±123*	321,387±7,997*
	db+/m+	3	3	F	887±63	192,265±17,589
	m+/m+	3	3	F	982±23	200,633±30,000
IV	db+/db+	6	4	M	1,452±223*	179,779±12,099*
	db+/m+	6	4	M	491±55	69,205±4,929

* The *P* values for comparisons of PFC responses of db+/db+ cells to db+/m+ or m+/m+ cells were <0.05. The responses of db+/m+ and m+/m+ were not statistically different.

TABLE III
Primary PFC Response by Spleen Cells after In Vitro *Immunization with SRBC*

Group	Age	In Vitro PFC	
		PFC/1 × 10^6	PFC/culture
	mo		
db+/db+ (9)‡	2–4	5,933±1,036*	15,301±1,871*
db+/m+ (9)‡	2–5	5,231±1,030 $P > 0.1$	18,204±2,674 $P > 0.1$

* Mean±SEM.
‡ Number of mice studied.

points after immunization and at each of the target cell to spleen cell ratios tested (1:25 and 1:100) db+/db+ spleen cells exhibited significantly less cytotoxicity (percent ^{51}Cr release) than did spleen cells from db+/m+ or m+/m+ mice. No consistent differences were noted in the cytotoxic response of db+/m+ and m+/m+ mice (Table IV, exps. III and IV). The cytotoxic response of db+/db+ spleen cells was also diminished when compared to db+/m+ or m+/m+ mice in 3- and 9-h cytotoxicity assays and was also found to be depressed when compared to db+/m+ mice after immunization with an increased dose (10 × 10^6) of EL-4 cells (data not shown). The latter results indicated that the cytotoxic deficiency in db+/db+ mice is not due to a low immunogenic dose of tumor cells.

In contrast with these findings, when spleen cells from db+/db+, db+/m+, m+/m+, and C57BL/Ks (all *H-2^d*) mice were sensitized in vitro to spleen cells from C57BL/6 (*H-2^b*) mice only minor differences were noted in cytolytic activity against ^{51}Cr-labeled EL-4 (*H-2^b*) cells (Table V). Spleen cells from all diabetic and nondiabetic mice generated high levels of cytotoxicity. The cytotoxicity generated was specific; ^{51}Cr-labeled LSTRA (*H-2^d*) lymphoma cells were not lysed (data not shown). The cytolytic activity of spleen cells from four of five db+/db+ mice (animals 1, 2, 8, and 13) and control db+/m+ or m+/m+ mice were equivalent at a target cell to spleen cell ratio of 1:8. The same four db+/db+ mice, however, demonstrated a slight but statistically significant ($P < 0.05$) decrease in percent ^{51}Cr release at low target cell to spleen cell ratios (1:1 or 1:2). One of five db+/db+ mice (animal 7) demonstrated diminished cytolytic activity at all target cell to spleen cell ratios examined. When compared with the diminished cytolytic activity of db+/db+ spleen cells after in vivo immunization (Table IV), the defect after in vitro immunization was distinctly less pronounced.

Allogeneic skin grafting (Table VI). When full thickness skin grafts from allogeneic C57BL/6 (*H-2^b*) mice were placed on db+/db+ (*H-2^d*) hosts, the rejection time was significantly ($P < 0.01$) prolonged as compared with db+/m+ (*H-2^d*) as hosts. The db+/m+ mice rejected C57BL/6 skin as well as did C57BL/KsJ (*H-2^d*) hosts.

Weights and in vivo DNA synthesis of thymus and spleen (Table VII). Both the spleen and thymus of db+/db+ mice weighed significantly less than organs from db+/m+ mice. Furthermore, in vivo DNA synthesis (as measured by [^{125}I]UdR uptake) in the thymuses of the db+/db+ mice was markedly less than that found in the thymuses of db+/m+ mice. In contrast no differences were noted in [^{125}I]UdR uptake of spleens from db+/db+ mice (despite their smaller weights) and db+/m+ mice.

DISCUSSION

The present studies demonstrate a markedly altered in vivo immune response in obese, diabetic db+/db+ mice characterized by a significantly diminished ability to reject allogeneic full-thickness skin grafts, a markedly diminished capacity to generate cytotoxic

TABLE IV
Cell-Mediated Cytotoxicity of Spleen Cells of Mice Immunized In Vivo with EL-4 Cells (5 × 10^6)

Exp. no.	Mice	Days after immunization	No. of Mice*	Percent ^{51}Cr release‡ at 6 h	
				1:25§	1:100§
			(Sex)		
I	db+/db+	10	3 (F)	11.4±3.4‖	14.4±2.7‖
	db+/m+	10	3 (F)	43.6±0.5	77.7±0.4
II	db+/db+	12	2 (M)	16.3±0.4‖	25.4±2.2‖
	db+/m+	12	2 (M)	42.9±9.0	58.4±10.6
III	db+/db+	12	1 (M)	—	5.3±1.2‖
	db+/m+	12	2 (M)	50.1±3.0	78.3±0.4
	m+/m+	12	2 (M)	37.5±3.9	70.9±0.9
IV	db+/db+	12	3 (M)	14.7±5.9‖	33.4±13.8‖
	db+/m+	12	3 (M)	49.7±2.6	78.9±0.9
	m+/m+	12	2 (M)	57.3±11.8	83.2±0.4
V	db+/db+	15	3 (M)	11.1±4.0‖	24.5±7.0‖
	db+/m+	15	3 (M)	32.3±7.1	63.5±9.2
VI	db+/db+	20	3 (F)	12.8±2.5‖	26.0±4.6‖
	db+/m+	20	3 (M)	26.0±5.4	51.4±4.7

* All mice were 3.5 to 5-mo old.
‡ Mean±SEM.
§ Target to spleen cell ratio—25 × 10^4 target cells per well.
‖ The *P* values for comparisons of the cytotoxic activity of db+/db+ cells to db+/m or m+/m+ cells were <0.05.

TABLE V
In Vitro Generation of Cell-Mediated Cytotoxicity of Spleen Cells against C57B1/6 Allogantigens

Exp. no.	Animal no.	Effector cells (H-2^d)	Percent ^{51}Cr release from EL4 (H-2^b) target cells in a 6-h assay			
			1:1*	1:2	1:4	1:8
I	1	db+/db+‡	26.9±1.3§	62.2±1.3	91.4±1.5	97.9±2.5
	2	db+/db+	27.3±0.5	69.9±2.3	92.9±1.4	92.4±4.4
	3	db+/m+	35.1±1.6	78.5±2.5	95.0±1.1	95.3±2.7
	4	db+/m+	35.9±0.5	72.8±0.5	94.7±1.2	92.5±1.6
	5	m+/m+	36.8±1.7	78.3±1.6	87.5±2.3	98.0±5.9
	6	m+/m+	30.1±1.4	68.9±0.4	89.5±2.1	91.3±2.7
II	7	db+/db+	10.5±0.3	21.6±0.4	41.2±0.3	61.1±1.6
	8	db+/db+	21.8±1.2	40.4±0.8	62.7±2.2	72.9±2.2
	9	db+/m+	26.0±0.3	46.9±0.3	65.0±1.4	75.1±0.6
	10	db+/m+	35.7±1.4	57.9±0.5	74.0±2.2	77.0±1.5
	11	m+/m+	27.8±2.0	50.8±1.6	67.8±2.0	70.3±0.3
	12	m+/m+	34.2±0.8	54.2±1.8	67.7±1.9	73.4±1.4
III	13	db+/db+	15.8±1.1	—	58.8±1.7	78.4±2.6
	14	db+/m+	21.7±1.8	—	64.4±0.4	76.2±2.8
	15	C57B1/KS	19.9±0.3	—	62.0±2.2	76.9±3.0

* Target to spleen cell ratio.
‡ All mice were 3-mo old.
§ Mean±SEM.

cells after in vivo sensitization with allogeneic EL-4 lymphoma cells, and a significantly enhanced in vivo PFC response to SRBC. In contrast, spleen cells from db+/db+ mice demonstrate only minimal alterations in in vitro responses to mitogens and allogeneic cells and no alteration in their capacity to generate PFC after in vitro stimulation with SRBC.

The discrepancies observed between in vivo and in vitro immunological assays suggest that the alteration in in vivo immune function of db+/db+ mice may be a consequence of the abnormal metabolic environment present in these animals. Whether the defect in immune function is related to persistent hyperglycemia, to the hyperinsulinemia and decreased insulin receptor activity on lymphocytes (13) or monocytes (27), to the metabolic consequences of obesity or to other metabolic factors is unknown; further work is needed to clarify this point. Pallavicini and Nichols (11) have described the existence of factor(s) in the plasma of alloxan-induced diabetic rats with suppressed in vitro blastogenic responses of both diabetic and normal rats. Thompson (28) has shown that in vivo administration of insulin can augment cell-mediated immune response to purified protein derivative in albino rats. An effect of insulin and glucose on the function of polymorphonuclear leukocytes of diabetic patients has been described (4, 5). Helderman and Strom (29) have reported that alloimmune T lymphocytes express insulin receptors and that brief (1–6 min) exposure of cytotoxic cells from nondiabetic rats to physiological concentration of insulin augments T-cell-mediated cytotoxicity (30). If cytotoxic cells were preincubated with insulin (1–0.01 nM) for a longer period of time (8 min) no augmentation occurred. It is possible that prolonged in vivo exposure of db+/db+ lymphocytes to elevated insulin level may, in fact, result in diminished in vivo cytotoxic activity as reported here. It appears, however, that the db+/db+ lymphocyte removed from its in vivo environment and place into a FCS supplemented culture medium can function in a normal or near normal manner in in vitro assays of both cellular and humoral immunity. These results suggest

TABLE VI
Allogeneic Skin Graft Rejection Time in db+/db+, db+/m+, and C57BL/Ks Mice

Host (H-2^d)	Donor (H-2^b)	Rejection time		P value vs. db+/m+
		Days	Mean±SEM	
db+/db+	C57BL/6	17, 16, 14, 10 12, 12*	13.5±1.1	<0.01
db+/m+	C57BL/6	7, 8, 8, 8, 8	7.8±0.2	
C57BL/Ks	C57BL/6	7, 9, 10, 9, 9, 9	8.0±0.4	>0.05

* Rejection time of individual mice.

TABLE VII
Weights of and In Vivo [^{125}I]UdR Uptake by Spleen and Thymus

Strain (no. mice)	Body weight	Spleen weight	*Thymus* weight	[^{125}I]UdR/cpm/organ	
				Spleen	Thymus
	g	*mg*	*mg*		
db+/db+ (8)*					
(6- to 8-wk old)	32.4±1.9‡	67.9±3.6	26.9±2.1	1,727±524	154±19
db+/m+ (8)*					
(6- to 8-wk old)	21.8±0.7	96.2±7.1	46.7±2.1	2,217±249	924±97
P value	<0.001	<0.005	<0.001	>0.05	<0.001

* Number of mice studied.
‡ Mean±SEM.

that the depressed functional activity of lymphocytes present in vivo can return to normal in vitro because of the more favorable metabolic environment or because the ratio and/or type of cells involved in in vivo and in vitro immunological assays may vary. The occurrence of both phenotypic and genotypic alterations in mammalian cells during in vitro cultures has been reported (31). These changes are thought to be secondary to changes in serum proteins, hormones, and/or macromolecular growth factors (32).

Our observation of altered in vivo immune function of db+/db+ mice is consistent with the recent report of delayed skin graft rejection and suppressed schistosoma granuloma formation in db+/db+ mice (6). Rodman et al. (8) have demonstrated that streptozotocin-treated diabetic mice have a diminished in vivo cell-mediated immune response to *Listeria monocytogenes* antigens and a diminished capacity to limit *L. monocytogenes* proliferation in vivo. Diminished skin test reactivity to purified protein derivative has been documented in alloxan-treated guinea pigs and is prevented by insulin administration (9). Webb et al. (7) recently reported that mortality in C57BL/Ks-db+ mice injected with group B coxsackie virus is related to the db gene rather than to the diabetic state per se. Nondiabetic heterozygous db mice had a greater mortality than mice of the non-db parental background strain (C57BL/Ks). Homozygous db mice, in turn, had a higher mortality than heterozygous db mice. In contrast, in the studies of in vivo immune function reported here, abnormalities were seen only in homozygous diabetic db+/db+ mice. The immune response of heterozygous db+/m+ mice were comparable to non-db m+/m+ of C57BL/Ks mice.

These data suggest that the influence of the db gene on immune function may be indirect and mediated through its metabolic effects. A more direct effect of the db gene in the homozygous state on immune function, however, cannot be excluded. Dolkert et al. (33) have demonstrated that the antibody responses to bovine serum albumin of alloxan-treated, hypoinsulinemic, hyperglycemic mice and normal controls are similar. We have documented an enhanced PFC response to in vivo immunization with SRBC in the spontaneous diabetic db+/db+ mouse. The reasons for this disparity are unknown but may reflect either differences in the in vivo metabolic environment in the experimental models or differences in the techniques used to evaluate humoral immunity.

The depressed in vivo generation of killer cells and the delayed rejection of allogeneic skin grafts in db+/db+ mice suggests a significant in vivo defect in T-cell cytotoxic function. The enhanced sheep erythrocyte PFC response in vivo in db+/db+ mice may be due to deficient T-regulatory or suppressor cell activity (34, 35). Recently both cytotoxic and suppressor T cells have been shown to carry Ly 2 and Ly 3 surface markers (36). The altered metabolic state in db+/db+ mice, therefore, may differentially affect subpopulations of lymphoid cells, impairing the function of Ly2+, 3+ T cells to a greater extent than B lymphocytes or Ly 1+ helper T cells. Furthermore, the thymus is altered in db+/db+ mice as evidence by a decrease in thymic weight and markedly diminished thymic [^{125}I]UdR uptake. The exact interrelationship of the thymic abnormalities to the diminished cytotoxic responses, augmented sheep erythrocyte PFC responses and alterations in regulatory or suppressor T cell in these mice remains to be elucidated.

The studies reported here are important from at least three points of view. First, the diabetic hyperinsulinemic db+/db+ mouse offers a unique opportunity to study abnormalities of the immune system that are detected primarily in in vivo immunological assays. This model will be useful in studies of the relationship between hormones, glucose metabolism, and lymphocyte function. Secondly, the results suggest that studies of immune function in human endo-

crinopathies should include both in vivo and in vitro assays. Finally, at the immunobiological level the experiments illustrate the fact that although in vitro assays are useful in understanding the many immunological phenomena they cannot totally replace the results obtained from an intact animal.

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