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Deficient Humoral Antibody Response of the Spontaneously Diabetic Chinese Hamster (41769)

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Abstract. The antibody responses of spontaneously diabetic Chinese hamsters to sheep erythrocytes was investigated. After primary challenge, total antibody production of the diabetics was only 50% or less than that of nondiabetic controls, and little or no switchover to IgG production occurred. After second challenge, total antibody of diabetics was still reduced compared with controls. Some switchover to IgG did occur after second challenge but was only 40% that of controls. Poor antibody response was not related to abnormal glucose metabolism since low titers and lack of IgG was found in euglycemic prediabetics. Additionally, genetic nondiabetics rendered hyperglycemic by streptozotocin responded normally, similar to euglycemic controls. The impaired humoral antibody response of the diabetic hamster is similar to that reported for some human diabetics and makes this animal model attractive for studying the consequences and possible causes of diabetes-dependent impairment of immune responses.

Although human diabetics are known to be more susceptible to bacterial and viral infections, and such infections are often more prolonged and severe, little has been published about their humoral antibody responses. In 1933, Richardson (1) showed that diabetics produce less agglutinin antibody after inoculation than do nondiabetics. Since then there have been some epidemiological studies which gave conflicting results. However, recently McCuish *et al.* (2) have shown that, although the lymphocyte transformation to PHA is normal in metabolically well-controlled patients, it was impaired in poorly controlled diabetics. Casey and Sterm (3) found the blastogenic responses of the lymphocytes of diabetics to *Staphylococcus* to be significantly lower than nondiabetic controls. Ludwig *et al.* (4) have demonstrated that diabetics showed decreased humoral immunity to *Escherichia coli* and *Staphylococcus.* Palmer *et al.* (5) have found that during outbreaks of Coxsackie B-3, B-4, or B-5 infections the antibody titers of diabetics were lower than controls for Coxsackie B-3 and B-4. Their responses to influenza A and B and Coxsackie B-5 were normal. The accumulated evidence suggests that in human diabetes the immune response is impaired and, perhaps, helps account for the increased severity of infections.

The spontaneously diabetic Chinese hamster has many characteristics which are similar to those of human diabetics. Although they are nonobese, diabetic hamsters display hyperglycemia, decreased pancreatic insulin levels, and relative or absolute plasma insulin deficiency. Hamsters have been bred for and against diabetes so that sublines are either essentially 100% diabetic or nondiabetic. Although this animal model has been well characterized and displays pathologies (6) similar to those of human diabetics, no studies on its immune response can be found. Therefore, this paper reports on the humoral immune responses of the genetically diabetic Chinese hamster.

Materials and Methods. *Animals.* Fifteen diabetic and fifteen nondiabetic Chinese hamsters, 3 to 4 months of age, were obtained from The Upjohn Company colony (6). Chinese hamsters were monitored for glycosuria with TesTape (Eli Lilly and Co., Indianapolis, Ind.) daily. Diabetics from inbred sublines displayed consistent daily 4+ glycosuria (nonfasting blood glucose > 200 mg/dl). Inbred nondiabetic Chinese hamsters displayed daily negative TesTape values (nonfasting blood glucose < 120 mg/dl). Prediabetics in this study were hamsters from the diabetic subline which carry the diabetic genes but have not yet displayed glycosuria and hyperglycemia. Both the diabetic and nondiabetic groups were comprised of equal numbers of males and females. They were housed

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singly in stainless-steel cages and maintained on Purina Rodent Chow (5015) and water *ad libitum*.

Antigens. Sheep erythrocytes were obtained in Alsever's solution from a single animal every 4 weeks from Colorado Serum Company and stored at 4°C. Cells were used for immunization within 1 week after arrival. Sheep erythrocytes were prepared for injection by washing three times in saline (0.9% NaCl), and were adjusted so that each injection consisted of 1×10^8 cells, given intraperitoneally. This dose was chosen since 1×10^8 sheep erythrocytes gave an optimum response in dose–response studies.

Reagents. Mercaptoethanol was purchased from Eastman Kodak Company, Rochester, New York 14650. Guinea pig complement was purchased from Grand Island Biological Company, Grand Island, New York. Modified barbital buffer was prepared by the method described by Campbell *et al.* (7). Streptozotocin (Lot No. 10518-GGS-37, The Upjohn Company, Kalamazoo, Mich.) was prepared in citrate buffer (pH 4.5–4.7) and injected less than 5 min after preparation of the solution at a dose of 125 mg/kg.

Assay for anti-sheep erythrocyte hemolysin antibodies. Twofold serial dilutions of whole serum which had been decomplemented by heating to 56°C for 30 min were prepared in modified barbital buffer. Equal volumes of a 1:10 dilution of guinea pig complement and 1% sheep erythrocyte were added, mixed, and incubated for 30 min at 37°C. After centrifugation the titer was recorded as the $\log_2$ of the reciprocal of the highest dilution exhibiting hemolysis.

Determination of IgG. Reduction of antibody was accomplished by treatment of serum with 0.1 *M* 2-mercaptoethanol. Resultant antibody titers were considered to be IgG.

Streptozotocin treatment. Streptozotocin (Lot No. 10518-GGS-37, The Upjohn Co., Kalamazoo, Mich.) was injected in citrate buffer (pH 4.5–4.7) less than 5 min after preparation at a dose of 125 mg/kg. As a streptozotocin control, NAD was prepared in saline at 500 mg/kg and injected 15 min prior to streptozotocin administration. Control animals received only NAD in citrate buffer. Animals were held 75 days prior to antigenic challenge as described above.

Results. Hemolysin antibody titers were followed daily in both nondiabetic and diabetic Chinese hamsters from days 1 through 16 showed that the kinetics of the antibody response were similar to those reported for both rats and mice (8–12). Additionally, the peak IgM response for both diabetics and nondiabetics was found to be Day 5 after immunization, and the peak IgG response to be Day 14.

Primary challenge with sheep erythrocytes. The primary total antibody response of diabetic Chinese hamsters was diminished compared with nondiabetic controls (Fig. 1A). The diabetic animals exhibited total titers only 60% that of nondiabetics at Day 5, about 50% of control on Day 10, and only 37% of normal by Day 14. This difference is even more remarkable when the dilutions are compared rather than $\log_2$. For example, on Day 5 a $\log_2$ of 7.7 represents a dilution of 1:250 for the nondiabetics contrasted with a $\log_2$ of 4.5 which represents a dilution of around 1:25 or a 10-fold decrease in antibody titer of diabetics.

The IgG antibody produced in response to primary challenge was determined by 2-mercaptoethanol reduction (Fig. 1B). Nondiabetic Chinese hamsters began to display IgG on Day 5 and reached a peak of $\log_2$ of 5.1 on Day 14. By contrast, none of the diabetic hamsters produced any IgG throughout the course of primary challenge.

Secondary challenge with sheep erythrocytes. A second challenge with sheep erythrocyte was administered 28 days following the primary challenge. Both the nondiabetics and diabetics reached peak total antibody titers considerably higher than at primary challenge (Fig. 2A). The peak titer at Day 5 for control animals was $\log_2$ of 10.1, representing a dilution of about 1:2000, whereas the peak of primary challenge was 7.7, representing a dilution of about 1:250. Indicating, after second challenge the diabetic hamsters still displayed total antibody titers that were considerably lower than control at all time points. Diabetic hamsters did respond to secondary challenge with a modest production of IgG (Fig. 2B). However, they were quite low compared with nondiabetics at each time point. The peak IgG production of the diabetics at Day 5 was 2.5, representing a dilution of 1:6 while the nondiabetics at Day 5 reached a titer of 9.3, rep-

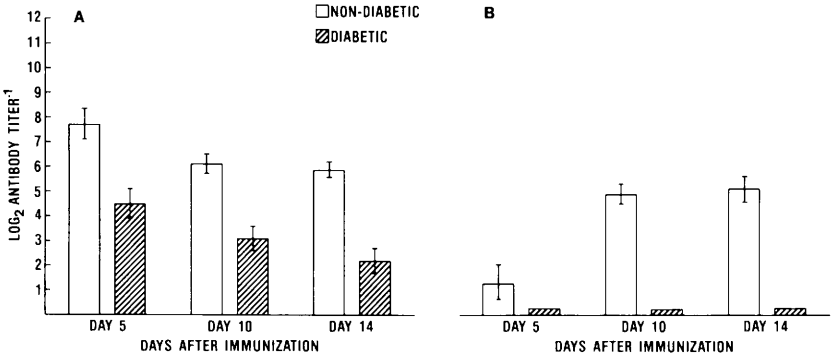

FIG. 1. Primary total (A) and IgG (B) antibody response of nondiabetic and diabetic Chinese hamsters to sheep erythrocytes.

resenting a dilution of 1:640. There was a 100-fold difference in titer between the diabetics and their controls.

Effect of fasting glucose levels on antibody response. Chinese hamsters of diabetic genotype were divided into two groups based on glucose levels. One group was markedly hyperglycemic with a mean fasting glucose level of 265 mg/dl. The other group was prediabetic with a mean fasted glucose value of 112 mg/dl. Data are shown on Table I. Both groups responded to challenge with sheep erythrocytes with a modest total antibody titer on Days 5 and 14, but neither group exhibited IgG antibody at either time point. Antibody titers and blood glucose data were tested by linear regression and no significant correlations were found. The correlation coefficient (r) between the Day 5 total antibody titers of high- and low-glucose hamsters was 0.3176 ($P = 0.199$). Correlation between Day 14 total antibody titers of high- and low-glucose hamsters was 0.2119 ($P = 0.399$). Abnormal antibody production did not correlate with elevated blood glucose concentration.

Primary total and IgG antibody response to streptozotocin-treated Chinese hamsters. To further investigate the possibility that the abnormal immune response of the diabetic Chinese hamster may have been related to hyperglycemia per se, genetically nondiabetic hamsters were rendered hyperglycemic by streptozotocin. Streptozotocin-diabetic hamsters responded to sheep erythrocytes in a manner similar to both untreated and NAD-treated controls at both Days 5 and 14 with respect to total antibody titer and also in respect to IgG production (Table II). The streptozotocin-treated hyperglycemic hamsters appeared to respond better than NAD-protected streptozotocinized controls with normal blood sugar. However, the data unequivocally show

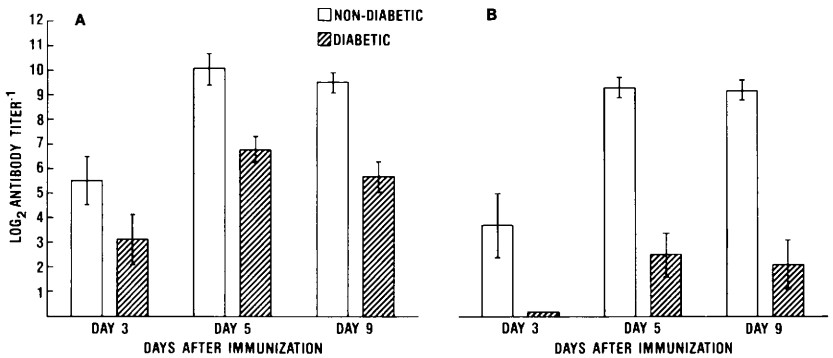

FIG. 2. Secondary total (A) and IgG (B) antibody response of nondiabetic and diabetic Chinese hamsters to sheep erythrocytes.

TABLE I. THE EFFECT OF BLOOD GLUCOSE CONCENTRATION ON THE ANTIBODY RESPONSE OF CHINESE HAMSTERS[a] WITH DIABETIC GENOTYPE

Fasting glucose (mg/dl)	AN/PT	Log_2 antibody[b] $titer^{-1}$ Day 5 Total titer	Day 5 IgG	Day 14 Total titer	Day 14 IgG
265 ± 11	10	4.9 ± 1	0	2.3 ± 0.8	0
112 ± 5	10	2.9 ± 1.3	0	1.7 ± 0.9	0

[a] Chinese hamsters were given a challenge with 1×10^8 SRBC via the ip route on Day 0.

[b] Anti-SRBC antibodies were determined by the hemolysin assay.

that hyperglycemia per se did not interfere with antibody production.

Discussion. The purpose of this study was to investigate the humoral immune response of the spontaneously diabetic Chinese hamster and to compare its response to the nondiabetic. The results indicate that diabetic hamsters have a depressed overall humoral immune response. The total antibody production of the diabetic was about 50% that of nondiabetics, and little or no switchover to IgG production occurred after the primary challenge. After second challenge, the total titers were still reduced and, although some switchover to IgG occurred, it was only about 40% that of nondiabetic controls. Even though modest, the IgG appearing after second challenge suggests that the B lymphocytes are capable of IgG production, but a defect in the mechanisms required for switchover apparently exists. Although no data are available, possible explanations are: (i) defective T- and B-cell interactions, (ii) reduction in number of receptors on lymphocyte membranes or (iii) a defect in the receptors in lymphocyte membranes, (iv) defective metabolism in the B and/or T lymphocyte.

The immune response of some inbred strains of mice can vary greatly to some antigens (13). Some strains are genetically determined high responders, and others low responders. In order to rule out the possibility that the spontaneously diabetic Chinese hamsters are genetic nonresponders to sheep erythrocytes and to determine if the poor humoral immune response was more general, their response to another antigen was determined. Although not shown, the response to chicken γ globulin was similar to the results stated above for sheep erythrocytes. This suggests that the defective antibody production is not an artifact due to a genetic nonresponse to sheep erythrocytes.

Depressed immune response did not correlate with blood glucose concentrations. Prediabetics exhibit the same depressed response as do the markedly hyperglycemic animals of the same inbred subline. It has been recently reported (14) that the lymphocytes of the spontaneously diabetic guinea pig have a defective proliferative response to the mitogens PHA and Con-A which appears to be related to hyperglycemia, since lymphocytes of diabetic guinea pigs in remission exhibit a normal response. Although the test systems utilized to determine immunological defects are different, it seems clear that the defect in the Chinese hamster is genetic, while it appears to be a metabolic rather than genetic defect in the guinea pig.

To further substantiate the possibility that the abnormal immune response of the diabetic Chinese hamster was not related to hypergly-

TABLE II. THE EFFECT OF STREPTOZOTOCIN-INDUCED HYPERGLYCEMIA ON THE ANTIBODY RESPONSE OF CHINESE HAMSTERS WITH NONDIABETIC GENOTYPE

No. animals	Treatment	Blood[a] glucose (mg/dl)	Log_2 antibody $titer^{-1}$ Day 5 Total	Day 5 IgG	Day 14 Total	Day 14 IgG
10	NAD	95 ± 8	5.6 ± 1.3	0.0	5.7 ± 0.6	3.6 ± 1.3
10	Strept.	400 ± 52	6.6 ± 0.6	0.5 ± 0.5	5.9 ± 0.4	5.5 ± 0.4
10	Untreated cont.	95 ± 4	6.9 ± 0.9	2.7 ± 0.8	7.5 ± 0.6	6.1 ± 0.4
10	NAD + strept.	129 ± 8	3.0 ± 1.2	0.0	4.0 ± 0.9	2.3 ± 1.1

[a] Measured 14 days postimmunization.

cemia per se, genetically nondiabetic hamsters were rendered hyperglycemic by streptozotocin. These animals responded normally to erythrocyte challenge suggesting that the defect in humoral antibody production is genetic and not related to abnormal glucose metabolism. Streptozotocin has been reported to have a depressive effect on the immune response (15–17). Saiki *et al.* (17) have shown that male mice injected with sheep erythrocytes about 3 weeks after streptozotocin treatment exhibit a markedly depressed humoral response after both primary and secondary challenge. In order to circumvent this problem, the Chinese hamsters in this experiment were treated with streptozotocin to induce hyperglycemia, and held 75 days prior to immunization to allow complete recovery from any possible toxic effects of this drug to the immune system.

It has not been possible to study the immune response of prediabetic humans due to the difficulty of identifying the susceptible individuals. However, some evidence has accumulated to suggest that humans with marked hyperglycemia have some impaired immune response (1–5). It is interesting to speculate that impaired immune responses could contribute to the etiology of the disease by allowing destruction of β cells by viruses or other agents. The Chinese hamster provides an excellent model to explore this possibility. Further research in the Chinese hamster should include determining the proliferative responses of splenic lymphocytes from prediabetics and diabetics to both PHA and Con-A as well as other mitogens. Since autoimmunity has been implicated in diabetics (2–5) it should be explored as an etiological factor in the Chinese hamster.

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