GENETICS

Protective Role of the Free Glucocorticoid Pool in the Development of Autoimmune Hemolytic Anemia

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The development of autoimmune hemolytic anemia in NZB mice aged 2-3, 6-7, and 10-12 months is associated with decreased corticosterone and increased corticosteroid-binding globulin concentrations in the blood. An increase in the pool of free glucocorticoids induced by dexamethasone decelerates the autoimmune processes. (NZB×DBZ/2) F_1 mice, which never develop the disease, are characterized by a high adrenal activity and a low blood level of corticosteroid-binding globulin. High level of free glucocorticoids is believed to prevent autoimmune reactions.

Key Words: *corticosterone; corticosteroid-binding globulin; autoimmune hemolytic anemia*

Adrenal hormones affect many immunological processes [3]. The role of endogenous glucocorticoids (GC) in the development of autoimmune diseases is virtually unknown. GC are believed to protect the organism from autoreactive cells in the course of immune response [4]. In addition, disorders in the immune system components normally regulated by adrenal hormones are typical of some autoimmune diseases [5,12].

NZB mice are a model of human autoimmune hemolytic anemia and systemic lupus erythematosus. Previously we revealed specific features in the pituitary-adrenal system of NZB mice: decreased plasma corticosterone (CS) content and adrenal sensitivity to adrenocorticotropic hormone anticipating the disease development [6]. We have suggested that a decrease in the blood GC concentration provokes autoimmune processes. The progeny of NZB mice mated with mice not prone to spontaneous autoimmune reactions never have autoimmune hemolytic anemia [9]. For more accurate elucidation of the contribution of adrenal hormones to the disease development, we compared blood content of CS and corticosteroid-binding globulin (CSG) and the production of CS by the adrenals of NZB mice and (NZB×DBA/2) F_1 hybrids at different ages and at different stages of disease.

MATERIALS AND METHODS

Experiments were carried out on male NZB and (NZB×DBA/2) F_1 hybrids aged 2-3 months (reproductive age at which no autoimmune reactions occur in NZB mice), 6-7 months (age before the disease), and 10-12 months (when the disease develops). At the age of 10-12 months NZB mice with an active autoimmune process were isolated as an experimental group.

A special group of NZB mice were intraperitoneally injected with dexamethasone (Sigma) twice

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a week in a dose approximating the physiological (5 μg/mouse). The injections were started from the age of 4 months and were continued till the appearance of antierythrocyte antibodies in the blood. The hormone was dissolved in 50% ethyl alcohol. For injections, dexamethasone solution in alcohol was diluted in sterile normal saline (1:19). An equal volume (0.1 ml) of 2.5% ethyl alcohol in normal saline was injected to control NZB mice.

Antibodies to erythrocytes were detected by Coombs' method [10]. The CS concentration in blood plasma and incubated adrenal specimens was measured by competitive protein binding [2]. Basal production of CS by the adrenals was studied *in vitro* [1], blood plasma CSG was measured by the radioligand method [6]. Results were statistically processed using Student's *t* test.

RESULTS

The antibodies were detected in 2% intact NZB males at the age of 6 months; by the age of 12 months 98% mice had developed autoimmune hemolytic anemia (Table 1).

In NZB mice aged 2-3 and 10-12 months without antibodies to erythrocytes, the blood concentration of CS was significantly higher than in hybrid animals (Table 2). In NZB mice aged 10-12 months with active autoimmune process blood CS level markedly decreased in comparison with 2-3-month-old mice and did not differ from that in (NZB×DBA/2) F_1 mice. A decrease in CS concentration in the blood of NZB mice was not caused by decreased functional activity of the adrenals, because their basal CS production in these animals did not change with age (Table 3). A decrease in adrenal sensitivity to adrenocorticotropic hormone occurring in NZB mice at the age of 6-7 months in NZB mice [6] may be the cause of low CS level.

Glucocorticoids not related to CSG possess immunoregulatory properties [7]. Blood content of CSG did not change in NZB mice without autoantibodies and increased considerably in mice with a developing disease in comparison with 2-3-month-old animals (Table 2). Similar data were obtained in studies on obese chicken with genetically determined autoimmune thyroiditis, in which CS level was two times higher than in normal chicken [8]. Increased blood CSG concentration, which may lead to a decrease in the content of free GC, seems to be characteristic of various autoimmune diseases. High content of active GC may prevent the development of an autoimmune disease.

TABLE 1. Incidence of Autoimmune Hemolytic Anemia in NZB Mice (% of Animals Developing the Disease)

Age, months	% of mice with autoantibodies	After prolonged administration of	
		normal saline	dexamethasone
6	2	18	—
7	11	38	—
8	28	65	9
9	51	65	18
10	81	82	29
11	98	88	47
12	98	88	57

This hypothesis is confirmed by experiments with prolonged administration of dexamethasone, a synthetic hormone not binding to CSG and selectively increasing the pool of free GC. In NZB mice treated with dexamethasone for a long time the antierythrocyte antibodies appeared at the age of 8 months, and by the age of 12 months more than half of the animals fell ill (Table 1). In 18% of control NZB mice injected with normal saline for a long time, antibodies appeared at the age of 6 months, and at the age of 12 months 88% of them developed the disease. It is obvious that the increase in the pool of free GC prevents the development of genetically determined disease. The protective role of a high

TABLE 2. Plasma Content of CS and CSG in Male NZB and (NZB×DBA/2) F_1 Mice ($M \pm m$)

Mouse group	CS, μg/100 ml			CSG, $\times 10^{-7}$ M		
	age, months					
	2-3	6-7	10-12	2-3	6-7	10-12
NZB	12.5±2.1*	7.9±1.1	6.3±1.4* 3.5±0.8**	6.4±0.45^x	6.6±0.5^x	7.0±0.25^x 8.1±0.25**
(NZB×DBA/2) F_1	6.4±1.8	5.0±1.1	3.2±0.4	2.96±0.26	1.77±0.23^+	0.72±0.14^+

Note. *$p<0.05$, ^x$p<0.001$ *vs.* (NZB×DBA/2) F_1 mice of the same age; **$p<0.05$ *vs.* 2-3-month-old NZB mice; ^+$p<0.001$ *vs.* (NZB×DBA/2) F_1 mice of previous age group.

TABLE 3. Basal Production of CS by the Adrenals *In Vitro* in NZB and (NZB×DBA/2) F_1 Mice (μg/100 mg tissue/h, *M±m*)

Mouse group	Age, months		
	2-3	6-7	10-12
NZB	0.42±0.05 (23)	0.79±0.23 (9)	0.89±0.25 (9)
(NZB×DBA/2) F_1	4.8±0.58 (15)*	4.32±0.40 (22)*	2.30±0.22 (18)**

Note. $p<0.01$: *significant differences between animals of the same age, *significant differences from (NZB×DBA/2) F_1 mice aged 6-7 months. The number of animals is given in parentheses.

level of GC in autoimmune en-cephalomyelitis has been demonstrated [11]. It was shown that the disease is easily induced in Lewis rats with pituitary-adrenal hypofunction, in contrast to PVG rats with a high level of CS.

From our findings and published reports it can be concluded that the disease development in autoimmune mice is associated with a decrease in the total blood CS level and an increase in the CSG level, which may decrease the pool of free GC. The dexamethasone-induced increase of the pool of active hormone markedly decelerates the development of autoimmune processes.

The complete clinical picture of the disease was not observed in (NZB×DBA/2) F_1 hybrids [9]. We did not detect antibodies to erythrocytes in these mice during the entire period of observation. However, besides the genetically mediated mechanisms preventing the disease, functional activity of the adrenals may be significant.

The steroid-producing capacity of the adrenals of (NZB×DBA/2) F_1 mice was much higher than that of NZB in all age groups (Table 3). By the age of 10-12 months the adrenal CS production decreased, but it did not affect the hormone content in peripheral blood. Blood concentration of the total CS did not change with age (Table 2) and corresponded to that in male DBA/2 mice [6]. We cannot explain the cause of high adrenal production of CS and of the discrepancy between the hormone production and its blood level. Presumably, the rate of CS metabolism is very high in hybrid animals, but this hypothesis needs experimental verification.

The level of CS in hybrid mice was lower than in NZB mice in all age groups (Table 2), indicating accelerated destruction of CS, because CSG protects the bound hormone from enzymatic oxidation in the liver, prolongs its half-life and decreases its renal excretion [13]. In addition, blood concentration of CSG in (NZB×DBA/2) F_1 mice significantly decreased by the age of 6-7 months and then by 10-12 months (Table 2), as a result, the content of GC in(NZB×DBA/2) F_1 at the age of 10-12 months was much higher than in NZB mice with autoantibodies.

Thus, the functional activity of the adrenals is high in (NZB×DBA/2) F_1 mice which do not develop autoimmune processes. A drop in the CSG level by the age of 10-12 months may appreciably elevate the pool of active hormone. It seems that along with the genetic factors, high level of free GC, which is typical of (NZB×DBA/2) F_1 mice, is a protective mechanism preventing the development of autoimmune reactions.

What is the nature of functional insufficiency of the pituitary-adrenal system in autoimmune animals? Do the shifts in the endocrine system result from pathological processes in immunocompetent cells or these changes develop in parallel and are related to primary genetic disorders? Published data and our findings indicate that the latter hypothesis is more probable. In any case, hormonal changes in the pituitary-adrenal system and decreased regulatory control realized by adrenal hormones favor unlimited activation of immune cells and provoke the development of hereditary autoimmune diseases.

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