MICROBIOLOGY AND IMMUNOLOGY

Patterns of Interaction between the Immune and Endocrine Systems in the Manifestation of Hereditary Autoimmune Disorders in NZB Mice

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Specific features of immune-endocrine interrelationships and of pituitary-adrenal system functioning are studied in NZB mice with hereditary autoimmune pathology. It is established that the development of disease is accompanied by a weakened response to stress, reduced blood corticosteroid level, and decreased adrenal reactivity to exogenous adrenocorticotropic hormone. Moreover, a loss of sensitivity to interleukin-2 is observed. These facts provide evidence of disturbed interaction between the immune and endocrine systems as well as of inhibition of pituitary-adrenal system activity as the autoimmune disease develops.

Key Words: *NZB mouse strain; antierythrocyte antibodies; corticosterone; interleukin-2*

That the immune and endocrine systems function closely together in order ot provide for the organism's homeostasis is beyond question [4]. Key players in this interaction are various hormones [6]. A study of the endocrine regulation of immune processes is of particular importance in the investigation of immunopathological states. Mice of the NZB strain represent one of the most best-studied experimental models of human autoimmune diseases, such as lupus erythematosus and hemolytic anemia. At a certain age these mice begin to produce antibodies to erythrocytes, nucleic acids, and thymocytes [7]. Massive autoantibody production leads to erythrocyte destruction, blood loading with immune complexes, deposition of the latter in the kidneys, and, as a consequence, to the early death of animals [13]. The hormones produced by the adrenal cortex are necessary for the normal functioning of the immune system [7]. In autoimmune pathology multiple glucocorticoid-dependent components of immune system are affected, including disturbance of both lymphokine synthesis and lymphokine receptor expression on the surface of lymphocytes, and alterations in thymic hormone synthesis [12]. All this makes it plausible to assume that in mice of the NZB strain the predisposition to autoimmune disease may have something to do with the functioning of the system responsible for maintaining the optimal glucocorticoid level in the organism. The goal of the present work was to study the functional activity of the pituitary-adrenal system (PAS) and its reactivity to signals from the immune system in NZB mice.

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MATERIALS AND METHODS

The experiments were carried out on male NZB mice with inherited autoimmune disease. Mice of

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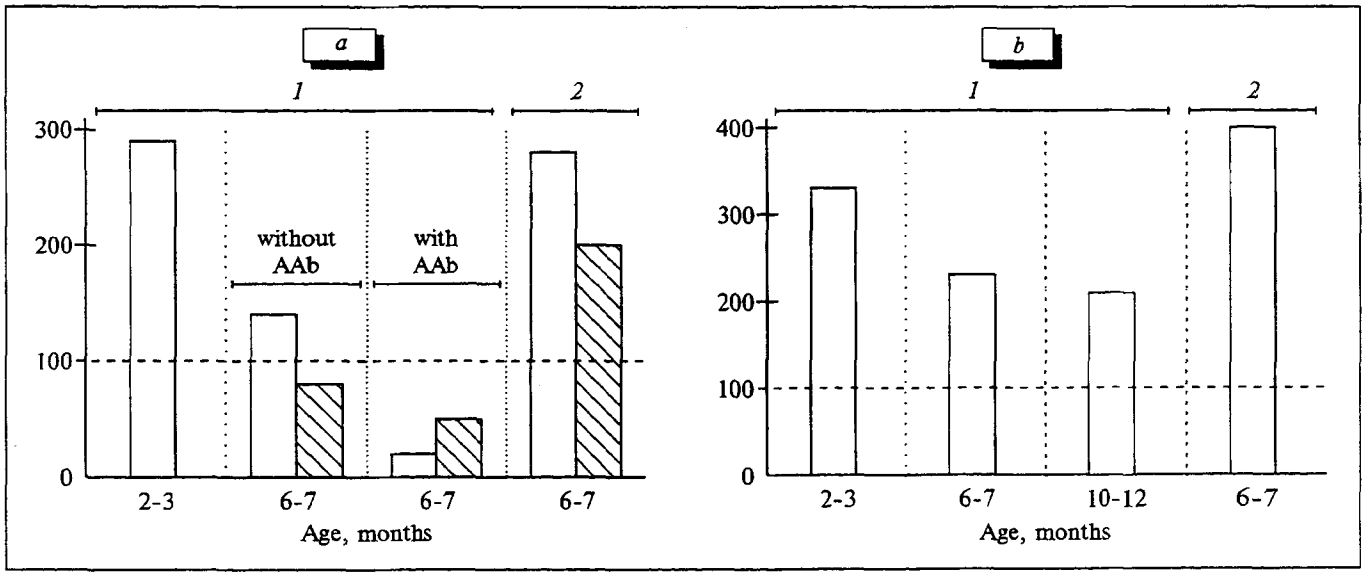

Fig. 1. Age-related changes in PAS responsiveness to IL-2 (*a*) and emotional stress (*b*) in NZB (*1*) and DBA/2 (*2*) mice. Ordinate: increment of blood corticosterone content as compared to the control, %. Plasma corticosterone level of control animals taken as 100%. *a*: the blood hormone level 1 hour after IL-2 injection is represented by white bars, and that 6 hours later by shaded bars.

the DBA/2 strain, which are not prone to autoimmune disorders, served as the control. Three age groups of experimental animals were examined: 2-3-month-old healthy mice, 6-7-month-old mice in the prodromal period, and 10-12-month-old mice with the full-blown pathological process. Animals were sacrificed by decapitation. Antierythrocyte antibody levels were estimated using the Coombs test [10]. The corticosterone concentration in the blood and in incubates of adrenals was evaluated by the method of competitive protein binding in a modification that does not require preliminary extraction of steroids from the plasma [3]. The adrenal response to exogenous adrenocorticotropic hormone (ACTH) was assessed by the method of gland incubation *in vitro* [1]. Emotional stress was induced by restricting animal mobility for half-an-hour [2]. Interleukin-2 (IL-2; a recombinant preparation of human IL-2, Institute of Organic Synthesis, Latvia) was administered intraperitoneally in a dose of 0.2 U per mouse. Control animals received Hanks solution. Mice were sacrificed by decapitation one or six hours following IL-2 injection. Statistical analysis of the results was performed using Student's *t* test.

RESULTS

The onset of the active phase of the disease in NZB mice is associated with the development of antierythrocyte autoantibodies (AAb). AAb were first detected at the age of 6 months, and by the 12th month the disease had developed in practically all animals (Table 1). Our results are in agreement with published data [13]. One of the key aspects in the development of the autoimmune process is a breakdown of the interaction between different elements of the immune system. An important role in disease development is ascribed to IL-2, since the disease is characterized by hyperactivation of the B-cell system, and IL-2 is necessary for the activation, proliferation, and differentiation of B cells [9]. Alterations in both the production of this lymphokine and responsiveness to it are intrinsic to many autoimmune disorders [9]. The sphere of IL-2 action is not restricted to the structures of

Table 1. Dynamics of Appearance of Antierythrocyte Antibodies in Intact Male NZB Mice

Parameter	Age, months								
	4	5	6	7	8	9	10	11	12
Number of animals (total)	129	126	126	120	54	73	82	86	95
Number of animals with AAb	129	125	121	98	25	27	14	2	2
Number of animals without AAb	–	–	–	22	29	46	68	84	93
Percentage of diseased animals	0	0.7	3.9	18.3	53.7	63.1	82.9	97.6	98.9

the immune system. During recent years it has been established that in *in vitro* systems IL-2 stimulates PAS, augmenting hypothalamic secretion of corticotropin-releasing hormone [5] and pituitary release of ACTH [8]. Moreover, IL-2 administration raises the blood level of ACTH and cortisol in patients with cancer and acquired immunodeficiency syndrome [8]. Our experiments revealed fundamental differences in the response to IL-2 in healthy and diseased NZB mice. One hour after IL-2 injection the blood concentration of corticosterone rose dramatically in both control mice (DBA/2) and NZB mice that did not produce autoantibodies (Fig. 1, *a*). However, 6-month-old NZB mice, regardless of AAb production, manifested a reduced PAS response to IL-2 as compared to young animals. The blood corticosterone level fell in mice of all age groups 6 hours after IL-2 injection. In 6-month animals with a developing autoimmune syndrome no response to IL-2 could be detected. These results point to a disturbance of the normal physiological relationship between the immune and endocrine systems that develops in NZB mice towards the time of AAb appearance. It is reported that chickens of the Obese strain suffering from autoimmune thyroiditis fail to express the PAS reaction in response to another transmitter of the immune system, namely IL-1 [11]. In addition, the ease of autoimmune syndrome induction in rats of the Levis strain as compared to other strains is also associated with disturbances of PAS activation during immune response [14].

The next series of experiments examined PAS functional activity during the development of autoimmune disease in NZB mice. The coordination of the functions of different elements of this system can be assessed by the response to stress. We subjected animals to emotional stress by restricting their mobility for half-an-hour. Young NZB mice responded with a considerable rise of the blood corticosterone level. Toward the 6th-7th month the response decreased significantly, while in the control mice of the same age it was quite high. In 10-12-month-old autoimmune mice the response to stress remained low (Fig. 1, *b*). Thus, in NZB mice PAS activity falls off at the time of AAb appearance. Analysis of the age-related dynamics of blood corticosterone showed that in young NZB mice the level of this hormone is extremely high, its absolute content exceeding that in control (DBA/2) mice 5-fold. When AAb appear, the blood level of the hormone drops, but is still reliably higher than in control animals of the same age. Only in old NZB mice with the fully developed autoimmune syndrome does the blood corticosterone level not differ from that in the control (Fig. 2). One of the possible causes of the age-related drop of the corticosterone level in NZB mice is a decline of adrenal sensitivity to ACTH, an adrenotropic hormone. Study of the adrenal response to exogenous ACTH (0.02 U/ml) *in vitro* showed that in the control animals this parameter undergoes no significant age-dependent changes. The adrenals of 6-7-month NZB mice expressed a reduced response to ACTH, while the adrenals of old mice exhibited practically no reaction (Fig. 3). Apparently, the high corticosterone level in young NZB mice indicates an increased adrenal sensitivity to ACTH, and the progressive decline of adrenal sensitivity is responsible for the age-related drop of the corticosterone level.

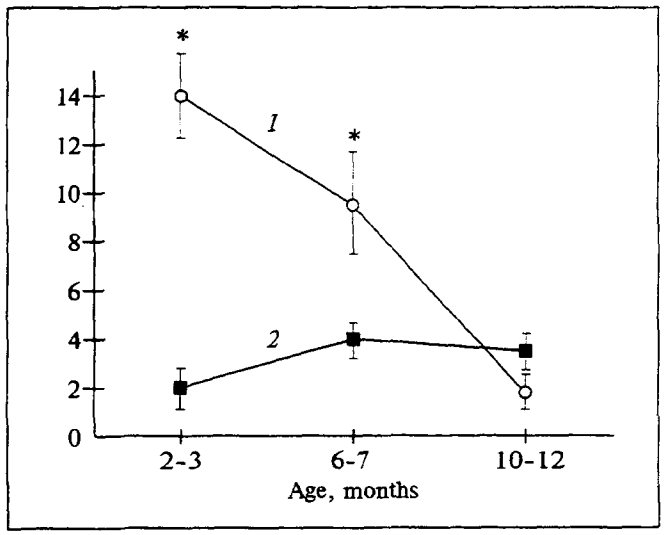

Fig. 2. Age-related dynamics of plasma corticosterone level in NZB (*1*) and DBA/2 (*2*) mice. Ordinate: plasma corticosterone concentration, μg/100 ml. *: significant differences between indexes of NZB and control mice of the same age.

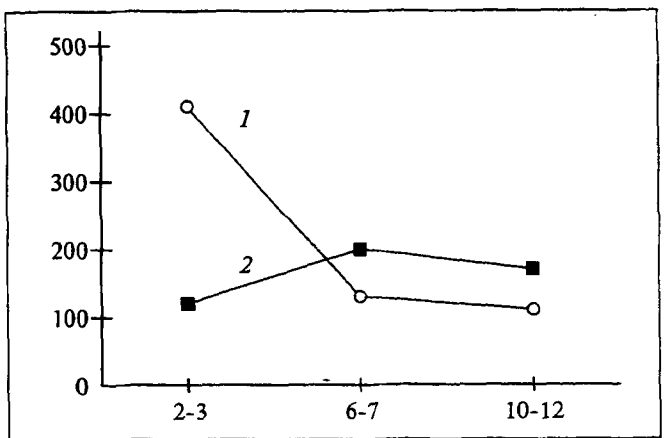

Fig. 3. Age-related changes of adrenal response to ACTH in NZB (*1*) and DBA/2 (*2*) mice. Ordinate: percentage increment of ACTH-induced (0.02 U/ml) corticosterone production as compared to basal level (100%).

Thus, high-level PAS functioning in conjunction with high responsiveness of the system to the signals produced by the immune system in young NZB mice postpones the development of the hereditary autoimmune syndrome up to a certain age. Our results, taken together with the data in the literature, suggest that the breakdown of the interactions between the immune and endocrine systems is an important pathogenetic factor in the development of autoaggression.

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