Pattern of Age Variability of the Autoantibody Levels in (NZB×NZW)F1 Mice

Yu. V. Nesvizhskii, D. V. Korogodin, and A. A. Vorob'ev

UDC 612.017.3:616-008.6

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.117, № 3, pp. 287-289, March, 1994
Original article submitted August 24, 1993

The age variability of the levels of antibodies against structurally different antigens is studied in female (NZB×NZW)F1 mice. It is established that the pubertal period is critical for the number of features studied and the degree of variability of autoantibody levels depends on the nature of the autoantigen.

Key Words: *(NZB×NZW)F1 mice; autoantibodies; age variability*

NZB mice and their first-generation (NZB×NZW) F_1 hybrids are widely known as a natural model of autoimmune pathology. The severity of their genetically determined disease depends characteristically on age, as has been well documented [4,7]. Another noteworthy feature of these animals is the broad spectrum of serum autoantibodies (AA). The levels of the latter, as well as other stigmata of the autoimmune process, manifest a definite age variability, especially in females. For example, the concentration of AA against DNA, collagen, and some other autoantigens increases markedly in the pubertal period, which correlates with the onset and further progress of an autoimmune disorder [1,2].

The phenomenon described is important for an understanding of the mechanism of development of autoimmune pathology in experimental animals. However, it is a moot point whether there is any regular pattern in the nature of age variability in the levels of AA against structurally different antigens of the organism proper in New Zealand mice. The present article is devoted to this topic.

MATERIALS AND METHODS

Serum from 57 female (NZB×NZW)F1 mice of three age groups (1, 5, and 9 months) was used for the study. The animals were obtained from the vivarium of the institute of Rheumatology, Russian Academy of Medical Sciences. Blood sampling was performed by puncture of the retroorbital sinus.

Antibodies were determined by an immunoenzymatic method [5] with the use of horseradish peroxidase-labeled staphylococcus A protein (Sigma, USA). The antibody level was measured in optical density units as related to indexes of reference serum.

The autoantigens used were nDNA, bovine thyroglobulin (Sigma, USA), chondroitin sulfate (Kochlight, Great Britain), and human myoglobin (made available by G. M. Rott, Moscow); mouse collagen type I was isolated from the skin of female (NZB× ×NZW)F1 mice by enzymolysis [6], followed by chromatographic purification of the preparation [8].

The data were statistically processed with the use of variational, correlation, and variance analysis [3].

RESULTS

Antibodies against the model antigens used in the study were found in the serum of all experimental animals. The distribution of individual values of the levels of AA against protein antigens approximates a normal curve, whereas AA against nDNA and chondroitin sulfate were characterized by an abnormal excess. The variational characteristics of the results obtained and other calculated indexes are listed in Table 1.

I. M. Sechenov Medical Academy, Moscow

0007-4888/94/0003-0290$12.50

The comparison of age groups (Fig.1) demonstrated a marked rise of the levels of parameters toward the 5th month ($p<0.01$), as is characteristic for female (NZB×NZW)F1 mice and it coincided with the manifestation of a lupus-like disease. The increase of the mean values of the features as a function of the nature of the antigen was 32-98% (1.58±0.15% on average). A remarkable stabilization of the rise of AA levels was noted aging, there being practically no difference ($p<0.05$) between the groups of 5- and 9-month-old animals.

The analysis of variance revealed a high significance of age in the variability of the levels ($p<0.01$). Susceptibility to this physiological factor attained quite high values (72.2±3.0%) and varied from 68.2 to 82.3% (Table 1). The correlation analysis also confirmed the positive correlation (0.746±0.030) between the levels of AA studied and the age.

As can be seen from the above data, individual peculiarities in the variability of AA levels in (NZB×NZW)F1 mice are clearly traced along with the general trend. The divergent factor may well be the nature of the autoantigen.

We explored this hypothesis. For convenience of analysis the data obtained were weighted according to the corresponding sampling means. A reliable difference between the type of distribution of AA levels against chondroitin sulfate and that for collagen, thyroglobulin, and myoglobin was found by the χ^2 method. The analysis of variance showed that the susceptibility of the variational characteristics studied to the nature of the autoantigen in 1-month-old animals is 17.6% ($p<0.01$) and in 5-month-old animals 27.5% ($p<0.01$). But the AA levels, as well as their age dynamics, did not correlate with the molecular weight of the autoantigens used ($r=0.101$ and $r=0.030$, respectively, $p>0.05$).

On the other hand, autoantigens may be logically categorized as substances of "protein" and "nonprotein" nature. In this case the most pronounced effect is found in 5-month-old mice. Thus, AA against nonprotein biopolymers differed markedly from AA against proteins according to the populational dispersion of their amount ($p<0.05$). The rise of the studied levels in aging

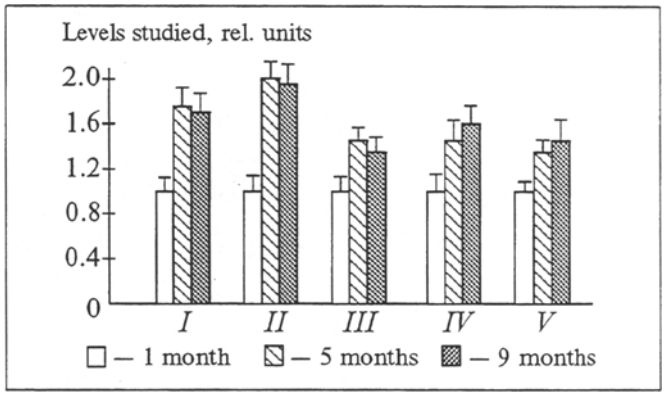

Fig. 1. Age dynamics of AA levels against different autoantigens in experimental animals. *1*) AA against nDNA; *2*) against chondroitin sulfate; *3*) collagen; *4*) thyroglobulin; *5*) myoglobin.

was also most pronounced in the case of AA against nonprotein biopolymers, namely chondroitin sulfate and nDNA (1.95- and 1.69-fold, respectively). The significance of the autoantigen nature for interindividual differences in AA levels attained 14.8% for nonpubertal animals ($p<0.01$) and 24.5% for pubertal mice ($p<0.01$).

During the study, it seemed of interest to clarify the nature of the relationship between age and autoantigen nature when determining the variability of AA levels in the experimental animals. For this purpose we drew on two-factor analysis of variance. The effects of both identified factors - the individual and cooperative effect of the animal's age and the autoantigen nature - and nonidentified factors were determined. (The specification of the latter lay outside the scope of the present study.) In the first experimental variant individual peculiarities of antigens were taken into account. In the second variant, antigens were divided into protein and nonprotein substances (see above).

It was established (Fig. 2) that the effect of the identified factors in both cases accounts for nearly 3/4 of the entire phenotypic variance of the values. In the first variant the population existed in cooperative form only (Fig. 2, *a*). In the second variant the phenomenon noted was the opposite: the factorial component was represented only by the isolated effect of age and antigen "nature."

TABLE 1. AA Levels in Female (NZB×NZW)F1 Mice

Autoantigen	$X\pm m$	Asymmetry	Excess	Age variability, %	Coefficient of age correlation
nDNA	0.69±0.02	−0.16	−1.06	69.6	0.693
Chondroitin sulfate	0.68±0.02	−0.08	−1.32	82.7	0.781
Collagen	0.60±0.01	−0.03	−0.71	68.2	0.671
Thyroglobulin	0.64±0.02	0.16	0.84	71.4	0.807
Myoglobin	0.70±0.01	0.11	−0.20	69.1	0.777

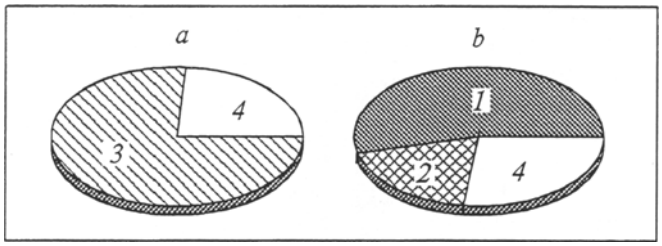

Fig. 2. Components of two-factor models of age variability: *a*) individual features of autoantigens; *b*) protein and nonprotein autoantigens. Factors: *1*) age; *2*) autoantigen nature; *3*) cooperative effect of age and autoantigen nature; *4*) nonidentified.

The contribution of age variability was 2.8-fold higher ($p<0.01$) than its alternative (Fig. 2, *b*).

Thus, the study confirmed the previously known fact of a peculiar ontogenesis of AA levels in female (NZB×NZW)F1 mice. It should be taken into account that the model antigens used in the study encompass the main types of antigen substances of the human organism. Therefore, it may be assumed that the features of variability of the levels of the test AA described in the study establish a pattern, and that the pubertal period in female (NZB× ×NZW)F1 mice is critical for the levels of all detected AA. The latter is significant for a practical study of autoimmunity in the given animals, as it restricts the investigation to narrow age limits. In view of the pattern described it is difficult to assume any difference in the fundamental development of immunoreactivity to structurally different antigens of the organism proper. Nevertheless, the nature of the autoantigen proved to be a rather powerful factor which can significantly affect the degree of variability of the studied immunological parameters, particularly in pubertal animals. That's why it is good practice to take into account the autoantigen molecular structure when designing experimental studies of the autoimmunity phenomenon.

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Effect of Thymosin Fractions on the Development of Toxic Swelling Edema of the Brain

I. A. Platonov and V. V. Yasnetsov

UDC 616.831-005.98-099-092.9-02:615.361.438]-07

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 3, pp. 290-291, March, 1994
Original article submitted December 29, 1993

Rat experiments have shown that thymosin fractions V and VI elicit an antiedemic effect by normalizing predominantly the density of brain tissues, the effect being independent of the preparation dose. It is demonstrated that a high dose of fraction VII has a pronounced antiedemic effect.

Key Words: *brain edema; thymosin fractions*

The problem of swelling edema of the brain (SEB) still awaits its solution [5,9,11]. The immune system may be implicated in this process [4,11]. At the present time it is recognized that the central nervous and immune systems communicate with each other, and various peptide hormones, for example those derived from the thy-

Scientific Industrial Center Hydrobios, Ministry of Public Health, Moscow. (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences) a

0007-4888/94/0003-0292$12.50