CUTANEOUS ALLERGIC SENSITIVITY IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

Yu. L. Zhitnukhin

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Guinea pigs of different groups were sensitized with myelin of homologous or heterologous (rabbit, bovine) spinal cord or with frog spinal cord homogenate together with Freund's complete adjuvant. The cutaneous reactions of delayed type to saline extracts of myelin isolated from homologous or heterologous spinal cord were studied 3, 5, 7, 10, and 14 days after sensitization. The skin tests began to be positive on the 5th day after sensitization. A regular decrease in the frequency of the skin reactions of the heterologous spinal cord compared with antigens from the homologous spinal cord of guinea pigs sensitized with myelin from the homologous cord was observed. The opposite relationship occurred in animals sensitized with myelin from heterologous spinal cord or with homogenate of frog spinal cord. No correlation was found between the frequency of the skin reactions to antigens of heterologous spinal cord and the incidence of disease among the animals; the latter correlated with the level of their sensitization to antigens of homologous spinal cord. Skin reactions to antigens of homologous spinal cord were of prognostic value in the inductive period as regards the subsequent development of experimental allergic encephalomyelitis. It is concluded that allergic mechanisms are concerned in the development of the pathological process.

Cutaneous allergic tests in demyelinating diseases of the nervous system can be used to investigate the level of sensitization of the affected organism.

Experiments on animals sensitized by various encephalitogenic preparations have shown that skin reactions can be obtained to antigens of both heterologous [6, 8, 12] and homologous [2, 5, 7, 11, 13] brain and also to antigens prepared from the brain at the height of experimental allergic encephalomyelitis (EAE) [1].

The object of the present investigation was to detect sensitization of guinea pigs to antigens of the myelin of the CNS obtained from animals of different species, to study the dynamics of development of cutaneous hypersensitivity, and to assess its prognostic value in relation to the disease in animals sensitized with encephalitogenic mixtures of varied composition.

EXPERIMENTAL METHOD

EAE was induced in noninbred adult guinea pigs (weighing 300-350 g) by inoculation with myelin from homologous spinal cord or from rabbit or bovine spinal cord mixed with Freund's complete adjuvant. The methods of preparation of the antigens and the encephalitogenic mixtures, of immunization of the animals, and of evaluation of the clinical manifestations of EAE were described earlier [4]. The antigens used for the skin tests were 1% saline extract of myelin isolated from the brain by differential centrifugation and centrifugation in a sucrose density gradient [3]. The reactions were assessed 24 h later by the degree of intensity of the erythema and the size of the area of induration.

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TABLE 1. Allergic Skin Reactions of Delayed Type in Guinea Pigs Sensitized with Myelin from Homologous Spinal Cord

Antigen for skin tests	Frequency of skin reactions (days after sensitization)					Total from 5th to 14 days
	3-rd	5-th	7-th	10-th	14-th	
Myelin of homologous spinal cord	0/4	8/11	7/10	3/9	2/7	20/37 (54%)
Myelin of heterologous spinal cord	0/4	0/8	4/6	2/6	1/6	7/26 (26,9%)
Extract of frog spinal cord	0/4	0/4	2/4	0/4	0/4	2/16

Note. Here and in Tables 2-4, the denominator gives the number of guinea pigs tested, and the numerator gives the number of guinea pigs with positive skin reactions.

TABLE 2. Allergic Skin Reactions of Delayed Type in Guinea Pigs Sensitized with Myelin from Heterologous Mammalian Brain

Antigen for skin tests	Frequency of skin reactions (days after sensitization)					Total from 5th to 14 days
	3-rd	5-th	7-th	10-th	14-th	
Myelin of heterologous mammalian brain	0/3	3/4	3/3	7/7	4/7	17/21 (81%)
Myelin of homologous brain	0/3	3/4	2/3	3/7	1/7	9/21 (43%)

TABLE 3. Frequency of Allergic Skin Reaction of Delayed Type in Guinea Pigs Sensitized with Various CNS Antigens

Antigen for skin tests	Sensitization with		
	myelin of homologous spinal cord	myelin of heterologous spinal cord	frog's spinal cord
Myelin of homologous spinal cord	20/37 (54%)	9/21 (43%)	2/15 (13,3%)
Myelin of heterologous spinal cord	7/26 (26,9%)	17/21 (81%)	Not tested
Frog's spinal cord	2/16 (12,5%)	Not tested	15/15 (100%)

EXPERIMENTAL RESULTS

In guinea pigs inoculated with myelin from homologous spinal cord (Table 1) skin reactions to this preparation were found on the 5th day in 8 of the 11 animals, whereas heterologous myelin and frog spinal cord did not induce this effect. The largest number of skin reactions to all test antigens used was observed on the 7th day after the beginning of the experiment.

On the 10th day the frequency of skin reactions to homologous and heterologous myelin fell, whereas the tests with frog spinal cord were negative.

On the 14th day some of the guinea pigs still continued to react to intradermal injections of myelin from homologous and heterologous spinal cord.

The results of analogous experiments on guinea pigs sensitized with encephalitogenic mixtures containing myelin of heterologous mammalian brain are given in Table 2. Skin reactions were noted in these animals not only with heterologous myelin used for sensitization, but also with myelin from guinea pig spinal cord.

It must be noted (these results are not included in Table 2) that inoculation of the guinea pigs with frog spinal cord plus adjuvant did not induce EAE, but it led to the development of skin reactions in all 15 animals tested to this material between the 5th and 14th days after sensitization.

Table 3 contains the combined data for the incidence of skin reactions in guinea pigs sensitized with CNS antigens from animals of different species.

TABLE 2. Comparison of Frequency and Times of Development of EAE with Results of Skin Tests with Myelin of Homologous and Heterologous Spinal Cord

Antigen for skin test	Days of performing skin tests	With positive skin tests			With negative skin tests		
		number of animals	number developing disease	mean time of onset of disease (days)	Number of animals	number developing disease	mean time of onset of disease (days)
Myelin of homologous spinal cord	5—7	20	19	14	8	2	19,5
	10—14	8	8	18,4	12	4	54,4
	Total	28	27	15,3	20	6	44,4
Myelin of heterologous spinal cord	5—7	11	9	14,5	10	9	15,7
	10—14	13	10	29,3	9	5	30,6
	Total	24	19	22,3	19	14	22,6

Note. Results of skin tests in animals with clinical features of EAE on the day of testing are not considered. Such guinea pigs did not react to intradermal injection of myelin.

In guinea pigs sensitized with myelin of the homologous spinal cord there was a regular decrease in frequency of the reactions to myelin from the heterologous cord and to antigens from the frog spinal cord (54, 26.9, and 12.5%). The corresponding figures for animals receiving injections of heterologous spinal cord myelin behaved in the opposite way (43.81%).

The development of skin reactions to myelin from the spinal cord of animals of different species can with advantage be compared with the frequency and times of onset of the disease (Table 4).

The high incidence of the disease among guinea pigs reacting to intradermal injection of myelin from homologous spinal cord (27 of 28 cases) and which developed clinical signs of EAE early (mean time of onset of the disease 15.3 days) will be noted, whereas of the 20 guinea pigs with negative skin tests with this preparation only 6 subsequently developed the disease, clinical manifestations of EAE first appearing on the average 44.4 days after sensitization, or more than 1 month after the skin test.

A relatively high percentage of animals developing the disease also was observed in the group of guinea pigs which developed cutaneous hypersensitivity to myelin preparations from heterologous mammalian spinal cord (19 of 24 animals, 79%). Meanwhile, in most animals not reacting to intradermal injection of these antigenic preparations (14 of 19; 73.7%), clinical signs of EAE also appeared; the mean period of onset of the disease was the same as in the previous group (22.3 and 22.5 days, respectively).

The frequency of local allergic reactions to myelin of the homologous spinal cord thus dependend on the species of animal from which the myelin used for inoculation was obtained. Skin reactions were observed most frequently after sensitization with myelin from the homologous spinal cord; they were less frequent if myelin from rabbit or bovine spinal cord were used as the encephalitogenic antigens. Although the greatest number of skin reactions after sensitization with myelin from the heterologous spinal cord was observed after intradermal injection of the same antigen, the level of sensitization to the heterologous antigens can hardly be regarded as reflecting the state of the pathogenic mechanisms responsible for the CNS damage. Lesions affecting structures of the CNS itself are implied in this respect; it will therefore be clear that the level of sensitization to homologous spinal cord is of prime importance. Inoculation with heterologous CNS antigens evidently facilitates the development of sensitization to the nonencephalitogenic components of the mixture. This conclusion is supported by the fact that injection of a mixture of frog's spinal cord with an adjuvant possessing no encephalitogenic properties into guinea pigs sensitized the animals at a certain period of time to saline extracts of frog spinal cord tissue. Comparison of the development of

EAE with the results of skin tests with heterologous spinal cord antigens showed that the incidence of the disease among the animals was sufficiently high irrespective of their activity to the test antigens. On the other hand, the incidence among animals inoculated with encephalitogenic mixtures containing myelin of both homologous and heterologous spinal cord correlated with the level of sinsitization of these animals to homologous myelin. The prognostic importance of the skin tests is noteworthy. Positive tests at times when the animals were in the inductive period predicted the development of clinical features of EAE in almost 100% of animals, whereas if the tests were negative, only 35% of the animals (6 of 20) developed the disease (at much later periods).

The observations described above are evidently support for the role of the allergic component (or factor) and, in particular, of the development of delayed hypersensitivity to homologous spinal cord antigens, in the development of the pathological process.

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