IMMUNOLUMINESCENCE STUDY OF THE SERA OF GUINEA PIGS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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The sera of guinea pigs with experimental allergic encephalomyelitis developed after intradermal injections of encephalitogenic mixtures of different composition were investigated by the indirect Coons' method. Antibodies capable of fixation in various brain structures (at the periphery of nerve fibers, in the cytoplasm of nerve cells) were detected in some sera. The results suggest antigenic differences between identical structures in the brain and spinal cord (nerve fibers), between different structures (nerve fibers and cells) in the same part of the central nervous system, and between identical structures (nerve cells) in animals of different species.

Complement-fixing antibodies have been found only rarely, and in low titers, in guinea pigs with experimental allergic encephalomyelitis (EAE) produced by inoculation with homologous brain homogenates together with Freund's adjuvant [1-4].

To demonstrate the presence of serological changes and to determine their characteristics in EAE, an investigation was carried out by means of an immunoluminescence method [5].

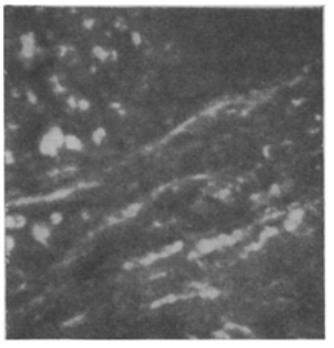

Fig. 1

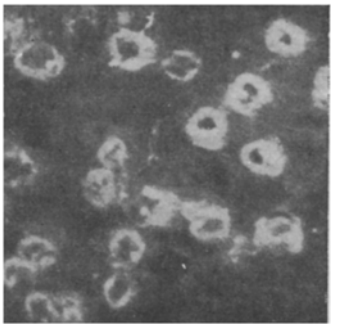

Fig. 2

Fig. 1. Specific fluorescence of fibers in section through spinal cord of a normal guinea pig after exposure with the serum of a guinea pig sensitized with homologous spinal cord (476 ×).

Fig. 2. Specific fluorescence of cytoplasm of nerve cells in section through the spinal cord of a normal guinea pig after exposure with the serum of a guinea pig sensitized with homologous spinal cord (476 ×).

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TABLE 1. Results of Investigation of Specific Fluorescence at the Periphery of Nerve Fibers in Sections Induced by Sera of Sensitized Guinea Pigs

Material for sensitization	Fluorescence of nerve fibers			
	in spinal cord sections of		in brain sections of	
	guinea pig	rat	guinea pig	rat
Guinea pig spinal cord	16/104	9/45	0/103	Not tested
Rat spinal cord	22/50	17/51	Not tested	0/50

Note: in Tables 1-4 the total number of sera investigated is shown as the denominator and the number of sera inducing specific fluorescence as the numerator.

TABLE 2. Comparative Numbers of Positive Sera on 3rd-7th Day after Inoculation of Guinea Pigs with Homologous or Heterologous Spinal Cord

Material for sensitization	Number of sera inducing specific fluorescence of nerve fibers in spinal cord sections of	
	guinea pig	rat
Homologous spinal cord	10/55 (18%)	14/22 (64%)
Rat spinal cord	1/22 (4.5%)	7/22 (32%)

EXPERIMENTAL METHOD

Experiments were carried out on adult noninbred guinea pigs of both sexes, divided into 3 groups. The animals were sensitized with encephalitogenic mixtures consisting of tissue homogenates of homologous spinal cord, and the spinal cord or brain of Wistar rats, emulsified in Freund's complete adjuvant, respectively. Blood for testing was taken at various times after sensitization (from the 3rd to the 21st day) by cardiac puncture; the sera were frozen in a mixture of dry ice and petroleum ether and stored at −20°C. Antibodies were detected by the indirect Coons' method, using a fluorescent rabbit antiserum against guinea pig γ-globulin, conjugated with fluorescein isothiocyanate. The fluorescent serum was diluted with 0.85% NaCl solution 16 times. The test sera (in a dilution of 1:3) were applied for 30 min to sections of the brain and spinal cord of normal guinea pigs and rats, prepared in a cryostat at between −15 and −20°C and fixed with 96° ethanol after preliminary washing with physiological saline and drying. The intermediate serum for the control sections was normal guinea pig serum. The unfixed serum was rinsed off with physiological saline, the sections were dried, and the fluorescent serum was applied to them. Incubation continued for 20 min in a humid chamber at room temperature. After rinsing, the sections were dried and examined in blue light (filters FS-1 and ZhS-18) by the ML-2 luminescence microscope with water immersion (magnification 40×; aperture 0.75). The luminescent sections were photographed under the same conditions (ocular Homal 1.7×) on RF-3 film. Specific luminescence at the periphery of the nerve fibers and diffuse fluorescence of the cytoplasm of the nerve cells were analyzed.

EXPERIMENTAL RESULTS

Altogether 114 sera from 29 guinea pigs inoculated with homologous spinal cord, 51 sera from 11 guinea pigs receiving rat spinal cord, and 48 sera from 10 guinea pigs receiving rat brain homogenate were investigated. As Table 1 shows, a few sera of guinea pigs inoculated with homologous spinal cord (15-20%) were fixed to the sections of guinea pigs and rat spinal cord and produced fluorescence at the periphery of the nerve fibers (Fig. 1). These sera were found appreciably more often (33-44%) in animals inoculated with heterologous (rat) spinal cord.

The differences observed were due principally to the large number of "positive" sera from animals within the group on the early days after inoculation (Table 2), but none of the sera of the two groups produced fluorescence of the nerve fibers in the brain sections.

TABLE 3. Number of Guinea Pig Sera Inducing Specific Cytoplasmic Fluorescence of Nerve Cells after Inoculation of Spinal Cord

Material for sensitization	Number of sera inducing fluorescence of nerve cells in sections through			
	spinal cord		brain	
	of guinea pig	of rat	of guinea pig	of rat
Homologous spinal cord .	28/104	12/45	32/103	Not tested
Rat spinal cord	36/51	31/51	Not tested	32/50

TABLE 4. Number of Guinea Pig Sera Inducing Specific Fluorescence in Brain Sections after Inoculation of Rat Brain

Character of fluorescence	Brain sections	
	of guinea pig	of rat
Fluorescence of nerve fibers	18/48	31/48
Fluorescence of cytoplasm of nerve cells	8/48	28/48

Results showing the specificity of fluorescence of the cytoplasm of the nerve cells are given in Table 3. They show that fluorescence of this type was observed after treatment of the spinal cord sections of both groups of animals (Fig. 2), and the number of positive sera was somewhat greater than in the previous experiments. In addition, many sera induced fluorescence of the cells in the brain sections.

With these results in mind the next step was to investigate the serum of animals of group 3 inoculated with rat brain homogenates. The corresponding results are given in Table 4. Clearly many of the sera induced specific fluorescence both of the cytoplasm of the nerve cells and at the periphery of the nerve fibers.

The material presented in this paper is insufficient to allow conclusions to be drawn regarding the role of the antibodies discovered in the development of the immunopathological process. However, it merits a more detailed study, for it was virtually impossible by means of the ordinary serological methods to detect antibodies against nerve tissue in EAE produced by injections of homologous brain antigens into guinea pigs. Meanwhile the results of this investigation point to possible antigenic differences: 1) between identical structures in the brain and spinal cord (nerve fibers), 2) between different structures (nerve fibers and cells) in the same part of the central nervous system, and 3) between identical structures (nerve cells) in animals of different species.

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