# REACTIVITY OF NEUROGLIA IN GUINEA PIGS SENSITIZED BY Mycobacterium tuberculosis

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In guinea pigs with experimental allergic encephalomyelitis (EAE) induced by sensitization with Mycobacterium tuberculosis cells mixed with petrolatum and Arlacel the reactivity of the glia was shown to change regularly depending on the stage and severity of the pathological process. Changes in reactivity determined by inhibition of glial migration in tissue culture were specific with respect to antigens from nerve tissue. The study of the mechanism of this phenomenon showed that similar changes in reactivity can be induced in a culture of glial cells from intact animals if blood serum or regional lymph gland cells taken from actively sensitized guinea pigs are added. It cannot yet be concluded from the available results that changes in reactivity of the glia to products of brain tissue destruction in guinea pigs with EAE are completely attributable to the action of humoral or cellular elements which penetrate into the CNS from the bloodstream or lymphatic tissue.

KEY WORDS: experimental tuberculus allergic encephalomyelitis; sensitization of the glia; brain antigen.

In the study of experimental allergic encephalomyelitis (EAE) little attention has so far been paid to the role of the neuroglia and, in particular, the oligodendrocytes, in its pathogenesis, although some workers have described morphological and functional changes in these structures preceding demyelinization, on the severity and extent of which the neurological symptoms depend [3, 4].

In the investigation described below the reactivity of the neuroglia to brain antigen was studied in the course of development of EAE induced by sensitization of guinea pigs by Mycobacterium tuberculosis in an adjuvant, and the effect of serum and cellular factors on the reactivity of the glia was examined.

## EXPERIMENTAL METHOD

Experiments were carried out on 94 guinea pigs weighing 300 ± 50 g, 80 of which were sensitized by injection of an autoclave-killed BCG vaccine in an oily mixture (petrolatum and Arlacel A in the ratio 8.5: 1.5) into the plantar pad in a dose of 0.5 mg of dried bacterial cells per animal. The control group consisted of 14 intact animals.

The reactivity of the glial cells (experiments of series I) isolated from the brain stem and containing chiefly oligodendrocytes was determined by the method described by the writers previously [1, 2], based on the change in their migration from capillary tubes into nutrient medium containing heterologous bovine brain and kidney antigens, used to exclude crossed reactions taking place on account of connective-tissue and vascular elements present in the homologous tissues. The antigens consisted of 20% saline extracts prepared by repeated freezing and thawing and containing 4.8-6.4 mg protein/ml. A 0.1% concentration of antigen was used (0.05 ml of 20% antigens to 10 ml medium).

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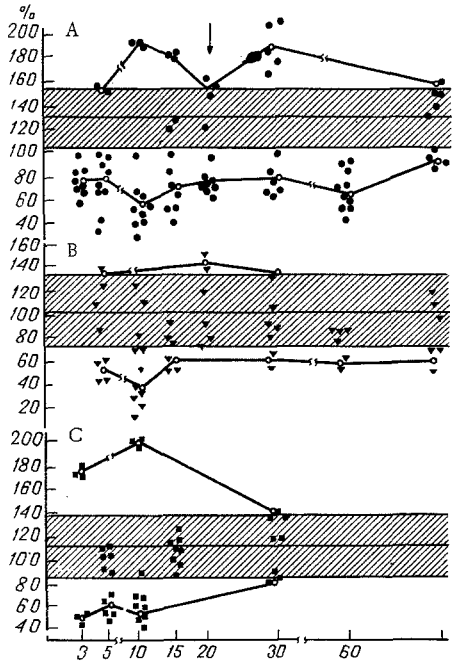


Fig. 1. Changes in migration activity of neuroglia: A) migration activity of neuroglia of guinea pigs actively sensitized by M. tuberculosis cells on the addition of 0.1% bovine brain antigen; B) migration activity of normal glia in the presence of a combination of 0.1% complement, 0.1% bovine brain antigen, and 5% serum from guinea pigs sensitized with M. tuberculosis cells; C) migration activity of normal glia in the presence of a combination of 0.1% bovine brain antigen and 1 million lymphocytes from regional lymph glands of guinea pigs sensitized by M. tuberculosis cells. Horizontal line and shaded zone represent control with confidence limits. Arrow points to mean time of appearance of clinical features of EAE. Abscissa, days after active sensitization of guinea pigs or day of taking sera or lymph glands after active sensitization with M. tuberculosis; ordinate, percentage of migration. Each of two curves in A, B, and C shows deviation of arithmetic mean values of inhibition and stimulation of migration from confidence limits ($I_{95}$).

In the experiments of series II to study the role of humoral factors in EAE, intact glia was incubated in a nutrient medium containing 0.1% bovine brain antigen, 5% heated serum, and 0.1% dry guinea pig complement (0.5 ml serum and 0.2 ml complement, diluted 1:20, to 9.5 ml medium).

In the experiments of series III to determine the effect of cellular factors on the reactivity of the neuroglia, viable cells from the regional, inguinal, and popliteal lymph glands of sensitized and normal guinea pigs, in a concentration of 1 million cells/ml, were incubated with intact neuroglia in medium containing 0.1% bovine brain antigen.

The nutrient medium was Eagle's medium with twice the concentration of glutamine and with the addition of 399 mg glucose to each 100 ml of medium. In the three series of experiments the cells were cultivated under sterile conditions at 37°C for 48 h.

Changes in the reactivity of the glial tissue were judged from the deviation of the migration indices for each animal of the experimental group from the confidence limits in the control. The migration indices were calculated for the experiments of series I by the equation

$$\text{Migration index (in \%)} = \frac{\text{Migration in medium with antigen}}{\text{Migration in medium without antigen}} \times 100.$$

For the experiments of series II and III the migration indices were determined similarly, the migration of the glia in one nutrient medium being taken as 100%.

## EXPERIMENTAL RESULTS

The results of the three series of experiments are illustrated in Fig. 1. The control in series I was the reactivity of the neuroglial cells of normal guinea pigs under the influence of a 0.1% concentration of bovine brain antigen in the nutrient medium (Fig. 1A). The arithmetic mean of the migration indices of the control experiments was 133 ± 13%. The animals of the experimental group were killed by decapitation in the early periods of sensitization and during development of the clinical picture of EAE in the course of 100 days of observation. The features of EAE developed on the 20th-30th day after sensitization, in the form of general sluggishness, atony of the hind limb muscles, and disturbances of movement coordination to a varied degree. In 14% of guinea pigs developing the disease pareses of the hind limb muscles were observed.

In the histological study of sections taken from different levels of the brain and spinal cord single perivascular foci of mononuclear infiltration were observed chiefly in the pia mater and the white matter of the brain. At all periods of observation the migration activity of the glia of the sensitized guinea pigs differed sharply from the control. This was expressed as inhibition or stimulation of cell migration. If the greater deviation of reactivity of the neuroglia (inhibition of migration) was taken as the guide, the following rule was observed. In most animals (83-100%) sharp inhibition of cell migration was observed on the 3rd-10th day after sensitization under the influence of brain antigen, before the appearance of clinical manifestations of EAE (deviation of the migration indices from the lower confidence limit for the control by 47.7-60%). At the end of the incubation period and also during the first two weeks after development of symptoms of EAE, a relative decrease in the number of animals (to 55.5-66.7%) in which migration of the glia was inhibited in the presence of brain antigen was observed. Migration of the glia of animals with pareses or with severe disturbances of coordination of movements, on the other hand, was constantly inhibited by brain antigen in 100% of cases (eight guinea pigs on the 60th day of sensitization) with deviation of migration indices from the control by 41.4%. Toward the end of the period of observation (100 days) the reactivity of the cells was gradually restored against the background of diminution of the clinical manifestations of EAE. In parallel tests for specificity, in which 0.1% bovine kidney antigen was used, slight inhibition was observed only on the 3rd day, evidently on account of a nonspecific increase in the functional activity of the glia. At later periods of sensitization no significant differences from the response of cells of normal animals could be detected.

In the experiments of series II (Fig. 1B) migration of the intact glia in the control experiments in the presence of heated sera of normal guinea pigs, complement, and bovine brain antigen was almost indistinguishable from its migration in nutrient medium without the addition of these substances. The arithmetic mean of the migration indices was 105 ± 14.8%. Starting from the 5th day of sensitization, blood serum of sensitized animals inhibited migration of the glia under the same conditions. In 50% of animals deviation of the migration indices from the lower confidence limit was 23.6%. Maximal inhibition was found in 70% of animals on the 10th day after injection of the adjuvant mixture into the animals. At the end of the incubation period and during development of the clinical picture the inhibitory action of the combination on normal glia was reduced.

It must be pointed out that heated sera, in the absence of complement and of antigen in the medium, had no such action on migration of the glia.

In the experiments of series III (Fig. 1C) migration of the glial cells in the control experiments in the presence of normal lymphocytes and antigen showed a small stimulating effect (arithmetic mean of the migration indices 109 ± 13.5%). Sensitized lymphocytes of 40% of the decapitated animals on the 3rd day of observation induced inhibition of migration with deviation of the migration indices from the lower confidence limit for the control experiments by 35%, whereas lymphocytes from 60% of animals induced stimulation with deviation of the migration indices from the upper confidence limit on the average by 40%. Toward the 10th day of sensitization lymphocytes of 65% of guinea pigs induced marked inhibition of glial migration. In the later periods of observation the ability of the senitized lymphocytes to inhibit migration of the glia whether in combination with brain antigen or without it was lost.

These results are evidence that one probable mechanism of the change thus demonstrated in the reactivity of the glia during the development of EAE could be its passive sensitization by humoral and cellular factors; the possibility of active sensitization of the glial tissue itself to products of nerve tissue destruction likewise cannot be ruled out.

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