PASSIVE TRANSMISSION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS BY SERUM ANTIBRAIN ANTIBODIES

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Intravenous injection of γ-globulin obtained from the serum of dogs in the preclinical period of experimental allergic encephalomyelitis into guinea pigs, followed by thermal coagulation of an area of the brain, leads to the development of fibrinoid necrosis of the vascular walls, perivascular and diffuse infiltration of a large zone of brain around the focus of necrosis, and demyelination. These changes develop 24 h after coagulation. γ-Globulin from intact dogs and from a dog with symptoms of encephalomyelitis did not cause the development of an inflammatory reaction.

KEY WORDS: experimental allergic encephalomyelitis; antibrain antibodies.

Experimental allergic encephalomyelitis (EAE) is an autoimmune process in whose development a leading role is played by hypersensitivity of delayed type [8].

The many unsuccessful attempts at passive transmission of EAE by the serum of animals with the disease, combined with the successful transmission by sensitized lymphocytes [8], constitute a powerful argument for the rejection of a role of humoral factors in the genesis of EAE. On the other hand, the cytotoxic action of the serum of animals with EAE on cultures of nerve tissue [5] suggest that it is theoretically possible to obtain passive transfer and that the attempt to find conditions for such transfer is justified. According to some authorities [1], both humoral and cellular factors take part in the development of allergic alteration of the brain. Analysis of the abundant data in the literature suggests that the chief difficulties in the way of passive transfer are connected with an inadequate concentration of antibrain antibodies in the recipient's blood and also with the presence of the blood–brain barrier, preventing the entry of antibodies into the brain tissue.

It was shown previously [3] that on the 7th-8th day of sensitization of dogs with encephalitogenic emulsion the titer of antibrain antibodies is at its highest, and during the development of clinical manifestations it falls considerably.

The possibility of the passive transmission of EAE by intravenous injection of γ-globulin obtained from dogs' serum on the 7th day of sensitization, followed by infliction of a thermal burn on the brain, was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on 22 guinea pigs weighing 250-300 g. Thermal coagulation of areas of the brain was carried out through the exposed parietal bone by means of a 50 W soldering iron for 10 sec [7]. The area of the heating surface was 3 times 2.5 mm. The γ-globulin was obtained by alcoholic precipitation and it was preserved with merthiolate (1:10,000).

Three types of γ-globulin were used in the experiments: immune – from the serum of dogs killed by exsanguination on the 7th day after sensitization with encephalitogenic emulsion when the titer of anti-

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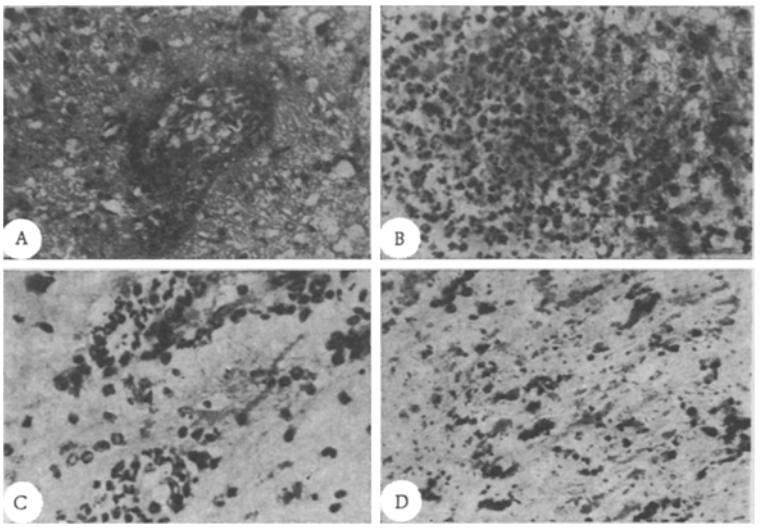

Fig. 1. Morphological changes in the brain of guinea pigs during passive transmission of EAE: A) fibrinoid necrosis of the wall of a blood vessel; B) diffuse infiltration with polymorphs; C) perivascular infiltration of polymorphs and monocytes; D) fragmentation of myelin into small granules. A-C) Hematoxylin–eosin; D) Marchi's stain, 280×.

brain antibodies was 1:160-1:320; normal – from the serum of intact dogs; γ-globulin from the serum of a dog with clinical features of EAE (antibody titer 1:40). Immunization was carried out by the method described previously [2]. γ-Globulin was injected intravenously in a dose of 1 ml at intervals of 24 h.

In the experiments of series I (6 guinea pigs), coagulation was carried out 24 h before injection of immune γ-globulin, 3 injections were given, and coagulation was then repeated in the opposite hemisphere. In series II (7 guinea pigs), 3 injections of immune γ-globulin were given, and 24 h later unilateral coagulation was carried out. In series III (3 guinea pigs), 3 injections of γ-globulin prepared from the serum of the dog with EAE were given, and this was followed by unilateral coagulation.

In the control group, coagulation was carried out without injections (3 guinea pigs) or with injection of normal γ-globulin as in the experiments of series II (3 guinea pigs). The animals were killed 1-3 days later. The brain of all the animals was examined histologically. Sections were stained with hematoxylin–eosin and by Marchi's method.

EXPERIMENTAL RESULTS

In four animals of series I, clinical features consisting of reduced motor activity, a forced position of the head ataxia, and weakness of the limbs appeared 1-2 days after the second coagulation only.

On histological investigation in the zone surrounding the area of coagulation carried out before injection of the immune γ-globulin the changes observed included edema, solitary foci of extravasation, a mild glial reaction and, in some blood vessels, leukostasis. Repeated coagulation in the opposite hemisphere led in every case to a widespread perifocal reaction. At the periphery of the focus of coagulation necrosis edema was sharply defined, the walls of many blood vessels were in a state of fibrinoid necrosis (Fig. 1A), and the brain tissue was diffusely infiltrated with degenerating polymorphs (Fig. 1B). In areas more distant from the focus of coagulation zones of perivascular infiltration consisting of polymorphs, histiocytes, and lymphocytes, were present (Fig. 1C). The blood vessels were dilated, with signs of plasmostasis and leukostasis and the walls of some vessels were in a state of plasma saturation and fibrinoid necrosis. Besides perivascular infiltration there was an ill-defined diffuse infiltration with polymorphs. The choroid plexus of the lateral ventricle and the meninges bordering the focus of destruction, as well as the submeningeal areas of brain substance, were also infiltrated with polymorphs and monocytes. Staining by Marchi's method revealed periaxonal fragmentation of the myelin which was black in color as a result of impregnation with osmium (Fig. 1D). The changes noted above took place 24 h after coagulation.

In the experiments of series II, in which the injection of immune γ-globulin preceded the thermal coagulation, in every case a marked inflammatory reaction identical to that described above was present. In three animals of this series motor activity was reduced, and the head was in a forced position.

After injection of γ-globulin obtained from the serum of a dog with acute encephalomyelitis on the 10th day after sensitization, in none of the three cases investigated were the characteristic inflammatory infiltration and neurological manifestations of the previous series of experiments found. Coagulation carried out after injection of normal γ-globulin likewise did not cause the development of inflammatory infiltration, although in these cases the manifestations of edema were more severe, the zones of extravasation were more extensive, and the proliferation of the glial cells was more marked than the morphological picture in guinea pigs with coagulation alone or with coagulation preceded by injection of immune γ-globulin.

Essential conditions for the harmful action of antibodies are a disturbance of the permeability of the blood-brain barrier and the presence of a sufficiently high titer of circulating antibodies in the blood. The latter condition is confirmed by the absence of the characteristic inflammatory reaction after injection of normal γ-globulin and of γ-globulin from a diseased dog with a low titer of antibrain antibodies. Finally, an important condition for detection of the harmful action of antibodies in the present experiments was their injection before injury to the brain. The phenomenon observed is evidently based on mechanisms characteristic of passive anaphylaxis and, in particular, preliminary fixation of the antibodies (possibly on the basement membranes of the brain vessels).

The morphological picture observed was similar to that described during the development of hyperacute forms of EAE in rats [7] and also in monkeys and dogs [2, 9], and which is characterized by features of fibrinoid necrosis of the blood vessel walls and diffuse infiltration of the brain with polymorphs. Perivascular infiltration with monocytes, so characteristic of the usual forms of EAE, must evidently be regarded as a primary nonspecific response to the action of antibrain antibodies, preventing them from penetrating further into the brain tissue. This hypothesis is confirmed by data obtained as a result of treating the recipients with cyclophosphamide, inducing a selective leukopenia. Injection of living lymphocytes from a specifically sensitized donor into such recipients also leads to the development of diffuse polymorph infiltration without the formation of perivascular cuffs of monocytes, despite the circulation of large numbers of specifically sensitized lymphocytes in the recipient's blood stream.

It can be concluded from the facts described above that the changes produced in the brain during cellular passive transmission, including the so-called "new form" of EAE [6], are due primarily to the action of antibrain antibodies produced in the recipient's body by living lymphocytes.

These facts are evidence of the harmful action of antibrain antibodies produced in response to the injection of encephalitogenic emulsion and of their important role in the pathogenesis of EAE.

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