

Immunofluorescent Staining of Rat Brain Glial Cells With Multiple Sclerosis Serum

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SUMMARY: *Antibodies directed against glial cells may be involved in autoimmunity in multiple sclerosis (MS). Using a tissue culture system, the presence of glial cell antibodies in MS-patient serum was detected through immunofluorescent technique. Thirty one of 73 MS-sera were strongly positive for anti-glial cells, 13 were equivocal and 29 were negative. The antibody staining was either cytoplasmic or associated with cell surface membrane, and involved IgG type of antibody.*

RÉSUMÉ: *Des anticorps dirigés contre les cellules gliales sont peut-être impliqués dans l'autoimmunité de la sclérose en plaques (SEP). Nous avons mis en évidence de tels anticorps aux cellules gliales dans le serum de patients avec SEP en utilisant une technique d'immunofluorescence dans un système de culture de tissus. Sur 73 sera de SEP, 31 étaient fortement positifs, 13 équivoques et 29 négatifs. La coloration des anticorps indiquait une localisation cytoplasmique ou associée à la membrane cellulaire de surface, et impliquait des anticorps de type IgG.*

INTRODUCTION

Multiple sclerosis (MS) is a chronic progressively degenerating disease, characterized by demyelination in the central nervous system. Demyelination appears to be an inevitable consequence of the patient's immune response involving some type of inflammatory autoimmune reaction in CNS (Paterson, 1973). Since oligodendroglial cells synthesize and elaborate myelin in CNS and the MS-plaque formation generally involves the destruction of oligodendrocytes (Raine, 1977), it has been suggested that these cells, instead of myelin that they produce, might be the target sites of demyelinating antibodies. In this respect the binding of specific antibodies directed against oligodendrocytes by indirect immunofluorescent technique has been reported in nearly 90% of the MS patients sera under examination (Abramsky et al., 1977). More recent studies, however, revealed that the immunofluorescent staining of oligodendroglial cells by MS—serum is nonspecific and indistinguishable from that of normal human serum (Traugott et al., 1979; Kennedy & Lisak, 1979).

This communication describes the presence of glial cell antibodies in MS-patient's sera which bind to rat brain glial cells in continuous cultures.

EXPERIMENTAL SECTION

All experiments were conducted with normal rat brain glial cell culture which was recently developed in this laboratory (Singh & Van Alstyne, 1978), and designated as the RG-cell line. These cultures contained mainly astrocytes (80-90%) and some 10-20% of the round-shaped oligodendrocytes and dividing glial cells. The growth

medium and the method of subculturing these cells are given elsewhere (Singh & Van Alstyne, 1978).

A total of 100 serum samples were analyzed. These included 73 patients diagnosed as multiple sclerosis, 8 with Huntington's chorea, 5 with encephalitis and 14 were normal human serum controls.

The detection of antibodies directed against glial cells was carried out by means of indirect immunofluorescent technique. The RG-cells were grown to confluency directly on glass slides (two chamber tissue cultures slides, Lab-Tek). The growth medium was aspirated, washed twice with PBS (10 mM sodium phosphate, pH 7.5 containing 0.9% NaCl) and then acetone-fixed at 4°C for 15 - 30 min. Alternately, the glial cells were gently scraped-off the tissue culture flasks (75 cm² size) using a rubber policeman, washed with PBS and kept in suspension in PBS. Both the fixed as well as the unfixed cells were incubated with different human sera (1/10 dilution) at 4°C for about 60 min. Towards the end of this incubation period, the cells were PBS washed and stained with FITC-conjugated goat antihuman total immunoglobulin fraction (IgG + IgM + IgA mixture; obtained from Cappel Labs. Inc.) for 45 min. at 4°C. The fluorescein conjugate was preabsorbed with RG-cell and was utilized at 1/15 dilution in these experiments. Subsequently, the glial cells were thoroughly washed with PBS-NaN₃ (0.002%) solution, mounted in 50% glycerin in PBS-NaN₃ solution and finally examined under epi-illumination of a Carl Zeiss Universal fluorescent microscope. The entire procedure with unfixed glial cells in suspension was carried out in borosilicate glass tubes (12 x 75 mm) and the washings were done by centrifu-

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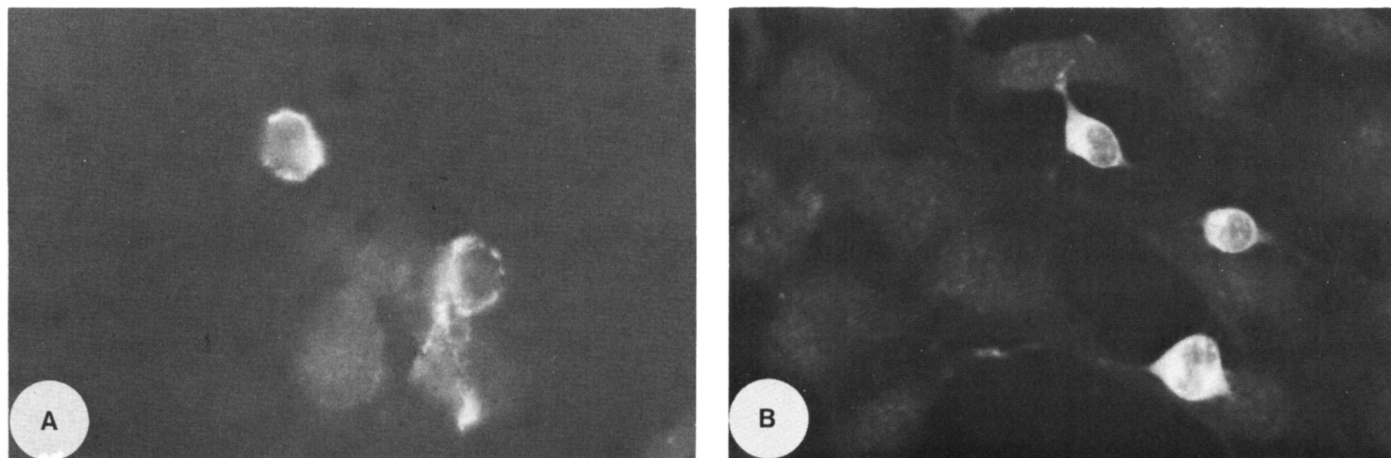


Figure 1 — Typical immunofluorescent staining of rat brain glial cells with MS-patient serum. The glial cells (RG-cell culture) were either in suspension for cell surface staining (A) or acetone-fixed for cytoplasmic staining (B). The MS-serum was used at 1/10 dilution. The astrocytes in the background did not stain (B). The similar tests with normal human serum were negative. (magnification X 635).

gation at 1,000 rpm for 10 min under refrigeration.

The preabsorption of the MS-patients sera (at 1/10 dilution) with RG-cells, rabbit kidney cells (RK-cell line) and human fibroblast was done for 60 min. at 4°C. This was followed by centrifugation at 3,000 rpm for 20 min. In some experiments, the acetone-fixed preparations of RG-cells were treated with bovine galactocerebroside (conc. 25 µg/ml for 30 min. at 4°C) before incubation with MS-patient serum. For the determination of immunoglobulin class of antibodies to glial cells, the staining was carried out by using monovalent conjugates of FITC-labelled goat antihuman IgG, IgM and IgA separately (Cappel Labs, Inc.).

RESULTS and DISCUSSION

The typical staining of RG-cells with positive MS-serum was localized with the cell surface membrane or cell cytoplasm of mainly round shaped glial cells (Fig. 1). The cell nucleus was not stained. Moreover, the astrocytic cells in these cultures were never stained. The galactocerebroside has been shown to be a specific cell-surface antigenic marker for oligodendrocytes in culture (Raff et al., 1978). The treatment of RG-cell cultures with galactocerebroside prior to staining with MS-serum completely blocked

the staining of RG-cells (Fig. 2). This finding suggests that the immunofluorescent staining of rat brain glial cells with MS-serum may be related to oligodendroglia and dividing glial cells in RG-cell cultures. The positive staining with patient serum can be depleted by preabsorbing the MS-serum with RG-cells, but not kidney cells or fibroblasts, suggesting that the antibodies are directed against glial cell antigens. Furthermore the class of immunoglobulins which stains glial cells appeared to be that of IgG class as the positive staining was seen with FITC-goat antihuman IgG only, and not with anti-human IgM or IgA.

The results summarized in Table 1 show that 31 out of 73 MS-patients were found to be strongly positive when the binding of antibodies to glial cells was examined with RG-cell line through the technique of indirect immunofluorescence. Thirteen patients sera were equivocal whereas the remaining 29 sera were completely negative. None of the 14 control sera exhibited any positive staining. Similarly 13 sera with other neurological disease (Huntington's chorea and encephalitis) showed no definite staining of RG-cells. These results were obtained at 1/10 dilution of all sera. Those MS-sera which showed positive staining at this dilution also exhibited slightly weaker staining at 1/100 dilution.

The presence of anti-oligodendro-



Figure 2 — Effect of galactocerebroside on the immunofluorescent staining of RG-cells with MS-serum. The RG-cells were acetone-fixed and treated with 25 µg/ml solution of galactocerebroside at 4°C for 30 min. After thorough washing with PBS, the incubations were done with MS-serum and FITC-conjugate as described in the text. (magnification X 158).

glial cells in MS-serum was originally demonstrated by using bovine brain cross-sections or isolated cells (Abramsky et al., 1977). In this study, 90% of

TABLE I

Summary of immunofluorescent staining of rat brain glial cells with MS-patient serum.

Clinical diagnosis	No. of patients analyzed	Immunofluorescent staining*		
		Positive	Equivocal	Negative
Normal Humans	14	N.D.	2	12
Huntington's chorea	8	N.D.	N.D.	8
Encephalitis	5	N.D.	N.D.	5
Multiple sclerosis	73 (100%)	31 (42.5%)	13 (17.8%)	29 (39.7%)

*Positive staining was scored based on the cell surface as well as the cell cytoplasmic staining without any doubt. Equivocal staining was generally very weak and inconsistent. N.D. = Not detectable. The observations were replicated in 2-5 experiments for each group.

the patient sera were found to be positive for oligodendrocyte specific immunofluorescent staining. Recently, Traugott et al. (1979) reported that approximately 63% and 37% of MS-sera showed positive staining for oligodendrocytes in bovine brain and human brain cross-sections, respectively. The astrocytes also showed some positive staining. Furthermore, they found positive staining with control normal human serum also, although only 2 - 4 out of 14 control sera were positive. These observations, along with some other results (Kennedy & Lisak, 1979), have been interpreted to suggest that the immunofluorescent staining of oligodendrocytes with MS-serum is not specific of this disease, unlike that reported by Abramsky et al (1977).

Our findings with rat brain glial cell cultures clearly suggest that the antibodies specific to round shaped glial cells (which are morphologically similar

to oligodendroglia and dividing glial cells, but not astrocytes) are present in about 43% of MS-sera examined. None of the 14 control normal human sera or 13 sera with other neurological disease showed any positive staining. The number of positive MS-sera is somewhat comparable to Traugott et al (1979), but not as high as reported by Abramsky et al (1977). The apparent inconsistency may be related to different species used by different researchers as resolved by the study of Traugott et al (1979), in which 63% of patients sera were positive with bovine brain as compared to only 37% with human brain. The detection of IgG class of immunoglobulins in our experiments is in agreement with others (Traugott et al, 1979).

From these observations it may be concluded that not all of the MS-patients sera contained antibodies to glial cells, but those which exhibit positive antibody staining retain cellu-

lar specificity for oligodendroglia and dividing glial cells. While more work is necessary to evaluate the role of anti-glial cells in MS, the presence of the antibodies in MS-patients serum may be related to some autoimmune reaction during the course of pathogenesis of the disease.

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