Anti-Neurofilament Antibodies in the Sera of Patients with Small Cell Carcinoma of the Lung and with Visual Paraneoplastic Syndrome

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ABSTRACT

The sera of patients with small cell carcinoma of the lung (SCCL) and an associated visual paraneoplastic syndrome (VPNS) have high titer immunoglobulins that react with retinal ganglion cells and with cloned lines of the SCCL. The immunoglobulins in the sera of two patients with SCCL and VPNS reacted with at least one common antigen shared by neural cells and cloned lines of the SCCL. The molecular weights of the predominant neural and tumor antigens were 205,000, 145,000, 65,000, and 20,000-24,000 as determined by Western blots. Three of the antigens from neural tissue copurify and comigrate electrophoretically with neurofilament proteins. Polyclonal antibodies that were prepared against authentic neurofilament proteins react with antigens having molecular weights identical to those of proteins that react with immunoglobulins from the SCCL-VPNS patients. Polyclonal antibodies that were prepared against isolated retinal ganglion cells and that were shown previously to cause the immunoablation of the ganglion cells in vivo reacted most intensely with the M, 205,000 antigen and weakly with the M, 145,000 and M, 70,000 antigens. Treatment of the Western blots with alkaline phosphatase from Escherichia coli did not affect the immunoreactivity between the immunoglobulins and the purified neurofilament proteins. It is proposed that the immunoglobulins in the sera of patients with SCCL-VPNS may be involved etiologically in the development of the VPNS.

INTRODUCTION

A subset of patients with carcinomas develops neurological dysfunction even when the nervous system is not invaded with the primary tumor or metastases. These dysfunctions are identified as PNS, and the syndromes were reviewed in 1982 by Minna and Bunn. It has been shown immunohistochemically that patients with ovarian carcinoma and cerebellar PNS have high titer immunoglobulins reactive with Purkinje cells. We have previously shown immunohistochemically that high titer immunoglobulins, reactive with retinal ganglion cells, were present in the sera of those patients with SCCL who had VPNS. These SCCL-VPNS immunoglobulins recognize a small number of protein antigens in extracts of both retina and small cell cancer tumors. The protein antigens have molecular weights of 205,000, 145,000, 65,000 and 20,000-24,000. The similarity of the molecular weights of the proteins in the neurofilament triplet to those of the antigens reported here (205,000, 145,000 and 65,000) suggested that the neural antigens may be neurofilaments. It has been demonstrated histochemically that neurofilaments are present in oat cell lung cancers.

MATERIALS AND METHODS

Human Serum Samples

The sera from seven patients with SCCL and VPNS were obtained over a 5-year period. Two of these seven sera were described earlier, and the clinical history from both of these patients was described briefly in (5). In addition to these seven patients, the present study examined the sera of five normal control subjects, of patients with SCCL and no VPNS, and of patients with different forms of lung cancer.

Polyclonal Antibodies Produced against Authentic Neurofilament Proteins

The polyclonal antibodies were produced in rabbits against purified neurofilament proteins by Dr. Doris Dahl and were a gift from her laboratory.

Preparation of the Neural Protein Extracts for Gel Electrophoresis

The neural tissues were placed in four volumes of sample buffer containing 2% SDS, 12% glycerol, and 63 mm Tris, pH 6.8. The samples were homogenized with a Potter-Elvehjem tissue grinder, and the homogenates were boiled for 2 min. The mercaptoethanol was added to a final concentration of 5%. These samples were used for gel electrophoresis and Western blot studies.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gels were prepared and run as described previously (12). The gels were prepared as either 5, 7.5, 10, or 15% polyacrylamide gel slabs. The resolving gel was 0.375 M Tris, pH 8.8, and the stacking gel was 0.125 M Tris, pH 6.8. The protein extracts in the sample buffers were applied to the gel (12 cm x 1.5 mm), and electrophoresis was done

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4 The abbreviations used are: PNS, paraneoplastic syndromes; SCCL, small cell carcinoma of the lung; VPNS, visual paraneoplastic syndrome; SDS, sodium dodecyl sulfate; PBS, phosphate buffered saline (0.01 M phosphate, pH 7.4); HA, hydroyxalapatite.
at 30 mA until the tracking dye reached the bottom edge of the gel. The tracking dye was 0.01% bromphenol blue. The gel slabs that were stained for protein were placed in 0.25% Coomassie blue R-200 dissolved in an aqueous solution containing 9.2% acetic acid and 50% methanol. The gels were destained with an aqueous solution containing 7.5% acetic acid and 12% isopropanol.

Western Blot Analysis

The gels that were transblotted onto nitrocellulose sheets (Bio-Rad) were not prestained with Coomassie blue. The proteins were transferred to the nitrocellulose membrane in a Bio-Rad apparatus by electrophoresis at 70 mA for 16 h in 20 mM Tris:190 mM glycine buffer (pH 8.3) containing 20% methanol. The blots were incubated for 60 min with either 10% normal goat serum or with 10% bovine serum albumin to block the non-specific adsorption of immunoglobulins to the nitrocellulose matrix. The immune serum samples were diluted 1:100 to 1:10,000 with PBS containing 5% normal goat serum and then placed on the blot and incubated for 16 h. Most of the serum dilutions were 1:100. The blots were washed four times with PBS and then incubated for 2 h with appropriate second antibody (goat anti-human IgG, IgM coupled to horseradish peroxidase; Kirkegaard and Perry). The second antibody was diluted 1:3000 with PBS containing 3% normal goat serum. The blots were rinsed four times with PBS and then developed with diaminobenzidine and hydrogen peroxide.

Preparation of Neurofilaments

Urea Extraction. A modification of the methods of Tokutake et al. (13) was used. Neural tissue samples were homogenized with a Polytron tissue grinder (Brinkman) in two volumes of buffer A. This buffer contained 10 mM sodium phosphate, pH 7.4, 1 mM EDTA, 0.1 M sodium chloride, 0.5 mM phenylmethyl sulfonyl fluoride, 0.85 M sucrose, and 1% Triton X-100. The homogenate was stirred for 16 h at 4°C and then centrifuged in a type 35 rotor at 100,000 x g for 30 min. The recovered pellets were suspended in one-half the volume of buffer A (but without sucrose) used in the original extraction. The suspension was layered on buffer A which contained sucrose but not Triton and was centrifuged for 30 min at 27,000 x g in an SW 27 rotor. The supernatant was used for gel electrophoresis.

Thiocyanate Extraction. An alternate procedure was used to purify the neurofilament proteins because urea carbamylates the proteins and because thiocyanate purification of the neurofilaments yielded increased amounts of the Mr 205,000 proteins compared with the urea. Neural tissue samples were placed in two volumes of buffer A described above. After homogenization with a Polytron tissue grinder and extraction as described above, the suspension was stirred for 90 min at 4°C and then centrifuged at 20,000 x g for 30 min. This final pellet was suspended in 10 volumes of 10 mM sodium phosphate, pH 7.4, containing 1% 2-mercaptoethanol and 1.5 M sodium thiocyanate. From 230 g brain tissue, 34 g of pellet (wet weight) were obtained.

Batchwise Elution of the Neurofilament Protein from Hydroxyapatite

The HA (purchased from Sigma) was equilibrated with 10 mM sodium phosphate, pH 7.4, containing 8 M urea. The neurofilament proteins were dissolved in 10 mM phosphate buffer, pH 7.4, containing 1% 2-mercaptoethanol and 8 M urea. The solution was added to 15 g of the HA in 25 ml of the equilibration buffer. A total of 500 mg protein were applied to the HA. The suspension was centrifuged at 10,000 g for 10 min. The supernatant was removed and the HA was then eluted with 70 ml of 130 mM sodium phosphate, pH 7, containing 8 M urea. The HA was finally eluted with 70 ml of 300 mM sodium phosphate, pH 7, containing 8 M urea. The final eluate contained 60–80 mg protein as determined by absorbance at 280 nm and by the Bradford method (14).

Chromatography of Proteins on DEAE Cellulose

DE-32 (Whatman) was prepared for chromatography and then equilibrated with phosphate buffer (10 mm, pH 7) containing 1 mm EDTA, 0.1% 2-mercaptoethanol, and 6 M urea. Thirty mg of neurofilament proteins that were extracted with urea were dissolved in 25 ml of the same phosphate:EDTA:urea:mercaptoethanol buffer and applied to the DEAE column (1.4 x 45 cm). The proteins were eluted at a flow rate of 18 ml/h with a 500-ml gradient of sodium phosphate; the gradient ranged from 10 to 300 mM phosphate. All elution buffers contained 1 mM EDTA, 0.1% mercaptoethanol, and 6 M urea.

The neurofilament proteins that were extracted with sodium thiocyanate were applied to the DE-32 in phosphate buffer (0.01 M, pH 7.4) and were eluted from the DE-32 with the same phosphate buffer containing a gradient of sodium thiocyanate (0 to 0.5 M thiocyanate).

RESULTS

Interaction of Neural Proteins with Immunoglobulins from Sera of Patients with SCCL and Visual PNS. The sera of seven patients with SCCL and visual PNS contained immunoglobulins that reacted with a limited number of proteins in retina and in cloned lines of SCCL. The molecular weights of the antigens were 205,000, 145,000, 65,000, and 20,000–24,000 as determined by western blots. Fig. 1 illustrates the reaction of the retinal antigens with immunoglobulins in the sera of two such patients at high serum dilutions. The serum from one of these two patients (patient 1) reacted with retinal antigens of molecular weight 65,000 and 20,000–24,000, while that from patient 2 reacted with the antigens of molecular weight 145,000 and 205,000. These two patients were described earlier (4, 5) and have been examined in detail in this report because (a) serum samples were obtained from them at different times during their clinical course and (b) retinal and neural tissue was obtained from patient 2 after his death. The sera from the other five patients with SCCL and VPNS also reacted with subsets of these four retinal proteins but not with other proteins at the dilutions examined. The serum immunoglobulins from control patients with SCCL and VPNS did not react with any of these retinal antigens.
subjects did not react with these antigens at the dilutions examined.

The sera from two of the patients with SCCL and VPNS (patients 1 and 2) reacted with antigens solubilized from cloned lines of SCCL obtained from Dr. John Minna (National Cancer Institute). The immunoglobulins from patient 1 reacted with a $M$, 65,000 protein shared by all four SCCL clone lines and human and chick retina (Fig. 2). The immunoglobulins from patient 2 reacted with a $M$, 150,000 protein shared by all four SCCL clone lines and human and chick retina. Immunoglobulins from patient 1 also reacted with a $M$, 90,000 protein in chick retina and a $M$, 20,000–24,000 protein in human retina that are not shared by the SCCL lines. Immunoglobulins from patient 2 reacted with a $M$, 205,000 protein in human and chick retina not shared by the SCCL lines; they also react with a $M$, 40,000–44,000 protein in two of the four SCCL clones that is not present in the other cell lines.

The immunoglobulins also reacted with frozen sections of retina. Neither the immunohistochemical nor the Western blot studies indicated species specific reaction of the immunoglobulins, since neural tissue sections and protein extracts from chick, human, cat, and dog retinas reacted similarly. Sera from patients with small cell carcinoma of lung but without visual PNS did not react immunohistochemically with the retinal ganglion cells. The sera of one of these SCCL patients, who had no visual or other PNS, contained immunoglobulins that reacted with protein antigens having molecular weights of 205,000 and 145,000 (Fig. 3); no reaction was detected with the $M$, 65,000 protein. The serum of a patient who had both a bronchoalveolar carcinoma of the lung and a squamous cell carcinoma of the parotid gland and who had weakness and dysphagia attributed to PNS but with no visual PNS had high titer immunoglobulins against a $M$, 65,000 neural protein (Fig. 3). The serum from this patient reacted weakly with the $M$, 205,000 protein but not with the $M$, 145,000 antigen. The serum from each of these two patients did not react immunohistochemically with retinal sections. The data from these two patients demonstrate that immune reactivity with the $M$, 205,000, 145,000, or 65,000 proteins in neural tissue does not necessarily indicate reaction of the serum immunoglobulins with retinal neurons.

Fig. 3. Reaction of neural antigens with antibodies from cancer patients who had no VPNS and effects of alkaline phosphatase treatment. Lane a, serum from SCCL patient without VPNS, dilution 1:100; lane b, serum from control, dilution 1:100; lane c, serum from patient with squamous cell carcinoma of the parotid and bronchoalveolar carcinoma, dilution 1:100; lane d, electrobolt treated with alkaline phosphatase from E. coli and then incubated with serum from the patient with SCCL but without VPNS (same as lane a); lane e, electrobolt treated with alkaline phosphatase from bovine intestine and then incubated with serum from patient with SCCL but without VPNS. The second antibody in all cases was goat-anti-human IgG, IgM coupled to horseradish peroxidase. $MW$, molecular weight.

The purification of the Neurofilament Triplet Proteins. Neurofilament proteins from bovine white matter were solubilized either using urea or sodium thiocyanate as described in "Materials and Methods." The urea solubilized proteins were purified further by batchwise elution from HA (Fig. 4) and by chromatography on DE-32 cellulose (Fig. 5). The neurofilament proteins were eluted from the HA at 0.3 M sodium phosphate and from the DE-32 at an ionic strength of 0.14–0.16, with a gradient of phosphate or sodium thiocyanate as the eluting solvent.

The neurofilament proteins from bovine brain were solubilized with 1.5 M sodium thiocyanate (Fig. 6A) and purified further by ethanol fractionation (Fig. 6B). Although a small portion of the high molecular weight ($M$, 205,000) component of the neurofilament proteins did precipitate in 50% ethanol, most of it remained in solution (Fig. 6B). The ethanol caused the removal of other proteins and nucleic acids by precipitation. The purified neurofilament proteins reacted with the immunoglobulins obtained from the sera of patients with SCCL and visual PNS; Fig. 7 illustrates the reaction with immunoglobulins from patient 2. The neurofilaments extracted with the thiocyanate were purified further by chromatography on a column of DE-32 as described in "Materials and Methods." The neurofilaments were eluted from the column at an ionic
Fig. 4. Purification of neural antigens by chromatography on hydroxylapatite. Lane a, molecular weight standards; lane b, total protein soluble in 8 M urea after Triton extraction; lane c, protein that did not bind to hydroxylapatite; lane d, protein eluted from hydroxylapatite at 130 mM sodium phosphate, pH 7.4; lane e, protein eluted from hydroxylapatite at 300 mM sodium phosphate, pH 7.4. Gel was stained with Coomassie blue. MW, molecular weight.

Fig. 5. Purification of neural antigens by chromatography on DEAE-32. Lane a, protein that did not bind to the DE-32; lane b, protein eluted with linear gradient of phosphate buffer at 70 mM, pH 7.0; lane c, molecular weight standards. MW, molecular weight.

Fig. 6. Purification of bovine neural antigens. A, extraction with sodium thiocyanate (SCN). Lane a, molecular weight standards; lane b, brain proteins from the Triton extract that were insoluble in 1.5 M SCN; lane c, proteins extracted by 1.5 M SCN and insoluble after dialysis against water; lane d, proteins soluble in 1.5 M SCN and soluble after dialysis against water; lane e, partially purified neurofilaments prepared by extraction with Triton and 8 M urea. B, ethanol precipitation of SCN soluble extract. Lane a, proteins soluble in 1.5 M SCN and insoluble in 35% ethanol; lane b, proteins soluble in 1.5 M SCN and insoluble in 50% ethanol; lane c, proteins soluble in 1.5 M SCN and 50% ethanol; lane d, molecular weight standards. MW, molecular weight.

strength of 0.14–0.16.

Reaction of Neurofilament Proteins Prepared from Specific Regions of Bovine Brain with the Immunoglobulins. Neurofilaments were prepared from bovine cerebral cortex, mammillary bodies, colliculi, hippocampus, cerebellum, brain stem, and retina. The neurofilament proteins were prepared by urea extraction as described in "Materials and Methods." They were subjected to electrophoresis (Fig. 8), and the resolved proteins were examined by Western blot methods; Western blots of the retinal extracts were shown in Fig. 1. The total amount of protein applied to the gel was similar from each of the brain regions. The immunoglobulins reacted similarly with the proteins of the different brain regions, with the exception of the hippocampus. For example, the immunoglobulins from patient 2 with SCCL and visual PNS reacted with a M, 205,000 protein derived from all brain regions examined except hippocampus (Fig. 9). In the hippocampus a M, 140,000 protein was most reactive (Fig. 9). The Coomassie blue stain patterns of the proteins from the different brain regions were also similar, except for the hippocampus, where the concentration of the M, 130,000-140,000 protein was increased compared to that of the M, 145,000 proteins in other brain regions (Fig. 8).
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MW $\times 10^{-3}$

205--

145--

70--

MW $\times 10^{-3}$

205--

116--

97--

68--

45--

29--

MW, molecular weight.

Fig. 7. Western blots of bovine neural proteins extracted with sodium thiocyanate (SCN) incubated with human serum and with rabbit anti-neurofilament immunoglobulins. Lane a, polyclonal rabbit serum prepared by Dr. D. Dahl against isolated $M$, 205,000 neurofilament protein, diluted 1:500; lane b, serum from a patient with SCCL-VPNS, diluted 1:100; lane c, control human serum, diluted 1:100; lane d, polyclonal rabbit immunoglobulins prepared against isolated large ganglion cells from bovine retina, diluted 1:250. The second antibody was goat-anti-rabbit IgG, IgM coupled to horseradish peroxidase for those lanes incubated with rabbit immunoglobulins and goat-anti-human IgG, IgM, IgA coupled to horseradish peroxidase for lanes incubated with human immunoglobulins. MW, molecular weight.

Fig. 8. Coomassie blue patterns of neurofilament fractions from various regions of bovine brain. Lane a, molecular weight standards; lane b, proteins from brainstem; lane c, from cerebellum; lane d, from colliculi; lane e, from cerebral cortex; lane f, from hippocampus; lane g, from mamillary bodies. Proteins were solubilized with urea and stained with Coomassie blue. MW, molecular weight.

A reaction of the Immunoglobulins with Neurofilament Proteins Prepared from Bovine and Rat Brain. Neurofilaments were prepared by urea extraction and by thiocyanate extraction from bovine and rat brains. The proteins were resolved on acrylamide gels and were then examined by the Western blot technique. The proteins recovered from each of the two species reacted similarly with the immunoglobulins from the sera of the patients with carcinomas. Fig. 10 illustrates the reaction of the serum immunoglobulins from a patient with SCCL and no VPNS with the $M$, 205,000 protein from rat and bovine neural tissue. Several patients with SCCL and VPNS, including patient 2, also reacted with the $M$, 205,000 protein.

Interaction of Anti-Retinal Ganglion Cell Immunoglobulins with Neural Proteins. Polyclonal antibodies were prepared in rabbits against large ganglion cells isolated from ox retinas (9). The immunoglobulin fractions of the rabbit sera were separated from the remaining serum proteins by chromatography on DE-32. The injection of the purified immunoglobulins into the vitreous of cats caused the immunoablation of the large ganglion cells in retina (10). The injections of the immunoglobulins also caused the marked inhibition of the electrophysiological activity of nerve cells in the lateral geniculate nucleus that received input from the large ganglion cells in the retina (11).

To obtain information about the molecular weights of the neural antigens, the purified rabbit immunoglobulins were incubated with Western blots of the bovine neural tissue and the partially purified neurofilaments (Fig. 7). It was found that there was an immunological reaction with the $M$, 205,000 protein (Fig. 7, lane d) and weaker reactions with the $M$, 70,000 and $M$, 145,000 proteins. The protein antigens that reacted with the polyclonal antibodies against authentic neurofilament protein (Fig. 7, lane a) reacted both with rabbit immunoglobulins and the human SCCL-VPNS immunoglobulins, as determined from their electrophoretic mobilities. The similarity of the molecular weights of the antigens and the copurification with neurofilament proteins suggest that the rabbit immunoglobulins and the human immunoglobulins are directed against similar proteins.

Alkaline Phosphatase Treatment of Neurofilaments Does Not Affect Interaction with Immunoglobulins. The neurofilament proteins were subjected to electrophoresis on polyacrylamide gels and then electroblotted onto nitrocellulose. The electrolots were treated with alkaline phosphatase prepared from either bovine intestinal mucosa (Worthington, similar to Sigma Type VII) or Escherichia coli (Sigma, Type III). Five units of each enzyme were used. The incubations were maintained in PBS (pH 7.4, 0.01 M phosphate) at 25°C for 2 h. The E. coli phosphatase had no effect on the interaction of the immunoglobulins with the neurofilament proteins (Fig. 3). From our studies with Coomassie blue stained polyacrylamide gels, the bovine enzyme is contaminated with protease. This resulted in the hydrolysis of the protein bands on the nitrocellulose. Elevated concentrations of phosphate (50 mM) did not inhibit the loss of immunoreactivity of the $M$, 205,000 neurofilament protein treated with bovine phosphatase, even though phospha-
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Fig. 9. Western blots of neurofilament proteins from various regions of bovine brain. Lanes a and b, cerebellar cortical antigens; lanes c and d, cerebral cortical antigens; lanes e and f, hippocampal antigens; and lanes g and h, bovine whole brain antigens. Lanes a, c, e, and g were from a patient with SCCL-VPNS and b, d, f, and h were from normal human controls. Primary sera were diluted 1:100. Second antibody was goat anti-human IgG, IgM, IgA coupled to horseradish peroxidase. MW, molecular weight.

tase activity is inhibited at this concentration of phosphate. These observations indicate that (a) the antigen is a protein and (b) the reaction of the immunoglobulins from the patients with SCCL and visual PNS is not dependent upon the phosphorylation of the neurofilament protein (15).

DISCUSSION

We have shown that the immunoglobulins from sera of patients with small cell carcinoma of lung and an associated visual paraneoplastic syndrome (SCCL-VPNS) react immunohistochemically with the retina. From Western blot analyses it is concluded that these immunoglobulins react with proteins of the neurofilament triplet and with a M, 20,000–24,000 protein. Three of the four primary antigenic species are neurofilament proteins because they copurify and comigrate electrophoretically with the neurofilaments. They also react with polyclonal antibodies prepared against the neurofilaments. At the high (>100) serum dilutions used in this study the neurofilaments and a protein of molecular weight 20,000–24,00 protein appear to be the primary antigens with which the immunoglobulins react as determined by Western blot analysis. The possibility must be considered that other retinal antigens react histochemically with the immunoglobulins but were not detected by the Western blot. For example, the antigenic determinants may have been altered by the SDS-polyacrylamide gel electrophoresis procedure, or the antigen may be of low molecular weight (less than M, 10,000) and therefore not retained on the gel. The experimental evidence to date indicates that the antigens that are detected histochemically are the same ones as revealed on the Western blots.

We propose that the anti-retinal immunoglobulins in the patients with SCCL-VPNS may be etiologically connected with the occurrence of the visual PNS. The criteria required for this hypothesis to be valid are as follows: (a) immunoglobulins that react specifically with neurons of the visual pathway must be present in the patients at sufficient titer; (b) these immunoglobulins must have access to the neurons in the central nervous system; (c) the immunoglobulins must recognize either the antigen against which they were induced or a cross-reacting molecule on the surface of the neurons in the visual system; (d) those neurons in the visual pathway that react specifically with the immunoglobulins should be reduced in numbers in the patients with the visual PNS; and (e) immunoglobulins against similar antigenic determinants should cause the immunoablation of the neurons in the visual pathway.

Most of these conditions have been satisfied in the cases of SCCL-VPNS, as will be discussed below. Patients with other tumors, malignant melanoma for example, were shown to have high titer serum immunoglobulins that reacted with the tumor (16, 17).

Immunoglobulin Specificity. We have demonstrated that the immunoglobulins of patients with SCCL-VPNS react immunohistochemically with ganglion cells of retinas from humans, macaques, dogs, cats, rats, mice, and chicks (4, 5). The immunoglobulins were detectable at high dilutions, and their reaction with the retinal ganglion cells was specific. The extent of serum
globulins in their sera that reacted with neurofilament proteins on Western blots at serum dilutions of 1:50; they state that all their studies were done at a serum dilution of 1:50. In our studies, the sera from SCCL-VPNS patients and controls were diluted extensively, from 1:100 to 1:8000 (5). The titer against the M, 205,000 protein, detected in the sera of SCCL-VPNS patients at these dilutions, was markedly elevated. Because of the difference in serum dilutions, our results are not contradictory to those of Stefansson et al. Although Stefansson et al. (18) concluded that none of the disease categories was associated with high titers of antibodies to the M, 200,000 protein, a single dilution of serum cannot reveal titer level, even though it may reflect the incidence of a specific immunoglobulin in a patient population.

The reaction between immunoglobulins from patients with SCCL-VPNS and retinal ganglion cells was another example of the specificity of the response. Other retinal neurons reacted weakly or not at all, and other central nervous system neurons (e.g., Purkinje cells) did not react with these immunoglobulins, as determined immunohistochemically. Of particular interest was the lack of reaction of cerebellar Purkinje cells with serum immunoglobulins obtained from the patients with SCCL-VPNS. It has been reported that patients with cerebellar PNS associated with ovarian carcinoma had high titer antibodies that reacted immunohistochemically with the Purkinje cells (2) but not with retinal ganglion cells (3). Therefore the immunoglobulins from the patients with PNS react immunohistochemically with neurons of the system affected by the PNS (e.g., retinal ganglion cells in the visual PNS and cerebellar Purkinje cells in the cerebellar PNS) but not with other neurons. Yet in this study it has been shown that the neurofilament fraction from different brain regions reacts on Western blots with the immunoglobulins from patients with SCCL-VPNS. These observations suggest that the antigenic determinants against which the SCCL-VPNS immunoglobulins react are masked during the immunohistochemical reaction but become available during the SDS-polyacrylamide gel electrophoresis procedure. There is evidence for the existence of neurofilament proteins with neurotypic regions that reflect either the masking of different antigenic determinants in different neuronal populations or the existence of variable domains in the neurofilaments. Goldstein et al. (19) observed that some monoclonal antibodies reacted with neurofilaments in one population of neurons, while different monoclonal antibodies reacted with the neurofilaments in a second neuronal population. This molecular heterogeneity or "neurotypy" was observed with monoclonal immunoglobulins prepared against the M, 200,000 and M, 155,000 neurofilament antigens. Drager et al. (20) also observed that a monoclonal antibody, reactive with the M, 205,000 neurofilament protein, interacted preferentially with particular retinal neurons and not with other neurons.

**Immunoglobulin Access to the Central Nervous System.** Frozen sections of the retinas from one of our patients with SCCL-VPNS revealed deposits of IgG or IgM in the retinal ganglion cell layer. This observation indicates that the immunoglobulins in this patient did cross the blood retinal barrier as required by the second condition. This patient's retina also had reduced numbers of retinal ganglion cells, indicating that these cells were lost in vivo. In this case there was an association of the loss of retinal ganglion cells with the localization of human immunoglobulins in the retina.

**Anti-Neurofilament Antibodies Interact with Neuronal Cell Surfaces.** The third requirement of the hypothesis is that antibodies must have access to the cells in situ and recognize one or more markers on the surface of the neural cells if they are to initiate a neuropathological process. Fields and Yen (21) demonstrated the interaction of the anti-M, 200,000 neurofilament antibodies with dissociated but apparently intact neurons from rat dorsal root ganglia. In our earlier studies on the immunoglobulins generated against the large ganglion cells isolated from retina, it was shown that the fluorescein labeled immunoglobulins reacted with intact but dispersed large ganglion cells (9). As is shown in this report, the polyclonal immunoglobulins from the rabbits immunized against the isolated retinal ganglion cells reacted with the proteins of the neurofilament triplet, as did the immunoglobulins from the patients with SCCL-VPNS. The antibodies may also have interacted with other antigens immunohistochemically that were not detectable on Western blots, as indicated at the beginning of the "Discussion."

The surface antigens with which the antibodies react may be the M, 205,000 antigen or a cross-reacting molecule. For example Drager et al. (22) have shown that antibodies prepared against α-melanocyte stimulating hormone react with the M, 150,000 neurofilament protein. These observations indicate that the patients' immunoglobulins may have interacted either with the high molecular weight neurofilament protein (M, 205,000) or with a protein that contains a similar antigenic determinant.

**Immunoaiblation of Neurons with Anti-Ganglion Cell Antibodies.** The fourth condition of the hypothesis is that the interaction of the immunoglobulins with the neuronal surface leads to immunoaiblation of the neuron. Our previous studies have demonstrated that the rabbit immunoglobulins prepared against the large retinal ganglion cells can cause the immunoaiblation of the large ganglion cells when injected into the vitreous (10, 11). The loss was demonstrated by anatomical analysis of the retina and by electrophysiological recordings from the lateral geniculate nucleus. As discussed above, retinal flatmounts from a patient with SCCL-VPNS, who also had high titer anti-retinal ganglion cell antibodies, revealed a loss of the large ganglion cells. These observations are related to the fourth and fifth conditions of the hypothesis and are consistent with the hypothesis presented.

**Anti-Neurofilament Antibodies and Neurological Dysfunction.** Immunoglobulins in the sera of patients with several neurological disorders and in some control subjects have been reported to react with the M, 205,000 protein of the neurofilament triplet. Increased levels of immunoglobulins against the neurofilament triplet proteins were observed in the sera of patients with kuru, Creutzfeldt-Jakob disease, Alzheimer's disease, subacute sclerosing panencephalitis, and Parkinson's disease (23-25). Toh et al. (25) observed antibodies in approximately 30% of normal control subjects and in 60-70% of the patients with the neurological disorders. The reaction of immunoglobulins, prepared against the M, 200,000 neurofilament protein, with the neurofibrillary tangles present in Alzheimer's disease (26) has resulted in the hypothesis that these antibodies may be involved etiologically in the neuropathological process (27). In the present study it was shown that the sera from two patients with carcinomas, but with no visual dysfunction, contained immunoglobulins against the M, 205,000 protein, even though the immunoglobulins did not react immunohistochemically with the retinal ganglion cells.

It is necessary to consider the apparent paradox that certain
control subjects with no evidence of neurological dysfunction also contain high titer immunoglobulins in their sera. These observations indicate one of four possibilities: (a) the anti-neurofilament and anti-M subunit proteins in the subjects without neurological dysfunction exist in the serum and do not cross the blood-brain or blood-retinal barrier in sufficient concentration to cause dysfunction; (b) the antibodies in the patients with SCCL-VPNS are directed against determinants that are accessible on the affected cells of the patient but not on controls; (c) the antibodies in the patients with SCCL-VPNS are directed against determinants that are different from those that bind the antibodies present in patients with other PNS or in control subjects; or (d) the immunoglobulins to these proteins are not involved in the PNS. To distinguish among these, it will be necessary to characterize the sequence of the amino acids involved in those antigenic determinants that react with SCCL-VPNS immunoglobulins.

One proposed mechanism by which the anti-ganglion cell antibodies may cause the VPNS is presented here: (a) the rapid growth of the SCCL is accompanied by central necrosis of the tumor; (b) tumor necrosis results in the release of the antigens from the tumor; (c) the antigens stimulate the production of immunoglobulins that react with the antigens or antigenically similar proteins on neuronal cell surfaces; (d) the permeability of the blood-brain or blood-retinal barriers increases in a subset of patients with the tumor. This results in the access of the immunoglobulins to the retina; and (e) the immunoglobulins react with the antigens on the surface of the retinal ganglion cells causing the immunooablation of the ganglion cells and visual dysfunction.

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