Examination of the number of plasma cells in lymph node and immunoglobulin levels in patient's serum

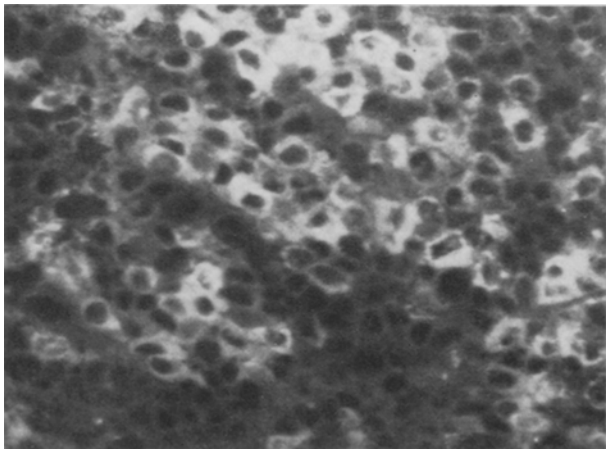
Ig	CDI[a] in lymph-node		Serum immunoglobulin levels in International Units	
	Examined case	13 cases of other colon cancer (mean values)	Examined case	Blood donors values
IgG	8.92	0.43	88	149 ± 43
IgA	0.47	0.45	68	139 ± 51
IgM	0.10	0.90	206	152 ± 48
IgE	0.33	0.06	n.t.	n.t.
K:L ratio	31:1	2.5:1	n.t.	n.t.

CDI, cell density index; n.t., not tested.

described the kappa: lambda-light chain ratio was 31:1, while in other cancer lymph nodes it did not exceed 2.5:1. Localization of kappa specific cells (Figure) corresponded closely to those of IgG and strongly favored the view that these IgG producing cells arose from single clone.

The lymph node contained a small metastatic foci, which did not change its structure. Germinal centres of lymphatic nodules were very few and inconspicious.

The patient displayed hypoproteinemia (total protein 3,4 g/100 ml). Quantitative serum immunoglobulin determinations revealed a decrease of IgG and IgA and an increase of IgM (Table). Both, paper electrophoresis and immunoelectrophoresis did not show monoclonal protein in serum.



Regional lymph node of cancer of the rectum. Specific staining of plasma cells with anti-human kappa antiserum labelled FITC. ×960.

These data show that regional lymph nodes in cancer may be a site of monoclonal Ig synthesis and that this phenomenon may not be detectable in the patient's serum.

The observed hypoproteinemia could be due to the loss of protein via the GI tract, a possibility suggested for cancer of the GI tract by JONES[4]. The failure to observe a monoclonal spike in the serum cannot be explained at this time but maybe due to the amount of IgG produced and the fact that it is produced focally.

The cause of local monoclonal immunoglobulin production and whether this Ig possesses anti-tumor antibody specificity remains unknown. Some observations hint however, that there exists a link between monoclonal immunoglobulin synthesis in the lymph node and an existing tumor. The absence of distinct inflammation in the immediate vicinity of the primary cancer site and the existence of metastatic foci as a source of antigenic material in lymph node, suggest this possibility. The described case indicates that monoclonal immunoglobulin production not related to general plasma cell dyscrasia may be manifested only on the local level.

Résumé. La technique directe de l'immunofluorescence a permis de mettre en évidence dans un ganglion lymphatique adjacent à la tumeur cancéreuse du rectum la présence de nombreuses cellules sécrétant de IgG monoclonal.

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[6] This work was supported by a grant No. 09.3.2. of the Polish Academy of Sciences.

Localizing Properties of Anti-Nervous Tissue Antibodies in Rat Cervical Ganglion

Since the demonstration that a rabbit anti-cat caudate nucleus serum affects the bioelectrical activity of caudate nucleus of the cat brain[1], the anti-nervous tissue antibody has become a valuable tool in structural and functional studies of the brain and the neuron[2]. The immunological investigations of the neuron concern, inter alia, the antigenic definition of neuronal components, and the mapping of antigens in the neuron and its microenvironment. The present report deals with the latter subject

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and describes the localization of rabbit anti-rat nervous tissue antibodies in the superior cervical ganglion.

Materials and methods. The cervical ganglia of Wistar rats were freed from connective tissue under a stereomicroscope, and then extirpated, homogenized in 5 vol (w/v) of distilled water and centrifuged at $5{,}000 \times g$ for 10 min. The supernatant fluid, which contained most of cytoplasmic materials, was discarded and the sediment resuspended in distilled water and lyophilized. The method of DE ROBERTIS[3] was employed for the isolation of the synaptic vesicles from brains of rats perfused with saline. The synaptic vesicles situated in the 'M_2A' layer of sucrose gradient after centrifugation at $50{,}000 \times g$ for 1 h in a Spinco ultracentrifuge were examined by means of a JEM-6C electron microscope[4]. The analysis of the 'M_2A' preparation indicated a homogenous fraction of synaptic vesicles slightly contaminated with synaptic plasma membranes[5].

Lyophilized cervical ganglion preparation and brain synaptic vesicle fraction were dissolved in saline and mixed with complete Freund's adjuvant. Each immunizing injection contained 18 mg of corresponding antigen and was administered s.c. into the toe-pads of hind legs of chinchilla rabbits. The second injection was given 1 month later, and serum samples were obtained 3 weeks after booster. Rabbit antisera were absorbed with rat serum, erythrocytes, liver and kidney to remove antibodies directed towards common organ antigens[6]. The globulin fraction was isolated with ammonium sulphate[7], and the protein concentration determined by a biuret reaction[8]. Antisera and antigens were tested by double diffusion[9] in 0.8% agarose at pH 8.4, and complement fixation reaction[10] using five 50% haemolytic units of complement.

The paraffin sections[11] of the rat superior cervical ganglion were examined by a standard immunofluorescence procedure[12] using a suitable dilution of fluorescein isothiocyanate-conjugated sheep anti-rabbit IgG. The following controls were included: sections treated with conjugate alone; sections exposed to anti-ganglion or anti-vesicle globulin previously absorbed with corresponding antigens; sections treated with specific antisera and then with unconjugated anti-rabbit IgG prior to staining with conjugated anti-IgG (blocking test); and sections exposed to normal rabbit serum and then to fluorescein conjugate. In addition, sections of rat liver and kidney treated with anti-nervous tissue sera and fluorescing reagent were also used as controls. The preparations were examined under a Leitz Orthoplan fluorescence microscope.

Results and discussion. Immunodiffusion assays of the anti-ganglion serum showed 3 well developed precipitin lines with ganglion antigen, and 1 faint line with the synaptic vesicle preparation. On the other hand, the anti-vesicle serum produced 2 precipitin bands with the corresponding antigen, and 1 band with ganglion homogenate. In the complement fixation reaction, the $\log_2$ titers of antibodies against corresponding antigens ranged

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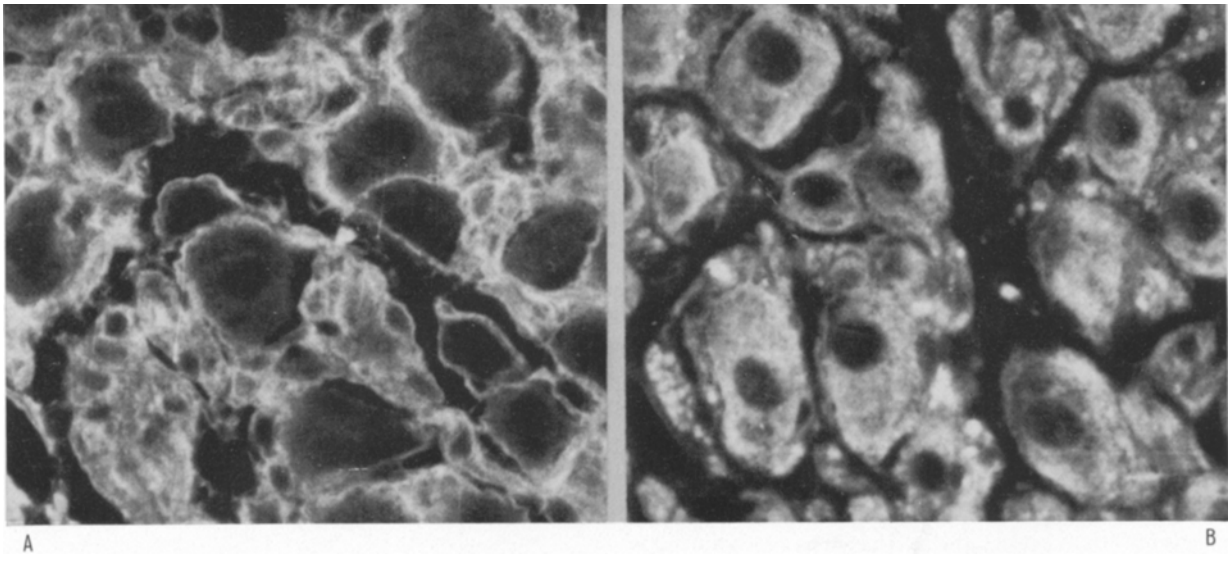

Fluorescence microphotographs (×440) of paraffin sections of the rat superior cervical ganglion. A) Section treated with anti-ganglion serum. The bright areas represent the intense staining of membranes and fibres. Negative shadows of neuronal cytoplasm and nuclei are visible within the fluorescent mass. B) Section exposed to anti-brain synaptic vesicle serum. The fluorescent material is accumulated in the whole cytoplasm. The nuclei, membranes and fibres are unstained.

between 9 and 11. A titer of 6 was observed when anti-vesicle serum was tested against ganglion. However, the anti-ganglion serum did not produce positive reactions with synaptic vesicles. These results would imply that the central nervous system shares some antigens with the peripheral nervous system[13,14].

The immunofluorescence analysis revealed different histological lacolizations of antibodies from anti-ganglion and anti-vesicle sera. Namely, the anti-ganglion serum reacted almost exclusively with cell membranes and the thick network of nerve fibres of the ganglion (Figure A), whereas the anti-vesicle serum combined preferentially with antigens situated within the neuron (Figure B). Large, round to oval nuclei, usually excentrically placed, remained unstained in all examined sections. The staining of ganglion sections was inhibited by absorption of antisera with corresponding antigens, thus pointing to the presence of organ-specific antigens in the preparations used for immunization. No staining was observed in control tests. A number of ganglion sections treated with anti-vesicle serum exhibited a faint fluorescence of inter-neuronal particulates other than blood vessels. This was probably due to the contamination of the synaptic vesicle preparation with other antigens[4,15]. Besides, antibodies other than those reacting with synaptic vesicles, e.g. anti-tubulin antibodies[16], might be contributing to the staining. This aspect of cross-reactivity between different neuronal components is now under investigation. Relevant to the problem of antibody specificity is the observation that a rabbit anti-nerve ending membrane serum affects the axoplasm and synaptic vesicles[17]. The present experiment does not establish evidence with respect to the antigenic similarity between brain synaptic vesicles and cervical ganglion antigens, and a variety of rat brain specific antigens[18–20].

In summing up, the immunofluorescence analysis described here provides some basic information about the localizing properties of antibodies from anti-cervical ganglion and anti-brain synaptic vesicle sera, and thus justifies the use of those immune reagents in a functional study of the rat superior cervical ganglion[21].

Résumé. On a démontré que les anticorps contre les vésicules synaptiques du cerveau du rat se fixent de préférence dans le cytoplasme des neurones, tandis que les anticorps contre les ganglions sympathiques cervicaux supérieurs réagissent presqu'exclusivement avec les membranes des neurones du ganglion.

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Ovarian Cyst Fluid Specific Antigens

The presence of ovarian cyst specific antigens have been detected earlier[1,2]. A preliminary report of our preparation and partial characterization of the antigens have shown that they are glycoprotein containing 12.3% fucose (FRGP)[2]. Furthermore, anti-FRGP antibody reacted with an extract of human spleen. Several reports have presented evidence of antigens with organ specificity for human heart[3,4], liver[5], and basement membrane[6,7]. Renal glomerular basement membrane glycoproteins shared their antigenecities with basement-membrane-rich organs such as lung, placenta and urinary glycoprotein[7]. Limited studies of ovarian cyst content were performed using immunological procedures. The present communication describes the chemical and immunological properties of FRGP of ovarian cyst origin.

Materials and methods. Approximately 200 l of ovarian cyst fluid from a patient with ovarian cystoma were collected and filtered. After 10 l of the fluid were mixed with 3.62 kg of solid ammonium sulfate and 30 ml of concentrated hydrochloric acid to pH 4.3, the mixture was allowed to stabilize for 20 h at 4 °C. The centrifuged supernate was acidified with concentrated hydrochloric acid to pH 3.7, and then 2.31 kg of ammonium sulfate were added for 100% saturation. After a 20 h period at 4 °C, the precipitated crude glycoprotein (CGP) was recovered by centrifugation, dialyzed and lyophilized. Ethanol was added to a 2% solution of CGP in stepwise fashion to give concentrations of 38%, 66% and 80% (v/v) at 4 °C. A glycoprotein fraction obtained at 38–66% (v/v) ethanol concentration (first stage) was refractionated using the ethanol procedure described above. The precipitate formed at 38–66% (v/v) ethanol concentrations was centrifuged at 24,000 *g* for 80 min at 4 °C. The supernatant (FRGP) was lyophilized. Frozen post-mortem tissues from pulmonary carcinoma patients were thawed and homogenized in cold distilled water. Soluble constituents were collected by centrifugation and lyophilized. Approximately 100 mg of the distilled water extract were dissolved in 30 ml of distilled water and subjected to 'first stage' ethanol fractionation. Pseudomucins were a gift of Dr. Y. Nagai, Fukushima Medical College. Rabbit antisera against FRGP were prepared by repeated injections of the antigen with Freund's complete adjuvant (Difco). The antisera were absorbed with lyophilized pooled normal human serum[8]. Immunological analyses

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