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DEPARTMENT OF PATHOPHYSIOLOGY

GENERAL SUMMARY

The large amount of serological data about organ-specific antigens has no chemical foundation to support the detailed analysis of specificities. Our understanding of the nature of the organ-specific antigen probably depends on the purification of the antigen. We have therefore embarked on a major effort in this direction.

Two general types of organ-specific antigens can be distinguished. The first type is soluble cell products such as hormones, albumin and globulins, or those which by a simple disruption technique are easily released from cells in a soluble form. The second type of organ-specific antigens are those which are either part of, or are intimately associated with, the insoluble membraneous structure of cells. This type of antigen seems to play a more important role than the first type in the pathogenesis of various diseases of autoimmune nature. However, methods for purification and characterization of this type of organ-specific antigen are few and arbitrary.

We demonstrated that the fraction (designated as LPfr 2) prepared from the cellular insoluble lipoprotein fractions of various organs by solubilization with desoxycholate and by chromatography on Sepharose 4B contained the organ-specific antigen with or without common antigens. The LPfr 2 preparations from the thyroid gland and the testis were substantially homogeneous as judged by the criteria of polyacrylamide gel electrophoresis and of gel-diffusion precipitin analysis. However, the similar fractions from many other organs were not homogeneous and contained the antigen which was organ-specific as well as several antigens which were shared by different organs, especially the liver and spleen. Our effort was subsequently directed to the isolation of the organ-specific antigen from these fractions. The adrenal-specific antigenic fraction (termed AdA) was isolated by sumbitting the LPfr 2 preparation of the adrenal gland to affinity chromatography on the CNBr-activated Sepharose 4B which linked with the globulin of the potent antisera to the cellular insoluble fractions of the liver and the spleen. This procedure promised to be a good general method for selective extraction of the organ-specific antigen from the cellular insoluble lipoprotein fractions of various organs.

The short rod-like particle demonstrated by electron microscopy as the principal substance of the LPfr 2 preparation from human thyroid glands appears to be similar to those of bovine AdA and to the LPfr 2 from bovine testis. All these particles had an apparent molecular weight of approximately 47,000 and were 3-4 percent lipids. Analysis by thin-layer chromatography indicated that the lipids consisted mainly of globoside I. The infrared spectrum and circular dichroism spectrum showed the particle to contain α-helix, β-configuration and disordered coil.

The determination of chlorination rate with *tert*-butyl hypochlorite showed that the disordered coil did not exceed more than 20 percent. Degradative studies emphasized the importance of the protein component in the organ-specific antigenicity of these fractions.

The actual presence of the organ-specific antigen in plasma membrane, mitochondrial outer membrane, endoplasmic reticulum and nuclear membrane was shown by peroxidase-antibody techniques using a monospecific antibody. The organ-specific antigens were obtained only after prolonged stirring of the cellular insoluble lipoprotein fractions with desoxycholate and thus presumably represents an intrinsic constituent of cellular membranes.

The structural analysis of molecules as large as the AdA or the LPfr 2 of the thyroid gland and the testis must begin with efforts to isolate smaller portions of the molecules which retain the antigenic activity. The LPfr 2 delipidated by a mixture of ether-ethanol was subjected to prolonged reduction with β-mercaptoethanol at about 20C for 7 days or more and chromatographed after alkylation or without alkylation. The subunit fraction which retained the organ-specific antigenicity was obtained. But, the fraction was not homogeneous on the disc electrophoregram. The isolation of the subunit which has only the organ-specificity is now under investigation.

Repeated hetero- or isoimmunization of guinea pigs with the organ-specific antigen incorporated in Freund complete adjuvant produced not only autoantibodies but some functional and histopathological lesions in the corresponding organ. Some of the lesions were transferred to other guinea pigs by passive transfer of antisera.

The isolation of tumor-specific antigens locating on cellular membranes is now in progress.

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ABSTRACT

(36) Immunological studies of insoluble lipoproteins: Characterization of the lipoprotein-bound thyroid gland-specific antigen.
S. Okada, Y. kurata, K. Konishi and T. Matsuda

The organ-specific lipoprotein fraction which appeared to be substantially homogeneous by electrophoretic criteria and yielded a single thyroid gland-specific line of precipitation in agar with antisera was obtained from the cellular insoluble lipoprotein preparations of human thyroid glands by solubilization with desoxycholate and gel filtration on Sepharose 4B. The electron micrography showed the fraction to consist of short rod-like particles (ca. 80×40Å) (Fig.). Degradative studies did not include lipid and carbohydrate from this role in the precipitating activity and they emphasized the importance of the protein

component, especially free amino groups, disulfide bonds and tyrosine residues of the antigen.

The antibody against this antigen was sensitively demonstrated in the sera of patients with chronic thyroiditis and also in the sera of immunized guinea pigs by tanned red cell hemagglutination technique.

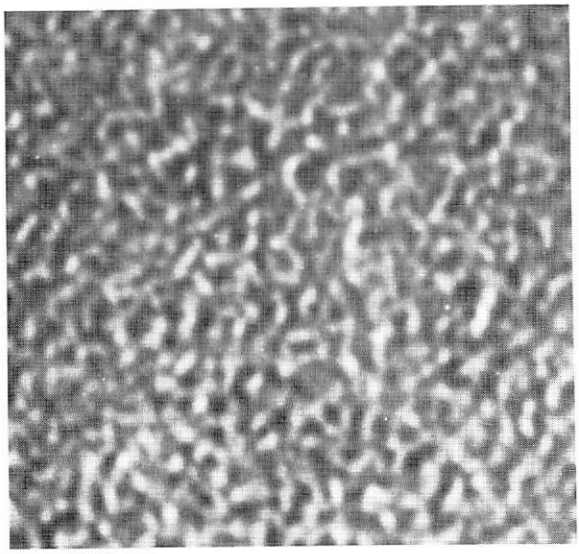

Fig. Electron micrograph of human thyroid gland LPfr 2. Negative staining with uranyl acetate (Magnif. 440,000).

(37) **On the liver-specific antigen locating on the cellular membranes.**

K. Konishi

The fraction extracted from the cellular insoluble lipoprotein fraction of the liver by solubilization with desoxycholate contained a liver-specific antigen, a liver-characteristic antigen and four to five antigens which were shared by many different organs. The liver-specific antigen migrated in the α-region on the immunoelectrophoregram (Fig.). This antigen was isolated by QAE-Sephadex column chromatography after treatment of the fraction with the globulin fraction of antisera against the insoluble lipoprotein fraction of the spleen. Short rod-like particles were demonstrated by electron microscopy as the principal substance of this fraction. The liver-specific antigen was located on the plasma membrane and intracellular membranes of the hepatic cells.

Some guinea pigs immunized repeatedly for 7 months with the fraction of bovine liver incorporated in Freund complete adjuvant developed piecemeal necrosis and slight fatty degeneration of the liver. The cytotoxicity of the liver-specific antisera was observed using Cr^{51}-labeled hepatic cells.

The loss or decrease of the liver-specific antigen was found in the cellular insoluble lipoprotein fractions of the cirrhotic livers.

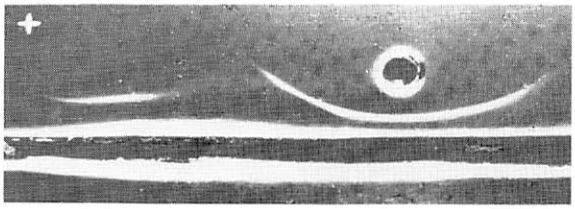

Fig. Immunoelectrophoresis. Left arc (α-globulin region) - a liver-specific antigen, Right arc (β-globulinregion) - a liver-characteristic antigen.

(38) A testis-specific antigen locating on cellular membranes.

M. Gokura

A testis-specific antigenic fraction which appeared to be homogeneous and contained rod-like particles (M. W. = ca. 48,000) was isolated from the cellular insoluble lipoprotein fraction of the testis. Peroxidase-antibody technique showed that the antigen was located on the cellular membrane of germinal cells (Fig. 1). Guinea pigs repeatedly injected with the fraction incorporated in Freund complete adjuvant developed circulating antibodies, delayed hypersensitivity and testicular lesions (Fig. 2). The antisera showed the sperm-agglutinating activity and the sperm-immobilizing activity in the presence of complement. Passive transfer of antisera produced testicular lesions in some recipeints.

Nine sera of 19 infertile men showed a precipitation line in agar with the LPfr 2 of human testis while 10 control sera were negetive.

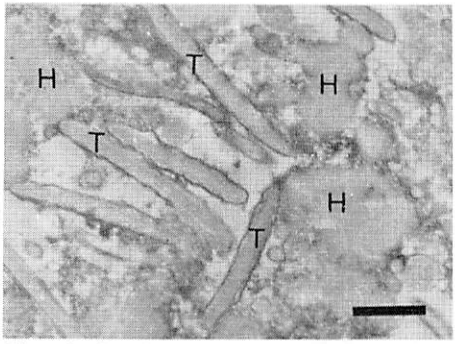

Fig. 1. Bovine spermatocytes stained with peroxidase-antibody technique. H - Head. T - Tail. Bar - 1 μ

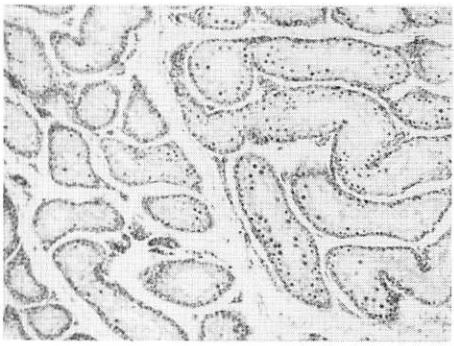

Fig. 2. Testis of the guinea pig immunized with the LPfr 2 of bovine testis (1 mg/injection, 5 times).

(39) On the tissue-specific antigens of thymus and skeletal muscle.

M. Mitsumata

The antigenic fraction (LPfr 2) which contained the organ-specific antigen as well as several common antigens was extracted from the cellular insoluble lipoprotein fraction of thymus and skeletal muscle, respectively. It was shown that hetero- and isoimmunization of guinea pigs with these antigens in Freund complete adjuvant were successful in producing the positive "waning" reaction on the electromyogram (Fig.) and circulating antibodies with no marked histopathological changes of

thymus and skeletal muscles. The antisera showed cytotoxicity to the thymus cells. A thymus-specific and a skeletal muscle-specific antigen which resided on the plasma membrane and intracellular membrane system of thymus cells and muscle cells, respectively, were demonstrated by the peroxidase-antibody technique.

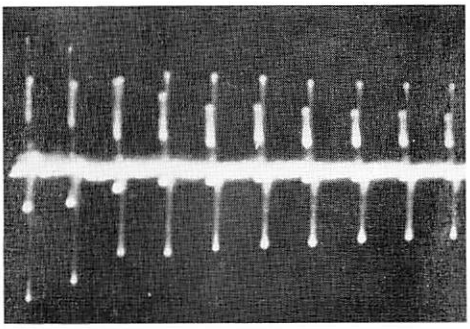

Fig. Waning reaction (+)
A guinea pig immunized with the LPfr 2 of bovine muscle (6 mg/injection, 5 times, 6 weeks).

(40) **Studies on the purification of an adrenal-specific membrane-bound antigen and its properties.**
M. Kamimoto

A lipoprotein fraction (LPfr 2) was isolated from cellular insoluble lipoprotein fraction of bovine adrenal glands by tratment with desoxycholate and gel filtration through Sepharose 4B. This fraction contained an adrenal gland-specific antigen and several common antigens. The common antigens were removed by affinity chromatography using antisera against the insoluble lipoprotein fractions of bovine liver and spleen. The identical adrenal gland-specific antigen was obtained from the cortex and the medulla of adrenal glands.

The electron micrograph showed the purified fraction (termed AdA) to consist of short rod-like particles. The particle contained about 4.4 per cent lipids (mainly globoside I). The molecular weight of the particle was determined to be 46,000 - 47,000. The circular dichroism spectra and the infrared absorption spectra showed the particle to contain α-helix, β-configuration and disordered coil. Chlorination of the particle with *tert*-butylhypochlorite suggested that the random coiling to be less than 20 per cent. The subunit fraction (M. W. = 3,550) was obtained by prolonged reduction of delipidated AdA. This fraction showed 10 or more components on disc electrophoregram.

Guinea pigs injected with bovine LPfr 2 or AdA preparations incorporated in Freund complete adjuvant developed a positive Thorn test, circulating autoantibody and adrenal lesions. But, the difference between histological lesions in these animals and those in adjuvant control animals was not significant. Intraveneous administration of sera from guinea pigs sensitized with LPfr 2 or AdA into normal guinea pigs gave a positive Thorn test and severer adrenal lesions than those of guinea pigs injected with adjuvant control sera.