

A Normal Organ Antigen and Its Corresponding Antibodies in Pathological Human Sera

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Key Words

Normal organ antigen · Pathological human sera · Wassermann antibodies · Bovine organ extract

Abstract

Previous studies on the appearance in pathological human sera of antibodies to an antigen of normal mammalian organs were continued. In gel precipitation reactions, antibodies combining with saline extracts of mammalian organs were found in 20 of 63 cancer sera and in 4 of 15 syphilis sera, but only in 3 of 56 other pathological sera. Furthermore, an identical antigen was demonstrated in 5 of 58 pathological sera. The antigen under study did not belong to any group of known heterophile antigens and it was devoid of organ and tissue specificity.

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Introduction

The idea that, under pathological conditions, autoantibodies are formed to intracellular antigens of normal organs was conceived in the 1910s in connection with the observation that in the Wassermann reaction antibodies do not react with microbial antigens (as originally be-

lieved) but with normal tissue antigen(s) [for bibliography up to 1930, see 1].

Starting with pioneering studies performed in 1950s by Witebsky and Rose [2] on experimental production of autoantibodies to thyroglobulin and subsequent demonstration of thyroglobulin antibodies in the Hashimoto disease [3], autoantibodies to a host of tissue antigens were described in patients with various pathological conditions including, importantly for our present study, cancer (this was recently reviewed extensively by Tan and Shi [4]).

In parallel with demonstration of antibodies to tissue antigens, studies were devoted to detection of such antigens in the circulation of patients with a variety of diseases, first of all cancer. These studies were crowned by successful demonstration of carcinoembryonic antigen in sera of patients suffering from colonic carcinoma and other malignancies [5]. Subsequently, many studies were devoted to the detection in the circulation of cancer patients of tumor-associated antigens, i.e. normal tissue antigens, released in increased quantities from tumors to circulation as well as tumor-specific antigens, i.e. antigens characteristic for tumors [for reviews, see 6, 7].

For several decades the senior author of this paper and his associates have been interested in studies of formation of autoantibodies to normal and altered organ antigens [for review, see 8]. Pertinent to the present study is a paper published in 1987 [9] that describes antibodies

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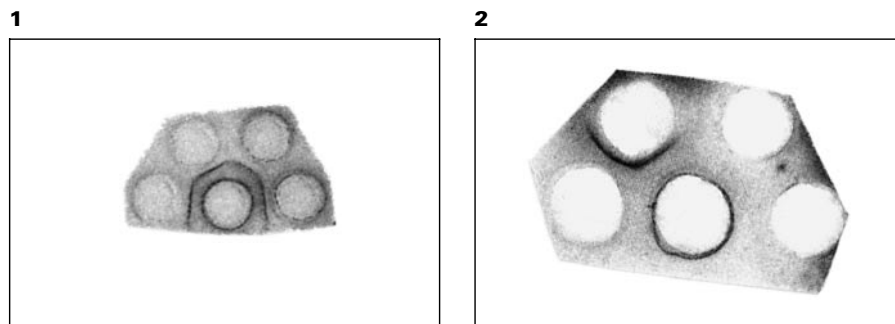
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Fig. 1. Central well: cancer serum St. Peripheral wells: 30% bovine organ extracts, clockwise from lower left well: kidney, liver, liver, kidney.

Fig. 2. Lower wells: cancer sera; Mal (left and right) and St (center). Upper wells: rabbit kidney extract, unaltered (left) and heated 15 min at 62°C (right).



reacting with saline extracts of mammalian organs, which were encountered in sera of patients with end-stage renal diseases. These antibodies detected by gel precipitation and complement fixation tests showed no significant species or organ specificity. In the present study, we continued examining reactions of various pathological human sera with mammalian organ extracts and, importantly, we found that the organ antigen under investigation could be detected in some such sera. For the preliminary studies to be reported here we employed mostly bovine organ extracts.

Materials and Methods

Animal organs were obtained from a local slaughterhouse. Saline extracts were prepared from a 30% suspension of homogenized organs. The procedures described by Martin et al. [9] were followed. Human sera originated from patients treated in the area hospitals. They were accumulated over a period of four decades and preserved at -20°C; many of them were used in previous studies of this laboratory.

Double diffusion gel precipitation tests were performed in 0.6% agarose gel in Petri dishes. Plates with charged wells were tightly closed and placed in the refrigerator at 4°C. Readings were taken after 24–72 h. To take pictures, plates were soaked for a few days in saline and for another few days in distilled water. The plates were then left at 37°C for 1–2 days and the dried agarose film was stained with amido black and 'destained' with acidic acid-methanol solution. Then the agarose film was removed, scanned into a computer and prints were obtained.

Results

As a standard antigen we employed a 30% saline extract of bovine liver. Against this extract we screened sera of 63 patients with cancer of the intestinal tract and obtained positive results with 20 sera. Figure 1 shows the reactions obtained with the serum St and two extracts of each, bovine kidney and bovine liver. It may be seen that

the reactions of complete identity were formed. Usually, however, kidney extracts had to be used at higher concentration than liver extracts in order to produce reactions of a comparable strength. The antigen under study was thermolabile in that it was completely destroyed by heating the extract at 62°C for 15 min. This is illustrated in figure 2 (note that in this particular experiment an extract of a rabbit rather than bovine origin was used). Reaction lines produced by individual cancer sera merged into reactions of complete identity, as illustrated in figure 2.

Screening 71 sera from diseases other than cancer detected antibodies identical with those in serum St in 7 sera; they appeared in significant frequency, 4 out of 15, in sera of patients with syphilis.

Subsequently we screened 58 pathological sera for the presence of the organ antigen under study. Reagents used for this screening were sera St and Mal. Positive results were obtained only with 2 of 11 Alzheimer disease sera, 1 of 12 rheumatoid arthritis sera, and 2 of 12 infectious mononucleosis sera. The remaining 53 were all negative. The antigen-containing sera produced identity reactions with each other as well as with the antigen present in organ extracts. This is illustrated in figures 3 and 4.

Consistently negative results were obtained when sera positive for the antigen in the present study were tested against infectious mononucleosis sera. This indicated that the organ antigen in the present study was unrelated to the antigens of the heterophile group which were found previously in some pathological sera [10–12].

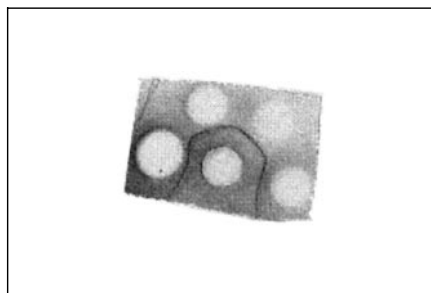
Discussion

The reactions of pathological human sera with extracts of mammalian organs described in the previous paper [9] and in this publication resemble closely the reactions of syphilis sera observed in the early era of syphilis serology [1]. Significantly, the antibodies described in the present

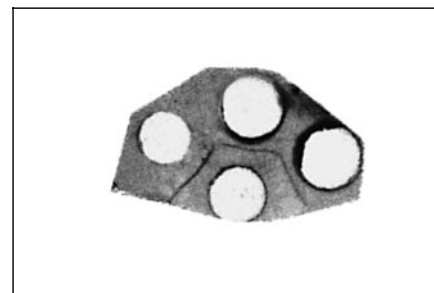
Fig. 3. Lower central well: cancer serum St. Lower left and right wells: Alzheimer disease serum Karp. Upper left well: bovine liver extract. Upper right well: Alzheimer disease serum Par.

Fig. 4. Lower well: cancer serum St. Left well: Alzheimer disease serum Karp. Upper well: Alzheimer disease serum Par. Right well: infectious mononucleosis serum Bret.

3



4



study were found in 20 of 63 patients with cancer, a disease in which 'false positive' results were frequently found when organ extracts were used as antigens for the Wassermann reaction. Also from 15 syphilis sera tested, 4 had these antibodies.

We believe that antibodies studied by us were akin to Wassermann antibodies and that their formation was elicited by a ubiquitous tissue antigen devoid of organ and tissue specificity, which had been released from pathological tissue in immunogenic form. Similar explanations for formation of Wassermann antibodies were proposed in the 1910s [1]. They were supported by experiments of Sachs et al. [13] who succeeded to produce, in rabbits, Wassermann-like antibodies by immunization with extracts of normal organs.

Detection of a free organ antigen in pathological human sera is of interest in view that such antigen was never demonstrated in syphilis. (However, in the previous study from our laboratory we obtained evidence that about 20% of serologically positive syphilis sera contained circulating immune complexes which might have contained such an antigen [13].)

Certainly it is highly unlikely that the antigen observed in this study was related to any known tumor-associated or tumor-specific antigen. The demonstration of a normal organ antigen in the circulation of patients with such diverse maladies as Alzheimer's disease, infectious mononucleosis and rheumatoid arthritis is of considerable interest and it may be of clinical significance if a more sensitive procedure (e.g. radioimmunoassay) is employed, which would permit the detection of the antigen under study in a larger number of sera.

Acknowledgement

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Appendix

Facts about Felix Milgrom at His 85th Birthday

Felix Milgrom was born in Rohatyn, Poland, on October 12, 1919. He obtained his MD degree from the University of Wrocław, Poland, in 1947 on the basis of a dissertation on serological mass examinations for syphilis. In 1951 he obtained the title of Privatdozent on the basis of work on the structure of natural antibodies. He was a faculty member at the School of Medicine, University of Wrocław, from 1946 to 1954, advancing from instructor to professor where he was associated with Ludwik Hirsztfeld, one of the creators of the field of immunogenetics. After Hirsztfeld's death in March 1954, Dr. Milgrom was appointed acting chairman of the Department of Microbiology and director of the Institute of Immunology and Experimental Therapy of the Polish Academy of Science (now Ludwik Hirsztfeld Institute). Following the reorganization of Hirsztfeld's school by the Polish communist regime, Dr. Milgrom was appointed professor and chairman of the Department of Microbiology of the School of Medicine in Zabrze where he stayed from 1954 to 1957.

In the summer of 1957, Dr. Milgrom left Poland with an invitation from Ernest Witebsky to join his department in Buffalo, New York. En route to the United States, Dr. Milgrom was employed as a research associate by Pierre Grabar at the Pasteur Institute in Paris. Dr. Milgrom started his work in Buffalo in April 1958 as a research fellow and again went through all the steps of an academic career, being appointed professor of microbiology in 1964, and chairman of the department in 1967. He chaired this department until 1985. In 1995, Dr. Milgrom retired from a salaried position but still carries his title of Distinguished Professor of the State University of New York and continues his research.

Dr. Milgrom is author or co-author of over 400 research papers and he has edited six books. He was editor-in-chief of *International Archives Allergy and Applied Immunology* from 1965 to 1991. He has honorary doctorate degrees awarded by the Universities of Vienna, Lund, Heidelberg, Bergen and New Jersey. He was the recipient of the Paul-Ehrlich-und-Ludwig-Darmstädter-Preis in 1987. He had over 100 students who are now working in various areas of immunology throughout the world.

Major Research Achievements

Studies on the structure of antibodies brought evidence for the existence of multispecificity of antibodies by showing that one antibody molecule may carry separate sites for two or more different antigens. Later studies demonstrated that the rheumatoid factor is a multispecific antibody.

Studies on antigenicity of antibodies led to: (1) description of 'anti-antibody' (Milgrom factor), an IgM serum factor which reacts with IgG antibody bound with its corresponding antigen – this provided evidence for molecular transformation of antibodies in the course of serological reaction; (2) production in rabbits of antibodies recognizing the antigenic configuration of the antibody combining site (later called idiotypic specificity), and (3) experimental production in rabbits of rheumatoid-like factor providing evidence that the rheumatoid factor is an antibody produced in response to antigen antibody reactions occurring *in vivo*.

Studies on transplantation led to the demonstration that hyperacute rejection of renal allografts is affected by humoral transplantation antibodies. This enabled application of pre-transplantation crossmatch that practically eliminated hyperacute rejection.

Studies on organ and tissue specificity led to discovery of the category of organ antigens of glycoprotein nature referred to as BE antigens for their resistance to boiling and ethanol insolubility.

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