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HANGANUTZIU-DEICHER ANTIGEN AND ANTIBODY IN PATHOLOGIC SERA AND TISSUES[1]

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Heterophile, Hanganutziu-Deicher (H-D) antigen was studied in pathologic sera by means of inhibition of agglutination of bovine erythrocytes by H-D antibodies. H-D antigen was demonstrated in 38% of random cancer sera, 25% of lymphoma or leukemia sera, 25% of leprosy sera, 8% of infectious mononucleosis sera, 6% of rheumatoid arthritis sera, and 27% of synovial fluids of rheumatoid arthritis patients. None of 134 normal human sera gave positive results. Some of the inhibition-positive cancer sera formed precipitation lines with H-D antibody-containing sera. Over 50% of various extracts of cancer tissues as well as spleens of lymphoma or leukemia patients were shown to contain H-D antigen by means of the inhibition test.

More than half a century ago, Hanganutziu (1) and Deicher (2) described "serum-sickness antibodies" that combined with sheep (SRBC) and bovine erythrocytes (BRBC)[3] and that were formed as a result of injections of human patients with foreign species sera such as horse antisera to diphtheria or tetanus toxin. Our previous studies (3) have shown that heterophile antibodies of this type were found, although infrequently, in sera of patients with various diseases who never received any injections of foreign species sera. Therefore, we used the term Hanganutziu-Deicher (H-D) antibodies and abandoned the misnomer "serum-sickness antibodies". More recently, we found H-D antibodies of high titers (4) and complexes composed of H-D antigen and antibodies (5) in some rheumatoid arthritis sera (RAS).

Our previous studies (6–9) showed that spleens of patients with lymphomas and leukemias frequently contained Paul-Bunnell (P-B) antigen detectable by reactions with infectious mononucleosis (IM) sera. We have also found H-D antigen in some of these tissues (7). More recently, it was demonstrated that P-B antigen is present in the circulation of patients with malignancies and rheumatoid arthritis as well as in extracts of malignant tissues (10).

In the present study, the appearance of H-D antigen and H-D antibodies in sera of patients with various malignancies and RAS was investigated. Attempts were also made to identify H-D antigen in extracts of various malignant tissues.

MATERIALS AND METHODS

Human sera. Sera of patients with various malignant diseases were gifts of several clinical colleagues from Buffalo and other areas in this county. RAS and synovial fluids (RASF) were obtained at the Veteran's Administration Hospital, Buffalo, N. Y. and E. J. Meyer Memorial Hospital, Buffalo, N. Y. Sera of patients with leprosy were kindly supplied by Dr. J. Convit of the National Institute of Dermatology, Caracas, Venezuela. Sera of patients with IM were kindly supplied by Dr. L. M. Musselman of the Student Health Service of this university. Sera of blood donors were kindly supplied by Dr. R. M. Lambert of the Buffalo Red Cross Center, Buffalo, N. Y. Normal human sera were obtained from apparently healthy staff members of this department. All human sera were stored at −20°C and were heat inactivated at 56°C for 30 min before use.

Animal sera. Bovine sera obtained at a local slaughterhouse were heat inactivated at 56°C for 30 min before use.

Human and animal RBC. Human RBC of blood group A or B as well as BRBC and SRBC were kept in acid citrate dextrose solution at 4°C for no longer than 2 weeks.

Human tissues. Human spleens were removed from patients with lymphomas or leukemias at surgery in the Roswell Park Memorial Institute, Buffalo, N. Y. Cancer and normal tissues were obtained at autopsy in the Buffalo General Hospital, Buffalo, N. Y.

Bovine spleens were obtained at a local slaughterhouse.

Purified H-D and P-B antigens. These were kindly supplied by Dr. J. M. Merrick of this department. The details of the procedures used for the preparation of these antigens were published elsewhere (11, 12).

Tissue extracts. Perchloric acid extracts (PAE) were prepared according to the technique of Krupey *et al.* (13) with a modification described in (14). These extracts were used in our previous studies on carcinoembryonic antigen (14).

Crude saline extracts (CSE) were prepared by mincing and homogenizing tissues by means of a Waring Blendor for 10 min in 2 volumes of 0.15 M saline. The homogenate was then centrifuged at 35,000 × G for 30 min. The supernatant was recovered and heated at 100°C for 60 min. After centrifugation at 35,000 × G for 30 min, the second supernatant, CSE, was recovered and lyophilized.

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[3] Abbreviations used in this paper: BRBC, bovine erythrocytes; H-D, Hanganutziu-Deicher; RAS, rheumatoid arthritis sera; P-B, Paul-Bunnell; IM, infectious mononucleosis; RASF, rheumatoid arthritis synovial fluids; PAE, perchloric acid extracts; CSE, crude saline extracts; CLL, chronic lymphocytic leukemia; HSA, human serum albumin; NGNA, *N*-glycolyl neuramic acid; UTA, ubiquitous tissue antigen; CEA, carcinoembryonic antigen.

Inhibition of agglutination of trypsinized BRBC. BRBC were washed three times with phosphate-buffered saline, pH 7.2 (PBS) and resuspended at a concentration of 2% (v/v). An equal volume of 2% (w/v) solution of trypsin (Trypsin 1-300, ICN Pharmaceuticals, Inc., Life Science Group, Cleveland, Ohio) in PBS was added to the cell suspension and the mixture was incubated for 30 min at 37°C. The treated BRBC were then washed six times with cold PBS and resuspended at a concentration of 1% in PBS.

Standard, H-D-antibody-containing serum 185 from the previous study (5) at a dilution corresponding to four agglutinating units was used for the inhibition test. This serum and the specimen to be tested (preabsorbed with untreated BRBC) were mixed each at 50 μl volume and the mixture was left for 2 hr at 20°C. Thereafter, 100 μl of a 1% suspension of trypsinized BRBC were added to the mixture. The tubes were left for another 2 hr at 20°C and were centrifuged at 1500 rpm for 2 min. The agglutination was then examined after shaking the tubes gently.

Double diffusion gel precipitation test. Agarose (SeaKem, Marine Colloids, Inc., Springfield, N. J.) was dissolved in saline at 100°C to give a 0.5% (w/v) solution; 4 ml of such solution were poured into a plastic Petri dish (Falcon Plastics, Culver City, Calif.). After the agarose solidified, circular wells, 5 mm in diameter, were cut at an edge-to-edge distance of 2.5 mm. Wells were filled with tested specimens and the Petri dishes, closed by cover lids, were left for 3 days at 4°C. Thereafter, pictures were taken.

Cytolysis in agar gel. The test was performed as described previously (15).

Absorption test. Pooled guinea pig tissues were homogenized, boiled, and centrifuged. The sediments were washed three times with PBS and used for absorption. Sediments of washed BRBC, SRBC, or human RBC of group A or B were also used. An appropriately diluted serum was mixed with an equal volume of each of these specimens. The mixtures were incubated for 1 hr at 20°C and centrifuged at 4,000 × G for 5 min. The absorption was repeated twice more with fresh materials and the supernatant was recovered.

Sucrose density gradient ultracentrifugation. Sera of cancer patients or RSF that showed strong inhibitory activities against H-D antibodies were fractionated by sucrose gradient centrifugation following the procedure described by Fudenberg and Kunkel (16). Briefly, 0.5 ml of a sample was overlayered on top of a 10 to 40% (w/v) sucrose gradient in PBS and centrifuged at 30,000 rpm for 16 hr at 4°C in a Spinco L-2 ultracentrifuge by using an SW50-1 rotor. Fractions were collected dropwise directly from the bottom of the centrifuge tube and assayed by the inhibition of agglutination of trypsinized BRBC. The P-B antibody of an IM serum was used as a marker for IgM, and incomplete Rh antibody (anti-CD) was used as a marker for IgG.

Gel filtration. Five milliliters of serum from a patient with chronic lymphocytic leukemia (CLL) were filtered through a Sephadex G-200 (Pharmacia, Uppsala, Sweden) column, 90 x 2.5 cm, equilibrated with PBS. Fractions of 70 drops each were collected dropwise and read for absorbance of light at a wave length of 250 nm and 280 nm. Fractions of each peak of protein concentration were pooled and lyophilized. Protein concentrations were adjusted to 10 mg/ml (w/v) in PBS and used for serologic tests.

Immunoelectrophoresis. The procedure of Scheidegger (17) with the use of an Immunophor apparatus from LKB Productor AB, Stockholm, Sweden, was employed.

RESULTS

Sera of cancer patients along with sera of random blood donors were, at first, studied for the presence of heterophile antibodies. As summarized in Table I, of 118 cancer sera, eight sera had lytic titers of 80 or more against BRBC and 12 sera had titers of 80 or more against SRBC. The frequency of sera with lytic titers of 80 or more against BRBC or SRBC among 298 blood donors was 4 and 15%, respectively. Of the eight cancer sera with BRBC lysin titers of 80 or more, two had SRBC lysin titer of 320, one with 160, and the remaining five with 40. The specificity of the BRBC lysins in these eight sera was then investigated by absorption tests. BRBC lysins of all these eight sera studied could be removed by absorption with either SRBC or guinea pig kidney, indicating that these lysins were H-D antibodies.

To ascertain further the H-D specificity of the heterophile antibodies in cancer sera, serum 15 was selected for additional studies. As reference sera, RAS 185 with H-D antibodies, which were used in our previous investigation (4, 5), and an IM serum G.C. with P-B antibodies were employed. Inhibition tests were performed with solubilized and purified H-D and P-B antigens obtained from BRBC stroma. Both antigens were prepared at an original concentration of 1 mg/ml. Dilutions of sera were adjusted to contain four agglutinating units against trypsinized BRBC and then various dilutions of inhibitors were added. As seen in Table II, H-D antigen inhibited agglutinins in the cancer serum as well as the RAS 185 at a titer of 64, whereas P-B antigen did not affect these agglutinins. In contrast, agglutinins of the IM serum were inhibited by P-B antigen at the titer of 128 but they were not inhibited by the H-D antigen. As expected, bovine serum albumin (BSA), a rich source of H-D antigen, showed inhibitory activity for agglutinins of the cancer serum and the RAS but not for those of the IM serum. Human serum albumin (HSA) did not affect agglutinating activity of any of these sera.

To demonstrate H-D antigen in sera of patients with various diseases including cancer patients and in extracts of various

TABLE I

Heterophile antibodies in sera of cancer patients

	No. of Sera with Lytic Titers of:						Total
	≤40	80	160	320	640	≥1280	
Sera of cancer patients							
BRBC	110	5	1	0	0	2	118
SRBC	106	1	6	4	1	0	118
Sera of random blood donors							
BRBC	286	10	1	0	1	0	298
SRBC	251	27	2	2	6	6	298

TABLE II

Characterization of heterophile antibodies in cancer serum: inhibition of agglutination of trypsinized BRBC by solubilized Hanganutziu-Deicher (H-D) and Paul-Bunnell (P-B) antigens

Antibodies from:	Inhibiting Titers of:			
	H-D antigen (1 mg/ml)	P-B antigen (1 mg/ml)	BSA[a] (30 mg/ml)	HSA[b] (30 mg/ml)
Cancer serum 15	64	<1	8	<1
RAS 185	64	<1	4	<1
IM serum G.C.	<1	128	<1	<1

[a] Bovine serum albumin.

[b] Human serum albumin.

malignant tissues, inhibition of agglutination of trypsinized BRBC by H-D antibodies was performed. Table III shows representative results obtained in inhibition tests. As seen in the table, cancer serum 10 inhibited agglutination up to a titer of 16. The remaining four cancer sera showed inhibitory titers between 1 and 8. CSE of CML spleen 157 showed a high inhibition titer of 64 and CSE of Hodgkin's disease spleen and another CML spleen had inhibition titers of 4 and 1, respectively.

As summarized in Table IV, 66 of 172 cancer sera (38.4%) and 6 of 24 lymphoma or leukemia sera (25%) inhibited H-D antibody activity of the standard serum. All these "H-D inhibition"-positive sera were tested for inhibition of agglutination of trypsinized Rh-positive group O RBC by anti-Rh serum, Ripley. None of these sera gave positive results. This would indicate that the observed inhibition of H-D antibody was not due to the presence of nonspecific factors in the sera. In addition, 6% of RAS, 27% of RASF, 25% of leprosy sera, and 8% of IM sera gave positive inhibition tests. Sera of 24 patients with IM-like symptoms gave negative results. In contrast, none of sera from 100 random blood donors or 34 healthy staff members of this department gave positive results. In studying extracts of various malignant tissues, it was found that 50% of PAE from cancer tissues, 50% of PAE prepared from spleen tissues of lymphomas or leukemias, and 60% of CSE of these spleens gave positive results. PAE and CSE of 18 apparently normal tissues gave negative results.

Double diffusion gel precipitation tests were employed to study H-D antigen in malignancy sera selected by inhibition of agglutination tests. For these experiments, H-D-antigen-containing sera with inhibition titer of 8 or greater were used. As seen in Figure 1, all these antigen-containing cancer sera formed a sharp precipitation line in their reaction with H-D antibody-containing cancer serum 15. These lines merged into a reaction of identity with each other and also with the line formed by bovine serum (a rich source of H-D antigen).

The specificity of the precipitation reaction between the two cancer sera was further investigated. As seen in Figure 2, the H-D-antibody-containing serum 15 formed precipitation lines with H-D-antigen-containing serum 24 and with bovine serum; these two lines merged into a reaction of identity. There were no reactions of serum 15 with P-B antigen. In contrast, when the serum 15 was replaced by an IM serum with P-B antibodies, no reactions were observed with the H-D-antigen-containing serum or bovine serum, but strong reactions were noted with P-B antigen. Immunoelectrophoresis was performed with antigen-containing serum 24. As seen in Figure 3, a precipitation arc was formed around the well in the reaction with antibody-containing serum.

Preliminary study was performed to obtain some information on the molecular size of the H-D antigen found in cancer sera and RASF. Four cancer sera and two RASF were fractionated by means of sucrose density gradient ultracentrifugation. H-D

TABLE III

Agglutination of trypsinized BRBC by standard H-D-antibody-containing serum, RAS 185: inhibition of reaction by cancer sera and spleen extracts

	Inhibition of Agglutination of Trypsinized BRBC by RAS 185[a] with Cancer Sera and Malignant Tissue Extracts at Dilution of 1:									
	1	2	4	8	16	32	64	128	256	512
Sera of cancer patients:										
10	−	−	−	−	−	+	++	++	++	++
24	−	−	−	−	+	++	++	++	++	++
145	−	−	−	−	++	++	++	++	++	++
16	−	−	++	++	++	++	++	++	++	++
153	−	++	++	++	++	++	++	++	++	++
Extracts of:[b]										
Spleen 157[c]	−	−	−	−	−	−	−	+	++	++
Spleen 149[d]	−	−	−	+	++	++	++	++	++	++
Spleen 150[c]	−	++	++	++	++	++	++	++	++	++
Bovine spleen	−	−		−		−	−	−	+	++

[a] Four agglutinating units of H-D antibody were used.
[b] Boiled saline extracts at a concentration of 5 mg (dry weight)/ml.
[c] Spleen from a patient with chronic myelocytic leukemia.
[d] Spleen from a patient with Hodgkin's disease.

TABLE IV

Hanganutziu-Deicher antigen in pathologic sera and tissues

	Positive	%	Total
Sera from patients with:			
Cancer	66	38.4	172
Lymphomas or leukemias	6	25.0	24
Rheumatoid arthritis	8	5.5	146
Leprosy	3	25.0	12
Infectious mononucleosis	1	8.3	12
Infectious mononucleosis-like symptoms	0	0	24
Sera from:			
Blood donors	0	0	100
Healthy staff members	0	0	34
Synovial fluids of rheumatoid arthritis patients	4	26.7	15
Extracts[a] of tissues from patients with:			
Cancer			
PAE[b]	6	50.0	12
Lymphomas or leukemias			
PAE[c]	4	50.0	8
CSE[d]	6	60.0	10
Extracts from apparently normal tissues			
PAE & CSE[e]	0	0	18

[a] Concentration was adjusted to 5 mg (dry weight)/ml.
[b] Perchloric acid extracts from four colon and eight liver specimens.
[c] Perchloric acid extracts of eight spleen specimens.
[d] Saline extracts from 10 spleen specimens obtained at 100°C.
[e] Perchloric acid extracts and saline extracts obtained at 100°C from liver, spleen, kidney, testicles, muscles, and pooled sera.

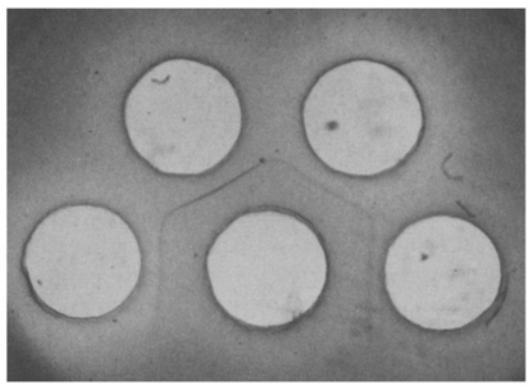

Figure 1. Lower left well, cancer serum 145; *middle well,* cancer serum 15 (H-D antibody); *lower right well,* bovine serum; *upper left well,* cancer serum 24; *upper right well,* cancer serum 74.

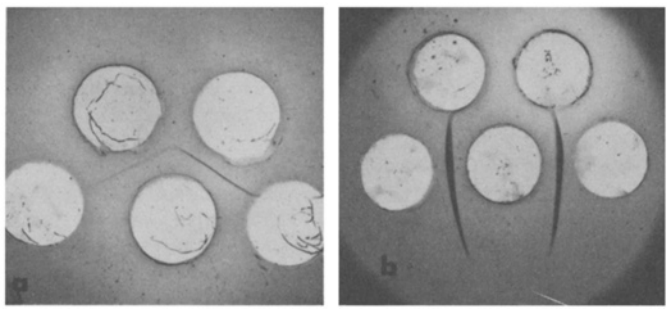

Figure 2. Lower middle wells: a, cancer serum 15 and *b,* IM serum G.C.; *lower right and left wells in a and b*: P-B antigen; *upper left wells in a and b*: bovine serum; *upper right wells in a and b*: cancer serum 24.

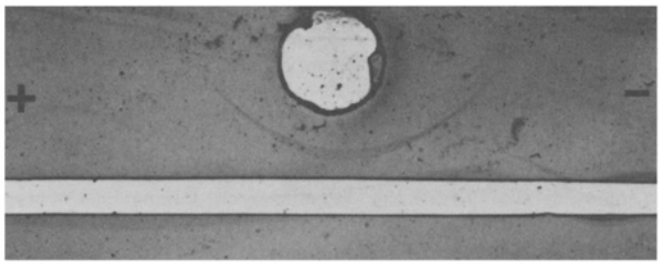

Figure 3. Immunoelectrophoresis: *well,* cancer serum 24 (H-D antigen); *trough,* cancer serum 15 (H-D antibody).

antigenic activities in two cancer sera and one RASF were found in fractions containing predominantly IgM or serum proteins of larger size and fractions containing albumin or serum proteins of smaller size. In the remaining two cancer sera, however, the antigenic activity was found only in the former fractions or in the latter fractions. When an H-D-antigen-containing CLL serum was fractionated by Sephadex G-200 gel filtration, H-D activity was found in IgM-rich fractions as well as in albumin-rich fractions.

DISCUSSION

As mentioned in the *Introduction,* H-D antibody was discovered in the 1920's (1, 2). With serum therapy of diphtheria and serum prophylaxis of tetanus largely replaced by active immunization, interest in H-D antigen and antibody has considerably decreased. More recently, a few investigators (18, 19) have studied heterophile antibodies in sera of patients who were injected with anti-lymphocyte or anti-thymocyte globulins from horse or goat. Our recent studies (3, 4, 9, 20) showed that H-D antibodies could be engendered in pathologic processes without any injections of foreign species sera. This would mean that H-D antigen is apparently formed as a result of various pathologic processes including malignant transformation.

As shown in our previous studies (3), the "H-D antigen" is not a single molecular species but a complex of several different molecules as evidenced by multiple precipitation lines formed by the H-D antibody-containing serum in reactions with various antigenic preparations. One of the antigenic molecules of the H-D complex was recently identified by Merrick *et al.* (12) and Higashi *et al.* (21) as *N*-glycolyl neuramic acid (NGNA) of bovine or equine hematosides.

Since the early studies of Deicher (2) confirmed by other investigators (see review 9) and ourselves (3), it has been known that bovine serum is a rich source of H-D complex. In the present study, bovine serum rather than purified NGNA preparation was used as a reference antigen in the double diffusion in agar gel because H-D antibody-containing tumor sera formed definite precipitation lines with bovine serum. On the other hand, only a few positive sera reacted with the NGNA preparation, which apparently represents only one of the specificities of H-D complex.

In the present study, we identified H-D antigen in sera of patients with cancer, lymphomas or leukemias, rheumatoid arthritis, leprosy, and IM. The highest incidence was observed in cancer, approximately 40% of random specimens. This was followed by RASF (27%), lymphoma or leukemia sera (25%), leprosy sera (25%), IM sera (8%), and RAS (6%). None of blood donors or healthy staff members possessed the antigen at a detectable level in their sera. The incidence of the antigen in malignant tissues was more than that of the patients' sera. It was found that 50% of PAE of cancer tissues and 60% of CSE and 50% of PAE of spleen tissues of lymphomas or leukemias contained the antigen at detectable levels. Numbers and nature of the antigens found in the patients' sera and their relationships to the NGNA are currently under investigation. Preliminary information indicates that the vast majority of sera studied contained a non-NGNA antigen(s) belonging to the H-D antigenic complex.

The frequent occurrence of H-D antigen in malignancy sera is certainly an interesting finding. Since the H-D antigen could be demonstrated in extracts of the majority of human tumors, it appears most likely that these tumors themselves shed off the antigen to the circulation. H-D antigen may be produced as a novel antigen by the tumor tissue and other pathologic tissues in an analogous way as P-B antigen is produced by abnormal cells in infectious mononucleosis (8, 9). This trend of thought certainly could apply to the ganglioside-containing NGNA, which has been identified as "the" H-D antigen. On the other hand, our previous studies left little doubt that H-D complex is composed of several antigenic molecules and some of them may well be normal human tissue components that possibly increase in quantity during the pathologic process. Still, these antigens are immunogenic and are detected by human sera. They resemble the ubiquitous tissue antigen (UTA), a previously described microsomal antigen that is a normal tissue component and that is released into the circulation under pathologic conditions (22, 23).

It should be stressed that in contrast to frequent demonstration of H-D antigen in malignancy sera, H-D antibodies were encountered only rarely, a finding very much reminescent of our previous observation on UTA (22, 23). It is tempting to speculate that both H-D and UTA are usually released in nonimmunogenic form to the circulation and possibly elicit tolerance. Only under rather unusual circumstances do these antigens stimulate antibody formation.

The possible practical application of the detection of H-D in malignancy sera should be further explored. In view of what is presented in this paper, it is rather obvious that the appearance of this antigen in the circulation can be also observed in pathologic conditions other than malignancies and, therefore, development of a diagnostic test based on the detection of this antigen can hardly be conceived. On the other hand, tracing the appearance and level of H-D in the course of malignancy may be helpful in diagnosing metastases and predicting the course of the malady. To this end, it should be stressed that detection of the carcinoembryonic antigen (CEA) in patients' sera could not be developed either as a diagnostic test because of the rather frequent occurrence of elevated CEA levels in pathologic conditions other than malignancy (24). On the other hand, detection and quantitation of CEA have served as a

valuable predictive test. In this connection, it should be stressed that very sensitive procedures of detection of H-D antigen may be based on a simple, inexpensive hemagglutination inhibition test rather than on the radioimmunoassay needed for quantitation of CEA.

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REFERENCES

1. Hanganutziu, M. 1924. Hémagglutinines hétérogénétiques après injection de sérum de cheval. C.R. Soc. Biol. 91:1457.
2. Deicher, H. 1926. Über die Erzeugung heterospezifisher Haemagglutinine durch Injection artfremden Serum. Z. Hyg. 106:561.
3. Kasukawa, R., K. Kano, M. L. Bloom, and F. Milgrom. 1976. Heterophile antibodies in pathologic human sera resembling antibodies stimulated by foreign species sera. Clin. Exp. Immunol. 25: 122.
4. Nishimaki, T., K. Kano, and F. Milgrom. 1978. Studies on heterophile antibodies in rheumatoid arthritis. Arthritis Rheum. 21:634.
5. Nishimaki, T., K. Kano, and F. Milgrom. 1978. Studies on immune complexes in rheumatoid arthritis. Arthritis Rheum. 21:639.
6. Milgrom, F., K. Kano, and A. Fjelde. 1973. Studies on heterophile antigen in lymphoma and leukemia spleens by means of absorption of infectious mononucleosis sera. Int. Arch. Allergy Appl. Immunol. 52:136.
7. Kano, K., R. Kasukawa, A. Fjelde, and F. Milgrom. 1976. Heterophile antigens on human lymphoid cells. Fed. Proc. 35:548 (Abstr.).
8. Kano, K., A. Fjelde, and F. Milgrom. 1977. Paul-Bunnell antigen in lymphoma and leukemia spleens. J. Immunol. 119:945.
9. Kano, K., and F. Milgrom. 1977. Heterophile antigens and antibodies in medicine. *In* Current Topics in Microbiology and Immunology. Springer-Verlag, Berlin-Heidelberg-New York. Pp. 44–69.
10. Nishimaki, T., K. Kano, and F. Milgrom. Detection of Paul-Bunnell antigen in malignancies and rheumatoid arthritis. Int. Arch. Allergy Appl. Immunol. In press.
11. Merrick, J. M., R. Schifferle, K. Zadarlik, K. Kano, and F. Milgrom. 1977. Isolation and partial characterization of the heterophile antigen of infectious mononucleosis from bovine erythrocytes. J. Supramolec. Struct. 6:275.
12. Merrick, J. M., K. Zadarlik, and F. Milgrom. 1978. Characterization of Hanganutziu-Deicher antigen. Int. Arch. Allergy Appl. Immunol. 57:477.
13. Krupey, J., P. Gold, and S. O. Freedman. 1967. Purification and characterization of carcinoembryonic antigen of the human digestive system. Nature 215:67.
14. Abeyounis, C. J., and F. Milgrom. 1972. Studies on carcinoembryonic antigen. Int. Arch. Allergy Appl. Immunol. 43:30.
15. Juji, T., K. Kano, and F. Milgrom. 1971. Antibody induced lysis of nucleated cells in agar gel. Int. Arch. Allergy Appl. Immunol. 41: 739.
16. Fudenberg, H. H., and H. G. Kunkel. 1957. Physical properties of the red cell agglutinins in acquired hemolytic anemia. J. Exp. Med. 106:689.
17. Scheidegger, J. J. 1955. Une micro-methode de l'immunoélectrophorèse. Int. Arch. Allergy Appl. Immunol. 7:103.
18. Kashiwagi, N., C. O. Brantigan, L. Brettschneider, C. S. Groth, and T. E. Starzl. 1968. Clinical reactions and serologic changes after the administration of heterologous antilymphocyte globulin to human recipient of renal homografts. Ann. Intern. Med. 68:275.
19. Pirofsky, B., J. C. Ramirez-Mateos, and A. August. 1973. 'Foreign serum' heterophile antibodies in patients receiving antithymocyte antisera. Blood 42:385.
20. Yoshida, H., K. Kano, and F. Milgrom. 1975. Reactions of murine myeloma cells with infectious mononucleosis. J. Immunol. 114:1449.
21. Higashi, H., M. Naiki, S. Matuo, and K. Okouchi. 1977. Antigen of "serum sickness" type of heterophile antibodies in human sera: identification as gangliosides with *N*-glycolyl neuraminic acid. Biochem. Biophys. Res. Commun. 79:388.
22. Kasukawa, R., R. Anthone, C. J. Abeyounis, and F. Milgrom. 1967. Studies on the serology of renal diseases. II. Unusual antibodies in glomerulonephritis sera. Int. Arch. Allergy Appl. Immunol. 32:563.
23. Palosuo, T., G. A. Andres, and F. Milgrom. 1976. A ubiquitous tissue antigen and its corresponding antibody in sera of patients with glomerulonephritidis. Int. Arch. Allergy Appl. Immunol. 52: 129.
24. Greenberg, D. M. 1976. Oncofetal and other tumor-associated antigens of the human digestive system. *In* Pathology of the Gastro-Intestinal Tract. Edited by B. Morson. Springer-Verlag, Berlin-Heidelberg-New York. P. 290.