Isolation of Lung Carcinoma-associated Antibodies from Immune Complexes and Production of Heterologous Antisera

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ABSTRACT

Immune complexes isolated from pleural effusions of lung carcinomas were dissociated by ion exchange chromatography in the presence of 8 M urea. The antibodies thus obtained from 2 lung adenocarcinomas and 2 squamous cell carcinomas were assayed by indirect immunofluorescence against a variety of target cells in fresh suspensions, tissue cultures, and paraffin-embedded sections. Strong cytoplasmic fluorescence was obtained with greater than 90% of the cell populations of all major histological types of lung carcinomas, and negative fluorescence was obtained with the cells of normal lung tissues and most of the nonpulmonary carcinomas. Antigen fractions prepared from the same lung carcinoma-associated immune complexes were used to immunize rabbits for the production of antisera directed against human lung carcinoma. The rabbit antibody preparations after proper absorptions were assayed in indirect immunofluorescence showing patterns of reactivity with normal and tumor cells of various derivations similar to those obtained with the human antibodies. Complete blocking of immunofluorescence staining with both allogeneic and xenogeneic antibody preparations was achieved by prior absorption with lung tumor tissue extracts or with various lung tumor antigen preparations.

The present study demonstrates the isolation from lung tumor effusion immune complexes of antibodies with high affinity for lung carcinoma cells and of antigens that can be used to produce tumor-directed heterologous antibodies. The immunofluorescence staining of lung carcinoma-associated antigens in situ on paraffin-embedded sections provides new, topographical information.

INTRODUCTION

The idea that human tumors, not unlike the experimental animal tumors, are able to generate cellular and humoral immune responses is supported by a large body of evidence (3, 8, 12, 15, 21, 28, 29, 42, 43). One of the strongest indications of reaction to neoplasia is the infiltration of tumor tissues by populations of lymphocytes, plasma cells, and macrophages (5, 16, 18, 22, 24, 38). In lung tumors, patterns of cellular infiltrates can be recognized that are associated with the various histological types, grades, and stages of carcinomas (18, 22). The abundance and the selectivity of cellular infiltrates suggest that their presence in the tumor tissues is in response to antigenicity expressed by the tumor cells. It is suggested further that they may produce and release antibodies with specificities directed against antigenic determinants of such tumor cells (19, 20). Immunofluorescence staining with antihuman immunoglobulin sera of lung carcinoma tissues showed the presence of heterogeneous immunoglobulins within the abundant plasma cell infiltrates (18, 22). To identify those antibodies associated with the lung tumor cells for their potential use in immunodiagnostic assays, we devised methods for their isolation (33). By the dissociation of antigen:antibody complexes present in the eluates of tumor tissues, pleural effusions (33), and bronchial washings (34) of patients with pulmonary neoplasms, we were able to obtain immunoglobulins reacting selectively with lung carcinoma cells in exclusion of normal lung cells and carcinoma cells of other organs.

The present study shows further refinements of these techniques leading to improved purification of tumor-associated antigens and antibodies. The antibodies thus obtained showed high affinity for lung carcinoma cells in fresh suspensions and tissue cultures as well as in paraffin-embedded sections. The antigens isolated from immune complexes were used for the production of heterologous antibodies which, after proper absorptions, demonstrated patterns of reactivity with tumor cells of various derivations entirely similar to those obtained with the human antibodies.

MATERIALS AND METHODS

Lung Tumors and Pleural Effusions

Pleural effusions of 2 squamous cell carcinomas and 2 adenocarcinomas of the lung were used to isolate tumor-associated antibodies. The antibodies obtained were assayed against fresh suspensions of lung tumor cells, lung tumor tissue cultures, and sections of lung tumors of various histological types.

The cell suspensions were derived from 2 squamous cell carcinomas and one adenocarcinoma of the lung, 2 carcinomas of the ovary, one carcinoma of the urinary bladder, one adenocarcinoma of the pancreas, one adenocarcinoma of the stomach, one adenocarcinoma of the colon, 2 normal lungs, and one normal placenta.

The tissue cultures assayed were 2 lines of adenocarcinoma of the lung (HuLT-59 and PC-3), 2 lines of squamous cell carcinoma of the lung (PC-1 and PC-10), 2 lines of small cell carcinoma of the lung (DMS-79 and DMS-114), and 2 lines of ovarian carcinomas (OT-54 and OT-72). Tissue culture lines PC-1, PC-3, and PC-10 were obtained by the courtesy of Dr. M. Takada of Roswell Park Memorial Institute (14); lines DMS-79 and DMS-114 were obtained by the courtesy of Dr. O. M. Pettengill, Department of Pathology, Dartmouth Medical School (38); and lines HuLT-59 and OT-72 were originated in our laboratory according to methods described previously (23).

The tissue sections examined included 8 lung tumors of various histological types, 12 tumors of other organs, and 6 specimens of different normal organs as detailed in Table I. In addition to sections of normal organs, the uninvolved tissues of various tumor sections served as controls.

Isolation of Immune Complexes from Pleural Effusions

Three hundred to 500 ml of fresh pleural effusions (2 squamous carcinomas and 2 adenocarcinomas of the lung) were collected by

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sterile technique, filtered through gauze to remove fibrin clots, and clarified by centrifugation at 5000 x g for 10 min. Cytological examination confirmed the presence of malignant cells in these specimens. Pleural effusions were adjusted to 33% ammonium sulfate with the addition of appropriate amounts of saturated ammonium sulfate (Sigma Chemical Co., St. Louis, Mo.). The precipitate that formed after 18 hr at 4°C was collected by centrifugation at 10,000 x g for 10 min and washed twice with 33% ammonium sulfate. The precipitate was then resuspended in PBS and dialyzed exhaustively against PBS. The immune complex-containing fraction was prepared by precipitation with 2.5% PEG, and then centrifuged again at 5000 x g for 10 min. The precipitate that formed after 18 hr at 4°C was collected by centrifugation at 10,000 x g for 10 min, washed once with 2.5% PEG, and then centrifuged again at 5000 x g for 10 min (39). The precipitate was then resuspended in 15 ml of PBS.

**Purification of IgG-containing Immune Complexes by the Use of Protein A**

Protein A:Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, N. J.) was swollen in starting buffer [0.01 M Tris:0.14 M NaCl: HCl (pH 7.2)], then packed, and equilibrated in a 30- x 1.5-cm column. Ten to 15 ml of immune complex-containing material were applied to the column and slowly run through three times. The column was then washed with starting buffer until A_{280} read <0.01. The IgG immune complex-containing fraction was then eluted using freshly prepared 8 M urea:0.1 M Tris:2.0 M NaCl (pH 5.0) (pK II) and dialyzed against PBS (39).

**Protein Assay**

Total protein was assayed by its absorbance at 280 nm or by the method of Bradford (6).

**Cytoplasmic Indirect Immunofluorescence**

**Cell Suspensions.** Cells for examination by indirect immunofluorescence were prepared as described previously (33). Briefly, cell suspensions from tissues, effusions, and tissue cultures were attached to Fluoroglide-sprayed slides. Antibody preparations were applied in increasing 2-fold dilutions to 7 of 8 wells, with Well 8 containing PBS as control. After 1 hr of incubation, the slides were washed, and FITC-conjugated goat anti-human IgG or goat anti-rabbit IgG (Kallestead Laboratories, Chaska, Minn.) was applied, incubated for 1 hr, washed, and coverslipped. End point titer of antibody activity was defined as the last 2-fold dilution showing definite immunofluorescent staining. Examination was done using a Leitz Ortholux fluorescence microscope equipped with an HBO-200 W mercury light source, blue-green exciter filter, and a K540 barrier filter.

**Tissue Section.** Immunofluorescence of Bouin’s fixed paraffin-embedded tissue sections from various normal and neoplastic tissue was performed as described previously (11). After embedding, 4-micron-thick sections were cut and processed for deparaffinization by heating for 30 min at 60°C. The slides were then deparaffinized and washed in 2 changes of 0.9% NaCl solution for 10 min each. Antibody was applied at appropriate dilutions in PBS:3% bovine serum albumin. Slides were then incubated for 1 hr at 37°C and washed, and appropriate FITC-conjugated antibody was applied. After a second incubation for 45 min at 37°C, the slides were washed and coverslipped with 10% PBS buffered to pH 8.6 in glycerol. Control tissue sections receiving only FITC-labeled antibody were run in parallel so that background staining could be evaluated.

**Quantitative Assay for Human Immunoglobulins**

The presence of human IgG was determined using the method of Ouchterlony (32). Quantitation of human immunoglobulins was assessed by performing RID tests on high- and low-level Quanta-plates (Kallestead) as described previously (33). Quantitative tests were performed for IgG, IgM, IgA, and IgD.

**Immunoelectrophoresis**

IEP analysis of antibody preparations was performed by electrophoresis of samples in 1% agarose in barbital buffer (pH 8.6) with a constant current of 40 mA at 4°C. A polyvalent anti-human serum (Hyland Laboratories, Los Angeles, Calif.) was added to preformed troughs and allowed to diffuse for 24 hr at room temperature (12).

**Column Chromatography**

A Bio-Gel A-1.5 m (Bio-Rad Laboratories, Richmond, Calif.) column (90 x 1.5 cm) was prepared and equilibrated with PBS. The column was calibrated for void volume with Blue Dextran 2000 (Pharmacia).

**Absorption of Antibodies**

Tissue extracts in 0.9% NaCl solution of normal human ovaries, placenta, liver, and lung as well as pooled normal plasma were prepared and cross-linked by the method of Avrameas and Ternynck (2). Tissue extracts in 0.9% NaCl solution of both adenocarcinomas of the lung and ovarian carcinoma were also prepared and polymerized to form an immunoabsorbent.

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Table 1

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<tr>
<th>Lung Carcinoma Antibodies and Heterologous Antisera</th>
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<td><strong>Cell suspensions and tissue culture immunofluorescence were considered positive only in titers above 1:128. Tissue sections immunofluorescence was read as positive or negative using antibody preparations diluted at 1:100.</strong></td>
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<td><strong>Indirect immunofluorescence staining of lung carcinoma-associated antibodies</strong></td>
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<td>Normal tissues of other organs</td>
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The abbreviations used are: PEG, polyethylene glycol; FITC, fluorescein isothiocyanate; RID, radial immunodiffusion; IEP, immunoelectrophoresis; PBS, 0.05 M phosphate-buffered saline.
Production of Rabbit Anti-Human Lung Tumor Antibody Preparations (RLTA-1 and RLTA-2)

RLTA-1. RLTA-1 was produced by immunization of rabbits with the antigenic fraction obtained by dissociation of immune complexes. According to a method described previously (13), QAE pK II from effusions of both adeno- and squamous carcinomas of the lung was pooled, and 250 μg of protein per ml were mixed 1:1 (v/v) with Freund’s complete adjuvant. New Zealand White rabbits then received injections s.c. with 2 ml of this mixture. On Days 21 to 23, the rabbits received boosters of 1 ml QAE pK II (250 μg/ml) and were bled 10 days later. The serum was harvested, decomplemented at 56° for 20 min, aliquoted, and frozen at −20°.

RLTA-2. RLTA-2 was produced by immunization of rabbits with lung tumor extracts eluted from an immunoabsorbent column prepared with human lung tumor antibody. Human lung tumor antibody (QAE pK I) was linked to a Sepharose 4B cyanogen bromide column (2 mg total protein) according to the manufacturer’s instructions (Pharmacia). Fresh adenocarcinoma tumor extract that had been sonicated in PBS was applied to the column and incubated for 1 hr at 37°. The column was then washed with PBS until the A28o was < 0.01, and the bound material was eluted using 8.0 M urea:0.1 M Tris (pH 7.2). The eluted material was dialyzed against PBS and adjusted to 100 μM/ml. The production of RLTA-2 with this preparation was done by immunizing rabbits as described previously.

RESULTS

Isolation and Dissociation of Immune Complexes (QAE pK I and QAE pK II)

Pleural effusions of 2 adenocarcinomas and 2 squamous cell carcinomas were collected, separately processed, and labeled HLAC and HLSC, respectively. Immune complexes were isolated from both pools of pleural effusions (HLAC and HLSC) by precipitation with 33% (NH4)2SO4 followed by reprecipitation with 2.5% PEG. Each preparation was chromatographed on Bio-Gel A-1.5 m, and one major peak was eluted corresponding to the void volume (Chart 1). The immune complex-containing fractions were then applied to a Protein A-Sepharose CL-4B column. The bound materials were eluted with 8 M urea:0.1 M Tris (pH 7.2), pooled, and dialyzed against 8 M urea:0.1 M Tris (pH 7.2) (Chart 2).

These fractions were then applied to an Bio-Gel A-1.5 m column. The bound materials were eluted with 8 M urea:0.1 M Tris:0.15 M NaCl (pH 7.2), pooled, and dialyzed against 8 M urea:0.1 M Tris (pH 7.2) (Chart 2).

Evaluation of Human Antibody Preparations HLAC pK I and HLSC pK I

Indirect Immunofluorescence Testing against Lung Carcinoma Tissue Cultures. HLAC pK I and HLSC pK I antibody preparations were adjusted to 1 mg total protein content per ml and assayed by indirect immunofluorescence against various tissue culture cell lines (Table 1). In all cell lines tested, greater than 90% of lung carcinoma cells showed strong granular cytoplasmic fluorescence with both antibody preparations at dilutions of up to 1:1024 (Fig. 2). In contrast, tissue cultures of 2 ovarian carcinomas showed positive fluorescence only at dilutions less than 1:8. The fluorescence staining of lung tumor cells could be blocked by prior absorption with QAE pK II obtained from either adeno- (HLAC) or squamous (HLSC) car-
cinoma pleural effusions. As a control, neat unprocessed pleural fluid from an adenocarcinoma of the lung was adjusted to 1 mg total protein per ml and tested in a similar manner. The unprocessed fluid reacted positively at dilutions of less than 1:8 against all tissue culture cell lines.

**Indirect Immunofluorescence Testing against Lung Carcinoma Cell Suspensions.** Freshly dispersed tumor cells were reacted with the same antibody preparations HLAC pK I and HLSC pK I and examined after immunofluorescence staining. All lung carcinoma cells tested (Table 1) were positive with stomach, positive at dilutions of 1:128 to 1:256, and one suspension of normal tissues and tumors of other organs was negative with the exception of one adenocarcinoma of the stomach, positive at dilutions of 1:128 to 1:256, and one normal placenta, positive at dilutions of 1:32 to 1:64.

**Indirect Immunofluorescence against Lung Carcinoma Paraffin-embedded Tissue Sections.** The 2 human antibody preparations HLAC pK I and HLSC pK I that had been absorbed with normal human lung, placenta, and ovarian tumor tissue extracts were tested against paraffin-embedded sections of 8 lung carcinomas (4 adenocarcinomas and 4 squamous cell carcinomas). All tissue sections showed bright granular cytoplasmic fluorescence present in the tumor cells only (Figs. 3 and 4). Normal lung tissue present in these sections did not stain. Of other tumors studied, only one gastric adenocarcinoma showed positive staining with both HLAC pK I and HLSC pK I. Both antibodies were used at 1:100 dilutions; however, dilutions as high as 1:1000 were still positive. At these higher dilutions, not all the tumor cells were stained, but fluorescence was restricted to areas of tumor cells that were positive. The reaction could be blocked by absorption of antibodies with QAE pK II or with lung tumor extract, after which all fluorescent staining was abolished (Fig. 5).

**Production of Rabbit Antibodies RLTA-1 and RLTA-2**

**Preparation of RLTA-1.** Multiple bands were observed when RLTA-1 was tested by gel diffusion by the method of Ouchterlony against various tissue extracts and normal plasma. Tested on IEP against normal human plasma, RLTA-1 showed 4 to 5 bands; however, no activity was noted in the IgG region. RLTA-1 was then absorbed with normal human plasma, placenta, lung, liver, and ovarian tumor extract for use in immunofluorescence assays.

**Preparation of RLTA-2.** RLTA-2 tested by gel diffusion by the method of Ouchterlony showed one faint band against normal human plasma. Tested by IEP against normal human plasma it showed one faint band corresponding to albumin. RLTA-2 absorbed with normal human placenta, plasma, lung, liver, and tissue extracts of ovarian carcinoma was tested by gel diffusion by the method of Ouchterlony against tissue extracts of 4 lung tumors (2 squamous and 2 adenocarcinomas), 4 tumors of other organs (2 carcinomas of the ovary, one adenocarcinoma of the stomach, and one adenocarcinoma of the colon), normal human plasma, QAE pK II used for the production of RLTA-1, and the eluate from the human immunoabsorbent column used to produce RLTA-2.

No bands were noted against normal plasma or the 4 nonlung tumors; however, 2 of 4 lung carcinomas shared one band of identity with both QAE pK II and the putative antigen fraction isolated from the immunoabsorbent column. Absorption of RLTA-2 with either QAE pK II used to produce RLTA-1 or the eluate from the human lung tumor-immunoabsorbent column abolished all activity when retested by gel diffusion by the method of Ouchterlony.

**Evaluation of Rabbit Antibodies RLTA-1 and RLTA-2 by Indirect Immunofluorescence**

In all cases, RLTA-1 and RLTA-2 were first absorbed with normal human plasma, placenta, lung, liver, and ovarian tumor tissue extracts prior to use.

**Cell Suspensions.** RLTA-1 and RLTA-2 were tested by indirect immunofluorescence against cell suspensions of 3 lung tumors (Table 1) and gave positive staining in titers greater than 1:128 (RLTA-1, 1:128 to 1:1024; RLTA-2, 1:256 to 1:4096). With cell suspensions of normal lung and placenta, both RLTA-1 and RLTA-2 showed positive fluorescence only in titers less than 1:4. Cell suspensions of 4 tumors of other organs (2 from ovaries, one from stomach, and one from urinary bladder) reacted positively with RLTA-1 in titers of 1:4 to 1:32 as did those of 2 tumors (colon and pancreas carcinomas) in titers up to 1:128. RLTA-2, however, showed positive fluorescence with all 6 carcinomas of other organs in titers less than 1:16.

**Tissue Cultures.** RLTA-1 and RLTA-2 tested against tissue culture lines of 6 lung carcinomas showed positive cytoplasmic fluorescence with titers greater than 1:128 (RLTA-1, 1:256 to 1:1024; RLTA-2, 1:512 to 1:4096) (Table 1; Fig. 6). With 2 tissue culture lines of ovarian carcinomas, titers of 1:8 and 1:16 with RLTA-1 and 1:8 with RLTA-2 were recorded. Subsequent absorption of both RLTA-1 and RLTA-2 with QAE-A50 pK II used to produce RLTA-1, the eluate of the human immunoabsorbent column used to produce RLTA-2, or with lung tumor tissue extract blocked positive cytoplasmic immunofluorescence against all lung tumor cell suspensions and lung tumor tissue culture lines tested.

**Tissue Sections.** RLTA-1 and RLTA-2 were diluted 1:100 and tested by indirect immunofluorescence against Bouin’s fixed, paraffin-embedded tissue sections. RLTA-1 showed positive cytoplasmic fluorescence with 8 of 8 different lung tumor sections and showed no fluorescence with normal lung tissues or normal tissues of other organs. RLTA-1 showed cytoplasmic fluorescence with 4 (stomach, ovary, breast, and pancreas) of 12 different tumors of other organs. Uninvolved tissues present in the sections of various tumors positive or negative showed no fluorescence and were considered as built-in controls. RLTA-2 similarly showed cytoplasmic fluorescence in 8 of 8 lung tumors and was negative with all other sections tested (Table 1; Fig. 7). Absorption of SP-1 and RLTA-2 with QAE pK II, eluate of the immunoabsorption column, or lung tumor tissue extracts entirely blocked the cytoplasmic staining of all 8 lung tumors when retested.

**DISCUSSION**

Cancer cells are antigenically distinguishable from their normal counterparts (31). According to this concept, now generally accepted, methods for the diagnosis and therapy of cancer based on the recognition and isolation of tumor-associated antigens could be developed. The identification of tumor antigens has been attempted in various ways. Our approach to the detection of tumor-associated antigens is related to their interaction with specific antibodies isolated from tumor-bearing
patients. We believe that the tumor site is the optimal location for the investigation of tumor-associated antibodies both in free form and locked in antigen:antibody complexes. The formation of immune complexes (4, 27, 35, 40) and their correlation with the extent of disease in different types of tumors (1, 7, 9, 17, 37) have been demonstrated. For their detection, multiple methods using different reactants are presently available (30).

In previous work, we used effusion fluids of ovarian (12) and lung carcinomas (33) to isolate immune complexes from which, by dissociation, tumor-reactive antibodies were obtained. PEG, an uncharged water-soluble linear polymer that at high concentrations (20%) precipitates most free immunoglobulins, was used at 2.5%, a concentration at which it precipitates immune complexes but not monomeric immunoglobulin or most other proteins (10). In contrast to our earlier work (12, 33, 34) in which acid dissociation was used to separate the antigen and antibody fractions of immune complexes, ion exchange chromatography in the presence of 8 M urea was used in the present procedure. This method is preferable because it results in almost complete dissociation of immune complexes and because urea which is non-ionic does not interfere with the separation of dissociated antigens and antibodies by ion exchange chromatography. In addition, 8 M urea is effective at pH 7.2, thus avoiding the harsh treatment with possible inactivation of antigens and antibodies by strong acid solutions. The denaturation induced by 8 M urea is reversible, and its removal by dialysis allows the recovery of antigens and antibodies with physical properties and immunological reactivities apparently unaltered (39, 41). The use of 8 M urea also prevents the reassociation of antigens and antibodies which rapidly occurs with the acid dissociation method when the dissociated immune complexes are returned to neutrality. Due to this advantage, considerably greater amounts of immune complex-containing fluids can be processed by this method. In our work, we found this procedure to be easily reproducible and to permit consistently the separation, isolation, and concentration of tumor-associated immunoglobulins.

The antibodies thus obtained from the pleural effusions of 2 adenocarcinoma and 2 squamous cell carcinomas of the lung were separately assayed by indirect immunofluorescence against a variety of target cells in both fresh suspensions and tissue cultures. The cells included lung carcinomas of all major histological types, a variety of carcinomas of other organs, and normal tissues of different origins. Strong cytoplasmic fluorescence was obtained with greater than 90% of the cell populations of all lung carcinomas when reacted with the antibody preparations obtained from both the adenocarcinoma and the squamous cell carcinomas. Totally negative fluorescence resulted with the cells of normal tissues, and only a few positive results occurred with the cells of non-lung carcinomas. However, while the fluorescence staining of lung carcinoma cells remained positive at dilutions up to 1:1024, that of non-lung carcinoma cells was present mostly at dilutions less than 1:8. Similar results were obtained when the antibody preparations were used against paraffin-embedded tissue sections. The brightness of staining and its selective restriction to the tumor cells were remarkable. The uninvolved tissues in the same sections were not stained. The fluorescence was blocked by prior absorption with the antigenic preparations (QAE pk II) of both adenocarcinoma and squamous cell carcinomas. When the antibody preparations were used at higher dilutions (1:1000) although the fluorescence was still strong, focal areas rather than the whole section were stained. The different staining at high dilutions of various tumor areas may be explained by the existence of more than one antigenic determinant in carcinoma cells, the variable amounts of antigens expressed by different tumor cells, or the clonal heterogeneity of tumor cell populations.

Using 2 different antigenic fractions prepared from lung carcinoma-associated immune complexes, we produced, by immunization of rabbits, antisera directed against human lung tumors. The first, (RLTA-1) was produced with the antigenic fraction obtained by the dissociation of immune complexes. Although the latter did not contain immunoglobulin IgG, it still contained normal components since the rabbit antibody preparation obtained with it showed several bands against normal plasma and tissue extracts when assayed in gel diffusion. The antibody preparation (RLTA-1) was absorbed with various tissue extracts before its use in immunofluorescence tests, reacted strongly with lung carcinoma cells, and also stained in lesser titer some carcinoma cells of other organs. The second antigenic preparation used to produce xenogeneic sera was comprised of the eluate of the immunoadsorbent column prepared with human lung tumor antibody (QAE pk I). As a result, the rabbit antibody preparation obtained (RLTA-2) proved to be superior upon immunofluorescence testing, as only lung carcinoma cells of all tissues assayed showed positive staining. With both xenogeneic (rabbit) and allogeneic (human) antibody preparations, complete blocking of immunofluorescence staining was achieved by prior absorption with lung tumor tissue extracts, the antigenic preparations from the ion exchange chromatography column, or the immunoadsorbent column prepared with human lung tumor antibody.

In the present work, we obtained selective immunofluorescence staining of lung carcinoma cells with both allogeneic and xenogeneic antibody preparations. The patterns of staining were entirely similar, and both were blocked with the same antigenic preparations.

A new development is the successful use of paraffin-embedded tissue sections in the immunofluorescence testing according to a new method described previously (11). The staining in situ of lung carcinoma-associated antigens with our antibody preparations provides important topographical information. This method is also applicable to the histological diagnosis of lung tumors of uncertain origin.

The recent advances made in the field of monoclonal antibodies (25, 26) open new prospects for this system (31). With the human antibody preparations described here, it is feasible through the use of an immunoadsorbent column to identify antigenic fractions of lung carcinomas to be used in the hybridoma production of monoclonal antibodies. At the present time, this technology appears to offer the best hope for the development of tumor immunodiagnosis.

REFERENCES

Lung Carcinoma Antibodies and Heterologous Antisera

Fig. 2. Lung adenocarcinoma, tissue culture line (HuLT-59), reacted with immunoglobulins isolated from pleural effusion of patient with squamous cell carcinoma of lung and FITC-labeled anti-human IgG. x 540.

Fig. 3. Lung adenocarcinoma, paraffin-embedded section, reacted with immunoglobulins isolated from pleural effusion of lung adenocarcinoma, absorbed with placental tissues and stained with FITC-labeled anti-human IgG. Tumor tissue is selectively stained. x 250.
Fig. 4. Lung squamous cell carcinoma, paraffin-embedded section, reacted with immunoglobulins isolated from pleural effusion of lung squamous cell carcinoma, absorbed with placental tissues and stained with FITC-labeled anti-human immunoglobulins. Tumor tissue is selectively stained. × 250.

Fig. 5. Section of lung adenocarcinoma reacted with immunoglobulins isolated from pleural effusion of lung adenocarcinoma, absorbed with antigen fraction of lung squamous cell carcinoma and stained with FITC-labeled anti-human IgG. Tumor tissue not stained. Plasma cells stained. × 250.
Fig. 6. Lung squamous cell carcinoma tissue culture line (PC-10) reacted with absorbed rabbit antibodies (RLTA-2) at 1:256 dilution and FITC-labeled goat anti-rabbit IgG antiserum. x 540.

Fig. 7. Lung adenocarcinoma, paraffin-embedded section, reacted with absorbed rabbit antibodies (RLTA-2) at 1:100 dilution and FITC-labeled goat anti-rabbit IgG antiserum. Tumor tissues not stained. x 100.
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