Immunoperoxidase Staining for Ia-Like Antigens in Paraffin-Embedded Tissues From Human Melanoma and Lung Carcinoma

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The human Ia-like antigens that are predominantly expressed by cells associated with immunologic function has been considered as a diagnostic marker of malignant transformation of some nonlymphoid tissues. Immunoperoxidase staining of formalin-fixed and paraffin-embedded tissue sections with a monoclonal antibody to Ia-like antigens was chosen for assessment of the value of this marker for diagnosis in surgical pathology. Monoclonal antibody LK8D3 developed against a human melanoma cell line bearing Ia-like antigens was found to react in serologic and immunochemical studies with an antigenic determinant of Ia-like antigens that was relatively stable to formalin fixation and paraffin embedding. Avidin–biotin complex peroxidase staining of formalin–paraffin sections with LK8D3 showed focal expression of Ia-like antigens in 3 of 12 melanomas, whereas all 8 cases of intradermal nevi were negative. Immunoperoxidase staining of formalin–paraffin sections of lung carcinomas with antibody LK8D3 was related to the histologic subtype of tumors. Thus, squamous cell carcinomas showed only very focal staining for Ia-like antigens in 5/9 cases, while widespread and intense Ia-like immunoreactivity was seen in 3/5 cases of lung adenocarcinomas, including two bronchioalveolar carcinomas. The presence of Ia-like antigens in lung adenocarcinoma may not be entirely associated with malignant transformation, because normal alveolar lining cells were stained with the antibody. (Am J Pathol 1984, 115:102–116)

Ia-LIKE ANTIGENS in humans are membrane-bound glycoproteins composed of 34,000 and 28,000 molecular weight polypeptides which are coded for by genes located in the major histocompatibility complex. These antigens are readily detected on macrophages, B-lymphoid cells, and activated T-lymphoid cells and are believed to regulate cell–cell interactions leading to immune effector function. Over the past several years, a number of reports have described Ia antigens on cells with no apparent immune function such as epithelial cells of kidney, gut, bronchi,[1–4] mammary gland,[5] skin, and vascular endothelium.[3,4,6,7] Although the importance of Ia expression in nonimmune cells is unclear, recent studies in which Ia antigens were induced in epidermis and gut epithelium by immunologic stimuli suggests an immunologic role for its expression.[4,7]

In addition to normal tissues, Ia-like antigens have been detected on certain tumors derived from nonimmunologic normal tissues that normally lack this antigen. This is an important concept, because it has been proposed that one may use Ia antigens as markers of malignant transformation. The most notable and thoroughly studied example of this phenomenon is the melanoma cell. Human Ia-like antigens are clearly detectable on the majority of cultured human melanoma cell lines and have not been found on normal skin melanocytes.[8–11] However, whether Ia can be used as a marker of malignant transformation of the melanocyte is presently unclear, because there is disagreement over the expression of Ia-like antigens on *in vivo* melanoma cells. Using monoclonal antibodies

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and frozen tissue sections, Natali et al[10] reported variable but significant staining for Ia-like antigens in 16 of 16 melanoma cases and no staining of 14 cases of benign nevi, while Thompson et al[12] found Ia-like antigens in all melanomas (15 of 15) and in some benign nevi (8 of 22). More recently, Ruiter et al[13] using monoclonal antibodies and frozen tissue sections, detected Ia-like antigens in only 1 of 12 cases of melanoma. Whether these discrepancies can be explained by differences in technique, antibody specificity, or patient differences is presently unknown.

Using a cultured human melanoma cell line as the immunogen, we have recently developed a monoclonal antibody to human Ia-like antigens that reacts not only in frozen tissue reactions (as in the above cited literature) but also in tissues fixed in formalin and embedded in paraffin. The great advantage of formalin–paraffin sections over frozen sections is their superior preservation of morphologic features, a factor that becomes critical when one is attempting to distinguish Ia-positive inflammatory cells, which normally infiltrate melanoma lesions from the tumor cells themselves. In this report we examined formalin-fixed and paraffin-embedded sections of skin lesions and lung carcinomas for Ia-like antigens using a monoclonal antibody and an avidin–biotin immunoperoxidase technique.

Materials and Methods

Human Biopsy Specimens

Formalin–paraffin blocks from 21 cases of melanoma, 20 cases of intradermal nevi, and 16 cases of lung carcinoma were selected randomly from the recent files of the Department of Pathology at the University of Michigan Hospitals. The diagnoses were made on conventional histologic sections stained with hematoxylin and eosin (H&E).[14]

Cultured Human Cell Lines

The cultured lymphoid cell lines RAJI, RPMI 1788, RPMI 7666, Molt-4, and CEM and the cultured melanoma cell line HT-144 were obtained from the American Type Culture Collection, Rockville, Maryland. All cells are routinely passaged in RPMI 1640 medium supplemented with 15% fetal calf serum and antibiotics. HT-144 melanoma cells grow as a monolayer in culture and are passaged after mild digestion in 0.05% trypsin. HT-144 cells exhibit strong staining by immunofluorescence with monoclonal antibodies to human Ia-like antigens.

Monoclonal Antibodies

A 75-sq mm culture flask containing approximately 5×10^6 HT-144 melanoma cells was washed with phosphate-buffered saline (PBS), and the adherent cells were released by scraping with a rubber policeman. A Balb/c mouse (Jackson Laboratories) was given an intraperitoneal injection of 5×10^6 HT-144 cells in 0.5 ml PBS every 2 weeks for a total of four times. Three days following the last injection, the mouse was sacrificed and the splenocytes isolated and fused at a 5:1 ratio with NS-1 myeloma cells with the use of 50% polyethylene glycol 3500 (Sigma Chemical Co., St. Louis, Mo) as described by Galfre et al.[15] After fusion, cells were cultured with spleen feeder cells in 96-well microculture plates containing hypoxanthine, aminopterin, and thymidine. Clones were initially screened for antibody activity by immunofluorescence staining of Lab-Tek microculture chamber slides (VWR Scientific) containing adherent acetone-fixed HT-144 cells. All hybridoma culture supernatants containing antibody activity were subsequently tested for immunoperoxidase staining of formalin-paraffin sections of a human nodular melanoma lesion (Case 1788, C.F.). Antibody LK8D3 was selected for further testing because it reacted principally with structures known to express human Ia-like antigens (eg, lymphocytes, endothelial cells, histiocytes, Langerhan's cells). The hybridoma cells secreting LK8D3 have been cultured for several months and have yielded culture fluids and ascites fluids; however, antibody secretion has not been maintained after subcloning, thus limiting permanent establishment of this hybridoma.

The monoclonal antibody to murine I-E^k/C^k antigens (14–4–4S) originally developed by Sachs and co-workers[16] was obtained from the American Type Culture Collection (Rockville, Md).

Immunoperoxidase Staining

Human biopsy specimens fixed in 10% neutral buffered formalin for 24 hours and embedded in paraffin were cut into 4–5-μ sections and attached to microscope slides with Sobo glue. After dewaxing in xylene and alcohol, sections were stained by the avidin–biotin complex method with the use of commercially prepared reagents and following the manufacturer's instructions (Vector Labs., Burlingame, Ca). The only differences were that all reagents were diluted with 4% bovine serum albumin in PBS (neutral pH) and that blocking serum and antibody were incubated only 30 minutes on slides. Visualization of

peroxidase was achieved with diaminobenzidine (0.02%), H_2O_2 (0.02%) in PBS for 10 minutes at room temperature. Slides were counterstained for 1 minute in Harris hemotoxylin, dehydrated, and mounted in Permount (Fisher). All sections tested were stained with the use of spent culture medium containing LK8D3 antibody because appropriate control culture media (from NS-1 cells or from non-cross-reactive hybridomas) failed to exhibit any background staining in our assay.

For cryostat sections, tissue embedded in OCT mounting medium was cut at 6 μ and then fixed in acetone for 10 minutes at 25 C. After washing the PBS, cryostat sections were stained by the avidin-biotin complex method essentially as described above for formalin-paraffin sections.

Immunoprecipitation and Gel Electrophoresis

Cultured RAJI B-lymphoid cells or HT-144 melanoma cells (1×10^8) were washed in PBS and were surface-labeled with 1 mCi ^{125}I by the use of the Iodogen-coated Petri plate method of Salisbury and Graham.[17] After labelling, cells were washed in PBS and extracted with 10 volumes of ice-cold PBS containing 0.5% NP40 detergent. The extract was cleaned of debris by centrifugation at 3000g, and the supernatant was stored at −20 C. Twenty microliters of Protein-A-conjugated Sepharose 4B (Pharmacia, Piscataway, NJ) loaded with rabbit anti-mouse immunoglobulin antibody was allowed to react with 400 μl spent culture medium containing LK8D3 antibody. After washing, the Sepharose was mixed for 2 hours at 4 C with 2×10^7 cpm of ^{125}I-labeled RAJI or HT-144 cell extract. After extensive washing, the labeled antigens were processed for standard slab gel electrophoresis using sodium dodecyl sulfate and the discontinuous buffer system of Laemmli.[18]

Results

Specificity of Antibody LK8D3 for Human Ia-like Antigens

Antibody LK8D3 was selected as a potential formalin-paraffin-reactive monoclonal antibody because it stained cells known to express Ia-like antigens (mononuclear inflammatory cells) in the initial tests with formalin-paraffin sections of a human melanoma. Subsequent immunofluorescence analysis showed that LK8D3 reacted strongly with virtually 100% of cultured human Ia-like-antigen-positive B-cell lines (RAJI, RPMI 1788, RPMI 7666) but did not stain cultured human Ia-like-antigen-negative T-cell lines (Molt-4, CEM). Additional evidence for Ia-like antigen specificity came from immunoperoxidase staining of formalin-paraffin sections of human lymph node biopsies where LK8D3 reacted with both germinal centers and interdigitating histiocytes in parafollicular areas. This exact pattern of staining was also observed in frozen sections of lymph nodes treated with monoclonal antibody LK8D3 or with other Ia-like antigen-specific antibodies that are only

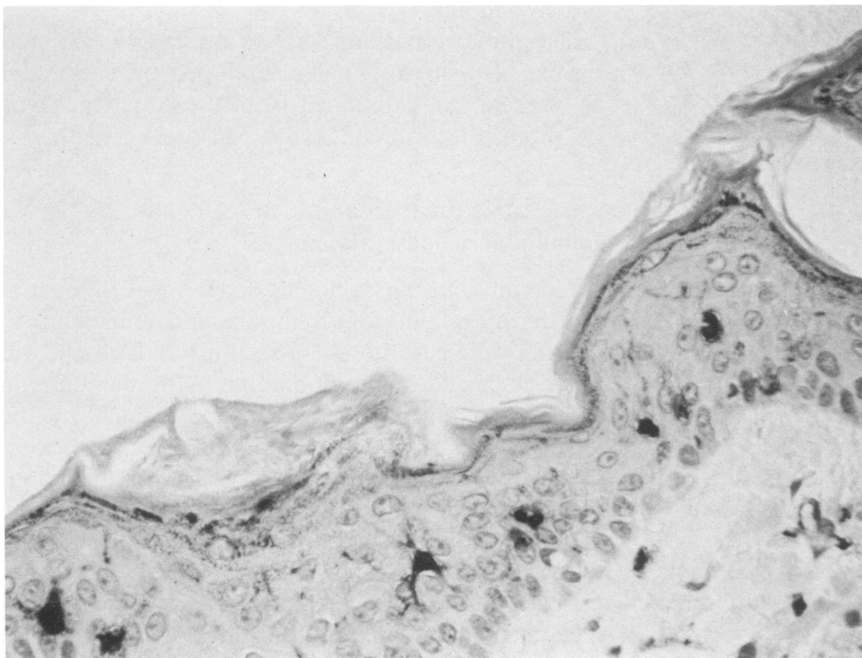

Figure 1—Immunohistochemical staining of a formalin-paraffin section of normal skin with monoclonal antibody LK8D3 against Ia-like antigens. The Langerhans' cells in the epidermis show intense cytoplasmic staining for Ia-like antigens. (Immunoperoxidase, ×330)

useful in frozen tissue sections. Immunoperoxidase staining of frozen sections and paraffin sections of normal human skin showed that antibody LK8D3 reacted with epidermal dendritic cells (Langerhan's cells) and with some mononuclear cells in the dermis, a pattern of staining also observed with other monoclonal antibodies to human Ia-like antigens (Figure 1).

Cell-surface radiolabeling of cultured human RAJI cells followed by immunoprecipitation and SDS-gel electrophoresis showed that LK8D3 reacts with the classical bimolecular complex of human Ia-like antigens (Figure 2). Antibody LK8D3 apparently reacts with the DR locus on the Ia-like antigen series because it reacts with the same 28,000 and 34,000 Mr structures on RAJI cells which are identified by a murine monoclonal antibody to mouse I-E/C antigens (14-4-4S). Whether LK8D3 also reacts with DS locus molecule is unknown.

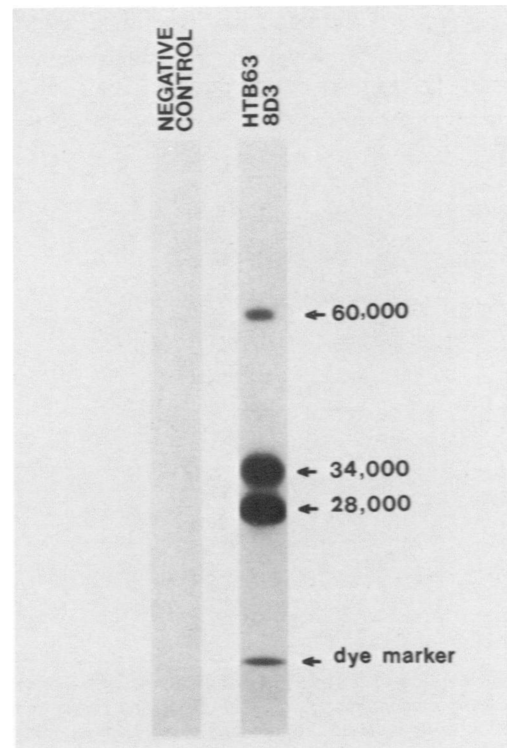

Figure 2 – Sodium dodecyl sulfate polyacrylamide gel electrophoresis of ^{125}I-labeled melanoma cell membrane proteins identified by monoclonal antibody LK8D3. The bimolecular complex of 34,000 and 28,000 molecular weight polypeptides typical of human Ia-like antigens were identified by antibody LK8D3, together with a small amount of a 60,000 molecular weight component. The latter may be a combination of the Ia-antigen subunits and is typically identified by antibodies to human Ia-like antigens.

Immunoperoxidase Staining of Human Skin Lesions

Formalin-fixed and paraffin-embedded sections of human melanoma and intradermal nevi were tested for expression of human Ia-like antigens by immunoperoxidase staining with spent culture medium containing LK8D3. A significant portion of melanoma cases (42%) and nevus cases (64%) were judged as unreactive sections and were excluded from Table 1 because no staining was observed with areas typically reactive with anti-Ia antibodies such as mononuclear inflammatory cells, histiocytes, Langerhan's cells, small blood vessels, and occasionally epidermal cells in these slides. Of the 12 reactive melanoma lesions tested (Table 1), only three showed positive staining for Ia-like antigens, and this was present focally in the tumor (Figure 3). In the great majority of melanoma cases in Table 1, some staining was seen in inflammatory cells both in and around the tumor, but not in tumor cells. In contrast to melanoma lesions, none of the 8 cases of reactive intradermal nevi exhibited nevus cell staining for Ia-like antigens.

Immunoperoxidase Staining in Human Lung Carcinomas

One case of lung adenocarcinoma among the 15 cases of lung carcinomas tested was excluded from the study because cells normally expressed Ia-like antigens were unreactive with our antibody, (eg, alveolar macrophages) in that slide. In general, lung squamous cell carcinomas did not express Ia-like antigens except for some rare cells dispersed within the tumor, and these were weakly reactive (Table 2). In contrast, the majority of adenocarcinomas tested, which included 2 cases of bronchioalveolar carcinoma, showed extensive staining for Ia-like antigens throughout most areas of the tumor (Figure 4). It was noted in many slides that Ia-like antigen immunoreactivity was visible in normal alveolar lining cells (Type II pneumocytes) and in submucosal bronchial glands, whereas no staining was seen in the ciliated pseudostratified columnar bronchial epithelium.

Discussion

The expression of human Ia-like antigens in skin lesions and lung carcinomas was investigated by immunoperoxidase staining of formalin-paraffin sections with a monoclonal antibody (LK8D3). Evidence for Ia-like antigen specificity of antibody LK8D3 came from 1) reactivity with cultured B- and not T-cell lines, 2) weak germinal center staining and strong staining of interdigitating histiocytes in T-cell areas of lymph nodes (both frozen and paraffin sec-

Table 1 — ABC Immunoperoxidase Staining of Human Ia-Like Antigens on Formalin-Fixed, Paraffin-Embedded Skin Lesions*

	Location	Histologic diagnosis	Positive melanoma or nevus staining†
Melanoma			
3574 ci	Skin	Superficial spreading	2+ (focal)
7973 ci	Skin	Superficial spreading	1+ (focal)
1788 CF	Skin	Nodular	2+ (focal)
6415 ci	Skin	Nodular	–
8021 CH	Skin	Nodular	–
11227 CF	Skin	Superficial spreading	–
11907 ci	Skin	Superficial spreading	–
10249 CH	Skin	Superficial spreading	–
8569 ci	Skin	Metastatic	–
8388 ci	Skin	Metastatic	–
1642 CH	Lymph node	Metastatic	–
3405 CH	Lymph node	Metastatic	–
Nevus			
6261 ci	Skin	Intradermal	–
8385 ci	Skin	Intradermal	–
8399 ci	Skin	Intradermal	–
7937 ci	Skin	Intradermal	–
7915 ci	Skin	Intradermal	–
8165 ci	Skin	Intradermal	–
8481 ci	Skin	Intradermal	–
6817 ci	Skin	Intradermal	–

* Slides were de-waxed in xylene and stained with monoclonal antibody LK8D3 to human Ia-like antigens in an avidin-biotin complex method using diaminobenzidine as substrate and Harris hematoxylin as counterstain.

† The following indicate the intensity of antibody staining: a negative sign means no staining, 1+ means weakly stained, 2+ means moderately stained, and 3+ means strongly stained.

tions), 3) reactivity with Langerhan's cells in skin epidermis and with mononuclear dermal cells (both frozen and paraffin sections, and 4) reactivity with the same 34,000 and 28,000 Mr structures of human RAJI cells identified by a murine monoclonal antibody to I-E^k/C^k antigens.[16] In skin lesions, Ia-like antigens were clearly demonstrated in focal areas of 3 of 12 cases of melanoma but were not seen in any of 8 cases of intradermal nevi. Adenocarcinoma of the lung was generally quite strongly and intensely

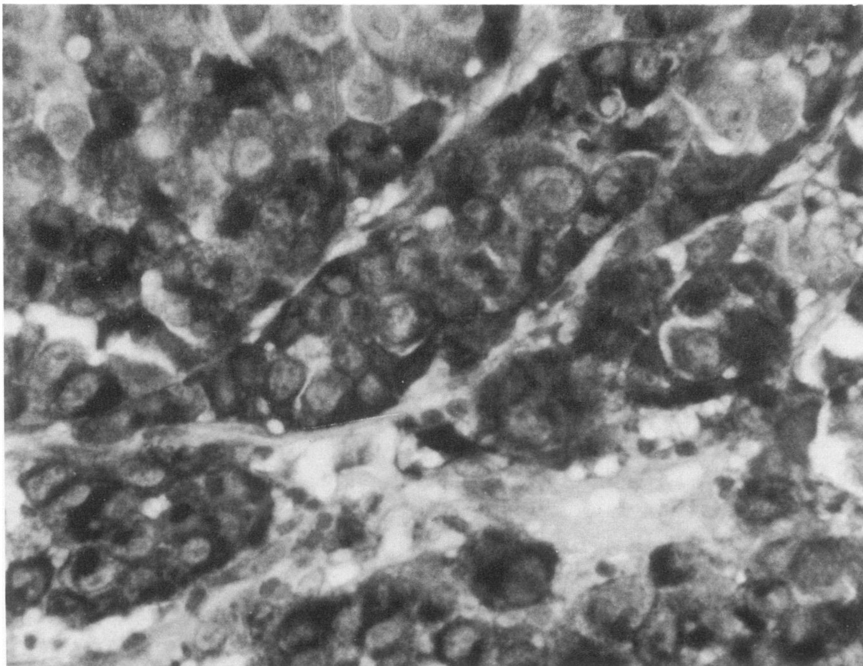

Figure 3 — Immunohistochemical staining of a formalin-paraffin section of a malignant melanoma with monoclonal LK8D3 antibody against human Ia-like antigens. Melanoma cells show a dark brown to black cytoplasmic staining, whereas the cell nuclei are unstained. (Immunoperoxidase, ×330)

stained for Ia-like antigens, whereas occasional tumor cells in only a portion of lung squamous carcinomas were positive. Staining of lung tumors paralleled the reactivity in normal lung tissue, with strong staining seen in alveolar lining cells or Type II pneumocytes.

For both skin lesions and lung tumors, a significant number of cases were excluded from the study because no LK8D3 reactivity was seen with normal cells in the section, known to express Ia-like antigens (ie, mononuclear infiltrates, Langerhan's cells, dermal macrophages, alveolar macrophages). Whether this loss of activity results from variations in the step involving formalin fixation or in the step involving paraffin embedding is presently unknown. The possibility that an antibody may not have reacted with the particular Ia-like antigens of these patients can be excluded because the antibody reacts with cells from all individuals tested which are either unfixed or are processed by frozen sectioning.

In previous studies employing monoclonal antibodies and frozen tissue sections, Natali et al,[10] using immunofluorescence, and Thompson et al,[12] using immunoperoxidase staining, reported variable but significant Ia-like antigen expression in all cases of melanoma examined, whereas a more recent study by Ruiter et al,[13] using immunoperoxidase, reported Ia-like antigen immunoreactivity in only 1 of 12 melanoma cases. In accordance with Ruiter et al,[13] we also found a small portion of melanomas which expressed Ia-like antigens (3 of 12 cases). The reason for the discrepant results remains unknown, because a large number of variables can effect these studies. Such variables include differences in antibody specificity, antibody dilution, samples studied, assay conditions, and interpretation of results. One advantage of this

Table 2 – ABC Immunoperoxidase Staining of Human Ia-Like Antigens in Formalin-Fixed, Paraffin-Embedded Lung Carcinomas*

Patient	Diagnosis	Positive tumor cell staining†
4094 ci	Squamous	2+ (<10%)‡
3193 ci	Squamous	1+ (<10%)
5039 ci	Squamous	1+ (<10%)
5863 ci	Squamous	1+ (<1%)
7719 ci	Squamous	1+ (<1%)
2943 ci	Squamous	–
3483 ci	Squamous	–
5885 ci	Squamous	–
4097 CH	Squamous	–
6951 ci	Adenocarcinoma§	3+ (>50%)
1111 ci	Adenocarcinoma§	3+ (>50%)
2903 ci	Adenocarcinoma	2+ (>50%)
643 ci	Adenocarcinoma	–
4993 ci	Adenocarcinoma	–

* Slides were de-waxed in xylene and stained with monoclonal antibody LK8D3 to human Ia-like antigens in an avidin-biotin complex method using diaminobenzidine as substrate and Harris hematoxylin as counterstain.

† The following indicate the intensity of antibody staining: a negative sign means no staining, 1+ means weakly stained, 2+ sign moderately stained, and 3+ means strongly stained.

‡ Indicates an estimate of the percentage of tumor cells stained.

§ Bronchioalveolar carcinoma.

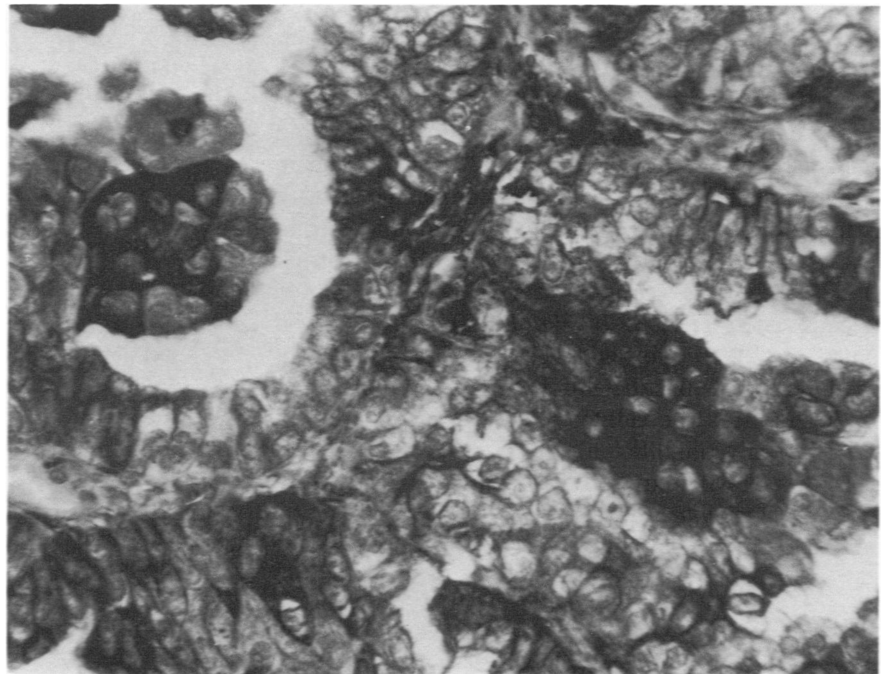

Figure 4 – Immunohistochemical staining of a formalin-paraffin section of a lung adenocarcinoma with monoclonal antibody LK8D3 against human Ia-like antigens. Most of the tumor cells exhibit cytoplasmic staining for Ia-like antigens. (Immunoperoxidase, ×330)

study over previous studies was the ability to use formalin-fixed and paraffin-embedded sections, which provide morphologic detail superior to that of cryostat sections. Because of this, we could distinguish several cases of melanoma where staining for Ia-like antigens was not associated with tumor cells. Thus, the results from this study and that of Ruiter et al[13] indicate that Ia-like antigen expression, although correlated with malignant transformation, may not be as extensively expressed by melanoma cells *in vivo* as earlier reports indicated. Since formalin-paraffin sections are routinely used to diagnose skin lesions in surgical pathology, it seems unlikely from our data that staining for Ia-like antigens will help solve controversial diagnoses of melanomas, as suggested by Natali et al.[10]

In a previous report by Thompson et al[12] human Ia-like antigens were identified in 1 of 2 cases of primary adenocarcinoma of the lung in which approximately 50% of the cells were stained. In a similar fashion, we have noted extensive Ia-like antigen expression in 3 cases of adenocarcinoma, whereas 2 cases were unreactive. The Ia-like immunoreactivity of adenocarcinoma cells may not be entirely related to malignant transformation, as with melanoma, because Ia-like antigens were also detected in normal submucosal bronchial glands and alveolar lining cells, which may give rise to some adenocarcinomas of the lung. Whether Ia-like antigen expression by lung adenocarcinomas is related to the clinical course of this disease is presently unknown. In contrast to adenocarcinoma, we found that the great majority of lung squamous carcinomas were Ia-like-antigen-negative, with only occasional positive tumor cells seen in a few cases. Ia-like antigens were also undetectable in the normal bronchial epithelium.

In conclusion, we have examined melanoma and lung carcinomas for expression of Ia-like antigen, which in previous studies has been considered as a marker of malignant transformation. Immunoperoxidase staining with a monoclonal antibody in formalin-paraffin tissue sections was chosen for this study because these sections are used routinely in surgical pathology. We found that Ia-like antigens were not readily expressed by melanoma tumor cells or by lung squamous carcinoma cells, whereas lung adenocarcinomas were either strongly positive or were negative. Thus, future efforts correlating clinical parameters of lung adenocarcinoma with Ia-like antigen staining may be warranted.

References

1. Wiman K, Curman B, Forsum U, Klareskog U, Malnnas-Tjernlund L, Tragardh L, Peterson PA: Occurance of Ia antigens on tissues of non-lymphoid origin. Nature 1978, 276:711
2. Scott H, Solheim BG, Brandtzaeg P, Thorsby E: HLA-DR antigens in the epithelium of the human small intestine. Scand J Immunol 1980, 126:2109
3. Natali PG, deMartino C, Quaranta V, Nicotra R, Frezza F, Pellegrino MA, Ferrone S: Expression of Ia-like antigens in normal human non-lymphoid tissues. Transplantation 1981, 31:75
4. Barclay AN, Mason DW: Induction of Ia antigen in rat epidermal cells and gut epithelium by immunological stimuli. J Exp Med 1982, 156:1665-1676
5. Klareskog L, Forsum U, Peterson PA: Hormonal regulation of the expression of Ia antigens on mammary gland epithelium. Eur J Immunol 1980, 10:958
6. Hirshberg H, Bergh OJ, Thorsby E: Antigen presenting properties of human vascular endothelial cells. J Exp Med 1980, 152:249s
7. Daynes RA, Emam M, Krueger GG, Roberts LK: Expression of Ia antigen on epidermal keratinocytes after the grafting of normal skin to nude mice. J Immunol 1983, 130:1536-1539
8. Winchester RJ, Wang C-Y, GiboFsky A, Kunkel HG, Lloyd RD, Old LJ: Expression of Ia-like antigens on cultured human malignant melanoma cell lines. Proc Natl Acad Sci (USA) 1978, 75:6235-6239
9. Wilson BS, Indiveri F, Pellegrino MA, Ferrone S: DR (Ia-like) antigens on human melanoma cells: Serological detection and immunochemical characterization. J Exp Med 1979, 149:658-668
10. Natali PG, Cordiali-Fei P, Cavaliere R, DiFilippo F, Quaranta V, Pellegrino MA, Ferrone S: Ia-like antigens on freshly explanted human melanoma. Clin Immunol Immunother 1981, 19:250-259
11. Houghton AN, Eisinger M, Albio AP, Cairncross JG, Old LJ: Surface antigens of melanocytes and melanomas: Markers of melanocyte differentiation and melanoma subsets. J Exp Med 1982, 156:1755-1766
12. Thompson JJ, Herlyn MF, Elder DE, Clark WH, Steplewski Z, Koprowski H: Expression of Dr antigens in freshly frozen human tumors. Hybridoma 1982, 1:161-168
13. Ruiter DJ, Bhan AK, Harrist TJ, Sober AJ, Mihm MC Jr: Major histocompatibility antigens and mononuclear inflammatory infiltrate in benign nevomelanocytic proliferations and malignant melanoma. J Immunol 1982, 129:2808-2815
14. Rosai J, Ackerman S: Surgical Pathology. 6th edition. St. Louis, C.V. Mosby, 1981
15. Galfre G, Howe SC, Milstein C, Butch GW, Howard JC: Antibodies to major histocompatibility antigens produced by hybrid cell lines. Nature 1977, 266:550
16. Ozato K, Mayer N, Sachs PH: Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J Immunol 1980, 124:533-539
17. Salisbury JG, Graham JM: Cell surface radioiodination with the sparingly soluble catalyst iodogen: Differences between dividing and non-dividing thymocytes. Biochem J 1981, 194:351-355
18. Laemmli VK: Clevage of structural proteins during assembly of the head of bacteriophage T4. Nature 1970, 222:680-685