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Immunohistologic Detection of Immunoglobulins in Malignant Lymphomas and Its Value in Histopathologic Diagnosis

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Summary. Formalin fixed, paraffin embedded material of malignant Non-Hodgkin lymphomas (132 cases), Hodgkin lymphomas (59 cases), angioimmunoblastic lymphadenopathy (2), lymphoepithelioid cell lymphoma (1) and lymph nodes with nonspecific lymphadenitis (8) were studied by a modified immunofluorescence technique for the presence and distribution of immunoglobulins (IgM, IgG, IgA, K, λ). Cells of lymphoblastic lymphomas of the convoluted type and of chronic lymphocytic leukemias were always Ig-negative. In immunocytic lymphomas tumor cells with morphologic features of plasma cells and plasma cell precursors always exhibited cytoplasmic Ig-staining showing monoclonality in two thirds of the cases (IgM/K, IgM/λ, IgG/K, IgM/IgG/K). In extramedullary plasmacytomas all cells displayed a bright cytoplasmic Ig-staining. In centrocytic lymphomas and in centroblastic-centrocytic lymphomas a honeycomb-like pattern of Ig-staining could be observed. Some centroblasts in centroblastic-centrocytic lymphomas and in centroblastic lymphomas showed a granular (surface) staining in addition. Some lymphoblastic lymphomas of the Burkitt type and some of the morphologically "unclassifiable" lymphoblastic lymphomas exhibited cytoplasmic fluorescence indicative of B-cell origin. In immunoblastic lymphomas almost 50% of the tumors investigated contained Ig-containing tumor cells again favouring their B-cell derivation. In cases of Hodgkin's disease some Hodgkin- and Reed-Sternberg-cells were positive for IgG/K. In angioimmunoblastic lymphadenopathy Ig-positive intercellular deposits were observed in addition to Ig-positive basement membranes of vessels, plasma cells and plasma cell precursors. All immunoglobulin classes could be detected in this localization though not in single cells. In lymphoepithelioid cell lymphoma (Lennert's

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lymphoma) only plasma cells and plasma cell precursors gave a positive staining and all immunoglobulin classes were present. In all lymphomas Ig-positive plasma cells and plasma cell precursors were found irrespective of the immunoglobulin content of the tumor cells and may, therefore, be interpreted as being reactive. On the basis of our studies it can be stated that immunomorphologic investigation of conventional biopsy material aids in characterization of Ig-producing lymphomas, and, hence, supplements the classical staining procedures.

Key words: Immunomorphology – Non-Hodgkin lymphomas – Classification.

Introduction

Classification of malignant Non-Hodgkin-lymphomas has been attempted by evaluating either the morphological features of the tumor cell alone (Bennett, 1975; Berard and Dorfman, 1974; Butler, 1970; Dorfman, 1972, 1975; Levine and Dorfman, 1975, Lukes, 1971; Rappaport, 1966; Sheehan and Rappaport, 1970; Nathwani et al., 1976) or by using morphological and functional variables (Aisenberg and Long, 1975; Braylan et al., 1975; Brouet et al., 1976; Catovsky et al., 1976; Cawley et al., 1976; Cossman et al., 1977; Davey et al., 1976; Garvin et al., 1976; Gérard-Marchant et al., 1974; Hansen and Good, 1974; Johansson et al., 1976; Lennert et al., 1975a, 1975b; Lukes and Collins, 1975; Lukes et al., 1978; Payne et al., 1977; Stathopoulos et al., 1977; Stein et al., 1976; Taylor, 1976) i.e. immunoglobulin receptors, Fc-receptors and erythrocyte receptors. Since B-lymphocytes, T-lymphocytes or "histiocytes" with similar morphologic appearance may be involved in these neoplasms, a diagnosis based on morphology alone can certainly be misleading.

In the present work a correlation between light microscopic appearance and immunoglobulin content of malignant lymphomas was sought and the significance of immunomorphology in the assessment of histopathologic diagnosis was evaluated. This was achieved by studying consecutive formalin fixed paraffin sections of different malignant lymphomas after application of immunofluorescence and conventional staining procedures.

Material and Methods

1. Specimens. 59 Hodgkin-lymphomas and 132 Non-Hodgkin-lymphomas were studied. The Hodgkin-lymphomas are classified according to Lukes et al. (1966a, 1966b), the Non-Hodgkin-lymphomas according to the Kiel classification (Gérard-Marchant et al., 1974; Lennert et al., 1975a) but reference is also given to the classification introduced by Rappaport (1966) and coworkers (Nathwani et al., 1976) (Table 1). In addition, two cases of angioimmunoblastic lymphadenopathy (Frizzera et al., 1974; Lukes and Tindle, 1975), one lymphoepithelioid cell lymphoma (Burke and Butler, 1976; Lennert and Mestdagh, 1968), and eight lymph nodes with nonspecific lymphadenitis were included.

Table 1. The table lists the Kiel classification and Rappaport's classification of Non-Hodgkin lymphomas. Corresponding entities are linked by lines

No. of cases	*KIEL CLASSIFICATION*	*RAPPAPORT'S CLASSIFICATION*
9	**ML, LYMPHOCYTIC**	**ML, WELL DIFFERENTIATED, DIFFUSE**
	ML, IMMUNOCYTIC	WITH MORPHOLOGIC MANIFESTATION OF DYSPROTEINEMIA
19	LYMPHOPLASMOCYTOID	**ML, POORLY DIFFERENTIATED**
4	LYMPHOPLASMOCYTIC	NODULAR
10	POLYMORPHOUS	NODULAR + DIFFUSE
14	**ML, CENTROCYTIC**	DIFFUSE
	ML, CENTROBLASTIC – CENTROCYTIC	**ML, MIXED (HISTIOCYTIC–LYMPHOCYTIC)**
16	FOLLICULAR	NODULAR
16	FOLLICULAR + DIFFUSE	NODULAR + DIFFUSE
2	DIFFUSE	DIFFUSE
3	**ML, CENTROBLASTIC**	**ML, HISTIOCYTIC**
	ML, LYMPHOBLASTIC	NODULAR
3	BURKITT'S TYPE	NODULAR + DIFFUSE
6	CONVOLUTED TYPE	DIFFUSE
8	OTHERS	**ML, UNDIFFERENTIATED, DIFFUSE**
19	**ML, IMMUNOBLASTIC**	BURKITT'S TYPE
3	**ML, PLASMACYTIC**	**ML, LYMPHOBLASTIC**
		CONVOLUTED
		NONCONVOLUTED
		EXTRAMEDULLARY PLASMACYTOMA

2. Methodology. The specimens were cut into blocks two to four millimeters thick, fixed in phosphate buffered 8% formalin, pH 7.3, for about 24 h and embedded in paraffin in the conventional way. Immunofluorescence was performed on 4 μ sections as previously described (Denk et al., 1976). Briefly, the dewaxed and rehydrated sections were covered with 0.3% celloidine and incubated with 0.1% pronase (type VII, Sigma Chem. Comp., MO) in phosphate buffered saline (PBS), pH 7.4, for 15 min at 37° C. Pronase treatment has been shown by Huang (1975) and Denk et al. (1977) to considerably suppress background staining and, in addition, to enhance specific reactivity of intracytoplasmic immunoglobulins in formalin fixed paraffin embedded material (Denk et al., 1977). Indirect immunofluorescence (IIF) was performed using anti-human globulin sera from the rabbit (Behring-Werke, Marburg, Germany) in dilutions of 1:8 (anti-IgA, anti-IgG and anti-K-chains), 1:16 (anti-IgM) and 1:4 (anti-λ-chains), respectively in the first layer. The working dilutions of the antisera corresponded to $^1/_2$ precipation unit as determined in Ouchterlony double immunodiffusion. After a 40 min-incubation period at room temperature in a moist chamber the slides were thoroughly rinsed in several changes of PBS and then covered with FITC-conjugated anti-rabbit globulin serum from the goat (Behring-Werke) diluted 1:10 (corresponding to $^1/_2$ precipation unit in double immunodiffusion; fluorescein-protein ratio 1.5:1) for 45 min. After rinsing the sections in PBS they were mounted in glycerol-PBS and read on a Leitz-Orthoplan fluorescence microscope with epiillumination (Ploem-Opak, filter combination 3/3). For control purposes, nonimmune rabbit globulins in comparable dilutions and PBS, respectively, were applied as the first layer. In addition, immunoglobulin antisera were used after absorption with the particular antigen insolubilized by the method of Avrameas and Ternynck (1969).

Consecutive paraffin sections were subjected to the following procedures: Hematoxylin-Eosin-, PAS-, Giemsa-, Gomori's reticulin-, and occasionally van Gieson-staining.

Results

Malignant Lymphomas, Lymphocytic Type

According to the Kiel classification (Gérard-Marchant et al., 1974; Lennert et al., 1975a) only chronic lymphocytic leukemia is included in this lymphoma group. This type is cytologically characterized by a dense infiltration of small lymphoid cells with a narrow rim of basophilic cytoplasm, round nuclei with dense chromatin, and rare mitotic figures. Growth centers (Braylan et al., 1975; Dick and Maca, 1978; Lennert et al., 1975a; Lukes and Collins, 1975) with larger prolymphocytes are occasionally present; a true follicular pattern, however, is always missing.

In IIF, the tumor cells were consistently negative for all immunoglobulins. However, occasionally plasma cells and plasma cell precursors containing IgG, or IgA, or IgM were present sometimes in juxtatrabecular position. These cells, apparently, did not belong to the lymphoma, but were reactive in nature.

Malignant Lymphomas, Immunocytic Type

Cytologically this variety consists predominantly of mature appearing lymphocytes, plasmacytoid lymphocytes and plasma cells in different relative proportions (Fig. 1A). Depending on the prevalence of plasma cells or plasmacytoid lymphocytes this lymphoma can be subdivided into the lymphoplasmacytoid and lymphoplasmacytic type. The polymorphous immunocytomas contain, in addition to cells mentioned above, cleaved follicle center cells (Lukes and Collins, 1975), non-cleaved follicle center cells (Lukes and Collins, 1975) and immunoblasts.

Immunofluorescence staining (Fig. 1B) for different immunoglobulins disclosed two subgroups: (1) One in which the neoplastic lymphoplasmacytoid cells and plasma cells contained only one light chain. In this category we found 10 cases with K-chains (3 with IgM/K; 5 with IgG/K; 2 with IgG/IgM/K) and 3 cases with λ-chains (IgM/λ). (2) In the second subgroup (20 cases) a less uniform immunomorphologic picture was found. 7 cases showed a predominance of one immunoglobulin light chain: 2 with λ-chains (1 with IgA; 1 with IgM) and 5 with K-chains (1 with IgA; 2 with IgM and 2 with no predominance of a heavy chain). In addition, some plasma cells positive for the other light chain, possibly reactive in nature, could also be detected. Occasionally nuclei with immunoglobulin inclusions (Dutcher and Fahey, 1959) (1 with IgG/K and 1 with IgG/λ) were found (Fig. 1). The residual cases classified as immunocytic lymphomas in light microscopy showed no predominance of a particular light or heavy chain expression. Cells positive in immunofluorescence were scanty and contained different immunoglobulins, and were stained with variable intensity.

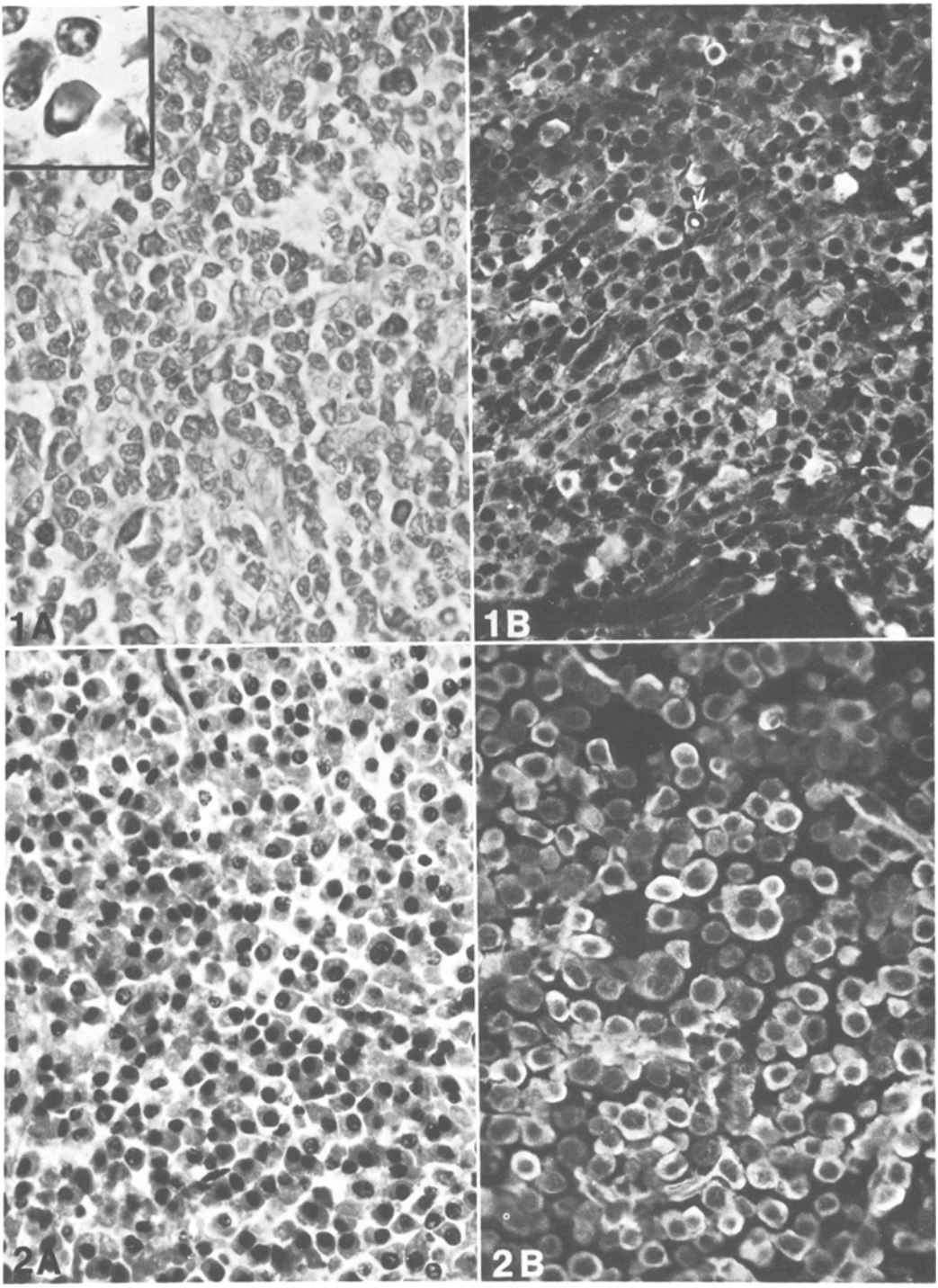
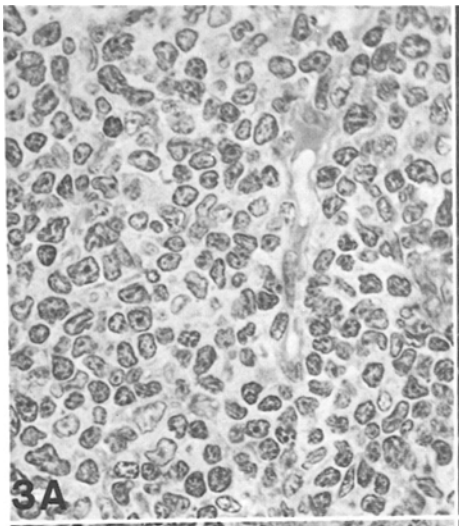


Fig. 1A and B. Immunocytic lymphoma. **A** Light microscopy. (Giemsa, ×340). Inset: nuclear immunoglobulin inclusion body = Dutcher body. (PAS, ×1,360). **B** Immunofluorescence with anti-human IgM serum (arrow: Dutcher body). (×340)

Fig. 2A and B. Plasmacytic lymphoma. **A** Light microscopy. (H & E, ×340). **B** Immunofluorescence with anti-human IgA serum. (×680)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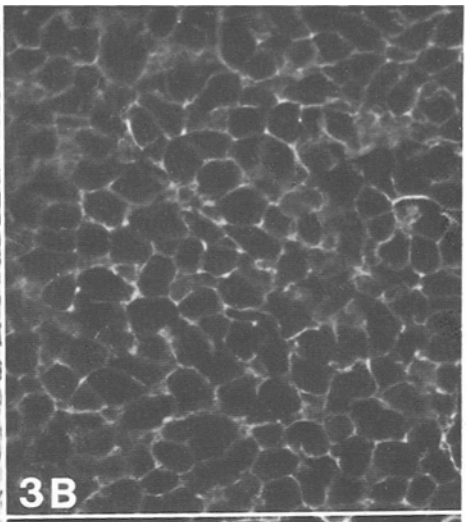
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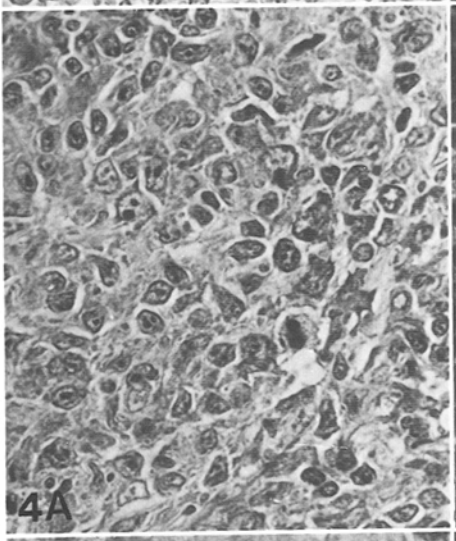
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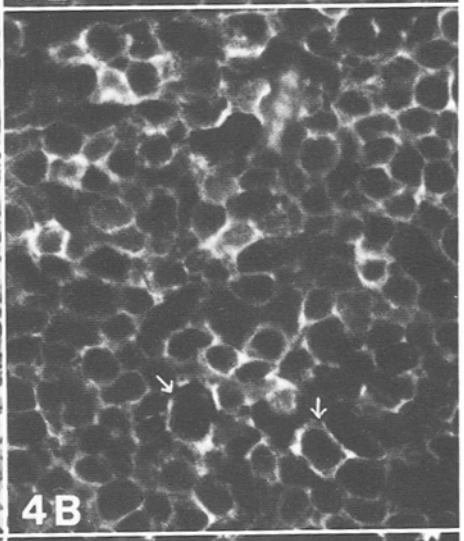
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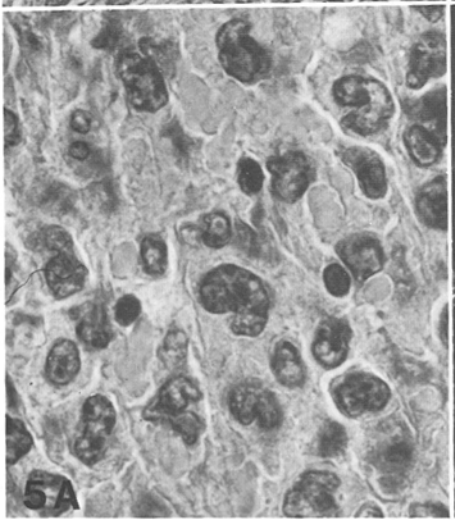
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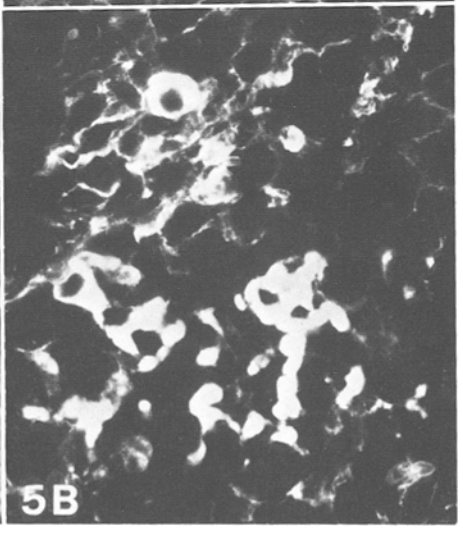

5B

Malignant Lymphomas, Plasmacytic

Constituent tumor cells are almost entirely plasma cells and occasionally plasmablasts (Fig. 2A). In our material, these tumors were all localized in the nasopharyngeal region.

Immunostaining revealed a fairly monomorphous pattern (Fig. 2B). The tumor cells of individual cases contained only one immunoglobulin class: one contained IgG/λ, one IgG/K and one IgA/K. However, at the margins of the malignant growth plasma cells, most of them containing IgA and only some with IgG, were present.

Malignant Lymphomas, Centrocytic

These lymphomas consist predominantly of small uniform atypical lymphoid cells differing from normal lymphocytes by slightly larger size and by cleaved nuclei with small nucleoli (Fig. 3A).

The immunofluorescence pattern of this type was the least clear cut of all lymphomas studied. In ten of 14 cases the tumor cells were outlined by rim-like fluorescence resulting in a "honeycomb-like" appearance (Fig. 3B). In all but 3 cases this fluorescence could be attributed to several light and heavy chains, predominantly to IgG, K and λ. Three specimens, however, appeared to be monoclonal, 2 of them showing IgG/K and 1 IgM/K. Plasma cells within trabeculae contained mostly IgM, IgG, and only rarely IgA, K- and λ-chains.

Malignant Lymphomas, Centroblastic-Centrocytic (Figs. 4 and 5)

The tumor consists mostly of small cleaved follicle center cells intermixed with varying amounts of large non-cleaved follicle center cells ("Centroblasten"; Lennert et al., 1975a). The latter are medium-sized to large cells with round nuclei poor in chromatin which is concentrated on the nuclear periphery. A small rim of darkly stained (Giemsa) cytoplasm surrounds the nucleus. The nucleoli are prominent and often located at the periphery of the nucleus. Dendritic reticulum cells are present in small amounts in the tumor areas. 16 of our 34 cases were follicular (6 of them with sclerosis; Bennett, 1975), 16 were

Fig. 3A and B. Centrocytic lymphoma. **A** Light microscopy. (Giemsa, × 540). **B** Immunofluorescence. (Anti-human IgG serum, × 680)

Fig. 4A and B. Centroblastic-centrocytic lymphoma of follicular and diffuse type without sclerosis. **A** Light microscopy. (Giemsa, × 540). **B** Immunofluorescence. (Anti-human IgM serum, × 680). Arrows indicate centroblasts

Fig. 5A and B. Anaplastic centroblastic-centrocytic lymphoma with intercellular immunoglobulin deposits. **A** Light microscopy. (H & E, × 850). **B** Immunofluorescence. (Anti-human IgA serum, × 680)

follicular and diffuse (Fig. 4A) (4 with sclerosis; Bennett, 1975) and 2 showed an entirely diffuse pattern. With respect to the localization, 11 were found in the stomach, 1 in the thyroid, 1 in the maxilla and the rest in lymph nodes.

The immunofluorescence pattern closely resembled that described in centrocytic lymphomas i.e. honeycomb-like appearance. However, a coarsely granular outline of some of the larger cells (presumably non-cleaved follicle center cells) was an additional finding (Fig. 4B). In 9 of our 34 cases a positive immunofluorescence was observed, IgG/K in 3 cases, IgG/λ in 3 cases and IgM/λ in 3 cases. All specimens including those which were immunoglobulin-negative in the tumor cells contained plasma cells mostly concentrated within the interfollicular space of the neoplastic follicles which were positive for different immunoglobulins.

Malignant Lymphomas, Centroblastic

This tumor consists of numerous large non-cleaved follicle center cells, whereas small cleaved cells are scanty. This type of lymphoma is rare in our material and only 3 specimens could be studied. One showed a follicular, one a follicular-diffuse and one a diffuse pattern. Sclerosis was absent in all cases.

Immunoglobulin containing cells were sparse in all 3 specimens and only in one case a specific weak fluorescence with IgG/λ was observed. In essence, the staining resembled that previously described for large non-cleaved follicle center cells, displaying a granular, "honeycomb-like" pattern. Plasma cells containing IgA and IgG were scarce.

Malignant Lymphomas, Lymphoblastic

The *Burkitt's type* is characterized by monomorphous medium-sized cells, often arranged in a cohesive-like pattern, with basophilic cytoplasm and round or oval nuclei containing one to four nucleoli. A "starry sky" appearance of the specimen (Fig. 6A) is caused by scattered phagocytes.

In immunofluorescence, all these tumors contained varying numbers of cells positive for IgG, IgM, K and λ in their cytoplasm. Only 1 case showed a prevalence of IgG/K (Fig. 6B).

Lymphoblastic lymphomas of the convoluted type (Lukes and Collins, 1975; Nathwani et al., 1976) contain medium sized cells with "convoluted" instead of round nuclei showing dense chromatin structures and narrow rim of lightly basophilic cytoplasm (Giemsa stain).

The bulk of the tumor cells was negative in immunofluorescence. However, small to medium-sized plasmacytoid cells containing IgG, IgA, IgM, K and λ were seen in the tumor. These cells were more frequent in subepithelial position in one tumor localized in the tonsil.

A third group is called *lymphoblastic lymphomas, type "others"* by Lennert et al. (1975a) since it neither fits the morphology of the Burkitt's nor that of convoluted type of lymphoblastic lymphomas. It contains small to medium sized cells with round or oval nuclei with reticular chromatin, sometimes promi-

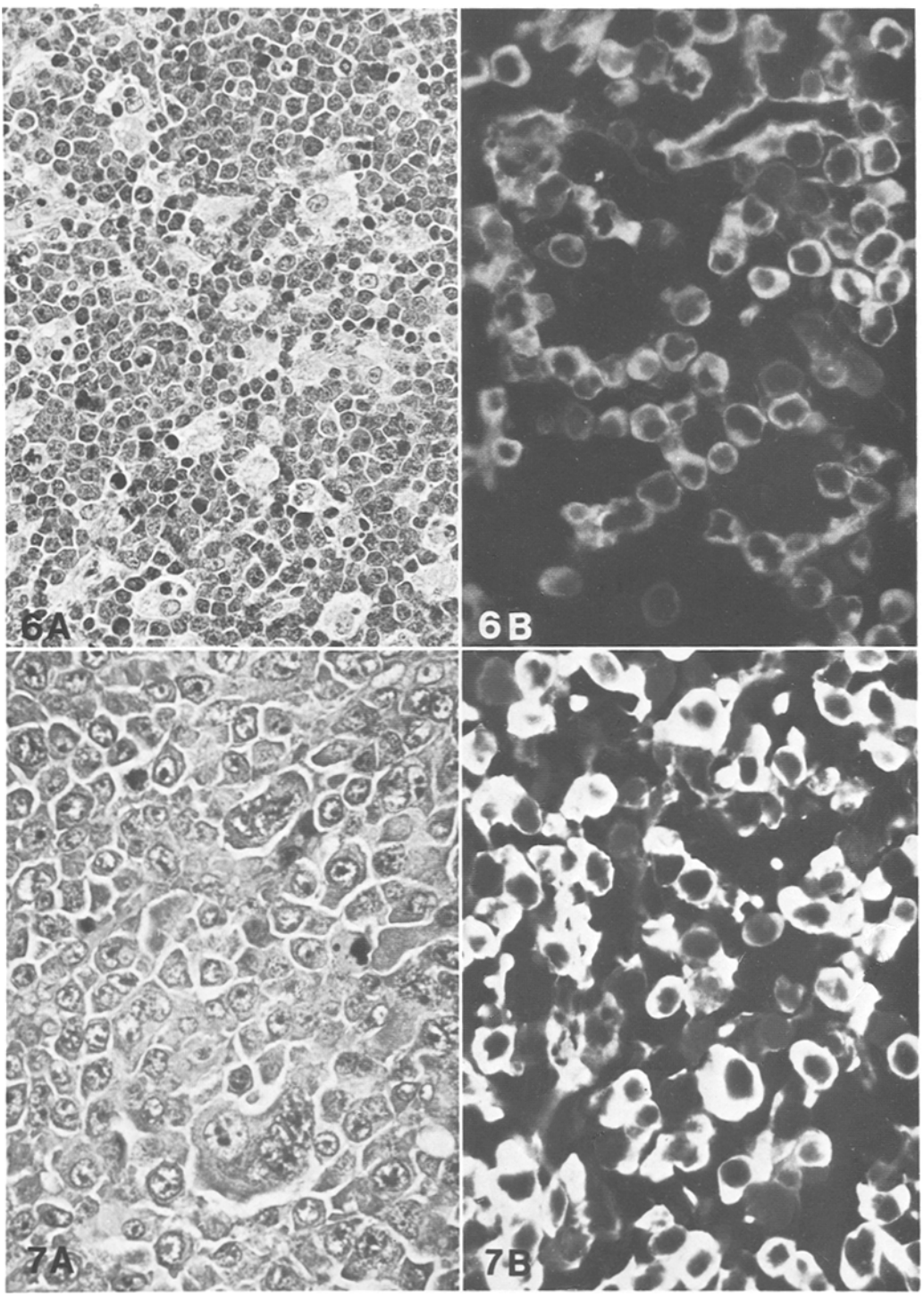
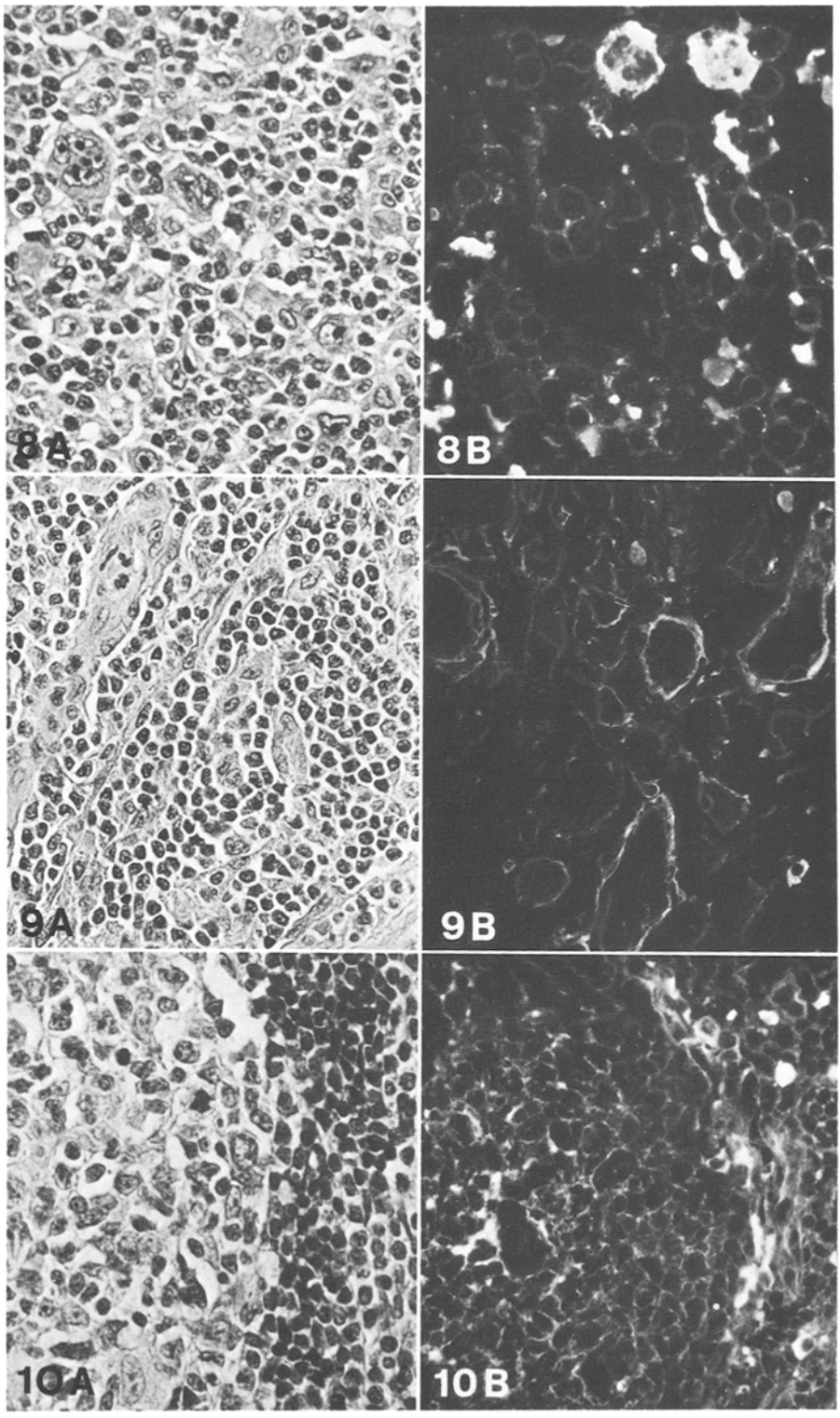


Fig. 6A and B. Lymphoblastic lymphoma of Burkitt type. **A** Light microscopy. (Giemsa, ×340). **B** Immunofluorescence. (Anti-human IgM serum, ×680)

Fig. 7A and B. Immunoblastic lymphoma. **A** Light microscopy. (Giemsa, ×440). **B** Immunofluorescence. (Anti-human IgG serum, ×680)


8A
8B
9A
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10B

nent nucleoli and a small rim of cytoplasm with varying staining intensity (Giemsa stain).

One of our 8 cases contained a conspicuous number of medium-sized cells with cytoplasmic IgG/K. One tumor showed a granular "honeycomb-like" staining positive for IgG/K. In the remaining 6 specimens all tumor cells were negative but constantly some plasma cells were detected at the tumor margins and also in the center of the tumor containing different immunoglobulins.

Malignant Lymphomas, Immunoblastic

This tumor consists of medium-sized to large cells with abundant basophilic cytoplasm and lightly stained large oval nuclei. The nuclei contain large prominent nucleoli in a central position. Occasionally binucleated giant cells are observed (Fig. 7A).

Five of the 19 specimens contained only a single immunoglobulin class (2 IgG/K, 2 IgM/K, 1 IgM/λ) in their tumor cells (Fig. 7B). Six specimens showed varying numbers of cells containing intracytoplasmic IgG, IgA, IgM, K and λ, in two a prevalence of IgA and in one of IgM was noted. Again, plasma cells and plasma cell precursors containing different immunoglobulin classes were present in the tumor in almost all instances. A prevalence of IgM in this group could not be detected in contrast to the results obtained by extraction procedures (Lennert et al., 1975b).

Hodgkin's Disease

The histologic appearance of lymphogranulomatosis is clearly defined (Lukes, 1971) and will not be discussed here.

Only 2 cases of Hodgkin's disease with *lymphocyte predominance* were studied. One was nodular and one diffuse. One case (diffuse type) contained a plasma cell infiltrate the individual cells of which were positive for different immunoglobulin classes. Positive Reed-Sternberg cells and Hodgkin cells could not be detected with certainty. In the other case (nodular type) most of the plasma cells contained IgG and K-chains and only a few IgA and λ-chains. In this case some but not all Hodgkin and Reed-Sternberg cells were positive for IgG/K. All lymphoid cells were negative.

Fig. 8A and B. Hodgkin's disease (mixed cellularity). **A** Light microscopy, (H & E, ×440). **B** Immunofluorescence. (Anti-human IgG serum). Note IgG positive Reed-Sternberg cells. (×680)

Fig. 9A and B. Angioimmunoblastic lymphadenopathy. Area of vascular proliferation is depicted. **A** Light microscopy. (H & E, ×340). **B** Immunofluorescence. Note positive vascular basement membranes. (Anti-human IgG serum, ×340)

Fig. 10A and B. Follicular hyperplasia in lymphadenitis. **A** Light microscopy. (H & E, ×340). **B** Immunofluorescence. (Anti-human IgG serum, ×340)

Specimens with the histologic picture of *nodular sclerosis* contained abundant plasma cells, positive with different immunoglobulin antisera, however IgG containing cells were predominant. In this group of 23 cases both Reed-Sternberg cells and Hodgkin cells were clearly positive for immunoglobulins in 12 cases, 7 with IgG/K and 4 with IgG/K and λ. In three cases only Hodgkin cells showed positive immunofluorescence with IgG whereas Reed-Sternberg cells were negative. Screening for light chains revealed K and λ in 2 and K only in 1 case. Lacunar cells were negative in all cases.

Hodgkin's disease with *mixed cellularity:* Despite plasma cells positive for all immunoglobulins with predominance of IgG the cases with positive Reed-Sternberg cells and Hodgkin cells (14 out of 22 cases) constituted about 50% like in nodular sclerosis (Fig. 8). The immunoglobulin content was also similar, i.e. mostly IgG/K and only occasionally λ-chains in addition. Two specimens contained only positive Hodgkin cells but the Reed-Sternberg cells were negative.

Only two cases belonged to the *lymphocyte depleted type:* Because of the high nonspecific background staining in diffuse fibrosis positive cells could not be detected with certainty in one specimen. In the second specimen (reticular type) Hodgkin and Reed-Sternberg cells were positive with respect to IgG/K and occasionally λ. Hodgkin associated mononuclear cells could not be identified in immunofluorescence.

Angioimmunoblastic Lymphadenopathy

The histologic picture (Frizzera et al., 1974; Lukes and Tindle, 1975) is characterized by a destruction of the normal lymph node architecture and proliferation of immunoblasts, plasma cells, eosinophils, lymphocytes and sometimes histiocytes and epithelioid cells. Reed-Sternberg cells and Hodgkin cells are absent. Vascular proliferation is a prominent feature predominantly in the paracortical area of the lymph node and the basement membranes of the vessels are thickened by amorphous PAS-positive material (Fig. 9A). Amorphous, slightly acidophilic material rich in mucopolysaccharides can be found in intercellular position.

In immunofluorescence, plasma cells and plasmacytoid cells and some immunoblasts were positive for IgG, and occasionally for IgM and IgA. The thickened basal membranes did not react with anti-IgA and anti-IgM, they were, however, clearly positive for IgG/K (Fig. 9B). The interstitial homogeneous material was slightly stained with anti-IgM and more pronounced with anti-IgG giving a filamentous appearance in immunofluorescence.

Lymphoma With High Content of Epithelioid Cells (Lennert's Lymphoma)

This disorder is characterized by effacement of lymph node structure due to infiltration with epithelioid histiocytes (Burke and Butler, 1976; Lennert and Mestdagh, 1968). These cells occur in clusters surrounded by varying amounts of lymphocytes, plasma cells, immunoblasts and eosinophils.

Immunofluorescence did not reveal specific staining of the epithelioid cells

and only interspersed plasma cells containing IgA and IgG, K and λ were observed.

Normal lymph nodes or those showing the histologic picture of *lymphadenitis* contained plasma cells and plasmacytoid cells in varying amounts which were positive with immunoglobulin antisera. In the germinal centers, a honeycomb-like pattern of Ig-specific fluorescence (IgA, IgG, IgM, K, λ chains) was clearly discernible (Fig. 10). Interstitial immunoglobulin deposits were also detected.

Discussion

Several attempts have been made in the past to apply immunomorphological techniques to routine material of malignant lymphomas (Braylan et al., 1975; Cawley et al., 1976; Dorfman, 1975; Garvin et al., 1976; Pinkus and Said, 1977; Skinner et al., 1976; Taylor, 1976) in order to characterize and identify the cellular constituents. In most instances frozen sections were used. Frozen sections, however, have considerable disadvantages in routine pathology, and, therefore, application of immunomorphologic techniques to conventional formalin fixed paraffin embedded tissue is desirable. In a previous paper (Denk et al., 1976, 1977) it was shown that treatment of dewaxed tissue sections with pronase, as originally proposed by Huang (1975) for the demonstration of hepatitis B-antigens in liver biopsy specimens, has a favourable effect on immunofluorescence by apparently increasing the antigenicity of intracytoplasmic immunoglobulins and suppressing nonspecific background staining.

Demonstration of immunoglobulin production of a cell is indicative of B-cell origin. Monoclonal immunoglobulin-containing tumor cells have been found in most immunocytic lymphomas, all plasmacytic lymphomas, some lymphoblastic and immunoblastic lymphomas but, in addition, plasma cells and plasma cell precursors, the individual cells positive for IgG or IgA or IgM, K or λ chains, were found in all lymphomas in varying numbers irrespective of the immunoglobulin content of the tumor cells. These cells are certainly polyclonal and considered to be reactive.

In chronic lymphocytic leukemia of the B-cell type the proliferating cells are blocked at an early stage of maturation (Seligmann et al., 1973). They are unable to produce intracytoplasmic immunoglobulins, and, hence, are negative in cytoplasmic immunostaining. Nevertheless, reactive plasma cells are nearly always present in the infiltrated lymph nodes.

Immunocytic lymphomas are characterized by plasma cells and plasma cell precursors with monoclonal immunoglobulin production in addition to lymphocytes. In the light microscope the presence of these cells is, therefore, considered as a finding of major diagnostic importance. On the basis of our results the discrimination between a monoclonal and a polyclonal plasma cell infiltration by immunomorphology is essential for differential diagnosis. Only the monoclonal type justifies the diagnosis "immunocytic lymphoma". However, in cases with plasma cells containing different immunoglobulins still the possibility exists that this cell population is made up by monoclonal tumor cells on one hand and by polyclonal reactive cells on the other. In this situation immunomorphology alone fails in discriminating immunocytic lymphoma from CLL and this

may apply the 13 cases listed in subgroup 2 diagnosed on the basis of characteristic light microscopic features. This example again indicates that immunomorphology cannot entirely substitute for light microscopy.

Centrocytic lymphomas and centroblastic-centrocytic lymphomas (Gérard-Marchant et al., 1974; Lennert et al., 1975a) unequivocally considered as B-cell neoplasms on the basis of light microscopy (Lennert et al., 1975a, 1975b; Lukes and Collins, 1975) and surface markers (Braylan et al., 1975; Jaffe et al., 1974) show a characteristic immunomorphologic pattern reminiscent of the normal non-neoplastic germinal center. This is in contrast to the findings of Braylan and Rappaport (1973) who did not find immunoglobulins in these tumors. Immunoglobulins, mostly IgG, were present in a honeycomb-like pattern; it is unclear whether this represents intra- or intercellular material or both, but in any case, the specific immunofluorescence pattern described can possibly be used as morphologic marker in tissue sections. With respect to the immunoglobulin classes prevailing in follicle center cell tumors, our results differ from those obtained by Lennert et al. (1975b) on the basis of extraction in that we were unable to find the prevalence of IgM.

In the category of lymphoblastic lymphomas all Burkitt type lymphoma cells show surface immunoglobulins in suspension (Binder et al., 1975; Epstein et al., 1976; Mann et al., 1976) but only a minority of these tumors actually produce and secrete immunoglobulins (Epstein, 1970; Sherr and Uhr, 1971; Van Furth et al., 1972) and this is also the experience from our immunomorphological studies. The T-cell nature of the convoluted type was assessed by spontaneous rosette formation (Lukes and Collins, 1975; Mann et al., 1975; Stein et al., 1976) and consequently immunoglobulin containing cells are missing in these neoplasms. Two cases of the third variety designated as "others" by Lennert et al. (1975a) could be characterized as B-cell derived by immunofluorescence. One showed a granular "honeycomb-like" immunofluorescence pattern indicative of its derivation from large follicle center cells.

The category of immunoblastic lymphomas is generally considered to be heterogeneous (Braylan et al., 1975; Brouet et al., 1976; Davey et al., 1976) although the B-cell-derived type seems to prevail (Davey et al., 1976; Lennert et al., 1975b; Lukes and Collins, 1975). Immunofluorescence supports this latter assumption in showing that some of the immunoblastic lymphomas contained monoclonal immunoglobulin positive cells. But even negative immunofluorescence does not exclude a B-cell lymphoma since surface immunoglobulins are not demonstrable by the technique used.

In the Hodgkin's lymphoma group some Hodgkin and Reed-Sternberg cells were positive for IgG/K. This is in agreement with the results of other authors obtained in tissue sections (Anagnostou et al., 1977; Garvin et al., 1974, 1976; Taylor, 1976). Lack of spontaneous rosette formation by Hodgkin and Reed-Sternberg cells (Kadin et al., 1976; Payne et al., 1976), negative immunofluorescence staining with anti-thymocyte globulin (Braylan et al., 1974; Kadin et al., 1976) and the findings of surface immunoglobulin on Reed-Sternberg cells (Kadin et al., 1974; Leech, 1973; Payne et al., 1976) further favours the B-cell nature of these cells. However, phagocytosis of immunoglobulins cannot be excluded particularly since phagocytosis by Hodgkin and Reed-Sternberg cells

has been demonstrated by Kaplan and Gartner (1977) in tissue culture. Recently, Curran and Jones (1978) also suggested that Hodgkin and Reed-Sternberg cells may derive from dendritic reticulum cells and suggested that detectable immunoglobulin probably results from absorption of antigen-antibody complexes. It is interesting that increased IgG- and IgA-levels in sera of Hodgkin's patients have been reported (Wagener et al., 1976) and this may be a reflection of the large numbers of IgG and IgA positive plasma cells in infiltrated lymph nodes.

Immunoblastic lymphadenopathy (Frizzera et al., 1974; Lukes and Tindle, 1975) and Lennert's lymphoma (Burke and Butler, 1976; Lennert and Mestdagh, 1975) did not reveal a clear-cut immunomorphologic pattern and no evidence was found for the presence of a monoclonal cell population. This agrees with the concept that these may be reactive lymphoproliferative disorders (Dorfman and Warnke, 1974; Frizzera et al., 1975; Gleichmann et al., 1976; Kreisler et al., 1977; Lukes and Tindle, 1975; Mathé et al., 1976; Pruzanski et al., 1976; Radaszkiewicz and Lennert, 1975).

The demonstration of immunoglobulins in routine material of malignant lymphomas facilitates diagnosis by providing additional objective criteria. It aids in the discrimination of immunoglobulin producing and secreting cells, in the evaluation of the degree of differentiation of a lymphoid neoplasm, and may, therefore, be relevant in the assessment of prognosis. Further subclassification of malignant lymphomas will only be meaningful on the basis of functional criteria in addition to cytology.

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