IMMUNOHISTOCHEMICAL STUDY OF DIFFUSE LARGE CELL HYMPHOMAS USING MONOCLONAL AND POLYCLONAL ANTIBODIES COMPARING ROUTINELY FIXED PARAFFIN EMBEDDED TISSUE AND FROZEN SECTIONS

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ABSTRACT

48 cases of diffuse large cell lymphomas (DLCLs) from Zurich were morphologically classified according to the Kiel Classification of non-Hodgkins lymphomas (NHL) and immunologically into large centrocytic 7 cases, centroblastic 23; B immunoblastic 10, peripheral T cell lymphomas (PTCLs) 6, and true histiocytic sarcoma 2 cases. 73% of the cases were of B cell, 23% of T cell and 4% of true histiocytic origin. Immunohistochemically Anti-leucocyte common antigen (LCA) PD7/26, 2B11 showed concordant immunoreactivity with 66% of DLCLs. LN2 and MB2 proved reliable B cell markers by reacting with 70% and 74% of B cell DLCLs respectively. None of the T cell lymphomas showed reactivity with these antibodies. UCHL1 proved a better T cell marker than MT1 by reacting with 72% of PTCLs against 45% of the latter. In addition 2 true histiocytic sarcomas were identified by immunophenotyping using appropriate markers. Light chain polytypic antibodies did not prove useful in detecting B cell DLCLs. Comparatively, immunophenotyping of 32 cases of DLCLs from Dar es Salaam showed 90% to be of B cell and 10% of T cell origin. Antibody PD 7/26, 2B11 showed immunoreactivity with 50% of the cases. LN1 and LN2 showed much lower percentage of immunoreactivity with B cell DLCLs than Zurich series. 2 of the 3 T cell DLCLs reacted with UCHL1.

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LIST OF ABBREVIATIONS

ACT	-	Alpha-1-antichymotrypsin
AT	-	Alpha-1-antitrypsin
Cb	-	Centroblastic lymphoma
CD	-	Cluster designation
DAB	-	Diaminobenzidine
DII	-	Double indirect immunoperoxidase
DLCLS	-	Diffuse large cell lymphomas
DRCS	-	Dendritric reticulum cells
EMA	-	Epithelial Membrane Antigen
FCC	-	Follicular Centre Cell
HLA	-	Human Leucocyte antigen
HE	-	Haematoxylin and eosin
IB	-	Immunoblastic lymphoma
IWF	-	International Working Formulation
KD	-	Kilo-dalton
LCA	-	Leucocyte Common Antigen
LCC	-	Large Centrocytic Lymphoma
ML	-	Malignant Lymphoma
NHL	-	Non-Hodgkin's Lymphoma
PAP	-	Peroxidase-anti-peroxidase
PAS	-	Periodic acid schiff
PBS	-	Phosphate Buffered Saline
PTCLs	-	Peripheral T Cell Lymphomas
TH	-	True histiocytic Lymphoma

DECLARATION

I declare that to the best of my knowledge that this thesis is a result of my work, and that it has not been submitted for a similar degree in any other University.

Signed.....

E.M. Mgaya

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INTRODUCTION

Diffuse Large cell lymphomas are neoplasms composed of lymphoid cells having a median nuclear diameter in excess of tingible-body macrophages or endothelial cells(9). In Rappaport's classification of non-Hodgkin's lymphomas (NHL) the term histiocyte was used for all large cell lymphomas in the belief that they arose from histiocytes (57). This has now been found to be incorrect, as the majority of these tumours are of B and T cell origin, and only a few cases arise from cells of the macrophage/monocyte series (true histiocytic) (8,37). The Lukes - Collins(42) and Kiel classifications(40) of NHL are the only classifications based on morphologic and immunologic features, and thereby differ from other purely morphological systems(7,23,45,59). These immunologically oriented classification systems recognize five variants of large cell lymphomas: large cleaved (large centrocyte), large non cleaved (centroblast), immunoblastic (T and B cell) and true histiocytic lymphoma. In addition to these, other variants of large cell lymphoma have been described including multilobate(13) and Ki-1 anaplastic lymphomas(68). Some peripheral T cell lymphomas also fall under the category of DLCLs(66).

DLCLs represent a heterogenous group of lymphoid neoplasms whose exact categorization cannot be determined by morphological appearance alone. Some are even difficult to differentiate from anaplastic epithelial tumours or sarcomas(3), particularly when they have metastasized to lymphnodes(28,29). The separation of NHLs into B and T cell

type is important because it is generally believed that T cell lymphomas behave more aggressively than those arising from B lymphocytes(12). Other authors, have the opposite view, that B cell lymphomas behave more aggressively than T cell lymphomas(56). These two notions have been rejected by some authors who think that immunophenotype is not an important prognostic indicator and that B and T cell lymphomas may show no differences in survival(16,35). Nevertheless, it is generally agreed that in some categories of DLCLs, long term survival can be achieved if more aggressive combination chemotherapy is instituted(19,26,39,60,63).

Frozen section immunostaining has been found useful in the diagnosis of DLCLs(33,36,41,71). Although this technique produces reliable and reproducible results, it is associated with a number of problems which limit its value in the context of routine pathologic diagnosis(44). Tissue morphology on frozen section is always inferior to that of paraffin sections. This may make it difficult to distinguish the labelling reactions of neoplastic cells from those of reactive cells. Tissue samples must be kept frozen at -70 degrees centigrade, a situation difficult to maintain in the developing countries, where electrical faults are frequent. Thus investigators have resorted to performing immunohistological analysis on fixed paraffin sections, which has the added advantage of superior morphology.

Although paraffin section immunostaining has been adequate for identifying cytoplasmic immunoglobulins in B cell lymphomas(70,72) only 15-20 percent of them will express this

marker(44,51,70). Surface immunoglobulin, present in the majority of B cell lymphomas B and T cell membrane antigens identified by monoclonal antibodies are either masked or destroyed by fixation and embedding procedures (15,34,36, 43,44,70)

Development of monoclonal antibodies reactive with antigens in fixed tissue:

The introduction of antibodies to leucocyte antigens resistant to routine fixation and processing has raised interest in the possibility of immunophenotyping NHLs. One of the first surface membrane antigens to be identified on leucocytes was the leucocyte Common Antigen (LCA)(5). This antigen is a 200 KD glycoprotein (T200) restricted to the membrane of leucocytes, including B and T lymphocytes, thymocytes, macrophages and granulocytes(5,74). Accumulating evidence suggests that LCA represents not a single molecule, but rather a group of four structurally related molecules with molecular weights of about 220, 205, 190 and 180 KD(6,55,74). Antigenically distinct epitopes have been mapped on the LCA molecule(11,17,30). A wide range of monoclonal antibodies which recognize antigens of the LCA family in fixed paraffin embedded tissue have been developed(52,53,65,77). Antibodies recognizing all molecular species (220,205,190 and 180 KD) are termed CD 45, while those recognizing the 220 and 205 KD forms are termed CD 45 R(14). The types of LCA expressed by T and B lymphocytes show a useful degree of lineage restriction, in that B cells largely express the 220 KD LCA, while peripheral T cells express small amounts of 220 KD form and high levels

of the 205, 190 and 180 KD types(3). It is on this basis that monoclonal antibodies capable of assigning a B, T cell or macrophage origin in paraffin sections were developed. These antibodies have been reported useful in immunophenotyping NHLs(18,47,53,61). Other monoclonal antibodies, which recognize antigens other than the LCA in fixed lymphoid tissue have also been described(24,53). Some of these antibodies react with antigens in nonlymphoid tissue including epithelia and endothelial cells(24).

HYPOTHESIS AND RESEARCH OBJECTIVES

In this study, an assessment of the immunoreactivity of some of these antibodies in formalin fixed and paraffin embedded tissue was made, to answer the following hypothesis: "It is possible to use immunohistochemistry to type malignant lymphomas into histiocytic, B and T cell types in a developing country like Tanzania using routinely fixed paraffin embedded tissue. This kind of typing in conjunction with routine histological typing have prognostic significance in the management of patients".

The objectives of this study were as follows:

1. To classify all DLCLs diagnosed at the Institute of Pathology, University of Zurich from 1985 to 1987, using the Kiel Classification of NHLs(40).

2. To characterize these cases of DLCLs immunohistochemically into B, and T or histiocytic type using formalin fixed, paraffin embedded tissue.

3. To carry out immunohistological staining of these cases of DLCLs in frozen section and compare the results with those of paraffin sections.

4. To perform immunohistochemical staining of routinely formalin fixed, paraffin embedded tissue of cases of DLCLs from Dar es Salaam and compare the immunophenotypic profile with those from Zurich.

5. To determine which of the various monoclonal antibodies are ideal for the diagnosis of NHLs in Tanzania.

MATERIALS AND METHODS

Tissue blocks and slides of 48 cases of DLCLs, diagnosed during the period 1985-1987 were retrived from the records of the Institute of Pathology, University of Zurich. Frozen tissue of these cases kept at -70 degree centigrade was available for immunostaining. 32 cases of DLCLs from Dar es Salaam were also included in the study. For all cases histological review of H/E, Giemsa, PAS and reticulin stains was made. DLCLs were classified according to the Kiel Classification of NHLs,(40) with modification on peripheral T cell lymphomas as proposed by Stansfeld(66).

Antibodies used in the study

Details of antibodies and their sources are shown in tables 3 and 4. Monoclonal antibodies PD7/26, and 2B11, combined together as Dako LC, were obtained from Dakopatts, Copenhagen, Denmark. They are all directed against the T200 LCA: This antigen is expressed in 90% of lymphoid malignancies(77).

LN, MB and MT series of monoclonal antibodies were obtained from Biotest, Dreiech, West Germany. Their usefulness in the diagnosis of NHLs has been confirmed in several studies(24,35,50,53,78). LN1 is an IgM monoclonal antibody that recognises a surface membrane antigen primarily found on germinal centre B lymphocytes and related lymphomas(24). T cell and histiocytes are unreactive. LN2 reacts with the nuclear membrane and cytoplasm of mantle zone lymphocytes, a subset of germinal centre cells and scattered interdigitating histiocytes(24,50). This antigen is expressed on HLA DR

positive tissues(24). LN3 is a murine antibody reactive with HLA class II antigens on the membranes of B cells, histiocytes and also activated T cells(46).

MB1 is a commercial murine monoclonal antibody that identifies normal B cells and related B cell lymphomas(22,78). MB2 is a monoclonal antibody reactive with a cytoplasmic antigen of all B cells but not T cells(22). MT1 is a murine monoclonal antibody strongly reactive with a membrane antigen on all T cells, myeloid cells, monocytes, macrophages but not with mature B lymphocytes(53). UCHL1 is an IgG2a monoclonal antibody which recognizes an antigen of 180 - 185 KD present on a subpopulation of T cells(73) and has been useful in the diagnosis of T cell lymphomas(48,49).

Anti-alpha-l-AT, anti-alpha-l- antichymotrypsin and antilysozyme are all polytypic antisera recognizing histiocytes.

For Dar es Salaam cases, antibodies used were anti-LCA PD7/261 2B11, LN1 LN2, UCHL1, Kappa and Lambda light chain antibodies, (Table 7).

Immunohistochemistry of DLCLs

Immunohistochemical study of DLCLs on paraffin as well as frozen sections was carried out using the double indirect immunoperoxidase reaction for monoclonal antibodies. The PAP method of Sternberger et al(69) as modified by Stein et al(67) was used for all polytypic antisera. Monoclonal antibodies

were diluted in PBS in a ratio of 1:30. For the polytypic antisera, dilution was as follows:

Anti-alpha-l-antitrypsin	1:200
Anti-alpha-l-antichymotrypsin	1:400
" Lysozyme	1:100
" Kappa light chain	1:400
" Lambda light chain	1:1000.

Five micron thick sections were prepared for staining by dewaxing, hydrating and washing in phosphate buffered saline(PBS). Endogenous peroxidase activity was blocked by exposing the sections to methanol containing 1% hydrogen peroxide for 15 minutes. To unmask antigens, enzyme digestion was carried out by incubating sections with 0.5% trypsin for 8 minutes for polytypic antisera, followed by washing three times in PBS. The initial step in both methods of immunostaining involved the incubation of the dewaxed sections with monoclonal and polytypic antibodies for 30 minutes in a humid chamber at 37 degrees centigrade. Sections were then washed in PBS three times for 5 minutes and incubated for 30 minutes with peroxidase-conjugated goat anti mouse Ig G, diluted 1:40 PBS (to which 5% normal human serum had been added to block cross reactivity against human Igs) for the double indirect immunoperoxidase method. For the PAP method, swine anti-rabbit antibody was applied and incubation carried out for 30 minutes, then washed three times in PBS. The third step consisted of incubating sections with peroxidase conjugated swine anti-goat Ig G and PAP complex for the double

indirect and PAP methods respectively. Peroxidase reaction was detected by incubating sections with 3'3 diaminobenzidine tetrahdrochloride 0.6 mg/ml and hydrogen peroxide 0.01% for 15 minutes. Sections were then rinsed in tap water, counterstained with Mayers' haematoxylin, dehydrated through a series of alcohol concentrations-75%, 90% and absolute alcohol) and finally cleared in xylene. Mounting was done using eukit.

RESULTS

There were 48 cases of DLCLs from the Institute of Pathology, Zurich. Of these, 23 were centroblastic, 10 B immunoblastic, 7 large centrocytic, 6 PTCLs and 2 histiocytic sarcoma (Table 5).

Of the 6 PTCLs, 3 were immunoblastic, 2 large monomorphic and 1 T-zone lymphoma.

Immunoreactivity to various markers

The results of immunostaining of these cases in paraffin as well as in frozen sections are shown in Tables 5 and 6. Monoclonal antibody PD7/26, 2Bll reacted with 30 out of 48 (64.5%) DLCLs in paraffin sections. Variation in staining intensity was noted not only from one case to another but even from one area of section to another area within same slide. Some areas were strongly stained while others were weak or not stained at all. Five out of 7 large centrocytic lymphomas, 16 out of 23 centroblastic lymphomas, and 5 out of 10 B immunoblastic lymphomas showed concordant immunoreactivity with PD7/26, 2Bll. The staining was characteristically membrane located (Fig. 1). Of the 17 cases that did not react with anti LCA, in paraffin sections, 11 showed immunoreactivity in frozen sections. The usefulness of this marker was exemplified by the strong immunoreactivity with an anaplastic large cell lymphoma that was morphologically thought to be a carcinoma of stomach (Fig. 2). PTCLs showed a wide variety of immunoreactivity. Whereas the small and medium neoplastic cells showed moderate staining, the large size

neoplastic cells showed weak or no staining at all (Fig. 3). Four out of the 6 cases of PTCLs reacted with PD7/27, 2B11. One of 2 cases of histiocytic sarcoma reacted with this antibody. The better morphology preserved in paraffin sections made it easier to differentiate between T and B cells in these cases which was not easy with frozen sections. From the Dar es Salaam series, only 16 out of 32 DLCLs (50%) showed immunoreactivity with PD7/26, 2B11 (Table 7). The Cb and LCC lymphomas showed the strongest immunoreactivity than the other categories (Fig. 4).

LN1, LN2 and LN3 immunostaining

LN1 was reactive with 23 out of 40 (57.5%) B cell DLCLs from Zurich, and 10 out of 29 (34%) B cell DLCLs from Dar es Salaam. LN1 reactivity was largely confined to centroblastic and large centrocytic lymphomas, and least with B immunoblastic lymphomas. The staining was localized to the membrane of neoplastic cells. LN2 showed a much higher reactivity with 29 out of 40 cases (72.5%) of B cell DLCLs from Zurich and 15 out of 29 (52%) B cell DLCLs from Dar es Salaam. Staining was cytoplasmic (Fig. 5). None of the PTCLs showed reactivity with LN1 or LN2. LN3 was reactive not only with B cell lymphomas but some T cell lymphomas as well. Twenty eight out of 40 B cell DLCLs (70%) and 4 of 6 PTCLs (66%) showed immunoreactivity with LN3.

MB1 and MB2 immunoreactivity

MB1 showed immunoreactivity in 13 out of 40 cases (32%) of B cell DLCLs. The staining intensity was generally weaker than

LN1 and LN2. In contrast MB2 proved a reliable B cell marker by reacting moderately with 26 out of 40 cases (65%). None of the PTCLs showed reactivity with MB1 and MB2, although non-neoplastic B cells in these tumours were strongly reactive and could easily be differentiated from the neoplastic cells.

MT1 reactivity

Monoclonal antibody MT1 was reactive with 2 of the 6 PTCLs(33%). The staining intensity was generally weak and patchy. Whereas the small and medium neoplastic cells showed concomitant immunoreactivity, there was variability in the staining intensity of the large neoplastic cell component. Some of the large cells stained weakly while others were not stained at all (Fig. 6). B cell DLCLs did not show immunoreactivity with MT1, although non-neoplastic T cells interspersed within the population of neoplastic B cells were strongly reactive.

UCHL1 reactivity

UCHL1 gave a stronger and more consistent immunostaining of PTCLs than that noted with MT1. The staining was membrane localized (Fig. 7). Five out of the 6 PTCLs from Zurich showed immunoreactivity with UCHL1. In contrast to MT1, UCHL1 stained the small, medium as well as the large cell neoplastic cells more or less equally. Two of 3 PTCLs from Dar es Salaam, showed immunoreactivity with UCHL1. We did not find any of the B cell DLCLs reacting with this antibody.

Anti EMA

All DLCLs did not show reactivity with monoclonal anti-EMA except one case of T immunoblastic lymphoma. In this case the staining was confined to large dispersed neoplastic cells resembling metastatic carcinoma cells in lymphnode (Fig.8). These large cells were also positive with anti LCA PD7/26, 2B11 but negative with other epithelial markers.

Alpha-1-antitrypsin, alpha-1-antichymotrypsin and lysozyme

Two cases, they were suspected to be of histiocytic origin showed strong reactivity with all three polytypic antisera. The staining intensity for all three markers was characteristically cytoplasmic.

Kappa and Lambda light chain immunoglobulin antisera

From the Zurich series 3 cases of DLCLs showed a kappa light chain restriction and 2 lambda light chain. The rest of the B cell DLCLs were unreactive. From Dar es Salaam cases, there was so much background staining that interpretation proved difficult. Even when we compared trypsinisation versus non trypsinization of sections there was no difference between the immunoreactivity. On the whole 2 cases showed kappa restriction and 1 case lambda light chain restriction.

Immunostaining of DLCLs in frozen sections

Markers for immunostaining of DLCLs in frozen sections are shown in Table 5. Monoclonal antibody PD7/26, 2B11 showed immunoreactivity with 36 out of 48 (75%) DLCLs from Zurich. Though consistent staining was noted, in the majority of cases

morphology was so poor that one could not say for sure whether it represented background staining or cellular immunoreactivity. B1, a B cell marker showed immunoreactivity with 27 out of 40 (67%) B cell DLCLs. Again background staining was encountered. Staining for kappa and lambda light chain immunoglobulins generally gave poor results. Twenty five of the 40 (63%) B cell DLCLs and 28 of them (70%) reacted with kappa and lambda light chain antisera respectively. The T cell marker, OKT3 was reactive with 5 of the 6 PTCLs, while 2 cases demonstrated reactivity with the antibody OKT4. The 2 histiocytic sarcomas showed immunoreactivity with the macrophage marker OKM1. In general the morphological appearance in these cases was inferior to that noted in paraffin sections.

DISCUSSION

Monoclonal antibodies reacting with antigens resistant to routine processing have proved useful in the diagnosis of NHLs. Monoclonal antibody PD7/26 and 2B11 combined, and marketed commercially as Dako LC, has shown immunoreactivity with 65% of DLCLs from Zurich and 50% from Dar es Salaam. In these cases, variation in staining intensity from one area of a section to another was noted which would suggest possible denaturation of antigens by the fixation and embedding procedure.

The lower percentage of immunoreactivity noted in this study contrasts with the findings by Kurtin et al(38) and Warnke et al(77), where over 90% of their cases showed immunoreactivity to this antibody. A close look at their cases, however, shows that the group of NHLs comprised all histological categories from low to high grade malignant NHLs. In this study, only DLCLs were included, a group which has not been extensively studied immunohistochemically.

A notable feature is that a higher percentage of immunoreactivity was noted with centrocytic, centroblastic and some PTCLs.

Immunoblastic lymphomas of both B and T cell type showed the least immunoreactivity. Reasons for the lower percentage of immunoreactivity to antibody PD7/26, 2B11 may be attributable to denaturation of some antigens by formalin fixative employed. Indeed better results with paraffin section

immunostaining are obtained when other fixatives, such as acetic acid and formol saline or formol sublimate-B5 are utilized(77). Indeed 5 of the 17 cases which did not react in paraffin sections, showed strong immunoreactivity with PD7/26, 2B11 in frozen sections. The finding of unreactivity with the large cell component, of DLCLs to anti LCA would unlikely suggest antigenic denaturation as the rest of cell population showed strong immunoreactivity. This would be compatible with loss of antigens in these neoplastic cells, as they become more undifferentiated. Indeed antigenic loss in some DLCLs has been described particularly in those with aggressive behaviour(32,50). Compared to frozen sections, paraffin section immunostaining gave better morphology, which could easily distinguish neoplastic cells from nonneoplastic cells. The high number of unreactive cases to antibody PD7/26, 2B11 indicates that caution is required in the interpretation of a negative result, as such cases may be misdiagnosed as nonlymphoid tumours. Even with frozen section, 12 cases did not show immunoreactivity with this antibody. The nonreactivity in these cases is most likely attributable to freezing and thawing artefacts which may compromise antigenic determinants.

The reliability of monoclonal antibodies to LCA as lymphoid discriminants is increased when epithelial markers are included in the panel of antibodies, since undifferentiated carcinomas are the ones most likely to be confused morphologically with DLCLs(28,29,38,64).

Antibodies to EMA, used to distinguish epithelial from non-epithelial tumours have however been recently found to react with some DLCLs(20,47,52). This property has been found useful in the diagnosis of a particular group of pleomorphic large cell lymphomas morphologically resembling histiocytic sarcomas or anaplastic carcinomas(4,21). These lymphomas have a unique feature of expressing the activation antigen Ki-1 in frozen section or BerH2 in paraffin section immunostaining(62,68). These lymphomas have been designated Ki-1 anaplastic lymphomas(68).

One of the pleomorphic PTCLs that showed immunoreactivity with anti EMA of large, singly dispersed cells, was a notable finding. These cells expressed OKT3 and OKT4 in frozen section, implying that they are of T cell derivation. These cells were also reactive with anti LCA. It is clear therefore, that other epithelial markers should be included in the panel of monoclonal antobidies in immunophenotyping tumours of uncertain origin.

LN1 and MB1 were less reliable as B cell markers as reactivity was noted in 57% and 32% of cases from Zurich and Dar es Salaam respectively. Better results were obtained with LN2 and MB2 which showed immunoreactivity with 73% and 65% B cell DLCLs respectively. Both these markers carried an added advantage of reactivity not only with FCC lymphomas, but also B immunoblastic lymphomas. These monoclonal antibodies represent more of pan B cell markers similar to Ia and B1 antibodies(24). Indeed LN2 has been found to span B cell

maturation from early pre B cells stage upto but not including the plasma cell stage(24). Though LN2 and MB2 have shown a consistently high percentage of immunoreactivity to DLCLs of B cell type, they carry the disadvantage that they also react with nonlymphoid tissue including epithelial and endothelial cells(24,53). These antibodies may be used to infer lineage only if a leucocyte specific marker is also positive.

Antibodies to Ia like antigens have been quite useful for phenotyping NHLs because of the consistent presence of the antigen in B cell lymphomas(1,2,27). LN3 is such an antibody with similar properties, having reacted with 88% of B cell DLCLs in this study. It was however also reactive with 75% of PTCLs, a finding observed in some studies(47). This is however not surprising as Ia antigens may be expressed on activated T cells as well as T cell lymphomas(1,32,58).

UCHL1 a T cell marker, showed intense immunoreactivity with 83% of PTCLs compared to 33% with MT1.

These findings are in contrast to other studies, where the two monoclonal antibodies were comparable in-terms of the percentage of immunoreactivity. The difference in immunoreactivity between these two markers may be attributable to decrease in potency of MT1 which even in reactive lymphoid tissue staining intensity was rather weak. UCHL1 proved useful not only in T cell lymphomas, but even in those lymphomas of B cell origin with a high content of reactive T cells. These reactive cells showed strong immunoreactivity

while the neoplastic B cells were unreactive, making differentiation of cells easier. This would have been difficult to interprete in frozen sections. We have not observed immunoreactivity of MT1 and UCHL1 with B cell DLCLs in this study, though some studies report such immunoreactivity(18,31,53).

Two of our cases which showed immunoreactivity with anti lysozyme, anti-alpha-one-antitrypsin and anti-alpha-one-antichymotrypsin were concluded to represent tumours of histiocytic origin. Though they were not tested with monoclonal histiocytic markers effective in paraffin sections, we had no doubt these were true histiocytic tumours as both reacted strongly with the macrophage marker OKM1 in frozen sections.

We have not been successful in the demonstration of cytoplasmic immunoglobulins in paraffin sections in cases of B cell DLCLs in this study. This is however not surprising as several other studies report the same findings(75,76). Even with and without enzyme predigestion results have been variable, sometimes the predigested section reacting strongly, while at other times the undigested section reacting better(77).

The PAP immunoperoxidase method which we employed is reported to be less sensitive for the detection of cytoplasmic immunoglobulins, and better methods include strepto avidin-biotin peroxidase complex(49,54).

SUMMARY

This study has shown that anti LCA PD7/26, 2B11 reacted with 63% of DLCLs from Zurich and 50% from Dar es Salaam. The staining reaction was characteristically membrane located. Five out 7 large centrocytic, 16 out of 23 centroblastic, 5 out of 10 B immunoblastic and 4 out of 6 T cell DLCLs showed immunoreactivity with PD7/26, 2B11. Although 75% of DLCLs showed immunoreactivity with PD7/26 2B11 in frozen sections, compared to 63% in routinely fixed tissue, the better morphology obtained with the latter method makes it the ideal diagnostic aid, easily applicable anywhere. The 37% of unreactive cases in paraffin sections is interpreted as a result of antigenic denaturation by fixation and embedding procedures. One case of T cell DLCL showed strong immunostaining with anti EMA of singly isolated large neoplastic cells. These cells showed immunostaining with anti LCA PD7/26, 2B11, and negative with other epithelial markers, a feature which has also been described in some studies.

LN2 and MB2 have proved reliable B cell markers by showing immunoreactivity with 72.5% and 65% of B cell DLCLs from Zurich respectively. From the Dar es Salaam series LN2 proved superior to LN1 by reacting with 52% of the B cell DLCLs.

UCHL1 immunoreactivity was noted in 5 out of 6 T cell DLCLs from Zurich, and 2 out 3 cases from Dar es Salaam. MT1 was found to be less useful in both studies.

Only 2 of the 40 B cell DLCLs demonstrated expression of Kappa light chains and one lambda chain immunoglobulins from Zurich series. From the Dar es Salaam series, 2 cases of B cell DLCLs showed Kappa light chain expression while one demonstrated lambda light chain. Although immunoreactivity of DLCLs from Dar es Salaam cases was in the majority of cases below 60%, this is interpreted as a great achievement, bearing in mind the fact that this is a newly introduced technique in our laboratory and that the biopsy materials were collected, fixed, processed and stored under variable environmental conditions. Certainly one should expect a higher degree of antigenic denaturation in such material.

The potency of the monoclonal antibodies might also be affected by the variable temperature ranges brought about by fluctuations in electric supply. The fact that a higher number of cases did not demonstrate immunoreactivity with the monoclonal antibodies employed calls for caution in the interpretation of negative findings. For antibodies to the LCA, a positive immunoreactivity would almost be diagnostic of lymphoma but a negative one does not exclude it. Antibodies of the LN and MB series are not specific for lymphoid cells. They are also expressed on nonlymphoid tissues including epithelia. For this reason, a positive immunoreactivity of one antibody should be supported by other markers known to identify such lymphoid antigens. Hence the importance of using a panel of monoclonal antibodies in immunophenotyping DLCLs.

We have introduced the technique in our laboratory, and with more experience, it will prove a very useful diagnostic aid.

CONCLUSIONS AND RECOMMENDATIONS

1. Immuno-histochemical staining of routinely processed paraffin sections using antibodies whose antigens resist fixation denaturation is a useful and reliable method in the diagnosis of DLCLS.

2. The ease with which the technique can be carried out even on routinely processed tissue, and the fact that no sophisticated equipment is required makes it an ideal diagnostic method in developing countries.

3. Bearing in mind the high cost of antibodies, a small panel of antibodies including PD7/26, 2BII, LN2, MB2, EMA and UCHL1 were found useful in this study.

TABLE 1: COMPARISON OF TWO CLASSIFICATION SYSTEMS OF DLCLs

Kiel-classification	I W F
ML, large centrocytic	ML, large cleaved cell
ML, centroblastic	ML, large noncleaved cell
ML, immunoblastic	ML, immunoblastic - plasmacytoid - clear cell - polymorphous - epithelioid cell component

TABLE 2: CLASSIFICATION OF PERIPHERAL T-CELL LYMPHOMAS/LEUKAEMIAS AFTER STANSFELD (1985)

	Monomorphic		Pleomorphic
Cells mainly uniform in size	small cell (lymphocytic)	- CLL-(T) - Mycosis fungoides - Sezary's Syndrome	small cell (intermediate)
	medium sized cell	- Prolymphocytic leukaemia of T-type - Monomorphic type with centrocyte like cells	medium sized cell
	large cell	- "Pale cell" large cell variant - T-immunoblastic lymphoma - "Multilobate" type	
Cells of varying sizes		- T-zone lymphoma - AILD-type - Lymphoepithelioid (Lennert's lymphoma)	mixed-medium and large cell type

TABLE 3: PANEL OF MONOCLONAL ANTIBODIES USED IN FROZEN SECTION IMMUNOSTAINING

NAME OF ANTIBOBODY	CD	ANTIGEN RECOGNISED	CELLULAR DISTRIBUTION	SOURCE
PD7/26,2B11	CD 45	Leucocyte common Antigen (LCA)	Leucocytes	Dako
B1	CD 20	-	B lymphocytes	Coulter clone
K127	-	Kappa chain	B lymphocytes	Own Lab.
< 24	-	B Lambda chain	"	"
Hb 57	-	IgM heavy/chain	"	"
OKT3	CD 3		Peripheral T cells	Ortho.
OKT4	CD 4	-	Helper\Inducer T cells	"
OKT8		-	Suppressor\ cytotoxic cells	"
OKM1	CD 8	-	Neutrophils and Monocytes	Ortho Diagno-stics

TABLE 4: LIST OF MONOCLONAL AND POLYTYPIC ANTIBODIES REACTIVE IN PARAFFIN TISSUE

Antibody	CD	Ig class	Lymphoid specificity	Antigen Mwt (kd)	Reference/Source
PD7/26, 2Bll	45	IgG	T cells, B cells myeloid cells	220,205,190	Warnke et al, 1983
OLN1	75	IgM	FCCs, epithelioid	45–85	Epstein et al, 1984
LN2	74	IgG1	FCCs, mantlezone, DRCs	31–35	Epstein et al, 1984
LN3		IgG2	B cells, activated T cells	29–33	Marder et al, 1985
MB1	45R	IgG1	B cells, some T cells	200, ll0, l00	Popemma et al 1987
MB2		IgG1	B cells, macrophages	28	" " "
MT1	43	IgG1	T cells, myeloid cells, macrophages, B cell subset	190, 110, 100	" " "
UCHL1	45RO	IgG1	T cells, B cells, myeloid cells	180	Smith et al 1986
Li-antitrypsin			Macrophages, granulocytes		
Li-antichymotrypsin			" "		Dako
Lysozyme			" "		

TABLE 5: IMMUNOHISTOCHEMICAL STAINING OF 48 CASES OF DLCLS IN FROZEN SECTIONS

Histologic type	No of cases	Antibody reactivity								
		LCA	B1	K127	< 24	M57	OKT3	OKT4	OKT8	OKM1
LCC	7	5	5	4	4	6	0	0	0	0
CB	23	18	14	17	18	20	0	0	0	0
IB	10	8	8	4	6	8	0	0	0	0
PTCLS	6	4	0	0	0	0	5	2	2	0
TH	2	1	0	0	0	0	0	0	0	2

TABLE 6: IMMUNOHISTOCHEMICAL STAINING OF DLCLS IN PARAFFIN SECTIONS

Histologic type	No of cases	Antibody immunoreactivity										
		PD7/26 2BII		EMA	LN1	LN2	LN3	MB1	MB2	MT1	UCHL1	LAT	LACT
LCC	7	5	0	2	5	4	2	3	0	0	0	0	0
CB	23	16	0	17	16	18	10	19	0	0	0	0	0
IB(B)	10	5	0	4	7	6	1	4	0	0	0	0	0
PTCLs	6	4	1	0	0	4	0	0	2	5	0	0	0
TH	2	1	0	0	1	0	0	0	0	0	2	2	0
	48	31	1	23	29	32	13	26	2	5	2	2	0

Histologic type	LYS	K.	L.
LCC	0	1	2
	2	3	2

TABLE 7: RESULTS OF IMMUNOSTAINING OF 32 CASES OF DLCLS FROM DAR ES SALAAM IN PARAFFIN SECTIONS

Histologic Classification	Number	Antibody Reactivity PD7/26 2B11	LN1	LN2	UCHL1	K	^
LCC	3	2	0	1	0	0	0
CB	18	9	6	9	0	0	1
IB(B)	8	3	4	5	0	2	0
PTCLS	3	2	0	0	2	0	0
TOTAL	32	16	10	15	2	2	1



Fig 1. Parafin Section of a centroblastic lymphoma (Cb) immunostaned with the antibody PD 7\26, 2B11 showing diffuse mainly membrane and cytoplasmic reaction products. (DII x 400)

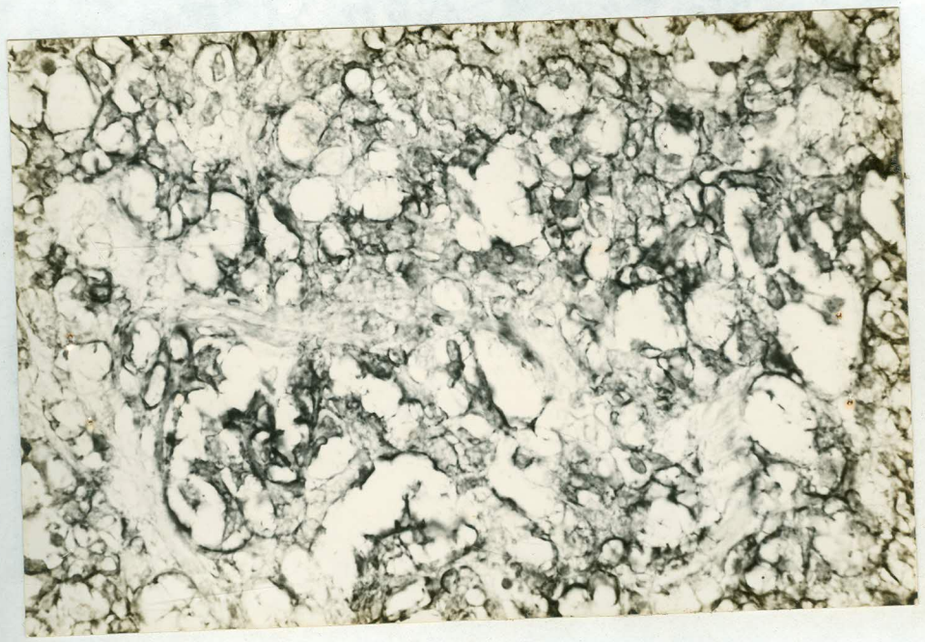


Fig 2. A paraffin section of an anaplastic large cell lymphoma of the stomach immunostained with the antibody PD7/26, 2B11. Reaction product indicating positivity is restricted to the cell membranes. (DII x 640)

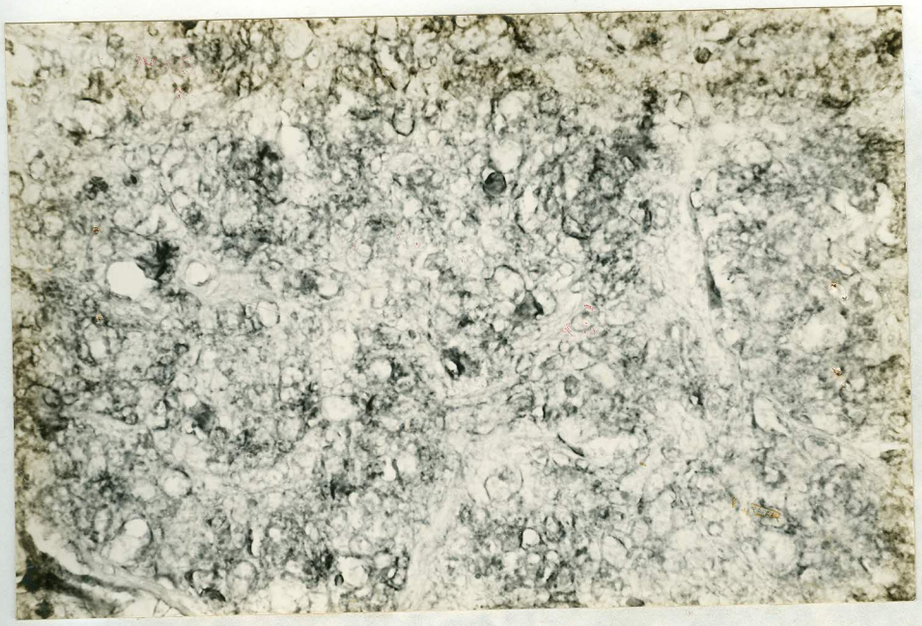


Fig 3. A histological section of a T immunoblastic lymphoma stained immunohistologically with PD 7/26 2B11. There is diffuse reaction products on the cell membranes, which is weak compared to Figs 1 and 2 (DII x 640)

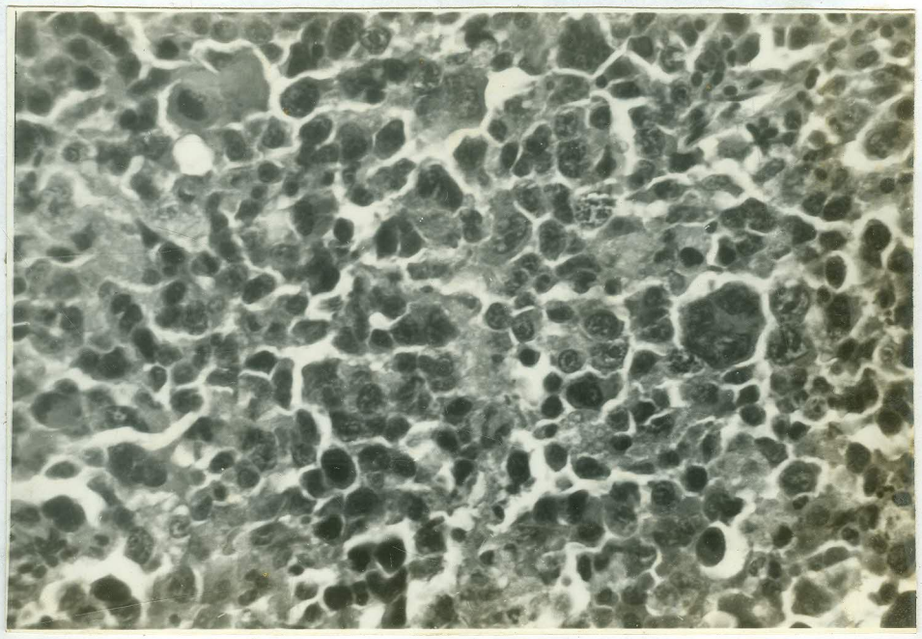


Fig 4. A histological section of a centroblastic lymphoma from the Dar es Salaam material immunohistologically stained with the antibody PD 7/26 2B11. There are diffuse dense reaction products in both the cytoplasm and cell membranes, (DII x 400)

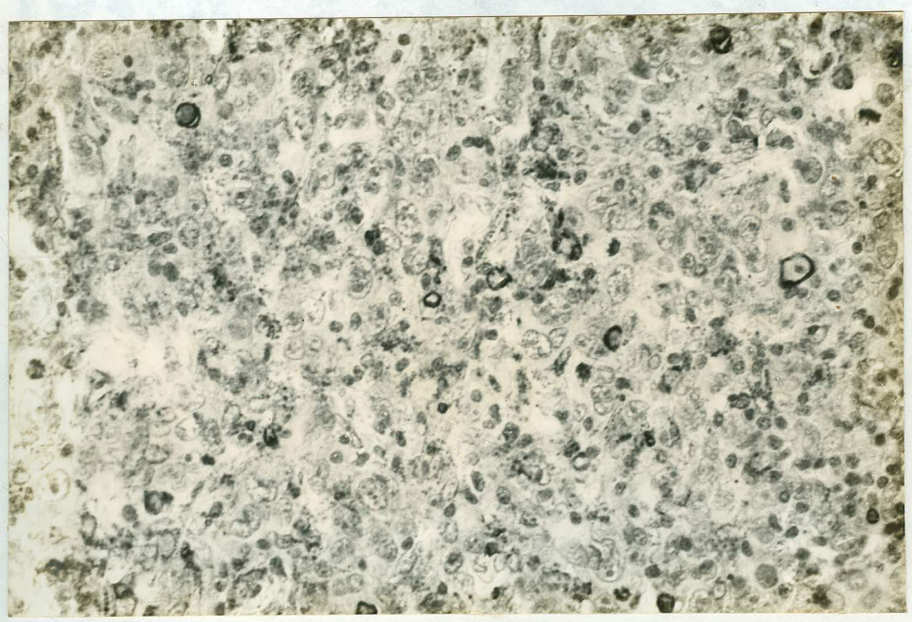


Fig 5. A histological section of another centroblastic lymphoma from Dar es Salam immuno-stained with the antibody LN2. Diffuse but weak reaction products are seen in the cell membrane and also in the cytoplasm. (DII x 100)

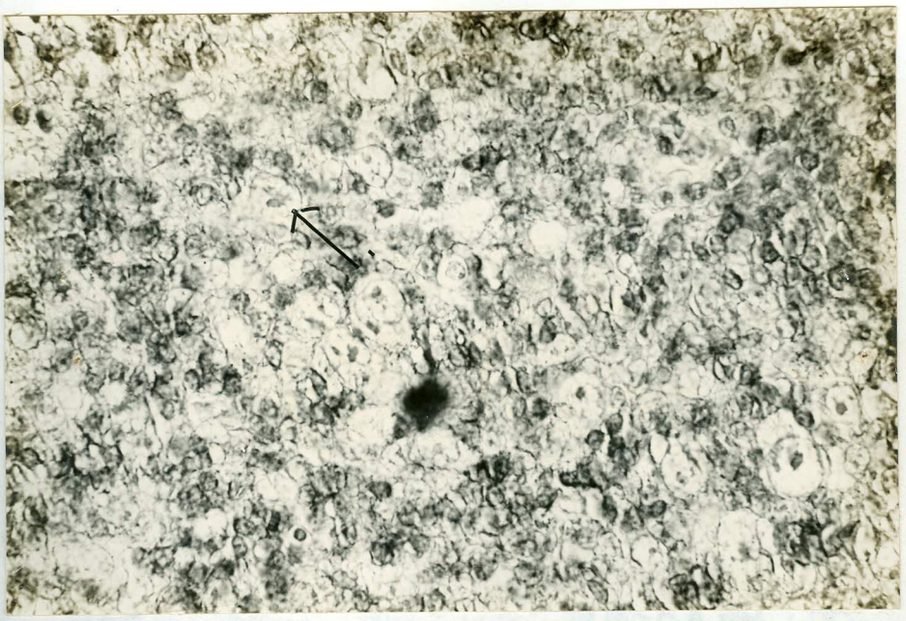


Fig 6. A histological section of T1b lymphoma from Zurich immunohistologically stained with the antibody MT1. Diffuse strong reaction products are seen in the small and medium sized cells, mainly in the cell membrane and cytoplasm. The large cells (arrows) are largely negative. (DII x 640.

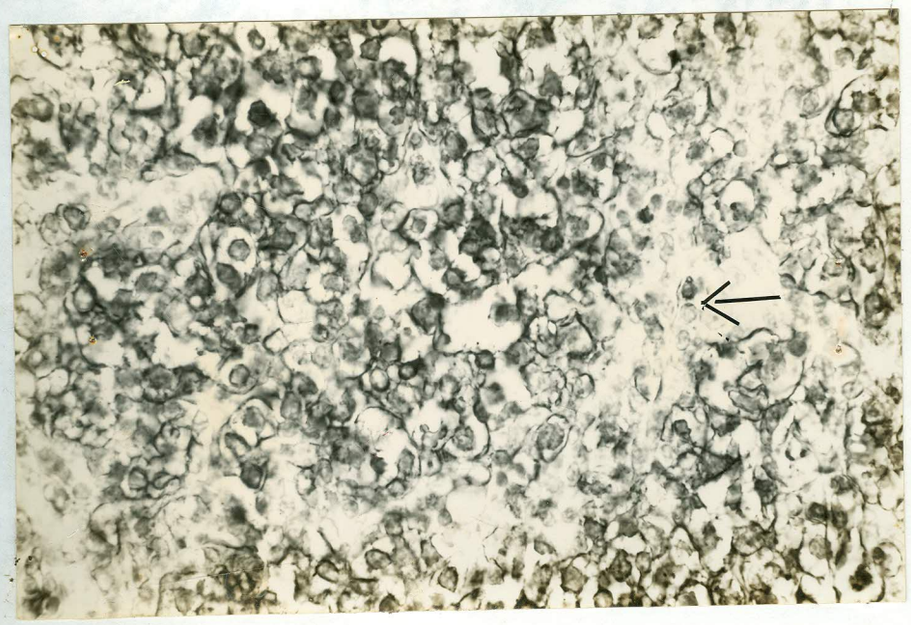


Fig 7. Another section from the same case as Fig. 6, immunostained with the antibody UCHLI. Diffuse strong reaction product which is mainly seen in the cell membrane is present. Non-neoplastic B cells are unstained (arrows) (DIIx 640)

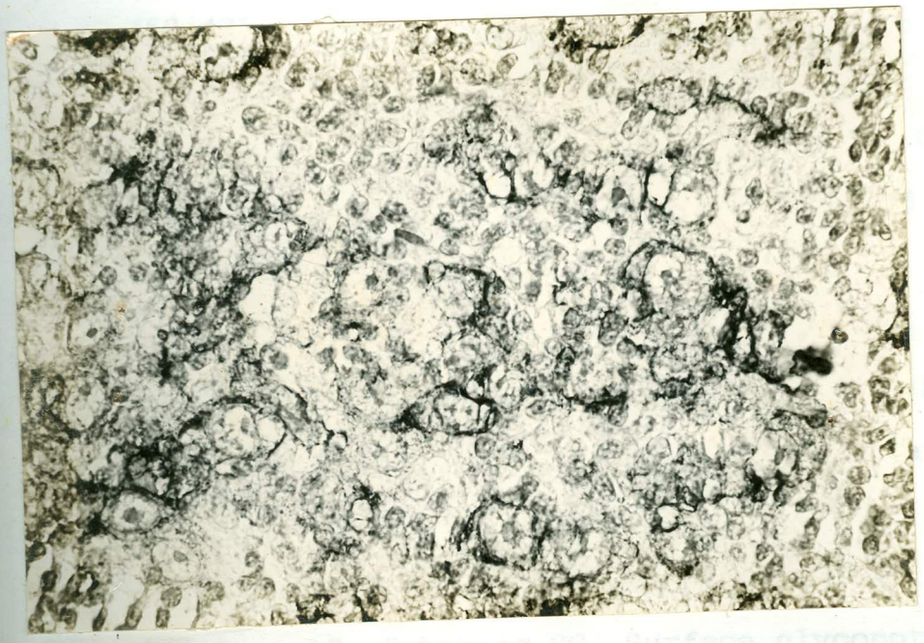


Fig. 8. Histological section of a T immunoblastic lymphoma immunostained with the antibody against Epithelial Membrane Antigen. There is a focal but strong reaction product confined to the cell membranes of large blast cells. (DII x 800)

REFERENCES

1. Aisenberg AC. Current concepts in immunology cell surface markers in lymphoproliferative disease. N Engl J.Med. 1981; 304: 331-336.

2. Aisenberg AC, Wilkes BM, Harris NL. Monoclonal antibody studies in non-Hodgkins lymphomas. Blood 1983, 61: 469-475.

3. Akbar AN, Terry L, Timms A et al. Loss of CD 45R and gain of UCHLI reactivity is a feature of primed T cell. J. Immunol. 1988;140: 2171-2178.

4. Alsaati T,Caveriviere P, Gorguet B et al. Epithelial membrane antigen in haemato-poietic neoplasms. Hum. Pathol. 1986; 17: 533- 534.

5. Anderson LC, Gahmberg CG. Surface glycopoteins of human white cell. Analysis of surface labelling. Blood 1978; 52: 57-68.

6. Barclay AN, Jackson DI, Williams AF. Lymphocyte specific heterogeneity in the rat leucocyte common antigen (T200) is due to differences in polypeptide sequences near the NH2-terminus. EMBO 1987; 6: 1259-1264.

7. Bennett MH, Farrer- Brown G, Henry K, et al. Classification of non-Hodgkins lymphomas. Lancet 1974; ii, 405- 406.

8. Berard CW, Jaffe E, Braylan R.C, et al; Immunologic aspects and pathology of malignant lymphomas. Cancer 1978; 42:911- 921.

9. Berard CW, Isaacson RG, Norman SN et al. Symposium on histiocytic or large cell lymphomas. In: Malignant lymphomas. Pathology Annual (ed) Sheldon CS and Rosen PP. Appleton - Century Crofts Norwalk Connecticut 1982; pp 109-168.

10. Bloomfied CD, Gaijl-Peczalska KJ, Frizzera et al. Clinical utility of lymphocyte surface markers combined with the Lukes-Collins histological classification in adult lymphoma. N. Engl. J. Med 1979; 301:512-520.

11. Brown VA, Smith SK, Maddy AH. Epitope mapping of the leucocyte common antigen (CD 45) in normal and leukaemia human lymphocytes. In: Leucocyte Typing III. White Cell Differentiation Antigens (eds). McMichael et al. Oxford. Oxford University Press, 1987; pp 808-810.

12. Brown DC, Heryet AG, Gatter KC and Mason DY. The prognostic significance of immunophenotype in high grade non-Hodgkin's lymphomas. Histopathol. 1989; 14: 621-627.

13. Cerrezo L. B-Cell multilobated lymphoma. Cancer 1983: 52: 2277-2280.

14. Cobbold S, Hale G, Waldmann H. Non- Lineage, LFA-family, and leucocyte common antigens; new and previously defined clusters. In:. Leucoyte Typing iii. White cell Differentiation Antigens. (eds) McMichael et al, Oxford; Oxford University Press, 1987; pp 788-803.

15. Colling LA, Poulter LW, Janossy G. The demonstration of cell surface antigens on T cell, B cell and accessory cells in paraffin-embedded human tissues. J Immunol 1984;75:227-239.

16. Cossmas J, Jaffe Es, Fisher RI. Immunologic phenotypes of diffuse aggressive non- Hodgkigns lymphomas. Cancer 1984;54:1310-1317.

17. Dalchau R, Flanagan BE, Fabre JW. Analysis by binding to pure antigen and by flow cytometry of the antigenic determinants recognized by the workshop antileucocyte common (LC) antigen antibodies. In: Leucocyte typing iii. White cell Differentiation antigens.(eds) McMichael et al, Oxford. Oxford University Press, 1987; 814-817.

18. Davey FR, Gatter KC, Ralfkiaer E et al. Immunophenotyping of non-Hodgkins lymphomas using a panel of antibodies on paraffin embedded tissues. Am J. Pathol. 1987:129:54-63.

19. De vita T Jr, Cannellos GP, Chabner B, et al. Advanced diffuse histiocytic lymphoma- a potentially curable disease. Results with combination chemotherapy. Lancet 1975; 1:248-252.

20. Delsol G, Stein H, Gatter KC, et al. Human lymphoid cells express epithelial membrane antigen. Implication for diagnosis of human neoplasms. Lancet 1984:2;1124-1129.

21. Delsol G, Al Saati T, Gatter KC, et al. Coexpression of epithelial membrane antigen (EMA), Ki-1 and interleukin-2 receptor by anaplastic large cell lymphomas. Diagnostic value in so-called malignant histiocytosis. Am J Pathol. 1988: 130: 59-70.

22. Dobson CM, Krajewski AJ, Myskow MW, et al. Immunohistochemical staining of non-Hodgkin's lymphoma in paraffin sections using the MB1 and MT1 monoclonal antibodies. J. Pathol 1987;153: 203-312.

23. Dorfman RF : Classification of non-Hodgkin's lymphomas Lancet 1974; 1:1295-1296.

24. Epstein AL, Marder RJ, Winter JN, et al. Two new monoclonal antibodies (LN1,LN2) reactive in B5 and formalin fixed paraffin embedded tissues with follicular centre cell and mantlezone in human B-lymphocyte derived tumours. J. Immunol: 1984; 133:1028-1036.

25. Feller AC, Wacker HH, Moldenhauer G, et al; Monoclonal antibody Ki-B3 detects a formalin resistant antigen on normal and neoplastic B cells. Blood 1987; 70:629-636.

26. Fisher RI, De Vita VT Jr, Hubbard JM, et al; Diffuse aggressive lymphomas increased survival after alternating flexible sequences of pro-MALE and MOPP chemotherapy. Ann Int. Med. 1983;98:304.

27. Foon KA, Schroff RW, Gale RP. Surface markers in leukaemia and lymphoma cells: recent advances. Blood 1982;60:1-21.

28. Gatter KC, Abdulaziz Z, Beverley P, et al. The differential diagnosis of routinely processed anaplastic tumours using monoclonal antibodies. Am J Pathol 1984;82:33-43.

29. Gatter KC, Heryet A, Alcook C, et al. Clinical importance of analyzing malignant tumours of uncertain origin with immunohistological techniques. Lancet 1985;1:1302-1305.

30. Halle G, Buckie C, Lovat P, et al. Epitope mapping of the human leucocyte common antigen by competitive binding and synergistic lysis. Leucoycte Typing III. White cell Differentiation Antigens Oxford. (eds). McMichael et al; Oxford University Press,1987; pp 811-814.

31. Hall PA, D'Ardennen AJ, Butler MG, et al; New marker of B lymphocytes, MB2: comparison with other lymphocyte subset markers active in conventionally processed tissue sections. J. Clin Pathol 1987, 40:151-156.

32. Halper JP, Knowles DM, Wang CY. Ia antigen expression by human malignant lymphomas. Correlation with conventional lymphoid markers. Blood 1980,55:373-379.

33. Hoffman FM, Yanagihara E, Byrne B, et al. Analysis of B cell antigens in normal reactive lymphoid tissue using four B cell monoclonal antibodies. Blood 1983; 62:775-781.

34. Holgate CS, Jackson P, Pollard K, et al. Effect of fixation on T and B Lymphocyte surface membrane antigen demonstration in paraffin processed tissue. J. Pathol 1986; 149:293-300.

35. Hornig SJ, Weiss LM, Crabtree GS, et al. Clinical and phenotypic diversity of T cell lymphomas. Blood 1986;67:1578-1582.

36. Hsu SM, Jaffe ES. Phenotypic expression of B lymphocytes. I identification with monoclonal antibodies in normal lymphoid tissues. Am J Pathol 1984;114:387-393.

37. Jaffe ES, Braylan RC, Nanba K, et al. Functional markers: a new perspective on malignant lymphomas. Cancer Treat Rep. 1977;61:953-962.

38. Kurtin PJ, Pinkus GS. Leucocyte common antigen - a diagnostic discriminant between haematopoietic and non-haematopoietic neoplasms in paraffin sections using monoclonal antibodies. Correlation with immunologic studies and ultrastructural localization. Hum Pathol 1985;16:353-365.

39. Laurence J, Coleman M, Allen SL. Combination chemotherapy of advanced diffuse histiocytic lymphomas with six drug COP-BLAM regimen. Ann Intern Med. 1982;97:190-198.

40. Lennert K, Mohri N, Stein H et al; The histopathology of malignant lymphomas. Br J Haematol. 1975;31:193-203.

41. Levy R, Warnke R, Dorfman RF. The monoclonality of human B cell lymphomas. J. Exp Med. 1977;145:1014-1019.

42. Lukes RJ, and Collins RD.; New approaches to the classification of the lymphomata. British J. Cancer 1975;31:1-28.

43. Mason DY, Biberfeld P. Technical aspects of lymphoma immunohistology. J. Histochem. cytochem. 1980;28: 731-745.

44. Mason D.Y., Gatter K.C., The role of immunocytochemistry in diagnostic pathology. J. Clin. Pathol, 1987; 40: 1042-1054.

45. Mathe G, Rappaport H, O'Connor GT, and Torloni H. Histological and cytological typing of neoplastic disease of haematopoietic and lymphoid tissues. International Histological Classification of Tumours 1976; No 14 WHO, Geneva.

46. Marder RJ, Variakojis D, Silver J, et al. Immunohistochemical analysis of human lymphomas with monoclonal antibodies to B cell and Ia antigens in paraffin sections. Lab Invest. 1985;52:497-503.

47. Norton AJ, Isaacson PG.; Immunohistochemical study of T cell lymphomas using monoclonal and polyclonal antibodies effective in routinely fixed wax embedded tissue. Histopathology 1986;10:1243-1260.

48. Norton AJ, Ramsay AD, Beverly PC, et al. Monoclonal antibody (UCHL1) that recognizes normal and neoplastic T cells in routinely fixed tissues. J. Clin Pathol. 1986;39:399-405.

49. Norton AJ, Isaacson PG. The diagnosis of malignant lymphoma using monoclonal antibodies effective in routinely fixed wax embedded tissue. J. Pathol 1987;151:183-184.

50. Okon E, Felder B, Epstein A, et al. Monoclonal antibodies reactive with B lymphocytes and histiocytes in paraffin sections. Cancer 1985;56:95-104.

51. Pangalis GA, Nathwani BN, Rappaport H. An immunocytochemical study of non-Hodgkin's lymphomas. Cancer 1981; 48:915-921.

52. Pinkus GS, Kurtin PJ. Epithelial membrane antigen a diagnostic discriminant in surgical pathology: Immunohistochemical profile in epithelial, mesenchymal and haematopoietic neoplasms using paraffin sections and monoclonal antibodies. Hum Pathol. 1985; 16: 929-940.

53. Poppema S, Hollema H, Visser L, Vos H. Monoclonal antibodies (MT1, MT2, MB1, MB3) reactive with leucocyte subsets in paraffin embedded tissue sections. Am J Pathol. 1987, 127: 418-429.

54. Pulford KAF, Falini B, Heryet A, et al. 4KB5, a new monoclonal anti B cell antibody for the routine diagnosis of lymphoid tissue biopsies. In: Leucocyte typing III. White Cell Differentiation Antigens. (eds) McMichael et al Oxford. Oxford University Press, 1987; pp 828.

55. Ralph SJ, Thomas ML, Morton CC, Trowbridge IS. Structual variants of human T200 glycoprotein (leucocyte common antigen). EMBO 1987; 6:1251- 1257.

56. Ramsay AD., Smith WJ., Earl HM, et al. T Cell lymphomas in adults: A clinicopathological study of eighteen cases. J. Pathol. 1987; 152: 63-76.

57. Rappaport H. Atlas of tumour pathology, 1966, sect. II, Fasc 8. Armed Forces Institute of Pathology, Washington DC.

58. Reinharz EL., Kung PC., Pesando JM, et al.; Ia determination of human T cell subsets defined by monoclonal antibody; activation stimuli required for expression. J Exp Med. 1979; 150:1472-1483.

59. Rosenberg SA, Berard CW, Brown BW, et al. NHL pathologic classification project, National Cancer Institute sponsored study of classification of NHLs: Summary and description of a working formulation for clinical usage. Cancer 1980, 49:2112-2135.

60. Rudders RA, Ahl ET Jr, DeLellis RA. Surface marker and histopathologic correlation with long term survival in advanced large cell non-Hodgkin's lymphomas. Cancer 1981; 47:1329-1335.

61. Salter DM, Krajewski AS, Dewar AE. Immunohistochemical staining of NHLs with monoclonal antibodies specific for leucocyte common antigen. J. Pathol 1985; 46:345-353.

62. Schwartig R, Gerdes J, Stein H. BER-H2: A new monoclonal antibody of the Ki-1 family for the detection of Hodgkin's disease in formaldehyde fixed tissue. In: Leucocyte Typing III. White Cell Differentiation Antigens (eds) McMichael et al. Oxford University Press, 1987, 574-575.

63. Skarin AT, Canellos S, Rosenthal DS, et al. Improved prognosis of diffuse histiocytic and undifferentiated lymphoma by use of high dose methotrexate alternating with standard agents (M-BACOD). J. Clin Oncol. 1983;1:91-102.

64. Sloan JP, Hughes F, Ormerod MG. An assessment of the value of epithelial membrane antigen and other epithelial markers in solving diagnostic problems in tumour histopathology. Histochem. J. 1983; 15:645-654.

65. Smith SH, Brown HM, Beverley PC, et al. Functional subsets of human helper inducer cells defined by a new monoclonal antibody; UCHL1. Immunology 1986; 58:63-70.

66. Stanfeld AG, Peripheral T cell lymphomas. In: Lymphnode biopsy,(eds) Churchill, Livingstone. Edinburgh,1985 pp 300-329.

67. Stein H, Heryet A, Gatter KC and Mason DY. Freeze dried paraffin embedded human tissue for antigen labelling with monoclonal antibodies. Lancet 1984; 141:71-74.

68. Stein H, Mason DY, Gerdes J, et al. The expression of the Hodgkin's associated antigen Ki-1 in reactive and neoplastic lymphoid tissue. Evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 1985; 66:848-858.

69. Sternberg LA, Hardy PH.J., Cuculis YJ, et al. The unlabelled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horse-raddish peroxidase) and its use in identification of spirochaetes. J. Histochem Cytochem. 1970; 18:315-333.

70. Taylor CR, Bunus J.; Immunohistological detection of intracellular immunoglobulin in formalin fixed paraffin embedded tissue using peroxidase labelled antibody. J. Clin Pathol 1974; 27:14-20.

71. Taylor CR: Results of multi-parameter studies of B cell lymphomas. Am J Clin Pathol 1979; 72:687-694.

72. Taylor CR, Mason DY; Immunohistochemical detection of intracellular immunoglobulin in formalin fixed paraffin sections from multiple myeloma using immunoperoxidase technique. Clin Exp Immunol 1974; 18:417-424.

73. Terry LA, Brown MH, Beverley PCL. The monoclonal antibody UCHL1 recognizes 180,000 molecular weight component of the human leucocyte common antigen, CD45. Immunology 1988; 64:331-336.

74. Trowbridge IS, Hyman R, Mazauskas C. Surface molecules of cultured human lymphoid cells. Eur. J. Immunol 1976; 6:777-780.

75. Tubbs RR, Fishleder A, Weiss RA, et al. Immunologic cellular phenotypes of lymphoproliferative disorders. Comprehensive evaluation of 564 cases including 257 non Hodgkin's lymphomas classified by the International Working Formulation. Am J Pathol. 1983; 113:207-215.

76. Warnke R, Levy R. Immunopathology of follicular lymphomas, a model of B lymphocyte homing. N.Engl.J. Med. 1978; 298:481-488.

77. Warnke RA, Gatter KC, Falin B, et al. Diagnosis of human lymphoma with monoclonal antileucocyte antibodies. N. Engl J. Med. 1983;309:1275-1281.

78. West KP, Warford A, Fray L, et al. The demonstration of B cell, T cell and myeloid antigens in paraffin sections. J. Pathol. 1986; 150:89-101.

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