

THE SPECTRUM OF LYMPHOMA IN MALAYSIA: A HISTOPATHOLOGICAL STUDY UTILIZING IMMUNOPHENOTYPING.

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Summary

A retrospective study was made to determine relative incidence of lymphoma subtypes in Malaysia. High grade non-Hodgkin's lymphoma was found to be common. Low grade non-Hodgkin's lymphoma and Hodgkin's disease were relatively rare in this Malaysian series. Non-Hodgkin's lymphoma of B-cell immunophenotype was four times as common as that of T-cell lineage. There was a high incidence of primary extranodal non-Hodgkin's lymphoma.

Keywords: Lymphoma, Hodgkin's, non-Hodgkin's, immunophenotype.

INTRODUCTION

During a sabbatical spent in Malaysia it became apparent that there was considerable difference in incidence of the various lymphoma types as compared to North America and Europe, the sources of most of the published experience. Accordingly, a retrospective histopathological study was initiated to determine incidence of the types of lymphoma in Malaysia.

MATERIALS AND METHODS

Paraffin blocks from cases diagnosed as lymphoma over the years 1983 to 1987 were retrieved from storage at the Medical Faculty of Universiti Kebangsaan Malaysia. Haematoxylin and eosin stained sections were classified without knowledge of the original diagnosis. Depending on the working diagnosis, various immunohistochemistry techniques were employed, these procedures being done in the Immunopathology Laboratory of the Hospital for Sick Children, Toronto. The monoclonal antibodies ML, MT1, MB2, MT2 and LN1 (Biotest) were used, with controls, to indicate non-Hodgkin's lymphoma as B or T immunophenotype. Leu M1 was used as an indicator of Reed-Sternberg cells when Hodgkin's disease was being considered. When the diagnosis of lymphoma was in doubt, the monoclonal antibodies to cytokeratin (Becton-Dickinson) vimentin (BIOGENEX), NSE (Dako), S-100 (Dako) were used, when indicated.

Hodgkin's disease was classified according to the Rye classification.¹ The presence of lacunar cells and birifringent collagen was taken as indicating nodular sclerosis subtype.²

Non-Hodgkin's lymphoma was classified according to the Working Formulation.³ An attempt was made to be as objective as possible in assignment of cases to the various subtype of non-Hodgkin's lymphoma. Nodular lymphoma was defined by presence of follicular or nodular histologic pattern. Large cells were those over three erythrocyte diameters (21 microns). More than fifteen of these per high power field qualified a tumour as large cell lymphoma. Small cells were defined as those less than two erythrocyte diameters. Less than five of larger than that size per high power field qualified as small cell lymphoma. The remainder of the tumours were considered as mixed small and large cell lymphoma. Non-cleaved cells have rounded nuclei and prominent nucleoli whereas the opposite features characterize cleaved cells. The presence of multinucleate cells indicated that a large cell lymphoma be classified as high grade.

RESULTS

Table 1 summarizes the 57 cases of lymphoma and indicates the relative incidence of Hodgkin's disease and non-Hodgkin's lymphoma.

Table 2 summarizes Hodgkin's disease cases by histological subtype. The biopsy that could not be further classified was a needle biopsy of liver that was too small to indicate a pattern for subclassification, but did show the diagnostic Reed-Sternberg cell, which was confirmed by the presence of the Leu M1 marker.

Table 3 summarizes 49 cases of non-Hodgkin's lymphoma, including the results of immunophenotyping.

The patterns of staining of normal tonsillar controls with the antibodies used for B- and T-cell immunophenotyping are indicated in Table 4. In our laboratory, the antibodies ML and MT1 were reactive to the lymphocytes of the zones occupied predominantly by T-cells in normal lymphoid tissue (Fig. 1). Reactivity of the malignant cells of a lymphoma with these antibodies was considered as evidence of T-cell origin of the malignancy. ML is actually a mixture of MT1 and MB1 antibodies. The sample used in this study did not have activity against B-cell areas of control tonsil, and behaved similarly to MT1. The degree of staining of T-cell areas by ML was slightly increased over the sample of MT1.

The antibodies LN1, MT2 and MB2 concentrated in the B-cell zones of normal tonsil. When they stained the malignant cells of a lymphoma, B-cell origin was indicated. LN1 concentrated in the lymphocytes of the

germinal centres (Figs 2 and 3). MT2 concentrated at the mantle zone (Fig. 4).

The reaction of the monoclonal antibody MB2 with normal tonsil simulated the combined effects of LNI and MT2, but with slightly lesser activity in the germinal centre than the mantle zone (Fig 5). It was considered a marker for all B-lymphocytes whereas mantle and germinal centre lymphocytes marked with MT2 and LN1 antibodies respectively. Activity of MT2 and LNI was most prominent at the periphery of the cell indicating that the marker antigen is located at the cell surface. An exception occurred in the case of LN1, which was found in some cases of lymphoma to also mark the cytoplasm in the form of a large dot at the hof of the cell (Fig. 6) This occurred in addition to the peripheral staining that was common to all the antibodies. MB2, reactive to a cytoplasmic antigen, concentrated in the entire cytoplasm.

TABLE 1
MALIGNANT LYMPHOMAS – 57 CASES

| | Number of cases | Age range | Median age |
|----------------------------|-----------------|-----------|------------|
| I. Hodgkin's disease | 1 | 34 | 34 |
| Lymphocyte predominance | | | |
| Mixed cellularity | 3 | 51 – 58 | 52 |
| Lymphocyte depleted | 1 | 27 | 27 |
| Nodular sclerosis | 2 | 15 – 48 | 31 |
| Unclassified | 1 | 52 | 52 |
| Total | 8 | 15 – 58 | 50 |
| II. Non-Hodgkin's lymphoma | | | |
| Low grade: 1 | | | |
| Follicular, mixed | 1 | 38 | 38 |
| Intermediate grade: 29 | | | |
| Follicular, large cell | 3 | 30 – 64 | 33 |
| Diffuse, small cleaved | 6 | 27 – 72 | 48 |
| Diffuse, mixed | 19 | 27 – 71 | 55 |
| Diffuse, large cell | 1 | 63 | 63 |
| High grade: 19 | | | |
| Immunoblastic | 13 | 4 – 70 | 56 |
| Lymphoblastic | 5 | 8 – 26 | 16 |
| Small, non-cleaved | 1 | 15 | 15 |
| Total | 49 | 4 – 72 | 50 |

TABLE 2
8 HODGKIN'S DISEASE CASES : CLINICAL DATA

| Histological subtype | Age | Sex | Race | Site of biopsy | Presenting symptoms and signs |
|---------------------------|-----|-----|---------|-----------------------|---|
| Lymphocyte predomiinancce | 37 | M | Chinese | nasal | |
| Mixed cellularity | 51 | F | Chinese | supraclavicular node | right hypochondrial pain (hepatosplenomegaly) |
| | 52 | M | Malay | axillary node | axillary mass |
| | 58 | M | Malay | cervical node | cervical mass |
| Lymphocyte depleted | 27 | F | Chinese | supraclavicular node | fever |
| Nodular sclerosis | 15 | M | Malay | axillary node | anorexia, loss of weight fever, generalized lyriiphadenopathy |
| | 48 | M | Indian | inguinal node | sweats, inguinal mass |
| Unclassified | 55 | F | Chinese | liver (needle biopsy) | hepatosplenomegaly |

When the antibodies were applied to lymphomas, the depth of staining was generally less than observed with controls, probably relating to decreased amount of antigen with dedifferentiation. Poorly differentiated lymphomas generally had lesser depth of staining than did well differentiated ones.

Nodular lymphomas retained the ability to react with the B-cell antibodies in a zonal fashion, with LNI concentrating centrally in the nodules and MT2 and MB2 concentrating at the periphery. As is observed with standard histological stains, the periphery of the nodule of a lymphoma is less sharply defined than the periphery of a germinal centre of normal lymphoid tissue (Fig. 6).

Diffuse lymphomas characteristically had immunoreactivity of a proportion of the component cells with each of the markers. It was important to observe immunolocalization in the malignant cells, generally the larger of the lymphoid cells comprising the malignant population. All lymphomas whether of B or T lineage tended to have a component of small normal T-lymphocytes.

Some larger apparently reactive T-cells were seen in some B-cell tumours. This feature was less prominent in the high grade tumours,

particularly lymphoblastic and Burkitt-type lymphomas and some of the more histologically homogeneous immunoblastic lymphomas.

Diffuse B-cell lymphomas varied in the marker present in the malignant cells, some marking predominantly as follicular centre cells, some as mantle cells, and some with components of both of these cell types.

Hodgkin's disease lesions were made up of mixed groups of cells of B and T lineage with T-cells predominating. In all but one of the examples of Hodgkin's disease, the Reed-Sternberg cells were reactive to **Leu M1**. The case that had non-reactive Reed-Sternberg cells was of lymphocyte predominance type. In two Hodgkin's disease lesions (both mixed cellularity), the Reed-Sternberg cells reacted to **MB2**.

Cases originally considered as lymphoma that were deleted from the series included examples of reactive lymphoid tissue, and metastatic non-lymphomatous neoplasms. The reactive lesions were sometimes very difficult to differentiate from lymphoma on routine histology. Immunohistochemistry was helpful

TABLE 3
49 NON-HODGKIN'S LYMPHOMA CASES: CLINICAL DATA:
HISTOLOGY AND IMMUNOPHENOTYPE

| W.F. classification | Age | Sex | Race | Site of biopsy | Presenting | Histology | Immunophenotype |
|---|-----|-----|---------|----------------------------|--|---------------------------------|-----------------|
| LOW GRADE Follicular, Mixed | 38 | F | Malay | cervical node | cervical mass | | B |
| INTERMEDIATE GRADE Follicular, Large cell | 30 | M | Chinese | node | generalized nodes, hepato- splenomegaly | | B |
| | 33 | M | Chinese | cervical node | cervical mass; fever, lethargy, loss of weight | | B |
| | 64 | M | Indian | cervical node | cervical masses | | B |
| Diffuse, Small cleaved | 27 | F | Malay | subman- dibular node | submandibular mass node | signet ring lymphoma | B |
| | 35 | F | Chinese | orbit | subconjunctival mass | | B |
| | 46 | M | Malay | inguinal node | inguinal mass | | B |
| | 50 | F | Chinese | node | | | B |
| | 64 | F | Chinese | cervical node | cervical mass | | B |
| | 72 | M | Malay | orbit | proptosis, anemia | | B |
| Diffuse, Mixed | 27 | F | Chinese | nasal node | nasal obstruction | | T |
| | 37 | F | Chinese | node | | | N |
| | 45 | F | Malay | trephine | | | B |
| | 48 | F | Malay | inguinal node | fever, loss of weight, anorexia, inguinal mass | | B |
| | 48 | F | Indian | skin | skin lesions for 4 yrs. | cutaneous T-cell lymphoma | T |
| | 48 | M | Chinese | cervical node, nasal | nasal and cervical masses | | B |
| | 48 | M | Malay | epitroch- lear | generalized nodes, hepato- splenomegaly | | B |
| | 50 | M | Malay | trephine | cervical, mass, hepatomegaly, pleural effusion | | B |
| | 52 | F | Malay | naso- pharynx | cervical and right tonsillar masses | | B |
| | 53 | M | Chinese | tonsil | tonsil mass | | B |
| | 55 | M | Malay | rectal | fungating anal mass | | B |
| | 56 | M | Malay | renal | acute renal failure abdominal mass | | B |
| | 57 | M | Malay | cervical node | cervical mass | | B |
| | 59 | F | Chinese | inguinal node | inguinal mass | | B |

| | | | | | | | |
|---|----|---|---------|--|--|--|---|
| | 60 | M | Malay | cervical node | cervical mass, abdominal pain | | B |
| | 62 | F | Malay | inguinal node | inguinal mass | | B |
| | 64 | F | Chinese | rectal | diarrhoea, cervical masses | | B |
| | 67 | F | Malay | nasal | epistaxis , nasal mass | | B |
| | 71 | F | Chinese | naso-pharynx | dysphagia | | B |
| Diffuse, Large | 54 | M | Malay | inguinal node | inguinal mass | | B |
| HIGH GRADE Large Cell, Immunoblastic | 4 | F | Malay | retroperitoneal node | vomiting, loss of weight, abdominal mass | clear cell | B |
| | 9 | M | Chinese | cervical node | cervical mass | poly-morphous | T |
| | 27 | M | Malay | submandibular node | orbital, submandibular masses | poly-morphous | T |
| | 29 | M | Malay | skin biopsy | skin lesions | poly-morphous (cutaneous T-cell lymphoma) | T |
| | 47 | M | Malay | nasal biopsy | nasal obstruction ; antrum mass | plasmacytoid | B |
| | 55 | M | Malay | tonsil | left tonsil mass | polymorphous | T |
| | 56 | M | Malay | cervical node | anorexia, loss of weight, cervical mass | plasmacytoid | B |
| | 60 | M | Chinese | cervical node | cervical mass | clear cell | B |
| | 61 | M | Malay | supra-clavicular node | anorexia, loss of weight, melena; gastric mass | clear cell | B |
| | 63 | M | Malay | nasal | nasal mass | polymorphous | B |
| | 63 | M | Malay | stomach | | polymorphous | B |
| | 64 | F | Chinese | stomach | anorexia, loss of weight, epigast. mass | clear cell | B |
| | 70 | M | Malay | cervical node | dysphagia, cervical mass | plasmacytoid | B |
| Lymphoblastic | 8 | M | Indian | axillary node | | | T |
| | 10 | M | Malay | supra-clavicular node | right hypo-chondrial pain, hepato-splenomegaly | | T |
| | 16 | M | Malay | cervical node | cervical mass | | T |
| | 21 | F | Indian | cervical | generalized lymphadenopathy | | T |
| | 26 | F | Indian | breast | masses, thyroid, breast, abdomen | | B |
| Small, non-cleaved | 15 | M | indian | Omentum ; retroperitoneal node | epigastric mass | non-Burkitt's | B |

TABLE 4
CHARACTERISTIC STAINING PATTERNS
OF ANTIBODIES IN NORMAL TONSIL

| Antibody | Germinal centre | Mantle | Interfollicular zone |
|----------|-----------------|--------|----------------------|
| ML | -- | -- | t |
| MT1 | -- | -- | t |
| LNI | t | -- | -- |
| MT2 | | t | -- |
| MB2 | t | t | -- |

in such cases in indicating the basically normal architecture of the lymphoid tissue, and the normal distribution of B- and T-cells.

Metastatic lesions that had originally been diagnosed as lymphoma included small cell carcinomas and neuroblastoma. These lesions failed to mark as T- or B-cells or with common lymphocyte antigen. In some cases other markers such as cytokeratin or S-100 protein were present.

TABLE 5
COMPARISON OF INCIDENCE OF NON-HODGKIN'S
LYMPHOMA SUBTYPES:
WESTERN AND UKM DATA

| Working formulation subtypes | Percentages | | | | |
|------------------------------|--------------------|--------------------|-------------------|-------------------------------|----------------------|
| | MSKCC USA n=456 | SEER USA n=6807 | NCI USA n=1014 | FINSEN INST. DENMARK n=632 | UKM MALAYSIA n=49 |
| LOW GRADE | 31.2 | 31.8 | 38.3 | 28.2 | 2.0 |
| Small lymphocytic | 9.2 | 11.4 | 4.0 | 8.1 | 0 |
| Follicular, small | 17.1 | 15.4 | 25.5 | 14.7 | 0 |
| Follicular, mixed | 4.8 | 5.1 | 8.8 | 5.4 | 2.0 |
| INTERMEDIATE GRADE | 56.4 | 63.8 | 42.1 | 28.6 | 59.2 |
| Follicular, large cell | 3.3 | 2.4 | 4.3 | 3.6 | 6.1 |
| Diffus small cleaved | 14.6 | 22.6 | 7.8 | 5.1 | 12.2 |
| Diffuse, small | 2.2 | 7.6 | 7.6 | 7.9 | 38.8 |
| Diffuse, large cell | 36.2 | 31.3 | 22.4 | 12.0 | 2.0 |
| HIGH GRADE | 12.5 | 4.4 | 19.5 | 38.4 | 38.8 |
| Large cell, immunoblastic | 6.1 | 1.9 | 9.0 | 23.1 | 26.5 |
| Lymphoblastic | 3.9 | 0.2 | 4.8 | 10.4 | 10.2 |
| Small non-cleaved cell | 2.4 | 2.3 | 5.7 | 4.9 | 2.0 |

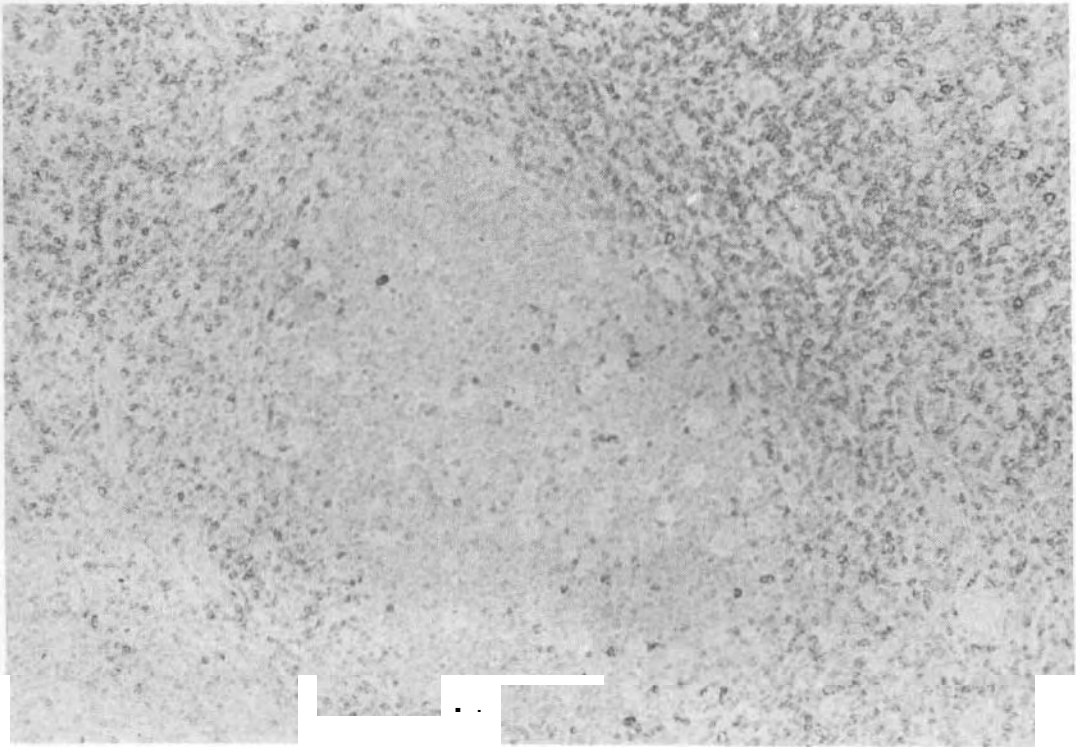


FIG. 1: Immunolocalization of ML antibodies in normal control tonsil: The T-lymphocytes are reactive to this combination of antibodies, and are situated in the interfollicular zone. Small numbers of T-lymphocytes are also found in the follicle (ML antibodies, immunoperoxidase, 115x).

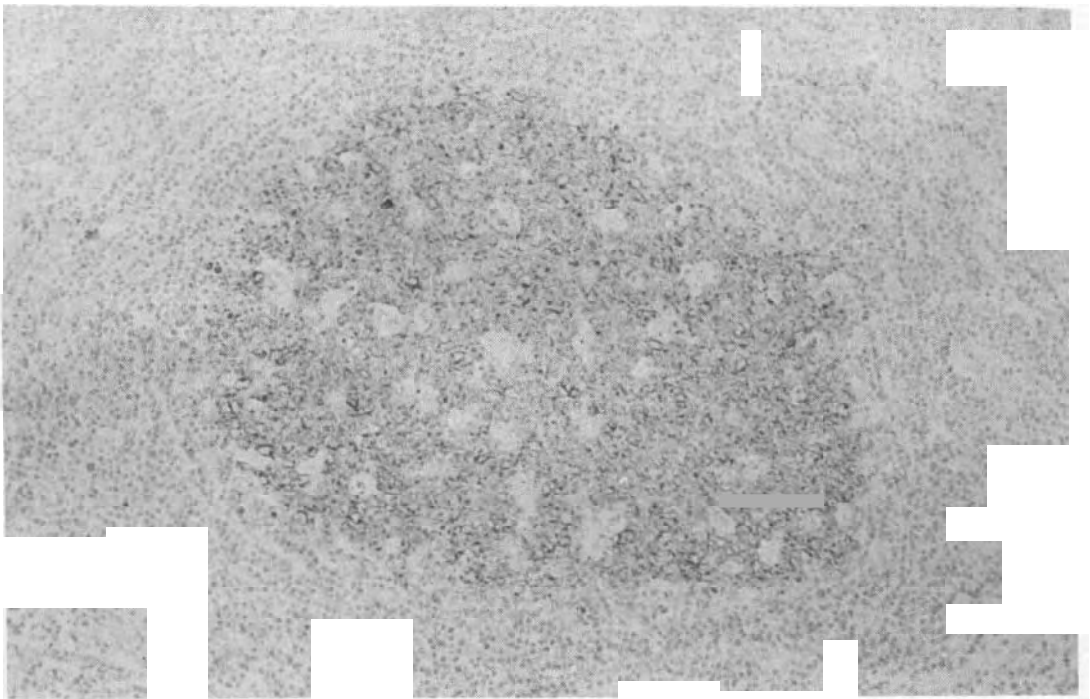


FIG. 2. Immunolocalization of LN1 antibody in normal control tonsil. The B-lymphocytes of the germinal centre show strong immunoreactivity (LNI antibody, immunoperoxidase, 115x).

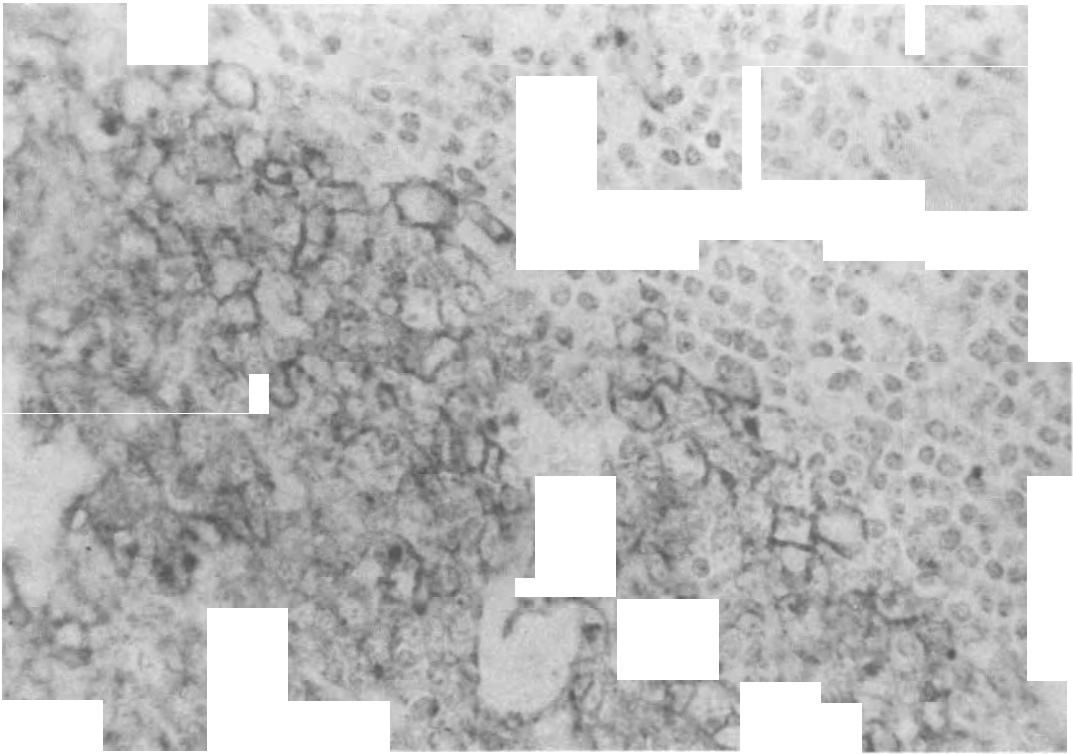


FIG. 3. Higher magnification of the edge of the germinal centre in Fig. 1. The antibody concentrates at the surface membrane of the B-cells. (460x).

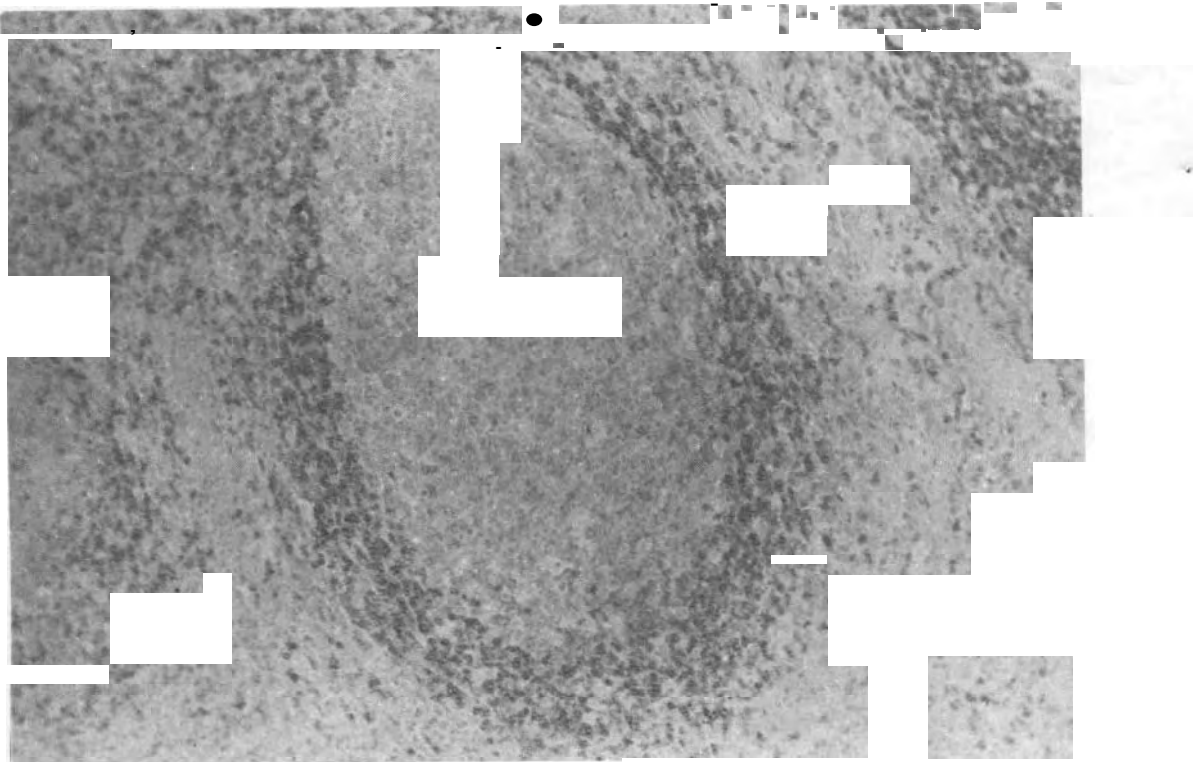


FIG. 4. Immunolocalization of MT2 in normal control tonsil. B-lymphocytes of the mantle zone show strong immunoreactivity; those of the germinal centre are only weakly reactive. (MT2 antibody, immunoperoxidase, 115x).

