

USE OF LeuM1 MONOCLONAL ANTIBODY FOR THE DIAGNOSIS OF HODGKIN'S DISEASE

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ABSTRACT

Monoclonal antibody to LeuM1, a granulocyte-related differentiation antigen, represents a highly effective reagent for detection of diagnostic "Reed-Sternberg" cells and variants in paraffin-embedded tissues of Hodgkin's disease. The "Reed Sternberg cell in all the cases of Hodgkin's disease except lymphocyte predominance variety revealed positive intracytoplasmic/paranuclear granular staining with LeuM1 marker. The A-S cells in lymphocyte predominance variety contain probably sialylated LeuM1 antigen. All the cases of non-Hodgkin's lymphohia and reactive lymphadenitis showed no staining with LeuM1 monoclonal antibody. Therefore this antibody represents a potentially helpful diagnostic discriminant in the assessment of Hodgkin's disease and its distinction from non-Hodgkin's lymphomas and morphologically similar reactive lymphoid lesions (JPMA 40 :156, 1990).

INTRODUCTION

Hodgkin's disease is a unique clinical and morphologic disorder with both neoplastic and reactive components. The reactive component consists of lymphocytes, histocytes, polymorphonuclear leukocytes, plasma cells, and fibroblasts. The neoplastic component consists of "Reed-Sternberg" & Hodgkin's cell¹. To guide the clinician for effective treatment an accurate diagnosis at an early stage of disease is of utmost importance. Early diagnosis and the effective treatment at an early stage has good survival rate². At present the histological criteria for diagnosis on hematoxylin and eosin (H&E) stain are based on criteria of Lukes and Buttlar³. To diagnose the different subgroups of Hodgkin's disease, the detection of different type of "Reed-Sternberg" cells alongwith other components of lymphocytes and sclerosis is important⁴⁻⁶. On H&E staining sometimes it becomes difficult to differentiate Hodgkin's disease from nonHodgkin's lymphoma and reactive lymphadenitis. To solve this problem immunoperoxidase techniques have been developed by which "Reed-Sternberg" cells can be demonstrated easily.^{7,8} In this connection many monoclonal antibodies Th5, Th6, Th9, 3C4 & Ki-1,⁹ LeuM1 and Peanut agglutinin and LeuM1 alongwith anti-EMA7 were used to detect "ReedSternberg" cells. Of these antigranulocyte antibodies LeuM1, is the best which reacts with all types of "Reed-Sternberg" cells in all the histological subgroups of Hodgkin's disease except lymphocyte-predominance variety⁷⁻¹⁰. The monoclonal antibody LeuM1 reacts with a sugar moiety, Lacto-N-fucopentaose III (LNF.HI), which is linked to membranous protein intracellular protein or lipids. This sugar is contained in a glycoprotein that is strongly expressed in "Reed-Sternberg" cells, therefore these cells can be stained with anti-LeuM1.

MATERIALS AND METHODS

Tissues

One hundred specimens of lymphnodes consisted of 36 specimens of current material received during the period from 1st March to 31st October, 1988 and 64 specimens were diagnosed in the department of Pathology, BMSI, JPMC, Karachi, during the period from 1st January, 1983 to 29th February, 1988.

All lymphnode biopsies selected for study were diagnosed on H&E staining. The statistical breakdown of these lesions were 50 cases of Hodgkin's disease, 30 cases of non Hodgkin's lymphoma and 20 cases of reactive lymphadenitis. The Hodgkin's disease cases consisted of eight with lymphocyte predominance, four with nodular sclerosis, 30 with mixed cellularity, six with lymphocyte depletion, and two cases were unclassified.

Reagents

Monoclonal antibody LeuM1 (Becton & Dickinson), Universal immunoperoxidase kit (Dakopatts), Trypsin (Sigma Corporation) and AEC (3-amino - 9 ethyl carbazole) was used as chromogenic substrate.

Staining Procedure

Tissue sections were mounted on Poly-L-Lysine coated slides, deparaffinized through xylene and rehydrated through graded alcohol, and placed in phosphate buffered saline (PBS), followed by methanolic hydrogenperoxide for 30 minutes at room temperature to reduce nonspecific background staining by blocking endogenous peroxidase. The slides were then washed and placed in PBS. The sections were incubated in a solution of trypsin for 20 minutes at 37°C for proteolytic digestion. The slides were washed and placed in PBS. The sections were treated with normal swine serum for 45 minutes at 37°C to avoid nonspecific staining. The sections were treated with primary antibody (mouse antihuman) LeuMi (1:50) and incubated in humidity chamber for 1.15 hour at 37°C. The slides were washed in three changes of PBS for 15 minutes. The sections were treated with secondary antibody (peroxidase conjugated rabbit antimouse immunoglobulin) and incubated in humidity chamber for 45 minutes at 37°C. The slides were washed in three changes of PBS for 15 minutes. The sections were treated with tertiary antibody (peroxidase conjugated swine antirabbit immunoglobulin) and incubated in humidity chamber for 45 minutes at 37°C. The slides were washed in three changes of PBS for 15 minutes. The sections were treated with AEC chromogenic substrate solution and incubated for 40 minutes in humidity chamber at room temperature. The slides were rinsed with distilled water and then counterstained with mayer's hematoxylin for 5 minutes. The slides were rinsed with distilled water. The sections were flooded with ammonia water and incubated for 10 seconds to develop counterstain. The excess water was wiped off and coverslips were mounted by using glycerine jelly. The positive control slides were prepared from tissue of acute suppurative appendicitis containing polymorphonuclear leukocytes and stained in similar manner. The negative control sections were stained with omission of primary antibody and replaced by normal nonimmune serum.

RESULTS AND OBSERVATIONS

All the cases of Hodgkin's disease, which were included in the study were classified on H&E stain according to Rye's classification⁵. Of the 50 cases of Hodgkin's disease, 40 cases showed positive staining with LeuM1 monoclonal antibody (Table).

TABLE showing LeuM1 staining in cases of Hodgkin's disease, nonHodgkin's lymphoma and reactive lymphadenitis.

Type of lesion on H&E staining	Total number of cases	Positive LeuM1 immuno staining		Negative LeuM1 immuno staining	
		Number of cases	percent- age	Number of cases	percent- age
Hodgkin's disease	50	40	80%	10	20%
Lymphocyte- predominance	8	0		8	
Nodular sclerosis	4	4		0	
Mixed-- Cellularity	30	30		0	
Lymphocyte- depletion	6	6		0	
Unclassified	2	0		2	
NonHodgkin's- Lymphoma	30	1	3.3%	29	96.7%
Reactive- lymphadenitis	20	1	5%	19	95%

All the cases of nodular sclerosis, mixed cellularity and lymphocyte depletion variants were found to be positive with LeuM1 antibody. None of the lymphocyte predominance variety showed positive staining. Two cases, which were diagnosed as unclassified on H&E staining because of the doubtful morphological appearance, showed no staining with LeuM1, suggesting that these two cases were not of Hodgkin's disease. Of the 30 cases diagnosed on H&E as non-Hodgkin's lymphoma, 29 cases showed negative staining with LeuM1 antibody. Only a single case of non-Hodgkin's lymphoma revealed positive staining with LeuM1 marker (Table), suggesting thereby that it was a case of Hodgkin's disease. Nineteen of the 20 cases of reactive- lymphadenitis showed negative staining of the histocytes with LeuM1 marker. One case which showed abnormal histocytes on H&E, revealed positive staining with LeuM1 monoclonal antibody (Table), suggesting that it might have been a case of Hodgkin's disease. In all the positive cases the staining was seen as intracytoplasmic, granular, reddish brown deposition mainly in the paranuclear region.

DISCUSSION

In this study all the cases of Hodgkin's disease except lymphocyte predominance variety showed

positive staining with LeuM1 marker. The "Reed-Sternberg" cells in lymphocyte predominance variety showed no positive staining with LeuM1 antibody because LeuM1 antigen in these cases was probably sialylated. Two unclassified cases showed no positive staining of their atypical histocytes because these probably were not true "Reed-Sternberg" cells.

In the present study 1/30 case of non-Hodgkin's lymphoma and 1/20 case of reactive lymphadenitis revealed positive staining of their abnormal histocytes. These cells took up the stain similar to that observed in true "Reed-Sternberg" cells, suggesting that these two cases most probably were of Hodgkin's disease but could not be definitely diagnosed previously on H & E stain. Twenty nine of the 30 cases of non-Hodgkin's lymphoma and 19/20 cases of reactive lymphadenitis showed no positive staining with LeuM1 antibody because atypical histocytes of these cases did not have LeuM1 antigen.

In the study reported by Hsu and Jaffe¹ except lymphocyte predominance variety, all the cases of Hodgkin's disease were positive with LeuM1 marker. None of the case of non-Hodgkin's lymphoma and reactive lymphadenitis showed positive staining with LeuM1 marker. In the study reported by Pinkus and Said¹¹, all the cases of Hodgkin's disease except lymphocyte predominance variety were shown positive staining with LeuM1 marker because these were histocyte origin, while lymphocyte predominance variety was B-cell origin. In the study reported by Hsu¹² the lymphocyte predominance variety were positive with LeuM1 marker, when these cases were pretreated with neuraminidase enzyme, which removes sialic acid from L & H variants of "Reed-Sternberg" cells. In other studies, all the cases of Hodgkin's disease except lymphocyte-predominance variety were positive with LeuM1 marker¹³⁻¹⁵. LeuM1 marker is helpful when "Reed-Sternberg" cells are rare or when they are so numerous that alternative diagnosis i.e. non-Hodgkin's lymphoma is considered. So this marker could be used to distinguish mononuclear "Reed-Sternberg" cells from atypical reactive lymphoreticular elements¹⁶. A case of a young boy who had four consecutive lymphnode biopsies, was reviewed by four experienced pathologists and diagnosed follicular hyperplasia. Several months later when this case was stained with LeuM1 marker, it was found to be positive. In this way LeuM1 marker proved useful to differentiate Hodgkin's disease from follicular hyperplasia¹⁷. LeuM1 marker is a specific marker in differentiating Hodgkin's disease from T-cell and B-cell lymphoma, having giant cells resembling "Reed-Sternberg" cells. This marker appears to be sensitive, when used with careful morphologic examination. On most occasions, positive staining of atypical "Reed-Sternberg" like cell in an abnormal lymphnode effectively rule out benign disorders and frequently non-Hodgkin's lymphoma. LeuM1 appears to be a stable antigen in well fixed specimens, since positive staining was observed in sections of tissues that had been present in paraffin blocks for longer period¹⁶. The indirect immunoperoxidase staining technique was used in this study which offers many advantages, including high specificity and more sensitivity.¹⁸ LeuM1 monoclonal antibody is therefore presumed to be a highly specific marker to differentiate Hodgkin's disease from non-Hodgkin's lymphoma and reactive lymphadenitis, because this marker localizes the LeuM1 antigen in "Reed-Sternberg" cells which is not present in R-S like cells or atypical histocytes and giant cells. It is not helpful to demonstrate L&H variants of "Reed-Sternberg" cells in lymphocyte predominance variety because LeuM1 antigen in these cells is probably sialylated. It is also presumed to be a specific and sensitive marker in retrospective study cases.

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