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Differential reactivity of HBME-1 and CD15 antibodies in benign and malignant thyroid tumours

Preferential reactivity with malignant tumours

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Abstract We studied a wide range thyroid tumours and non-neoplastic conditions (total 463 cases) immunohistochemically to evaluate the possible diagnostic potential of HBME-1 and CD15 antibodies. HBME-1 monoclonal antibody recognizes a biochemically unknown epitope present in mesothelioma and variably present in some adenocarcinomas. CD15 antibodies recognize a sugar epitope also included in Lewis X blood group antigen. All papillary (145/145) and follicular carcinomas (27/27) were HBME-1 positive, usually in the the majority of tumour cells. In contrast, cases of nodular goitre and papillary hyperplasia either showed no reactivity or were focally positive (in a third of cases). The patterns of CD15 reactivity were generally similar, although smaller numbers of tumour cells were positive in papillary carcinomas, and only 50% of follicular carcinomas were positive. Because fetal thyroid also showed CD15 reactivity, this antigen appears to behave as an oncofetal antigen in relation to thyroid tissue. Anaplastic carcinomas were negative with both antibodies, indicating the loss of these epitopes upon high grade malignant transformation. We conclude that HBME-1 and CD15 immunohistochemistry may be helpful in the histological differential diagnosis between benign lesions and differentiated thyroid carcinomas, especially papillary tumours. Although the biochemical basis of HBME-1 reactivity is unknown, increased CD15 reactivity in malignant thyroid tumours probably reflects changes in thyroid follicular epithelial glycoconjugates related to malignant transformation.

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Introduction

The differential diagnosis of thyroid tumours is often difficult because of the wide histological spectrum of both non-neoplastic thyroid tissue and thyroid carcinomas. Although immunohistochemistry is helpful in tumour typing, the differential diagnosis of benign and malignant conditions is essentially based on cytohistological features [6, 35]. In particular, the differential diagnosis between papillary thyroid carcinoma and papillary hyperplasia may be difficult when papillary carcinomas lack typical cytological features, such as nuclear grooves and optically clear nuclei [19, 26, 34]. It is also true that the nuclear grooves are not specific for papillary carcinoma, but may be present in benign thyroid lesions including nodular goitre and follicular adenoma [30]. Following our initial observation that HBME-1 and CD15 monoclonal antibodies reacted with papillary thyroid cancers but not with benign thyroid epithelium, we studied a large series of thyroid tumours and non-neoplastic lesions immunohistochemically in order to evaluate the biological significance and potential diagnostic value of these findings.

HBME-1 monoclonal antibody was raised by Battifora to cultured mesothelioma cells. The target antigen of the HBME-1 is a yet unknown membrane antigen of mesothelioma cells, as the antibody neither immunoblots nor immunoprecipitates [32] (H. Battifora, personal communication, November 1994). However, HBME-1 antibody has been found useful in the differential diagnosis between mesothelioma vs. adenocarcinoma, as the former is positive and the latter typically negative [20, 32].

CD15, a cluster antigen of myeloid cells, contains a sugar sequence similar to that of Lewis X (LeX) blood group antigen and stage specific embryonic antigen (SSEA) [1]. CD15 immunoreactivity is widely used as a

diagnostic marker of Hodgkin's cells [5, 7, 11, 12, 14, 24, 36]. Because many adenocarcinomas are CD15 positive but mesotheliomas are negative, CD15 is also commonly used in the differential diagnostic panel for evaluation of possible mesothelioma [22, 23, 31, 32, 37, 39, 40]. The presence of CD15 (LeuM1) reactivity has been suggested as an unfavourable prognostic sign in thyroid medullary and papillary carcinoma [17, 21, 28, 29]. However, no comparative evaluation of benign and malignant thyroid tissue has been reported.

In this study we show that HBME-1 and CD15 antibodies react with most differentiated thyroid carcinomas, especially papillary carcinomas, but do not react significantly with benign tumours suggesting, that these antibodies may be useful in the differential diagnosis of thyroid lesions, especially papillary lesions.

Materials and methods

Representative thyroid tumours and non-neoplastic lesions (total 463 cases) were obtained from the files of the two institutions (Department of Pathology and Cell biology, Jefferson Medical College of Thomas Jefferson University, and Department of Pathology, University of Helsinki). Normal thyroid was obtained from surgical specimens, and 15-week fetal thyroid was obtained from a surgical specimen (intrauterine fetal death). The tissues had been fixed in phosphate-buffered formaldehyde (8 to 72 h) and embedded in paraffin. The tumour diagnoses were based on published criteria [2, 6, 26]. Previously frozen tissue was avoided, as it showed reduced immunoreactivity for HBME-1 and markedly reduced reactivity for CD15. Follow-up information of selected cases was obtained from local tumour registries. Hürthle cell lesions were excluded from the study; they showed weak to moderate diffuse background-like staining most probably attributable to endogenous biotin/avidin binding; in addition, the staining patterns were similar in metaplastic and neoplastic Hürthle cells.

The primary mouse monoclonal antibodies HBME-1 (Dakopatts Carpinteria, Calif.) and CD15 (LeuM1, Beckton-Dickinson, Mt View, Calif.) were used at dilutions of 1:40–1:50. HBME-1 mouse IgM hybridoma antibody was raised by Battifora to a suspension of cultured human mesothelioma cells, and it reacts with an incompletely characterized membrane component of mesothelioma cells [20, 21]. Based on our experiments, pepsin digestion [0.05% crude preparation of pepsin (Merck, Darmstadt, Germany) in HCl, pH 2.0 for 20–30 min at +37°C] was used prior to the immunostaining for both antibodies; we found nonenzymatic microwave antigen retrieval or no pretreatment often ineffective for optimal immunostaining for both of these antibodies. The primary antibody was incubated overnight at +4°C, followed by secondary and tertiary reagents (Vectastain Elite, Vector Laboratories, Burlingame, Calif.) in an automatic immunostainer (Shandon Cadenza, Pittsburgh, Pa.). The secondary antibody was a goat anti-mouse IgM-specific antiserum (1:200) applied for 30 min, since both of the primary antibodies are of IgM isotype. The secondary antibody was followed by avidin combined with biotinylated peroxidase (both 1:500) for 30 min, and diaminobenzidine (1 mg/ml), with hydrogen peroxide (three consecutive 100 µl applications, 5 min each). A haematoxylin counterstain was used. In the negative control the primary antibody was omitted. Normal lung or a mesothelioma was used as positive control for HBME-1, and normal tonsil for CD15.

Results

All results are summarized in Table 1.

Normal thyroid follicular epithelium in adults was typically negative for both HBME-1 and CD15. However, in a 15-week fetus about 50% of the thyroid follicles showed intense CD15 reactivity in a luminal and cytoplasmic pattern, and there was focal HBME-1 positivity in fetal thyroid showing a similar distribution. In adult thyroid, HBME-1 also reacted with scattered histiocytes and CD15 with granulocytes, most of the latter being present intravascularly. Crushed tissue and surgical margins were often diffusely CD15 positive, presumably because of antigen released from granulocytes; such areas were not evaluated.

Table 1 Immunohistochemical distribution of HBME-1 and CD15 reactivities in thyroid lesions [– no positive cells found, + positive cells found; for positive staining: *trace* (less than 5% of positive cells); *1+* less than 10% of positive cells; *2+* 10–50% of positive cells; *3+* more than 50% of positive cells; * papillary and follicular carcinoma components positive, figures in parenthesis are percentages]

	Number of cases			Quantification of positive staining			
	–	+	Total	Trace	1+	2+	3+
Nodular goitre - HBME 1	57 (63)	33 (37)	90	22	6	5	0
Nodular goitre - CD15	75 (83)	15 (17)	90	15	0	0	0
Papillary hyperplasia (Graves) - HBME1	6 (55)	5 (45)	11	3	2	0	0
Papillary hyperplasia (Graves) - CD15	11 (100)	0 (0)	11	0	0	0	0
Chronic thyroiditis - HBME1	6 (15)	33 (85)	39	8	13	12	0
Chronic thyroiditis - CD15	29 (74)	10 (26)	39	4	5	1	0
Follicular adenoma - HBME1	53(72)	21 (28)	74	6	5	4	6
Follicular adenoma - CD15	40 (87)	6 (13)	46	2	1	1	2
Atypical adenoma -HBME1	2 (25)	6 (75)	8	1	0	1	4
Atypical adenoma - CD15	5 (63)	3 (37)	8	1	0	1	1
Papillary carcinoma - HBME1	0 (0)	145 (100)	145	4	4	16	121
Papillary carcinoma - CD15	2 (1)	136 (99)	138	28	18	29	61
Follicular carcinoma - HBME1	0 (0)	27 (100)	27	1	2	4	20
Follicular carcinoma -CD15	6 (32)	13 (68)	19	5	3	1	4
Medullary carcinoma - HBME1	9 (75)	3 (25)	12	1	1	1	0
Medullary carcinoma - CD15	4 (57)	3 (43)	7	1	1	1	0
Anaplastic carcinoma - HBME1	19 (100)	0* (0)	19	0	0	0	0
Anaplastic carcinoma - CD15	11 (100)	0* (0)	11	0	0	0	0

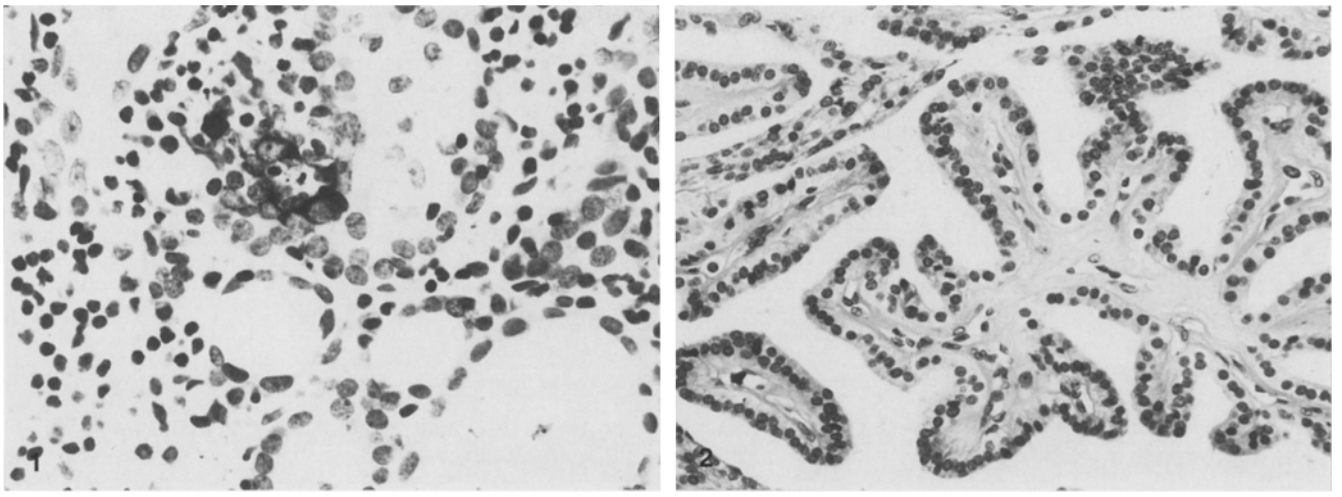

Fig. 1 Nodular goitre shows a small follicle with CD15-positive epithelial cells surrounded by focal lymphocytic infiltration (*upper left centre*). ABC immunoperoxidase with haematoxylin counterstain, ×360

Fig. 2 Papillary thyroid hyperplasia is CD15 negative; this case was also HBME-1 negative. ABC immunoperoxidase with haematoxylin counterstain, ×180

Nodular goitres were generally HBME-1 and CD15 negative. However, HBME-1- and CD15-positive follicular cells were seen in 37% and 17% of the cases, respectively. Both HBME-1 and CD15 reactivity was usually seen in connection with epithelial alterations, including foci of atrophic follicles, cysts or cystically dilated follicles, or foci of inflammation (Fig. 1). Only in 11 cases (12%) were there more than scattered HBME-1-positive cells, and in only 5 cases (6%) was there positivity in a cell population over 10%. In none of the nodular goitres did HBME-1-positive cells account for more than 50% of all follicular cells. CD15 reactivity in goitres was always limited to a few scattered cells.

Papillary hyperplasia with Graves' disease was typically HBME-1 negative. However, in nearly half of the cases (5/11, 45%) there were scattered HBME-1-positive cells, but these never amounted more than 10% of the cellular population. None of the papillary hyperplasias with Graves' disease showed CD15 reactivity (Fig. 2). However, occasionally a few papillary foci in goitres were focally CD15 positive.

There was a higher number of HBME-1-positive follicular cells in chronic thyroiditis than in other benign thyroid lesions. Such positive cells were seen in atrophic follicles and clusters of them, and 30% of cases showed more than 10% of HBME-1-positive follicular epithelial cells. CD15 reactivity in thyroiditis was more limited, being present in more than 10% of the cells in only 1/39 cases.

The HBME-1 reactivity in thyroid neoplasms is summarized in Table 1. Follicular adenomas were typically HBME-1 and CD15 negative, similar to the surrounding normal thyroid tissue. However, a few adenomas (13%) showed significant HBME-1 reactivity (more than 10% of tumour cells); the surrounding thyroid in these cases was HBME-1 negative. The HBME-1-positive tumours had a benign course during follow up without evidence of later metastases (median follow up 12 years). Similarly significant CD15 reactivity was found only8% of the adenomas; generally the CD15-positive cases were also HBME-1 positive.

Atypical adenomas showing cytological atypia, increased cellularity and inconclusive evidence of vascular invasion showed more HBME-1 and CD15 reactivity than ordinary adenomas; in fact, the reactivity in these cases approached that seen in follicular carcinomas. However, follow up of these cases did not show evidence of malignant evolution.

In this study, papillary carcinoma was defined as a tumour composed of larger than normal cells with enlarged, overlapping, often grooved nuclei showing either focal or diffuse papillary pattern with one-cell layer covering the papillae. Cases with entirely follicular pattern with the cytological features of papillary carcinoma were always classified as papillary carcinoma. Most cases of papillary carcinoma showed strong and widespread HBME-1 reactivity, and 97% of cases had more than 10% of tumour cells positive. All but one showed CD-15 positive cells, and 67% of the cases had more than 10% tumour cells that were CD15 positive. Both HBME-1 and CD15 reactivity often appeared as luminal staining, but in many cases the lateral cell membranes were also stained. In some cases, there was general cytoplasmic reactivity for HBME-1 and CD15. The HBME-1 and CD15 reactivity was in contrast to the surrounding normal thyroid tissue, which was typically negative (Figs. 3–6). Follicular variants showed reactivity similar to that of the tumours with a papillary appearance. Lymph node metastases (7 cases) generally showed strong HBME-1 and CD15 reactivity similar to that of primary tumours. In many cases, minimal satellite lesions outside of the main tumour mass were highlighted with HBME-1 and CD15 immunostaining (Figs. 3, 5). Three incidental tumours (5 mm in diameter) that were studied were all intensely HBME-1 positive, whereas 20–40% of tumour cells were CD15 positive. Previously frozen tissue

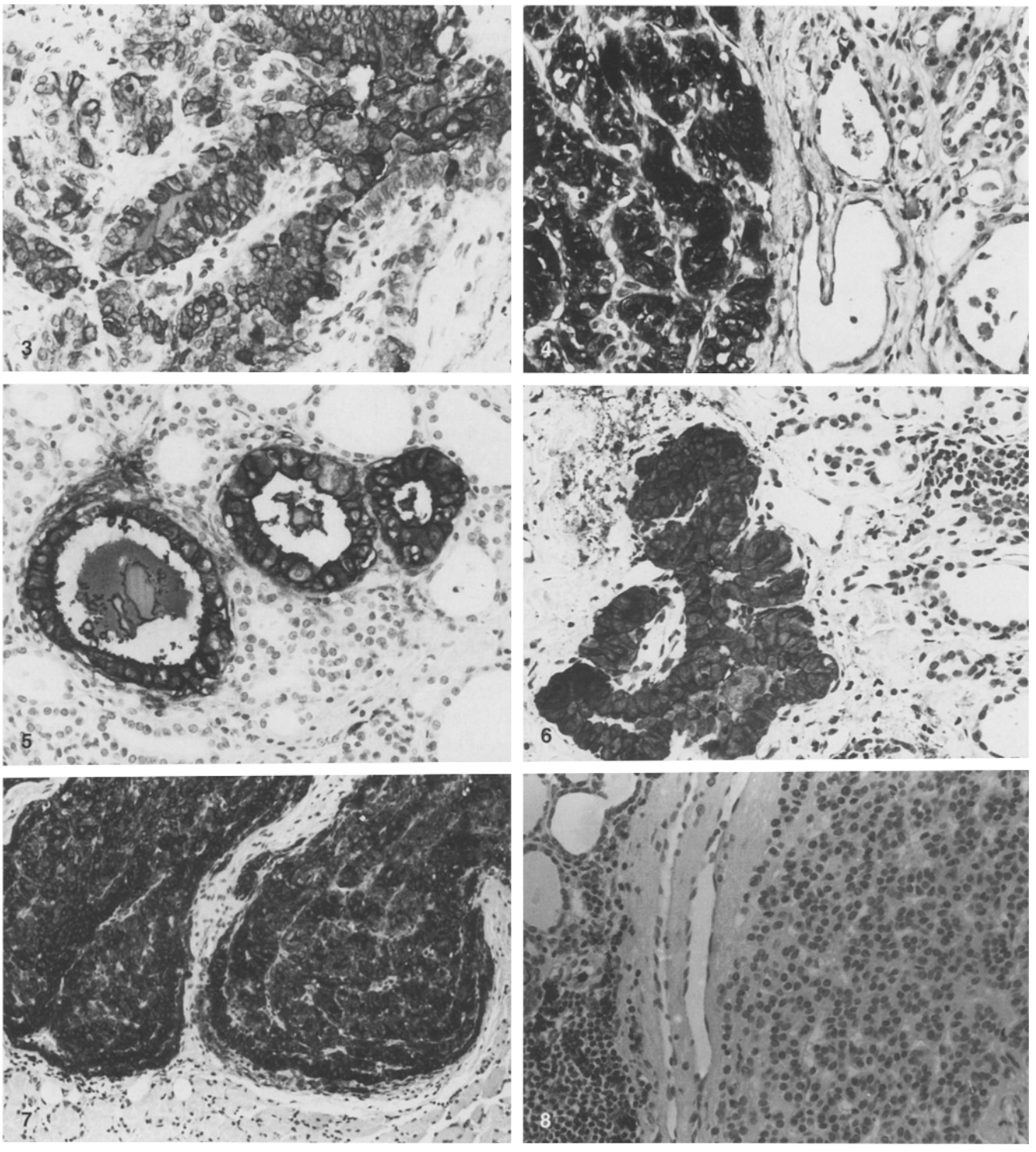

Fig. 3 Thyroid papillary carcinoma is strongly CD15-positive (about 80% of tumour cells positive). ABC immunoperoxidase with haematoxylin counterstain, ×180

Fig. 4 Thyroid papillary carcinoma of follicular type is strongly HBME-1 positive, whereas the surrounding thyroid tissue is negative. ABC immunoperoxidase with haematoxylin counterstain, ×180

Fig. 5 Three follicular structures representing a small satellite lesion of thyroid papillary carcinoma with follicular pattern are strongly HBME-1 positive. The surrounding thyroid is negative, but scattered histiocytes are positive. ABC immunoperoxidase with haematoxylin counterstain, ×180

Fig. 6 Satellite lesion of thyroid papillary carcinoma is CD15-positive, in sharp contrast to the surrounding thyroid tissue, which is negative. A few granulocytes are also positive. ABC immunoperoxidase with haematoxylin counterstain, ×180

Fig. 7 The vascular invasion elements of thyroid follicular carcinoma show strong HBME-1 reactivity. ABC immunoperoxidase with haematoxylin counterstain, ×90

Fig. 8 In contrast to follicular carcinomas, most follicular adenomas are HBME-1 negative. ABC immunoperoxidase with haematoxylin counterstain, ×180

showed reduced HBME-1 and markedly reduced CD15 immuno-reactivity, although granulocytes remained CD15 positive, apparently illustrating partial antigen loss in such tissues. Therefore, previously frozen tissue was excluded from scoring of the results.

All follicular carcinomas evaluated in this study showed vascular (venous) invasion outside of the tumour. Most cases showed strong HBME-1 reactivity in the tumour cells, and metastases outside of the thyroid (soft tissue, bone, 7 cases) showed reactivity equal to that in the primary tumours. CD15 reactivity was variable, about half of the follicular carcinomas showing significant positivity; this was in contrast with most adenomas, which were negative (Figs. 7, 8).

Medullary carcinomas were typically HBME-1 negative. However, amyloid stroma was HBME-1 positive. In 2 cases there were moderate numbers of HBME-1-positive medullary carcinoma cells in tumours that also showed follicle formation. A varying degree of CD15-reactivity was seen in 3 of 7 medullary carcinomas.

The anaplastic giant-cell, epithelial-pleomorphic and sarcomatous components were consistently HBME-1 and CD15 negative. In 2 cases, HBME-1-positive residual papillary and follicular carcinoma components were identified in the background of anaplastic carcinoma, indicating that the anaplastic carcinomas had arisen in the background of papillary and follicular carcinomas; in 1 of these cases some differentiated foci were CD15 positive.

Discussion

In this study, we evaluated a large series of benign and malignant thyroid tumours for HBME-1 and CD15 immunoreactivity that appeared preferentially in malignant tumours. Since the differential diagnosis between benign and malignant thyroid lesions is essentially based on cytohistological interpretation, immunohistochemical reagents that help this distinction would be welcome. HBME-1 and CD15 antibodies therefore appear to have the potential to complement the histological diagnosis of thyroid tumours.

HBME-1 monoclonal antibody was raised by Battifora to cultured mesothelioma cells, and it identifies an incompletely characterized mesothelioma cell membrane antigen [32]. This antibody shows reactivity with mesothelioma, and adenocarcinomas react variably [20]. Although HBME-1 is not specific for mesothelioma, it is presently one of the few markers that can be used for a positive identification of mesothelioma in paraffin sections, whereas most other differentiation markers are negative in mesothelioma and often positive in adenocarcinoma [22, 32, 40].

The results indicate that benign tumours, such as nodular goitres and follicular adenomas, have very limited HBME-1 reactivity, whereas differentiated carcinomas of both papillary and follicular types are usually strongly positive. Remarkably, many reactive lesions that show papillary features and may resemble papillary carcinoma, appear HBME-1 negative, while papillary carcinomas, almost without exception, are strongly HMBE-1 positive. Although the biological basis for HBME-1 reactivity is unknown, this antibody may be useful in the evaluation of thyroid lesions.

Generally, the patterns of CD15 immunoreactivity appear similar to those of HBME-1. Reactivity is present in differentiated thyroid carcinomas, especially the papillary ones, and absent in benign papillary lesions in a highly contrasting pattern. In follicular lesions, the absence of CD15 in adenomas and presence of follicular carcinomas shows a less contrasting pattern, as only two thirds of the carcinomas are positive. Insofar as they show reactivity in many clinically benign follicular lesions, both HBME-1 and CD15 appear less useful for the distinction between benign and malignant follicular lesions; a greater contrast is observed between the reactivities of benign and malignant papillary lesions.

Although CD15 reactivity has been suggested as an unfavourable prognostic sign in thyroid papillary cancer [28], our results, showing marked CD15 reactivity in the majority of papillary carcinomas (44% of cases showing more than 50% of tumour cells positive, 67% of cases showing more than 10% of tumour cells positive) indicate the limited prognostic value of this finding. Published follow-up data referring to the majority of cases in the present series showed distant metastasis only in 5.5% of the cases and tumour-related death in 16% of the cases [16]; these figures are significantly lower than the frequency of marked CD15 positivity. The more frequent detection of CD15 in thyroid papillary carcinoma in our series may result from a higher immunohistochemical detection sensitivity. In our study, we optimized the immunostaining by using protease digestion, and we also used IgM-specific secondary antibodies corresponding to the IgM isotype of the primary antibodies; this is known to increase the detection sensitivity of CD15 [18]. The previous study showing significant CD15 reactivity only in in 32% of thyroid papillary carcinomas did not mention the use of enzyme digestion or the use of a secondary antibody of IgM class [28].

The differential expression of CD15 in benign and malignant thyroid follicular epithelial cells apparently results from the different glycoconjugate patterns in benign and malignant follicular epithelial cells. Extensive biochemical studies by Hakomori et al. [9, 10] have suggested that CD15-related antigens might represent oncofetal tumour antigens that in some organ systems, such as the gastrointestinal tract and the lung, are generally absent in normal adult tissue but are present in fetal tissue and are acquired by tumour cells by the process of abnormal glycosylation. Although the early data on tumour antigens were based on biochemical studies, immunohistochemical studies have confirmed the suggestion that CD15 (Le X) is a tumour-associated antigen that has limited expression in normal colonic and lung alveolar epithelia and urothelium, but is widely expressed in corresponding carcinomas and also corre-

sponding fetal epithelia [3, 13, 25]. Our results showing that CD15 is also present in fetal thyroid suggest that in the context of thyroid tissue CD15 behaves as an oncofetal antigen.

Although HBME-1 and CD15 react with most cases of differentiated carcinomas, both of these epitopes are apparently lost in anaplastic carcinomas that are immunohistochemically negative. Similarly, lack of HBME-1 reactivity in poorly differentiated and sarcomatous mesotheliomas, in contrast to well-differentiated ones, suggests that the recognized epitope may be lost upon high-grade malignant transformation [20].

The differences in cellular glycoconjugates can also be evaluated by lectins, animal or plant proteins that have antibody-like affinity with certain specific sugar sequences [4]. Studies on binding patterns of some of the most commonly used lectins, such as WGA, SBA, DBA and RCA, in thyroid tissue yielded the conclusion that the lectin-binding patterns in benign and malignant thyroid epithelia were not sufficiently different to be useful in the differential diagnosis [8, 27, 33]. However, one study suggested that thyroid cancer epithelium shows more intense binding of lectins from jack fruit, peanut and winged bean than does benign epithelium [38]. Intensity-based differences, however, are difficult to standardize in immunohistochemistry, and are less likely to be clinically useful.

Other markers have also been suggested in the differential evaluation of benign vs malignant thyroid tissue. Recently Xu et al. demonstrated immunoreactivity for galectin I and III, galactose residue binding proteins (endogenous lectins), in malignant but not in benign thyroid tissue [41]. The difference of expression was dramatic and it was suggested that it could of diagnostic value. Kotani et al. [15] in turn showed that antibodies to dideptidyl aminopeptidase IV had a preferential immunoreactivity in thyroid papillary and follicular carcinoma vs follicular adenoma. However, 27% of follicular adenomas were also positive, limiting the potential diagnostic value of this finding.

In conclusion, malignant thyroid tumours show significantly greater HBME-1 and CD15 reactivity than benign lesions. These patterns may be useful in the evaluation of histologically difficult thyroid lesions, especially the papillary ones. However, caution must be exercised in the interpretation, because the results in benign (usually negative) vs malignant (usually positive) lesions may overlap; these findings also await independent confirmation. Although the biochemical bases of HBME-1 and CD15 are different according to the different tissue distribution patterns of immunoreactivity, these antibodies may highlight the changes in cellular glycoconjugates related to malignant transformation.

References

1. Arber DA, Weiss LM (1993) CD15. A review. Appl Immunohistochem 1:17–30
2. Carangiu ML, Zampi G, Rosai J (1985) Papillary thyroid carcinoma: a study of its many morphologic expressions and clinical correlates. Pathol Annu 20:1–44
3. Cordon-Cardo C, Reuter VE, Lloyd KO, et al (1988) Blood group-related antigens in human urothelium: enhanced expression of precursor, LeX and LeY determinants in urothelial carcinomas. Cancer Res 48:4113–4120
4. Damjanov I (1987) Lectin cytochemistry and histochemistry. Lab Invest 57:5–20
5. Dorfman RF, Gatter KC, Pulford KAF, Mason DY (1986) An evaluation of the utility of anti-granulocyte and anti-leukocyte monoclonal antibodies in the diagnosis of Hodgkin's disease. Am J Pathol 123:508–519
6. Franssila KO, Ackerman LV, Brown CL, Hedinger CE (1985) Follicular carcinoma. Semin Diagn Pathol 2:101–122
7. Frierson HF, Innes DJ (1985) Sensitivity of anti-Leu-M1 as a marker in Hodgkin's disease. Arch Pathol Lab Med 109:1024–1028
8. Gonzalez-Campora R, Sanchez Gallego F, Martin Lacave I, et al (1988) Lectin histochemistry of the thyroid gland. Cancer 62:2354–2362
9. Hakomori SI (1989) Aberrant glycosylation in tumors and tumor-associated carbohydrate antigens. Adv Cancer Res 52:257–331
10. Hakomori SI, Nudelman E, Kannagi R, Levery SB (1982) The common structure in fucosyllactosaminolipids accumulating in human adenocarcinomas, and its possible absence in normal tissue. Biochem Biophys Res Commun 109:36–44
11. Hall PA, D'Ardenne AJ (1987) Value of CD15 immunostaining in diagnosing Hodgkin's disease: a review of published literature. J Clin Pathol 40:1298–1304
12. Hsu SM, Yang K, Jaffe ES (1985) Phenotypic expression of Hodgkin's and Reed-Sternberg cells in Hodgkin's disease. Am J Pathol 118:209–217
13. Kim YS, Itzkowitz SH (1988) Carbohydrate antigen expression in the adenoma-carcinoma sequence. Prog Clin Biol Res 279:241–250
14. Kornstein MJ, Bonner H, Gee B, Cohen R, Brooks JJ (1986) LeuM1 and S100 in Hodgkin's disease and non-Hodgkin's lymphomas. Am J Clin Pathol 85:433–437
15. Kotani T, Asada Y, Aratake Y, et al (1992) Diagnostic usefulness of dipeptidyl aminopeptidase IV monoclonal antibody in paraffin-embedded thyroid follicular tumours. J Pathol (Lond) 168:41–45
16. Kukkonen ST, Haapiainen RT, Franssila KO, Sivula A (1990) Papillary thyroid carcinoma: the new, age-related TNM classification system in a retrospective analysis of 199 patients. World J Surg 148:837–841
17. Langle F, Soliman T, Neuhold N, et al (1994) CD15 (LeuM1) immunoreactivity: prognostic factor for sporadic and hereditary medullary thyroid cancer? Study group on multiple endocrine neoplasia of Austria. World J Surg 18:583–587
18. LeBrun DP, Kamel OW, Dorfman RF, Warnke RA (1992) Enhanced staining for Leu M1 (CD15) in Hodgkin's disease using a secondary antibody for immunoglobulin M. Am J Clin Pathol 97:135–138
19. Livolsi VA (1986) Pathology of the thyroid. Major problems in surgical pathology. Saunders, Philadelphia
20. Miettinen M, Kovatich AJ (1995) HBME-1 monoclonal antibody useful in the differential diagnosis of mesothelioma, adenocarcinoma and soft tissue and bone tumors. Appl Immunohistochem 3:115–122
21. Neuhold N, Langle F, Gannt M, Hollenstein U, Niederle B (1992) Relationship of CD15 immunoreactivity and prognosis in sporadic medullary thyroid carcinomas. J Cancer Res Clin Oncol 118:629–634
22. Ordonez NG (1989) The immunohistochemical diagnosis of malignant mesothelioma. Differentiation of mesothelioma and lung adenocarcinoma. Am J Surg Pathol 13:276–291

23. Otis CN, Carter D, Cole S, Battifora H (1987) Immunohistochemical evaluation of pleural mesothelioma and pulmonary adenocarcinoma. A bi-institutional study of 47 cases. Am J Surg Pathol 11:445–456
24. Pinkus GS, Thomas P, Said JW (1985) Leu-M1 – a marker for Reed-Sternberg cells in Hodgkin's disease. An immunoperoxidase study of paraffin-embedded tissues. Am J Pathol 119:244–252
25. Rao SK, Fraire AE (1995) Alveolar cell hyperplasia in association with adenocarcinoma of the lung. Mod Pathol 8:165–169
26. Rosai J, Carangiu Ml, DeLellis RA (1992) Tumors of the thyroid gland. Armed Forces Institute of Pathology, Washington, DC
27. Sasano H, Rojas M, Silverberg SG (1989) Analysis of lectin binding in benign and malignant thyroid nodules. Arch Pathol Lab Med 113:186–189
28. Schröder S, Schwarz W, Rehpenning W, Loning T, Böcker W (1987) Prognostic significance of Leu-M1 immunostaining in papillary carcinomas of the thyroid gland. Virchows Arch [A] 411:435–439
29. Schröder S, Schwarz W, Rehpenning W, et al (1988) Leu-M1 immunoreactivity and prognosis of medullary carcinomas of the thyroid gland. J Cancer Res Clin Oncol 114:291–296
30. Scopa CD, Melachrinou M, Saradopoulou C, Merino MJ (1993) The significance of the grooved nucleus in thyroid lesions. Mod Pathol 6:691–694
31. Sheibani K, Battifora H, Burke JS (1986) Antigenic phenotype of malignant mesotheliomas and pulmonary adenocarcinomas. An immunohistologic analysis demonstrating the value of LeuM1 antigen. Am J Pathol 123:212–219
32. Sheibani K, Esteban JM, Bailey A, Battifora H, Weiss LM (1992) Immunopathologic and molecular studies as an aid to the diagnosis of malignant mesothelioma. Hum Pathol 23: 107–116
33. Sobrinho-Simoes M (1995) Tumours of the thyroid: a brief overview with emphasis on the most controversial issues. Curr Diagn Pathol 2:15–22
34. Sobrinho-Simoes M, Damjanov I (1986) Lectin histochemistry of papillary and follicular carcinoma of the thyroid gland. Arch Pathol Lab Med 110:722–729
35. Stanta G, Carangiu ML, Rosai J (1988) The biochemical and immunohistochemical profile of thyroid neoplasia. Pathol Annu 23:129–157
36. Stein H, Uchanska-Ziegler B, Gerdes J, Ziegler A, Wernet P (1982) Hodgkin and Sternberg-Reed cells contain antigens specific to late cells of granulopoiesis. Int J Cancer 29:283–290
37. Strickler JG, Herndier BG, Rouse RV (1987) Immunohistochemical staining in malignant mesotheliomas. Am J Clin Pathol 88:610–614
38. Vijayakumar T, Augustine J, Mathew L, et al (1992) Tissue binding pattern of plant lectins in benign and malignant lesions of thyroid. J Exp Pathol 6:11–23
39. Warnock ML, Stoloff A, Thor A (1988) Differentiation of adenocarcinoma of the lung from mesothelioma. Periodic acid-Schiff, monoclonal antibodies B72.3, and Leu M1. Am J Pathol 133:30–38
40. Wick MR, Loy T, Mills SE, Legier JF, Manivel JC (1990) Malignant epithelioid pleural mesothelioma versus peripheral pulmonary adenocarcinoma: a histochemical, ultrastructural, and immunohistologic study of 103 cases. Hum Pathol 21:759–766
41. Xu XC, El-Naggar AK, Lotan R (1995) Differential expression of galectin-1 and galectin-3 in thyroid tumors. Potential diagnostic implications. Am J Pathol 147:815–822