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Prognostic significance of Leu-M1 immunostaining in papillary carcinomas of the thyroid gland

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Summary. Leu-M1 antigen is a monocyte/granulocyte-related marker known to be consistently expressed in the Reed-Sternberg cells of patients with Hodgkin's disease. Recently, however, the presence of Leu-M1 has also been noted in tumour cells of a variety of non-haematopoietic neoplasms, most of them adenocarcinomas. The biological significance of this aberrant reaction has not been clarified. We have been able to demonstrate marked epithelial Leu-M1 immunoreactivity (>15% tumour cells positively stained) in 24 out of 76 (32%) papillary carcinomas of the thyroid gland (PC). This phenomenon was more frequently observed among PCs at an advanced stage of disease (pT_4 vs. pT_{1-3} and M_1 vs. M_0 $p<0.05$). The degree of epithelial Leu-M1 positivity was also shown to be significantly correlated to the clinical course of PC. Irrespective of other morphological and clinical features, death resulting from cancer occurred 17 times more frequently among PCs with marked Leu-M1 positivity (8/24) when compared with tumours with only slight or absent immunoreactivity (1/52) ($p<0.00005$). These findings suggest that Leu-M1 immunostaining provides significant prognostic information for patients with papillary carcinoma of the thyroid gland.

Key words: Leu-M1 antigen – Immunocytochemistry – Thyroid carcinomas – Prognosis

Introduction

The presence of myelomonocyte antigen Leu-M1 was initially established in adherent mononuclear cells, granulocytes, some T-cell lines, mitogen-activated T-cells and neoplastic cells of patients with acute and chronic myelocytic leukaemia (Hanjan et al. 1982). Subsequently, the Leu-M1 antigen was shown to be consistently expressed in the Reed-Sternberg (RS) cells in Hodgkin's (HD) disease (Hsu and Jaffe 1984; Pinkus et al. 1985). Since positive Leu-M1 immunostaining was also recorded for RS-like cells of occasional non-Hodgkin's lymphomas (Hyder and Schnitzer 1986; Kornstein et al. 1986), this antigen is now regarded to be a sensitive though not specific marker for HD. Recently, however, Sheibani et al. (1986a) also established immunoreactivity for Leu-M1 antibodies in a variety of non-haematopoietic neoplasms, which included 113 of 119 carcinomas, most of them (58%) adenocarcinomas. As stated by these authors, those epithelial tumours which revealed positive staining in respect of Leu-M1 comprised a number of primary thyroid carcinomas. The purpose of the present study was to obtain these findings on a large selection of clinically well-defined papillary carcinomas of this organ and to search for its potential biological significance.

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Materials and methods

Paraffin blocks of 76 papillary thyroid carcinomas (PC) were retrieved from the surgical pathology files of the Institute of Pathology at the University of Hamburg, and of the Department of Pathology at the General Hospital Hamburg-Harburg, FRG. All were cases of primary thyroid neoplasms which were surgically treated from 1970 to 1979. The specimens had been fixed in 10% buffered formalin and routinely processed for embedding and sectioning. Using the criteria laid down by the WHO (Hedinger and Sobin 1987) and by Tscholl-Ducommun and Hedinger (1982), the tumours were subtyped into variants of growth pattern (17 microcarcinomas (MPC), 8 encapsulated carcinomas (EPC) and 51 widely invasive carcinomas (WIPC)) and groups of various degrees of differentiation (42 type Ia–c, 11 type IIa, 12 type IIb, 7 type III and 4 oxyphilic). Each of the 76 tumors was categorized according to its TNM-stage (Spiessl et al. 1982). The medical records were reviewed, and all patients were monitored until summer 1985.

Immunocytochemical studies for Leu-M1 antigen (comercially available monoclonal antibody purchased from Becton-

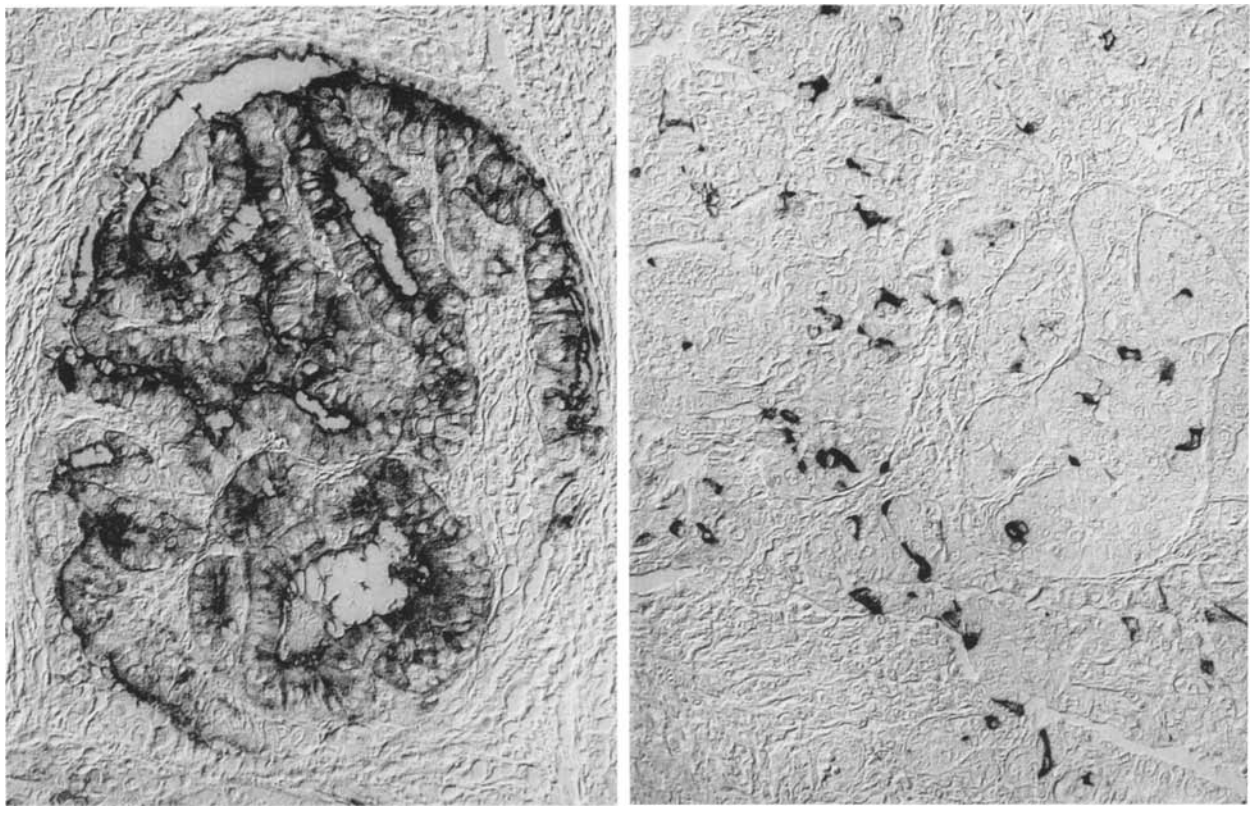

Fig. 1. Leu-M1 immunocytochemistry in PC. *Left*, highly differentiated tumour (Ia) with strong positivity (score: 72%); *right*, moderately differentiated tumour (IIb) with slight positivity (score: 6%) (differential interference contrast optics, ×280 and ×220)

Dickinson, Heidelberg, FRG, dilution 1:30) were carried out using the avidin-biotin-peroxidase complex method (Hsu et al. 1981). Leu-M1-positive tumour cells and unstained tumour cells were counted at 100 fold magnification with the aid of an eyepiece micrometer (120 × 120 mm) which was superimposed on the histological section. The whole tumour cut surface was thus examined, and the mean immunoreactivity was recorded as the proportion of positively stained cells per 100 tumour cells.

The Leu-M1 immunoreactivity was related to various clinical and morphological features, including tumour subtype, degree of differentiation, stage of disease, age and sex of patients and density of Langerhans cells (LC) infiltrating with the tumour tissue. Dense infiltrates of LC have recently been demonstrated to be significantly correlated with a favourable prognosis in patients with PC (Schröder et al. 1988). The significance of differences between groups was evaluated by Student's *t*-test at the 5% level. The influence of the immunocytochemical findings and the aforementioned clinicomorphological findings on the prognosis was considered in a multivariate analysis using Cox's forward-stepping proportional hazard model (Cox 1972). In addition, the probability of survival and tumour recurrence was analysed using the Kaplan-Meier method (Kaplan and Meier 1958), employing the Mantel-Cox test and the generalized Wilcoxon test. Statistical analyses were carried out using the computer programs BMDP (Hopkins 1981) and SAS (Ray 1982).

Results

Epithelial Leu-M1 immunoreactivity was marked (>15% tumour cells positively stained) in 32% (24/76), slight (≤15%) in 14% (41/76) and absent in 54% (41/76) of all PC cases (Fig. 1). In a given Leu-M1-positive case, immunoreactivity either resulted in a strong diffuse cytoplasmic staining or revealed an enrichment near the cell plasma membranes. An analysis of the immunocytochemical results revealed a correlation between the percentage of Leu-M1-positive cells and several clinical and morphological factors of PC. Leu-M1 immunoreaction was significantly more pronounced among WIPCs (23.3 ± 30.3) as compared to EPCs and MEPs (including all PCs of stage pT_1: 9.0 ± 18.7; $p < 0.02$). The same was demonstrated for WIPCs of stages pT_4 (29.2 ± 31.6) as comparded to pT_{2+3} (15.2 ± 22.9; $p < 0.02$). PCs presenting distant haematogenous spread at the time of diagnosis (M_1: 27.3 ± 36.8) were more intensely stained than tumours lacking this feature (M_0: 17.7 ± 26.7; N.S.).

No differences existed when comparing PCs with and without initial regional lymph node involvement (N_1 vs. N_0), PCs at various degrees of differentiation or tumours among patients of different age and sex. Leu-M1 positivity was not observed in residual nonneoplastic thyroid parenchyma adjacent to PC at all.

Table 1. Epithelial immunoreactivity for Leu-M1 antigen ($\leq 15\%$ vs. $>15\%$) and prognosis (recurrence rate and survival rate) in papillary thyroid carcinomas

Risk group	Cox's regression		Kaplan-Meier curves *P* values	
	Chi^2	*P* value	Breslow	Mantel-Cox
Recurrence rate				
PC	7.72	0.0054	0.0676	0.0168
WIPC	1.41	0.2357	0.4385	0.1778
WIPC T_4	0.22	0.6416	0.8654	0.7568
Survival rate				
PC	16.66	<0.00005	0.0017	0.0017
WIPC	11.68	0.0006	0.0144	0.0174
WIPC T_4	4.11	0.0426	0.0549	0.0500

(PC: all 76 papillary carcinomas; WIPC: 51 widely invasive papillary carcinomas; WIPC T_4: 23 WIPCs invading the cervical soft tissue)

In a study of recurrence rate and survival, the degree of epithelial Leu-M1 immunoreaction was shown to be significantly related to the course and outcome of disease. Using Cox's regression, dichotomizing the proportion of Leu-M1-positive tumour cells at the level of 15/100 cells was revealed to produce the most significant results. Tumour recurrence and death resulting from cancer were significantly more frequent among PCs with marked immunoreactivity ($>15\%$ positivity) as compared to PCs with no or only slight immunostaining ($\leq 15\%$). As shown in Table 1 and Fig. 2 (stating the actual numbers of patients at risk), Leu-M1 was significantly correlated to survival rate even when only comparing WIPCs of the locally advanced stage pT_4. Assuming that there were no complex interactions between Leu-M1 expression and other clinicomorphological factors possibly influencing the prognosis of PC (including age and sex of patients, pTNM-stage, degree of histological differentiation and density of LC infiltrates), all these features were tested by Cox's forward stepping proportional hazard model. The results, which are listed in Table 2, indicate the significant impact of Leu-M1 expression on prognosis in comparison with other factors.

Discussion

The present study indicates that the detection and quantification of epithelial Leu-M1 immunoreactivity is a reliable method in assessing the prognosis of patients with papillary thyroid carcinoma. Irrespective of other morphological and clinical features, death resulting from cancer occurred

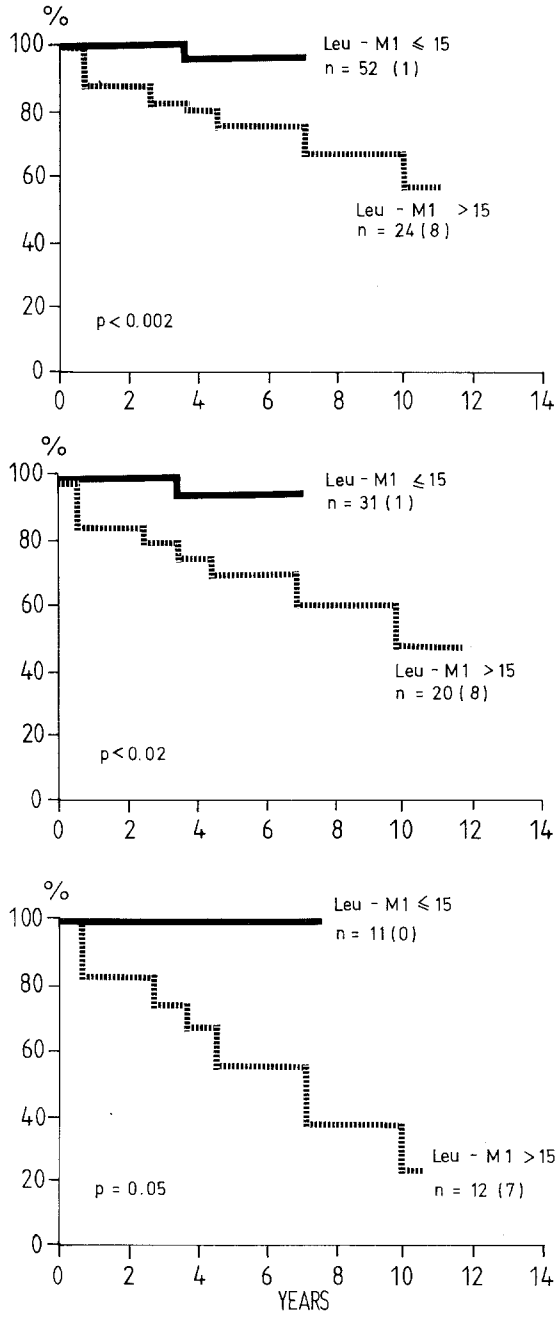

Fig. 2. Adjusted survival (Kaplan-Meier) curves for patients with (*top*) PC ($n=76$), (*middle*) WIPC ($n=51$) and (*bottom*) WIPC T_4 ($n=23$) comparing tumours of different degree of epithelial Leu-M1 immunoreactivity ($\leq$ vs. $>15\%$). Numbers of patients at risk (and of cases of death) are stated for the respective tumours groups. The solitary PC case with lethal outcome and slight Leu-M1 positivity represents an oxyphilic WIPC with widespread haematogenous metastasis present at the time of diagnosis ($pT_2N_1M_1$)

17 times more frequently among PCs with marked Leu-M1 positivity (8/24) when compared with tumours with only slight or absent immunoreactivity (1/52) ($p<0.00005$). We recently demonstrated that the density of S-100 protein-positive LCs infil-

Table 2. Cox's foward stepping proportional hazard model: Summary of stepwise results to evaluate the influence of age and sex of patients, pTNM-stage, degree of histological differentiation (Diff.), density of Langerhans cell infiltration (S-100$^+$-LCs) and epithelial Leu-M1 immunoreactivity (Leu-M1$^+$) on survival among 76 patients with PC

Improvement		
Parameter	Chi^2	*P* value
M_x	13,797	0,0002
Diff.	9,771	0,0018
T_x	6,327	0,0125
Leu-M1$^+$	8,007	0,0047
S-100$^+$-LCs	5,099	0,0239

Age, N_x: N.S.

trating the tumours tissue is also significantly correlated to the clinical behaviour of these neoplasms (Schröder et al. 1988). A favourable effect of marked LC infiltration was anticipated since these cells have been shown to play an important role in immunological defense mechanisms of the host against the tumour in a variety of neoplasms found in different organs (Furukawa et al. 1985; Igisu et al. 1983; Nomori et al. 1986; Tsujitani et al. 1987). The demonstration of marked Leu-M1 positivity, however, was revealed to be even more effective in determining the probability of death in an individual case of PC when compared with the finding of absent LC infiltration. We are unable to offer any plausible explanation for this observation.

The carbohydrate residue recognized by anti-Leu-M1 antibodies belongs to the lacto series of complex cell membrane glycolipoproteins (Hakomori and Kannagi 1983) and was recently determined as lacto-N-fucopentaose III (LNF III) (Hsu et al. 1986). Complex glycolipoproteins containing fucose have been found particularly in epithelial cells of the intestinal wall during early embryogenesis and are considered to indicate and determine blood group activity (Kim et al. 1982). In adults, synthesis and organization of cell membrane molecules including blood group determinants are highly disturbed during oncogenic transformation, the biochemical basis of which lead to the definition of oncofetal antigens (Hakomori and Kannagi 1983). Expression of glycoconjugates containing fucose has been demonstrated by detecting Ulex europeus lectin-binding sites on epithelial neoplasms, and – more interestingly – by observing LNF III moieties in human adenocarcinomas of the gut, pancreas, kidney and lung (Raedler and Raedler 1985).

Although the molecular characteristics of the antigen recognized by anti-Leu-M1 have not yet been fully determined it appears that some normal cells, as well as the neoplastic cells of a variety of haematopoietic and nonhaematopoietic neoplasms, share the epitope recognized by the antibody (Sheibani et al. 1986a). These findings are analogous to the demonstration of epithelial membrane antigen (originally assumed to be expressed only on epithelial cells) on reactive and neoplastic plasma cells, on some tumour cells of non-Hodgkin's lymphomas and on RS cells of HD (Delsol et al. 1984). Since Leu-M1 positivity has recently been observed in 47/50 (94%) pulmonary adenocarcinomas, but not in any of 28 malignant pleural mesotheliomas investigated for this antigen by Sheibani et al. (1986b), these authors consider Leu-M1 immunostaining to be a useful diagnostical method of distinguishing between these two conditions. Our results infer that Leu-M1 immunostaining might be of clinical relevance in another respect; the selection of different aggressive adjuvant therapeutic procedures to be applied in widely invasive PCs with high or low malignant potential.

The adverse implications of epithelial Leu-M1 expression for the prognosis of an individual tumour have so far not been described for any other neoplasm. We have, however, reason to believe that a similar correlation also exists for further human malignancies. In a preliminary study of 10 primary medullary thyroid carcinomas (MC) we found a considerably higher Leu-M1 immunoreactivity among 8 tumours with unfavourable clinical course (lethal outcome or occurrence of recurrent disease: 28.4 ± 32.6) as compared to 2 tumours which remained symptom-free for at least 9 years (7.5 ± 10.6). Since PC and MC differ entirely regarding their histogenetic background, the search for the probable prognostic significance of Leu-M1 immunostaining among carcinomas found in different organs ought to be continued.

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