

Mast cell infiltration correlates with poor prognosis in Hodgkin's lymphoma

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Received 15 February 2002; accepted for publication 19 April 2002

Summary. Hodgkin's lymphoma (HL) is characterized by a few Hodgkin, Reed–Sternberg cells (HRS) surrounded by benign cells. We recently reported that mast cells were the predominant CD30L-positive cells in HL tumours, and that they activate HRS *in vitro* through CD30L–CD30 interaction. Here, we investigated the clinical importance of mast cell infiltration in the tumours of 123 patients. Tumour specimens were stained with a mast-cell-specific antibody

that detects tryptase. Mast cells were detected in virtually every case and increasing numbers of mast cells correlated to nodular sclerosis histology ($P = 0.008$). Patients with higher mast cell infiltration had a worse relapse-free survival ($P = 0.01$).

Keywords: Hodgkin's lymphoma, mast cells, prognosis, histology, bystander cells.

Hodgkin's lymphoma (HL) is characterized by a few tumour cells, the Hodgkin and Reed–Sternberg (HRS) cells, surrounded by many benign cells. Interactions between these are probably of importance for disease progression. The HRS cells express CD30, a member of the tumour necrosis factor/nerve growth factor receptor superfamily, and we have focused on the role of the bystander cells for the CD30–CD30 ligand (CD30L) (CD153) interaction. The presence of one of the bystander cells, the eosinophilic granulocyte, has prognostic significance (Enblad *et al.*, 1993; von Wasielewski *et al.*, 2000), although this is debated (Axdorph *et al.*, 2001). The poor prognosis in eosinophil-rich cases has been proposed to depend on the eosinophilic expression of CD30L (Pinto *et al.*, 1996). However, recently we reported that the predominant CD30L-positive cell in HL is the mast cell and that mast cells stimulate HRS cell lines via a CD30L–CD30 interaction *in vitro* (Molin *et al.*, 2001a).

The role of mast cells in the pathogenesis of many inflammatory disorders is well established. Mast cells also have important immunoregulatory functions through their expression of cytokines and accessory molecules (Nilsson *et al.*, 1999). Mast cells are often present in tumours (Dimitriadou & Koutsilieris, 1997), but their role in HL has not been explored.

The aim of this study was to investigate the clinical importance of mast cells in HL.

PATIENTS AND METHODS

Patients and clinical characteristics. One hundred and twenty-three patients diagnosed in the Uppsala/Örebro region, Sweden, 1989–1994, were included in the study. All cases were re-classified according to the Revised European–American Classification of Lymphoid Neoplasms (REAL classification). Patients were treated according to National Guidelines (Glimelius *et al.*, 1996). Staging was according to the Ann–Arbor system. Bulky disease was a tumour diameter exceeding 10 cm or a mediastinal tumour more than one-third of the thorax diameter at the thoracic (Th.) 5–6 level. A complete response was defined as disappearance of all known disease. Clinical characteristics and histology are seen in Table I.

Immunohistochemistry. The tissue specimens were fixed in formalin, embedded in paraffin and sectioned in 3 µm thick sections. The monoclonal antibody G3, recognizing the mast-cell-specific protein tryptase, was provided by Dr L. B. Schwartz, MCV, Richmond, VA, USA (Irani *et al.*, 1989). The avidin–biotin–horseradish peroxidase complex (ABC/HRP) and diaminobenzidine (DAB) techniques were used to visualize the tryptase-positive cells. The positive cells were counted in 10 randomly selected high power fields (HPF, 400×), by one author. The ocular was equipped with a lattice square net. Thirty-three of the

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Table I. Clinicopathological characteristics and number of tryptase positive cells $\pm$ SEM.

Clinicopathological variable	n	Mean number of tryptase positive cells $\pm$ SEM	P-value
Male	72	18.5 $\pm$ 2.5	n.s.
Female	51	20.0 $\pm$ 2.8	
NS I,II histology	78	22.6 $\pm$ 2.5	0.008
Non-NS histology	45	13.2 $\pm$ 2.6	
MC	32	12.5 $\pm$ 3.2	
LPn	11	14.6 $\pm$ 4.2	
LD	2	14.0 $\pm$ 12.0	
A (No B symptoms)	82	17.0 $\pm$ 1.9	0.3
B symptoms	41	23.5 $\pm$ 4.1	
Bulky disease	22	22.6 $\pm$ 5.1	0.9
Non-bulky disease	102	18.4 $\pm$ 2.0	
Stage I-IIA	74	14.9 $\pm$ 1.7	0.07
Stage IIB-IV	49	25.6 $\pm$ 3.8	

NS, nodular sclerosis; MC, mixed cellularity; LPn, nodular lymphocyte predominance; LD, lymphocyte depletion.

cases were re-counted by another author. In the analyses, both the absolute number of positive cells and a grouping into few (< 10) versus more (> 10) mast cells/10 HPF were used.

Statistical methods. The Mann–Whitney *U*-test was used to compare groups and the Spearman test to compare different continuous variables. The Kaplan–Meier method was used for survival curves and the log-rank test to compare these. The proportional Cox hazards regression model was used to compare the importance of different prognostic variables.

RESULTS AND DISCUSSION

Tryptase-positive mast cells were present in 113/123 (92%) cases and in every histopathological subgroup (Table I). The mean number was 19.2 (range 0–101) mast cells/10 HPF. A cut-off point of 10 mast cells/10 HPF was chosen because of the distribution of mast cell numbers seen in this and our previous study (Molin *et al*, 2001a). Seventy-one (58%) cases had > 10 mast cells/10 HPF and 52 (42%) had < 10.

Patients with > 10 mast cells/10 HPF had a significantly worse disease-free survival ($P < 0.01$) and a tendency to worse overall survival ($P = 0.06$) than those with < 10 (Fig 1A and B). Further attempts to subgroup those with > 10 positive cells did not yield any additional prognostic information (data not shown). Two of the 10 patients with no mast cell infiltration relapsed.

In patients with stage IIB–IV disease and bulky disease, both disease-free and overall survival tended to be worse in patients with > 10 mast cells/10 HPF. This implicates a stimulatory effect of the presence of mast cells, not accentuated by the presence of an abundance of these cells. Mast cells can stimulate proliferation of HRS cells via the CD30L–CD30 interaction *in vitro* (Molin *et al*, 2001a). They

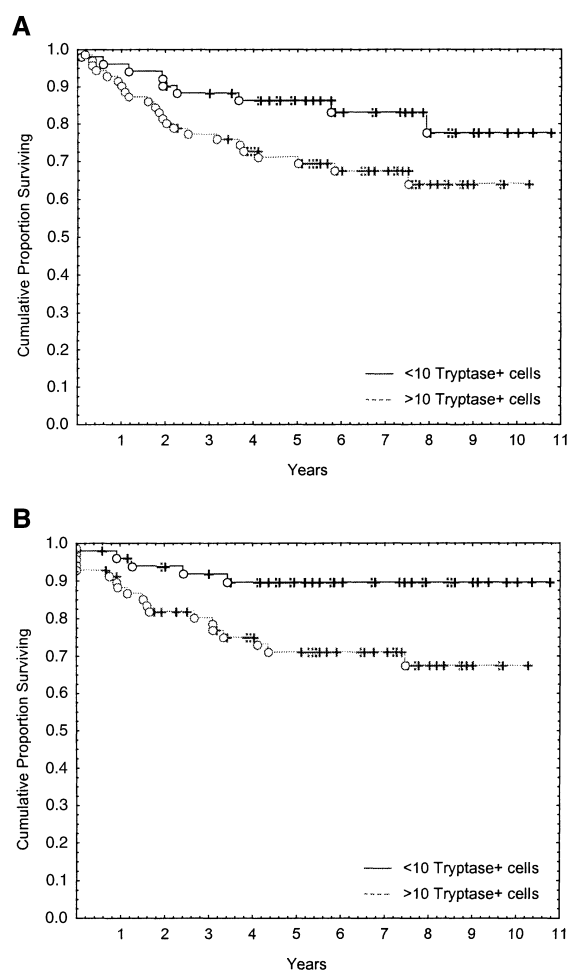


Fig 1. (A) Overall survival according to number of tryptase-positive mast cells in the tumours. $n = 123$, $P = 0.06$. (B) Disease-free survival according to number of tryptase-positive mast cells in the tumours. $n = 123$, $P = 0.01$.

are also the predominant CD30L-expressing cells in HL and we propose that the CD30L–CD30 interaction is of importance also *in vivo*. In the tumours, mast cells were seen both in proximity to the HRS cells and in areas of fibrosis. For the relatively small numbers in this study, the Cox regression analysis did not add any further information, as the prognosis was comparatively good with few events. The only variable that significantly affected the outcome in a multivariate analysis was age.

The number of mast cells also correlated to known prognostic factors such as high white blood cell count (B-WBC), (< 10 mast cells/10 HPF had a mean B-WBC of $7.3 \pm 0.4 \times 10^9$; > 10 mast cells/10 HPF, had a mean B-WBC of $9.5 \pm 0.5 \times 10^9$, $P = 0.002$) and low blood haemoglobin (B-Hb), (< 10 mast cells/10 HPF had a mean B-Hb of 13.24 ± 0.28 g/dl; > 10 mast cells/10 HPF, had a mean B-Hb of 12.53 ± 0.21 g/dl, $P = 0.03$). This implicated a correlation between the proportion of mast cells and the aggressiveness of the disease. However, the number of mast cells did not correlate to the erythrocyte sedimentation

rate (ESR), (< 10 mast cells/10 HPF had a mean ESR of 37.3 ± 5.0 ; > 10 mast cells/10 HPF had a mean ESR of 43.0 ± 4.9 , $P = 0.4$) or serum lactate dehydrogenase levels ($P = 0.8$). Nodular sclerosis (NS) histology also correlated to the mast cell infiltration (Table I), indicating a role of mast cells in the progression of this histological type.

Research concerning the role of the surrounding cells can contribute to the understanding of HL. There are similarities between the role of the eosinophils and mast cells in HL; abundance of both correlates to NS histology and a worse prognosis (Enblad *et al.*, 1993; von Wasielewski *et al.*, 2000) and both cells express CD30L (Pinto *et al.*, 1996; Molin *et al.*, 2001a). Increased serum levels of eosinophil cationic protein is associated to negative prognostic factors (Molin *et al.*, 2001b). This knowledge may create new prognostic factors to aid further tailoring of treatment. Confirmatory studies of these findings in large prospective patient materials are therefore necessary.

Although the association of mast cells with cancer is not new, mast cells have until recently been ignored as part of tumour development. The accumulation of mast cells in different tumours has been well documented, but the role of these cells is uncertain. In HL, mast cells and tumour cells can probably interact not only through CD30L–CD30 but also through soluble factors like interleukin 9 (IL-9) (Gruss *et al.*, 1992) and IL-13 (Skinnider *et al.*, 2001). Further knowledge about these interactions may provide useful information both for diagnosis and for the development of new therapeutic strategies.

ACKNOWLEDGMENTS

We wish to thank Anders Kreuger and co-workers at the Children's Hospital for providing the patient material. This work was supported with research grants from the Swedish Cancer Society and Lion's Cancer Research Foundation.

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