Virchows Arch [Pathol Anat] (1985) 405:503–509

Virchows Archiv A

Bone marrow mast cell reaction in preleukaemic myelodysplasia and in aplastic anaemia

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Summary. The relationship of bone marrow mast cell counts to prognosis was investigated in 48 patients with preleukaemic myelodysplasia, in 59 patients with aplastic anemia and in a DMBA induced myelodysplasia/leukaemia rat model. In patients with myelodysplasia terminating in overt leukaemia the number of mast cells per square millimeter was not correlated to duration of the preleukaemic course. Leukaemia development probabilities of patients at risk were not different for low and elevated mast cell counts. In aplastic anaemia, however, a lower bone marrow mast cell count was related to a higher survival probability and longer survival time. In the animal model no significant differences could be found between myelodysplastic, leukaemic, and control animals.

Key words: Mast cells – Preleukemia – Myelodysplasia – Aplastic anemia

Introduction

Recently, increased bone marrow mast cell content has been noticed in patients with acute leukaemia and preleukaemic syndromes (Prokocimer and Polliack 1981; Yoo and Lessin 1982). The functional significance of this reaction, however, is unclear. It has been suggested that it may represent an expression of the host's defence against neoplasia, and, accordingly, bone marrow mast cell quantitation has been proposed as an additional diagnostic and prognostic tool (Yoo and Lessin 1982). This hypothesis was tested in a retrospective patient study and in an experimental rat model.

Certain forms of preleukaemic syndromes show some overlap with aplastic anaemia (Dameshek 1967; Fohlmeister et al. 1979, 1983; Killmann 1968; Te Velde and Haak 1979), in which an increase of bone marrow mast cells is a common finding (Burkhardt 1975; Duhamel et al. 1978; Fischer and Fohlmeister 1983; Naeim et al. 1978; Sale and Marmont 1981; Savage

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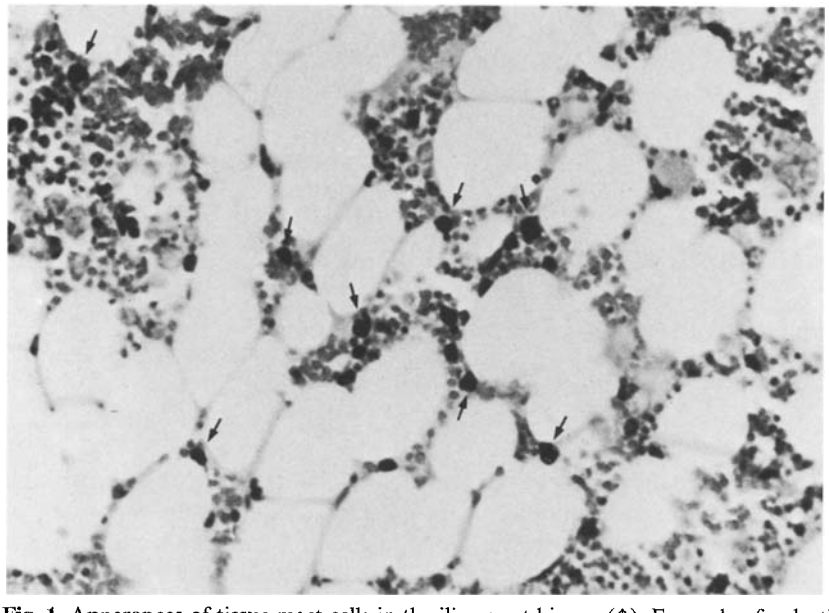

Fig. 1. Apperances of tissue mast cells in the iliac crest biopsy (↑). Example of aplastic anemia with high mast cell count. Giemsa, × 300

et al. 1979; Te Velde and Haak 1979). This prompted us to investigate, in addition, if bone marrow mast cell numbers are related to prognosis in aplastic anaemia.

Materials and methods

Iliac crest biopsy specimens from 48 patients with myelodysplasia terminating in acute non-lymphatic leukaemia and from 59 patients with aplastic anaemia, obtained at first clinical presentation, were evaluated for tissue mast cell content (Fig. 1). The cases of myelodysplasia consisted of 26 with a normocellular to hyperplastic and of 22 with a hypocellular bone marrow. Four patients with hypocellular myelodysplasia and 13 patients with normocellular to hyperplastic myelodysplasia had small blast nests in the biopsy or elevated blast cell counts in bone marrow smears (6–30%). Overt leukaemia developed within one to 35 months. Twenty two of the patients were female, 26 male. They ranged in age from 20 to 81 years, with a mean age of 58.3 years. None of these patients had received chemotherapy before the development of overt leukaemia.

The 59 patients with aplastic anaemia consisted of 26 men and 33 women, ranging in age from 7 to 86 years, with a mean age of 43.5 years. In 20 the disorder was idiopathic, in 39 secondary. Thirty-two patients died within one week to 78 months, 27 were still alive at termination of the study. They had been followed-up for at least 20 months.

The animal material originated from male Wistar rats which had received up to five pulses of 7,12-dimethylbenz(a)anthracene (DMBA) (35 mg/kg body weight) at ten-day intervals. This dose regimen and injection route of DMBA is known to produce acute myeloid leukaemia (Huggins and Sugiyama 1966) preceded by myelodysplasia (Fohlmeister et al. 1981). Fifteen rats were killed or died 5 to 156 days after the first DMBA injection. Five of them had developed overt leukaemia at the time of death, which occurred on day 47, 65, 85, 104, and 156, respectively. Fifteen animals, which had received only the solvent of DMBA, and five uninjected rats served as controls. Tissue mast cell number was evaluated in the femoral bone marrow. Human iliac crest biopsies and rat femora were fixed (30 ml formalin, 20 ml glutaraldehyde and 15.8 g calcium acetate per 1000 ml aqua dest.) and decalcified (EDTA)

Table 1. Mast cell count per square millimeter (mean ± standard error) in different subgroups of myelodysplastic cases

Blast cells	Cellularity		
	Normal to increased	Decreased	Total
Not increased	44.4 ± 12.1	62.9 ± 11.2	55.2 ± 8.3
Increased	35.5 ± 9.9	29.5 ± 10.4	34.1 ± 7.8
Total	39.9 ± 7.7	56.9 ± 9.7	47.7 ± 6.2

Table 2. Frequency distribution of myelodysplastic cases in relation to duration until overt leukemia, and bone marrow mast cell number per square millimeter

Mast cell number	Duration until overt leukemia (months)				
	<3	3<6	6<12	>12	Total
0–19	4	2	2	4	12
20–49	7	5	2	5	19
50–99	3	2	3	3	11
≧100	4	1	0	1	6
Total	18	10	7	13	48

in a neutral milieu (pH 7.0–7.4). After conventional paraffin embedding 3 μ thick dewaxed sections were stained with Giemsa. In addition mast cells were made visible with the naphthol-AS-D-chloracetate esterase reaction. Tissue mast cells have very coarse granules in this reaction in contrast to neutrophil granulopoietic cells. Mast cells were counted with a grid ocular at 480 times magnification in 8 to 16 randomly selected fields, equalling 0.5 to 1 mm^2, and expressed as number per 1 mm^2. Data were compared with the Friedman or Wilcoxon rank test. Statements about frequency distributions were verified with the Yates test after forming contingency tables.

The life table method was used to calculate the probability of survival in aplastic anaemia and the probability of overt leukaemia development in myelodysplasia for patients with different mast cell counts (Peto et al. 1977). For this purpose the range of the mast cell count was arbitrarily subdivided into four classes with about equal patient numbers. The probability values of these subgroups were compared with the observed numbers of death or overt leukaemia, respectively, with the logrank test. Adjacent classes, for which different values were not apparent were recombined and survival probabilities for the combined classes calculated anew.

Results

Mast cell counts ranged from 0 to 192 per square millimeter in patients with preleukemic myelodysplasia. Subdivision of cases with respect to marrow cellularity and blast cell content revealed no statistically significant differences. Mean values and standard errors are given in Table 1. Classification of cases according to duration until overt leukaemia and mast cell number shows a homogeneous frequency distribution. There is, especially, no trend towards lower mast cell numbers with shorter duration of preleukaemic myelodysplasia (see Table 2). In the animal model the range of

Table 3. Number of tissue mast cells in the femoral bone marrow of male Wistar rats after one to five pulses of DMBA or of the solvent of DMBA. For comparison mast cell counts of uninjected age-matched controls are included. Injections were on day 1, 11, 21, 31, and 41. Cases marked with asterix had developed acute myeloid leukemia at the time of death

Time period after start of experiment (days)	Agent injected		
	DMBA	Solvent	None
1–20	4	16	7
	387	28	
21–40	12	173	26
	98	105	225
	252	17	
	105	6	
41–60	247	102	10
	166	266	
	12*	8	
	35	261	
	0	24	
>60	104*	0	177
	41*	15	
	220*	21	
	88*	128	

mast cell number was 0 to 387 per square millimeter. The experimental group had a mean value and standard error of 118 ± 30, compared to 78 ± 26 in injected and 75 ± 42 in uninjected controls. Statistically significant differences could not be demonstrated. Among the experimental animals the mast cell number of the five leukaemic animals did not differ from that of the myelodysplastic rats (Table 3).

With 0 to 205 per square millimeter the mast cell count in patients with aplastic anemia is within the range of myelodysplastic cases. There are no statistically significant differences. Mean and standard error are 59 ± 5 per mm^2.

Classification of cases, which were followed-up till death, according to survival time and mast cell number disclosed a statistically significant trend towards lower mast cell numbers in longtime survivors ($P < 0.01$) (see Table 4).

In myelodysplasia the prognostic relevance of bone marrow mast cell number may best be estimated by comparison of leukaemia development probabilities for different classes of mast cell counts. We classified cases into those with a mast cell count below 20 and those with 20 and more per square millimeter. Between both classes the probability of developing leukaemia within up to 20 months was not statistically different. If separate curves are calculated for cases with hypocellular or normo- to hypercellular marrow, the result is the same.

In contrast to this, in the aplastic anaemia cohort a significantly higher survival probability ($P \ll 0.01$) was found for patients who had less than 20 mast cells per square millimeter in the bone marrow biopsy (see Fig. 2).

Table 4. Frequency distribution of patients who died from aplastic anemia in relation to duration of survival, and bone marrow mast cell number per square millimeter

Mast cell number	Duration of survival (months)				
	<3	3<6	6<12	>12	Total
0–19	0	0	0	4	4
20–49	5	0	2	3	10
50–99	7	5	2	0	14
≧100	1	2	0	1	4
Total	13	7	4	8	32

Result of the YATES test for trend: $\chi^2 = 7.742$; df = 1; $P < 0.01$

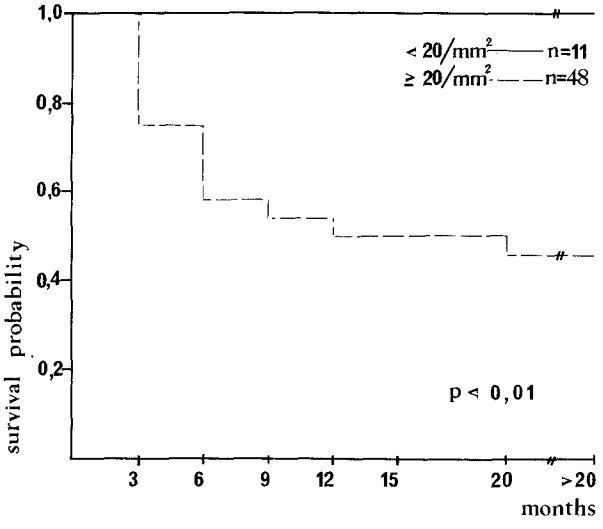

Fig. 2. Survival probabilities in aplastic anemia according to the mast cell count in the initial iliac crest biopsy. Logrank test: $\chi^2 = 8.299$; df = 1; $P \ll 0.01$

Discussion

Recently bone marrow mast cell quantiation has been suggested as an additional diagnostic and prognostic tool in preleukaemic syndromes with regard to the development of leucaemia (Yoo and Lessin 1982). From our results, however, no confirmation could be found for such a prognostic role of bone marrow mast cell numbers. Mast cell counts were not correlated with duration of the preleukaemic course in cases of myelodysplasia terminating in leukaemia and the probability of patients at risk to develop frank leukaemia was not different according to mast cell count.

These findings argue against the hypothesis that the mast cell reaction in myelodysplasia might be the expression of an immunological reaction directed against neoplastic or transformed haemopoietic cells (Yoo and Lessin 1982). But no positive conclusion regarding the role of myelodysplasia associated tissue mast cell reaction can be drawn from these negative results.

In aplastic anaemia, in contrast, the bone marrow mast cell reaction was related to prognosis. Cases followed-up till death showed an inverse

correlation between mast cell count and survival time, and the probability values for survival of patients at risk to die were significantly lower for the lower class of mast cell counts. Bone marrow mast cell reaction in aplastic anaemia mostly appears in combination with an "inflammatory" lymphoplasmocytic infiltrate (Burkhardt 1975; Duhamel 1978; Fischer and Fohlmeister 1983; Fohlmeister et al. 1983; Te Velde and Haak 1979). Recent studies as well as successes with immunotherapy indicate that an immuno-linked regulatory system for stem cell proliferation and/or differentiation influences severity and prognosis of aplastic anaemia (Amare et al. 1978; Bacigalupo et al. 1980; Gordon et al. 1982; Hartmann et al. 1982; Kagan et al. 1976; Speck et al. 1977; Spitzer and Verma 1982). So far, however, only certain T-cell sub-sets have been identified as the operating principle (Amare et al. 1978; Bacigalupo et al. 1980; Gordon et al. 1982; Spitzer and Verma 1982), while mast cells are known to be related to certain forms of the B-cell reaction. Also in this disorder, therefore, the role of tissue mast cells remains to be clarified.

Acknowledgements. The authors wish to thank Mrs. Angelika Porr for excellent technical assistance.

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Accepted December 17, 1984