Multiple Myeloma: Immunodeficient, Osteolytic, Renal, and Amyloid Syndromes

V. A. Almazov, E. I. Podoltzeva, E. V. Morozova, X. O. Sitskaya, V. M. Kravzova, and B. V. Afanasiev [1]

Introduction

Multiple myeloma (MM) is a malignant clonal B-lymphoproliferative disease with typical immunodefficient, osteolytic, renal, amyloid syndromes. Disturbance in the immunologic system appears early and influences the outcome of MM [1]. Light chain isotype suppression (LCIS) [2] and imbalance in T-cells [3] are the factors determining the immunodeficiency syndrome. There are no convincing data about changes in natural killer (NK) activity in MM [4, 5]. Osteolysis is the second most frequently observed syndrome, and interleukin-1_β (IL-1_β), plays the main role in its development [6, 7]. IL-1_β probably is also responsible for chronic renal insufficiency (CRI) [7, 8], which is of great prognostic value im MM [9]. Amyloidosis rarely appears in MM patients (6%–15%) [10]. The aims of this study were to clarify the role of LCIS, to determine NK activity of peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMC) and IL-1 production by peripheral blood monocytes (PBM) in MM, and to assess any possible correlation between these data and the course of MM.

Material and Methods

140 patients with MM were investigated. Amyloidosis was diagnosed in 15 patients according to the typical pathology of skin specimens with Congo Red staining.

Clinical Classification. MM was classified according to previously described criteria [11].

Light Chain-Isotype Suppression. The presence of cell antigens was determined by an indirect immunofluorescence assay [12]. LCIS was determined from the ratio of kappa lymphocytes to lambda lymphocytes (kappa/lambda ratio). The normal range for the kappa/lambda ratio was determined by testing 30 blood donors, and was found to be 0.6–3.0. Patients with kappa myeloma were regarded as having LCIS if the kappa/lambda ratio was less than 0.67; patients with lambda myeloma and a kappa/lambda ratio of more than 3.0 were also regarded as having LCIS. The presence of LCIS was detected by anti-kappa and anti-lambda monoclonal antibodies, which were provided by the Central Research Institute of Roentgenology and Radiology, Leningrad, USSR.

NK Activity. Effector cells were obtained from heparinized peripheral blood and bone marrow by centrifugation over Ficoll-Hypaque. Bone marrow cells were preparated and cultured as described by Yoda et al. [13] with recombinant IL-2 200 U/ml (Institute of Organic Synthesis, Riga, Latvia, USSR). K-562 cells were used as target cells. Cytotoxity was measured in a standard 18-h [^{3}H]uridine microcytotoxicity assay [14]. NK activities (CTX%) were calculated using the following formula:

[1] 1st Leningrad Medical Institute, USSR Petrov Research Institute of Oncology, Leningrad, USSR.

Haematology and Blood Transfusion Vol. 35
Modern Trends in Human Leukemia IX
R. Neth et al. (Eds.)

$$\text{CTX \%} = \left(1 - \frac{\text{experimental cpm}}{\text{control cpm}}\right) \times 100\%$$

Effector: target ratio = 25:1

IL-1 Activity. PBM were cultured in RPMI 1640 in the presence or absence of lipopolysaccharide (LPS; "Pyrogenal", USSR, 40 mg/ml). After 24 h incubation, supernatants were removed, filtered, and frozen at −20°.

Mice thymocytes (C3H/j) ($1 > 10^7$ cells/ml) were incubated in RPMI 1640, supplemented with 1% fetal calfserum (FCS), gentamycin (0.2 mg/ml) L-glutamine, and PGA (1 mg/ml; Difco), with various dilutions of the tested supernatants in a CO_2 incubator for 72 h. The level of cell proliferation was measured by the incorporation at 16 h of [^{3}H]thymidine added to thymocytes. One unit per milliliter of IL-1 activity was defined as the reciprocal of the dilution causing 50% of the maximal response [15, 16].

Statistics. Data were evaluated using Student's *t* test.

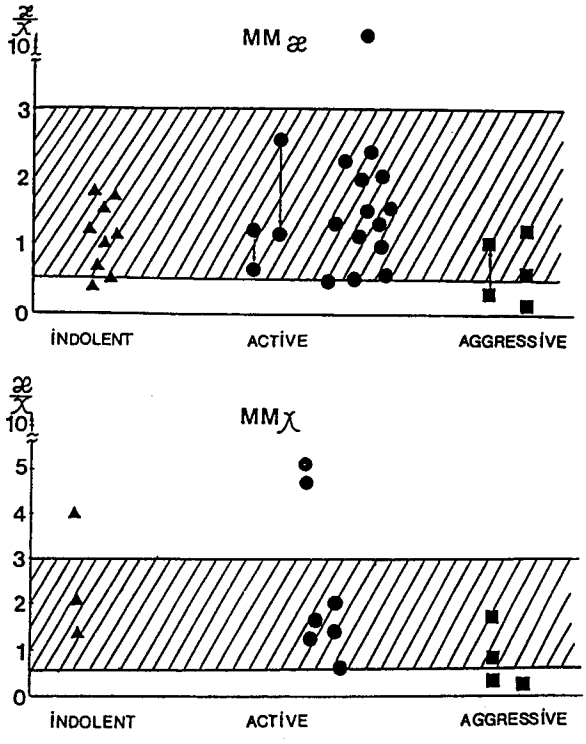

Fig. 1. The relationship between light chain isotype suppression (LCIS) and the course of disease in patients with kappa MM (*top*, $n = 29$) and with lambda MM (*bottom*, $n = 14$). Kappa MM patients with LCIS (kappa/lambda ratio < 0.67): 2 with indolent course (▲), 2 with active course (●), and 2 with aggressive course (■). Lambda MW patients with LCIS (kappa/lambda ratio > 3.0): 1 with indolent course, and 2 with active course (●)

Results

Immunodeficiency Syndrome. Some patients with indolent, active, or aggressive MM had LCIS (Fig. 1). There were no differences in NK activity of PBMC and BMMC in all of MM patients as compared to normal donors (Fig. 2). However, a significantly lower NK activity was found in active and aggressive MM than in normal donors (Fig. 3). The NK activity of BMMC increased after cultivation in media with IL-2 for 72 h (Fig. 4). Pyrogenal-stimulated IL-1 production by PBM showed a tendency to be higher in active and aggressive MM than in indolent MM and normal donors (Fig. 5).

Osteolytic Syndrome. Survival was higher in patients with no or minimal bone changes (osteoporosis) than in patients with moderate or extensive bone lesions (Fig. 6). Patients with indolent MM had mild osteoporosis and no bone lesions (Fig. 7). More than half of the patients with active and aggressive MM had moderate or extensive bond lesions. Pyrogenal-stimulated IL-1 production by PBM differed significantly between patients with no bone lesions or with osteoporosis and patients with moderate or extensive bone lesions (Fig. 8).

Renal Syndrome. Patients with CRI at presentation had shorter survival timer than patients without CRI (Fig. 9). No patient with indolent MM had CRI at presentation, whereas CRI was diagnosed in 4.7% of patients with active MM and in 44.4% of those with aggres-

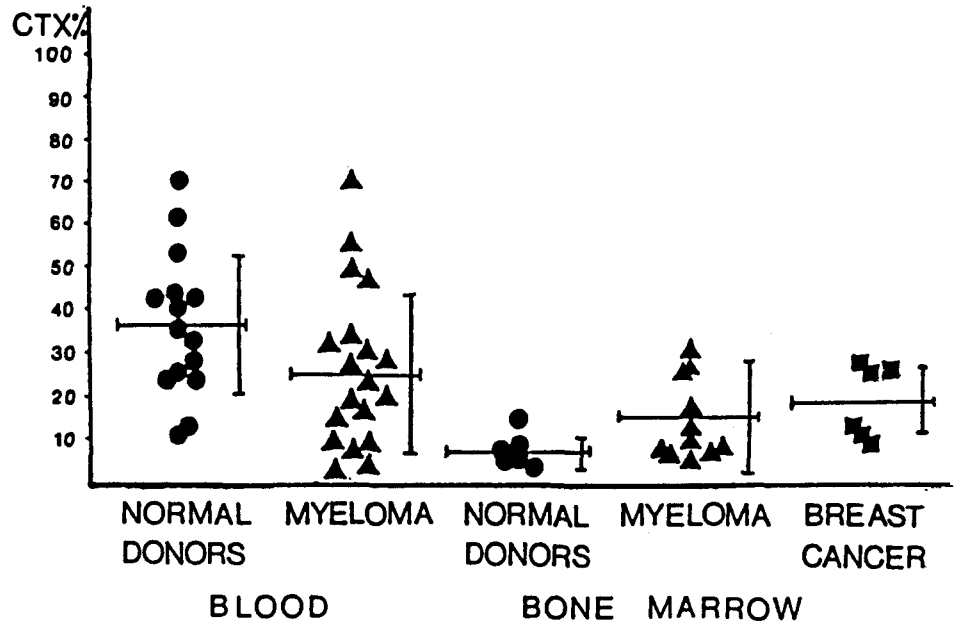

Fig. 2. NK activity (CTX%) of peripheral blood mononuclear cells (PBMC) and bone marrow cells in patients with MM and breast cancer.
Mean ± SD level of CTX% of PBMC: in MM patients, 24.9 ± 18.5 ($n = 19$); in normal donors, 36.2 ± 16.4 ($n = 15$) ($p > 0.05$). Mean CTX% in bone marrow: in MM patients, 17.5 ± 12.6 ($n = 11$); in normal donors 7.3 ± 4.1 ($n = 6$) ($p > 0.05$); in breast cancer patients, 17.95 ± 8.7 ($n = 6$) ($p < 0.05$ with respect to normal donors)

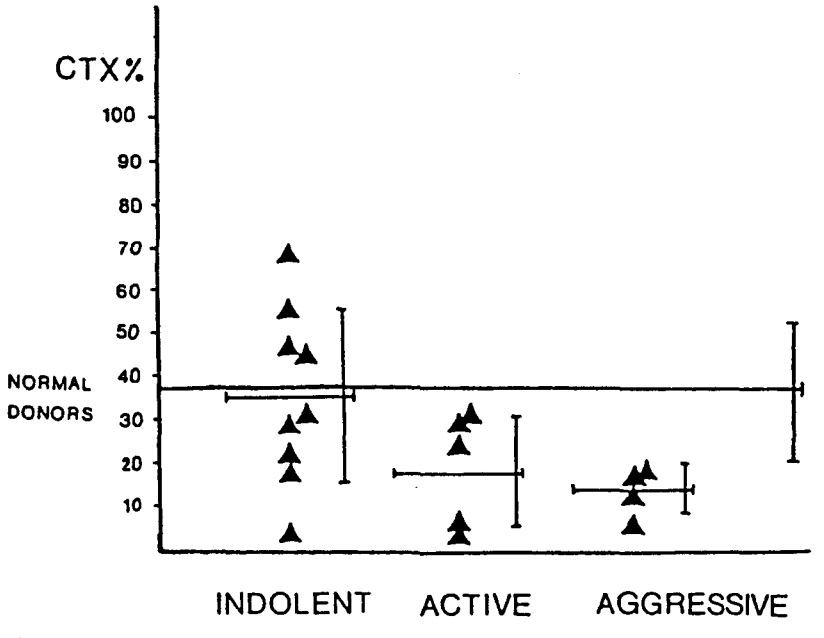

Fig. 3. NK activity of peripheral blood mononuclear cells (PBMC) in MM patients with different courses. Mean ± SD level of CTX%: in indolent course, 35.6 ± 20.0 ($n = 9$) ($p > 0.05$ with respect to normal donors); in active course 18.1 ± 13.4 ($n = 5$); in aggressive course, 13.8 ± 6.35 ($n = 4$) ($p < 0.05$ with respect to normal donors)

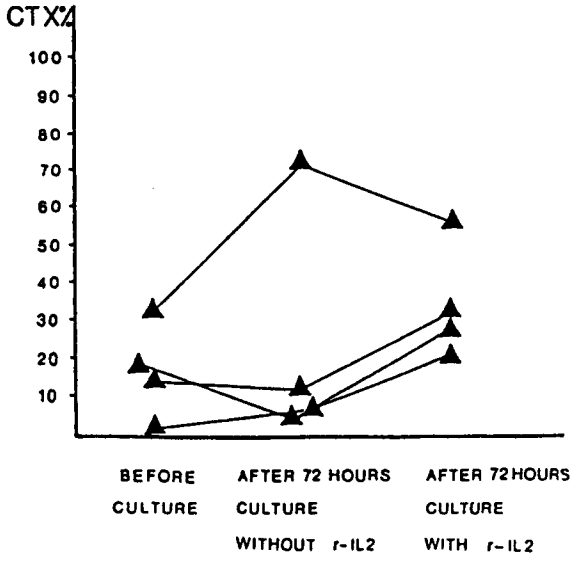

Fig. 4. Effects of recombinant IL-2 (*r-IL2*) on NK activity of bone marrow cells in MM. Bone marrow mononuclear cells were cultivated with IL-2 200 U/ml over 72 h

sive MM. The number of patients without monoclonal protein secretion was higher in aggressive MM (Fig. 10). Pyrogenal-stimulated IL-1 production by PBM was significantly higher in patients with CRI than in patients without CRI (Fig. 11).

Amyloid Syndrome. A difference was found in survival between active MM patients with amyloidosis compared to all MM patients (Fig. 12). None of patients with amyloidosis in indolent and active MM died during the period of observation. NK activity of PBMC in MM patients with amyloidosis was 41.2 $\pm$22.4% (mean$\pm$SD), which is significantly higher than in MM patients without amyloidosis (19.1$\pm$13.4%) ($p < 0.05$). There were no differences in pyrogenal-stimulated IL-1 production by PBM in MM patients with or without amyloidosis (Fig. 13).

Discussion

LCIS plays a certain role in development of immunodefficiency in MM, but it has also been shown to be a factor inhibiting tumor growth [1]. The development of LCIS is considered to be a favorable sign [17]. According to our data, this phenomenon is a rare one, and is more typical for indolent and active MM. It may be present in aggressive MM, and is probably not the basic factor in genesis of immunodefficiency syndrome and in suppression of tumor growth. There are many data about the antitumour effects

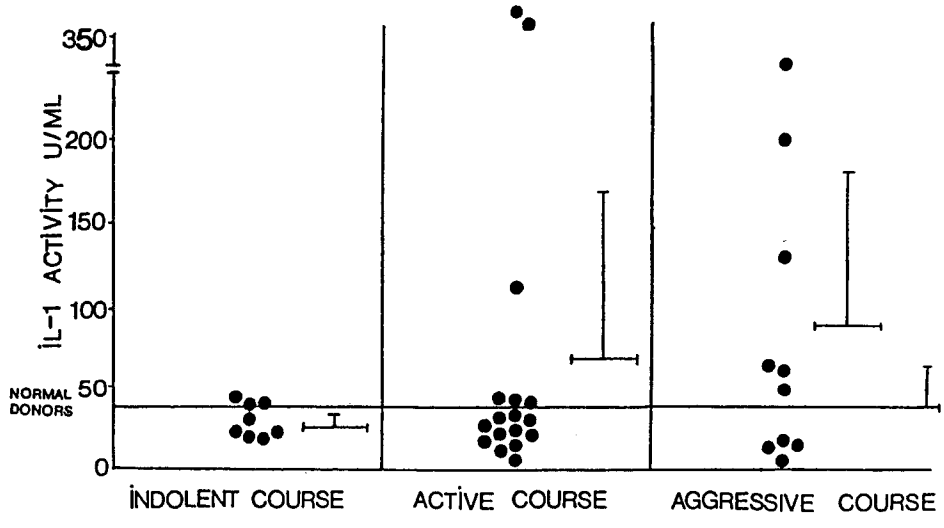

Fig. 5. Pyrogenal-stimulated production of IL-1 by peripheral blood monocytes (PBM) in MM patients with different types of course. Mean$\pm$SD level of IL-1 production by PBM: normal donors, 39.6$\pm$26.03 U/ml ($n = 11$); patients with indolent course, 27.05$\pm$8.2 U/ml ($n = 8$); patients with active course, 67.4$\pm$102.2 U/ml ($n = 17$), patients with aggressive course, 80.8$\pm$84.25 U/ml ($n = 10$) ($p > 0.05$)

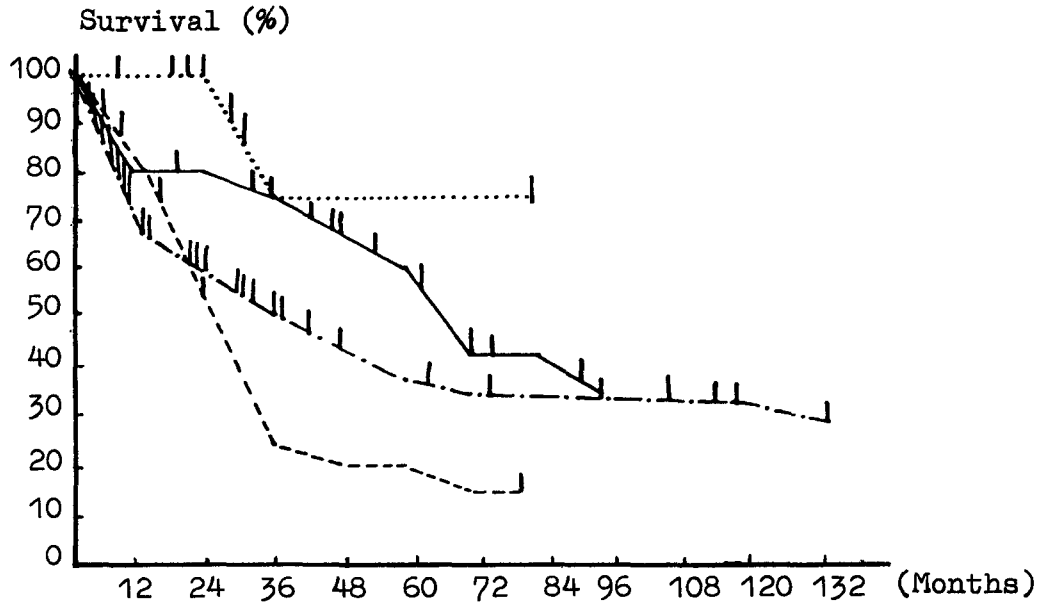

Fig. 6. Bone lesions and the survival of patients with MM. ..... no lesions ($n = 8$), —— minimal lesions (osteoporosis) ($n = 24$), median survival 62.7 months; –.–.– moderate lesions ($n = 71$), median survival 36 months; ---- extensive lesions ($n = 20$), median survival 24 months

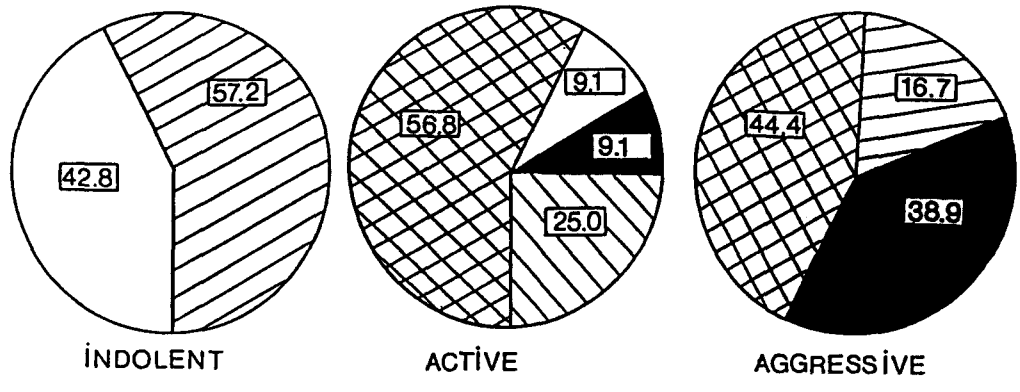

Fig. 7. Bone lesions in MM patients with different courses. ▭, no lesions; ▨, minimal lesions; (osteoporosis); ▩, moderate lesions; ■, extensive lesions

of NK-cells [18, 19]. As described previously [4, 5], there were no differences in NK activity of PBMC between MM patients and normal donors. In this study, NK activity of PBMC varied widely between MM patients and did not differ from that in normal donors. At the same time, NK activity of PBMC in active or aggressive MM was significantly lower than in normal donors. This shows that there is a decrease in natural antitumor resistance in MM patients with active and, especially, aggressive MM. The possibility of increasing NK activity of BMMC in culture with recombinant IL-2 enabled us to use this drug in MM treatment [20–22]. It was shown that IL-1 stimulates T-cells to synthesize IL-2 and receptors to IL-2 [22]. The increase in IL-1 production by PBM in active and aggressive MM may indicate the activation of antitumor mechanisms that are, however, still insufficient to stimulate IL-2 synthesis. The frequency of extensive bone lesions was higher in active and, particularly, in aggressive MM. There is also a positive correlation between the degree of bone lesions and the level of IL-1

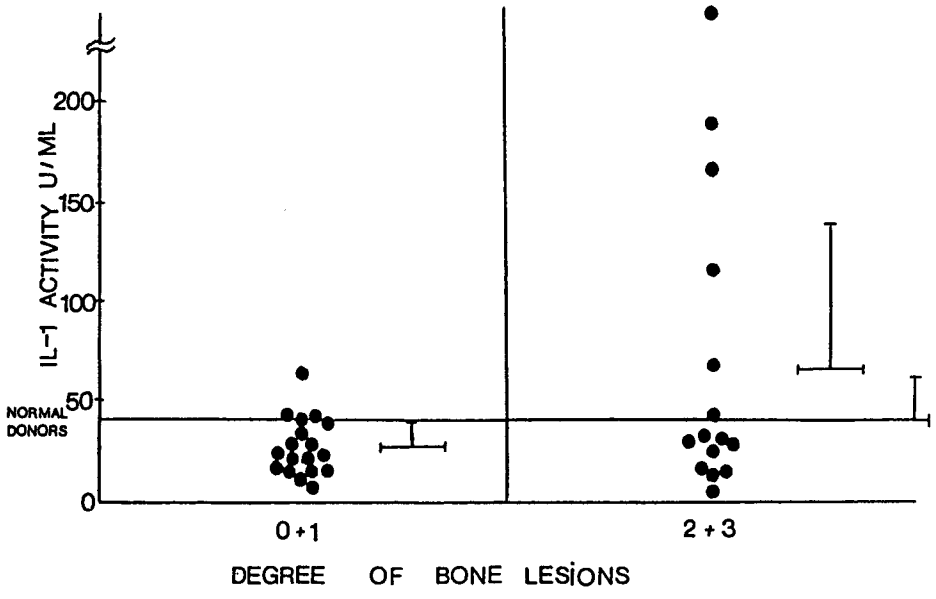

Fig. 8. Pyrogenal-stimulated IL-1 production by peripheral blood monocytes (PBM) in MM patients with different degrees of bone lesions: 0 no bone lesions; 1 osteoporosis; 2 moderate bone lesions; 3, extensive bone lesions. Mean $\pm$ SD IL-1 production by RBM; 0 + 1 patients, 27.9 $\pm$ 13.8 U/ml ($n = 18$); 2 + 3 patients, 67.1 $\pm$ 73.4 U/ml ($n = 15$) ($p = 0.05$)

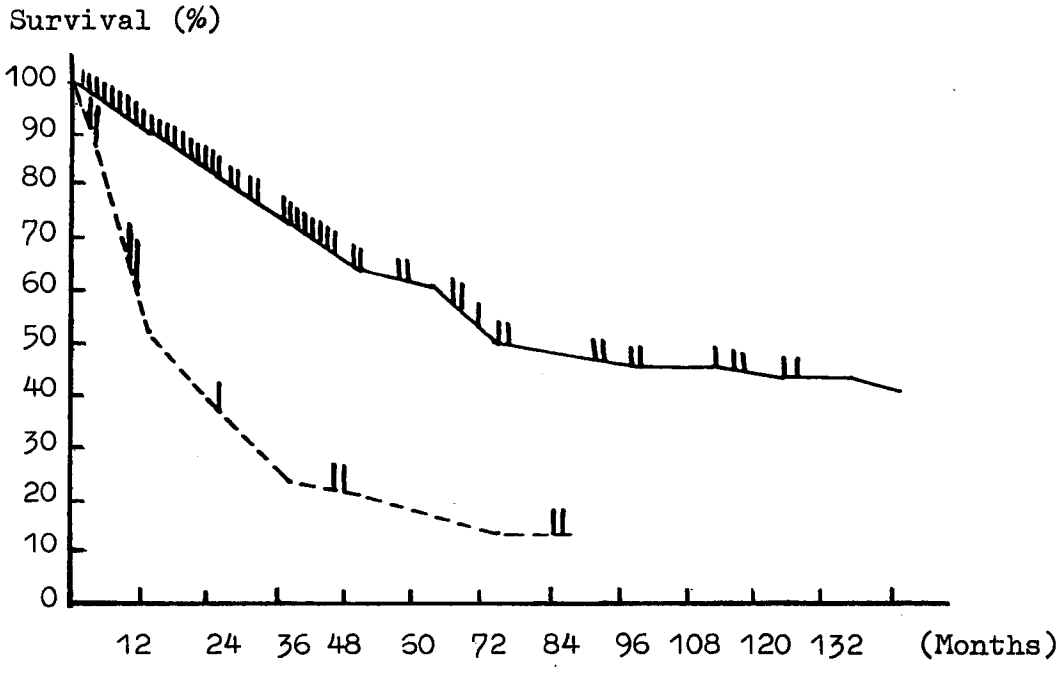

Fig. 9. Survival of MM patients with and without chronic renal insufficiency (CRI). ——, survival of MM patients without CRI ($n = 9$), median 72 months; ----- survival of MM patients with CRI ($n = 43$), median 14.4 months

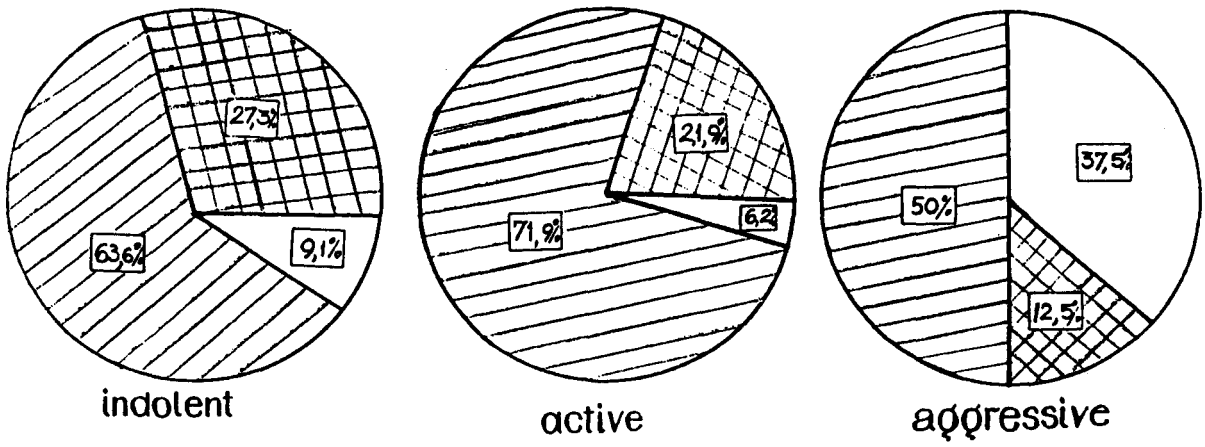

Fig. 10. Immunoglobulin light chain type in MM with different courses: ☐, nonsecreting; ▨, kappa chain; ▩, lambda chain

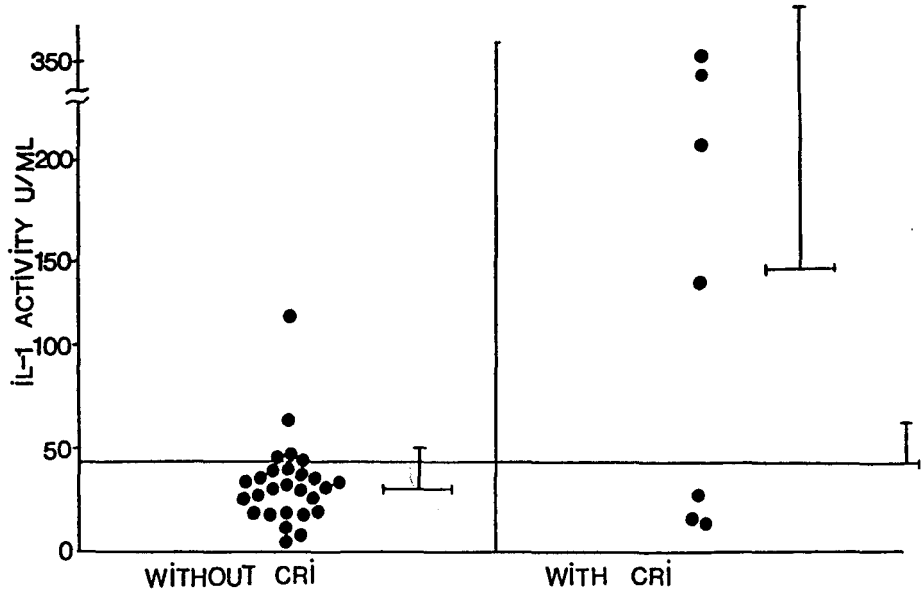

Fig. 11. Pyrogenal-stimulated production of IL-1 by peripheral blood monocytes in MM patients with and without chronic renal insufficiency (CRI). Mean ± SD IL-1 production by PBM in MM patients without CRI ($n = 27$), 32.3 ± 22.3 U/ml; in MM patients with CRI ($n = 7$), 141.5 ± 131.7 U/ml ($p < 0.01$)

production by PBM in MM patients. These data indicate that IL-1$_\beta$, produced leading role in the genesis of osteolytic syndrome in MM [6, 7]. In this study, monocytes, known to be precursors of osteoclasts, increased IL-1 production. IL-1 is probably an autocrine factor that stimulates osteoclast function Myeloma with CRI and nonsecretory myeloma are of a more aggressive type.

There is a positive correlation between the development of CRI and the level of IL-1 production by PBM in MM patients, and this may play a certain rol in the genesis of CRI [7, 8]. The increased survival of patients with active MM with amyloidosis in comparison with all active MM patients without amyloidosis may be the result of activation of antitumor immunity. NK activity of PBMC in MM patients with amyloidosis was significantly higher that in MM patients without amyloidosis. Perhaps that makes the MM more favorable. Further investigation to clarify the pathogenesis of MM clinical syndromes are necessary.

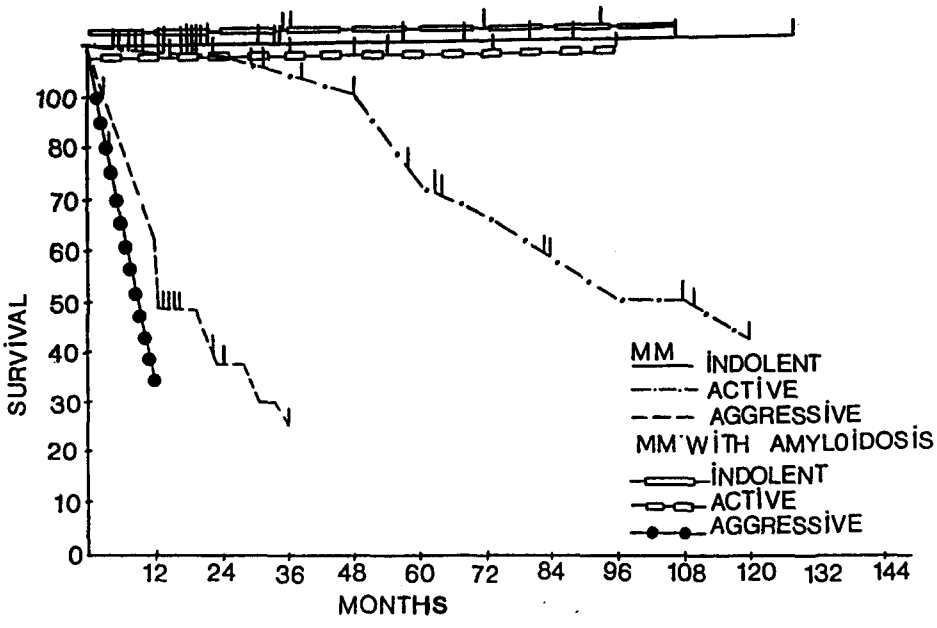

Fig. 12. Survival of patients with MM in different courses with and without amyloidosis. ——, indolent MM ($n = 11$); –.–.–, active MM ($n = 32$) (median survival 96 months); – – –, aggressive MM ($n = 23$) (median survival 12 months); –□–, indolent MM with amyloidosis ($n = 5$); –□–□–□, active MM with amyloidosis ($n = 3$)

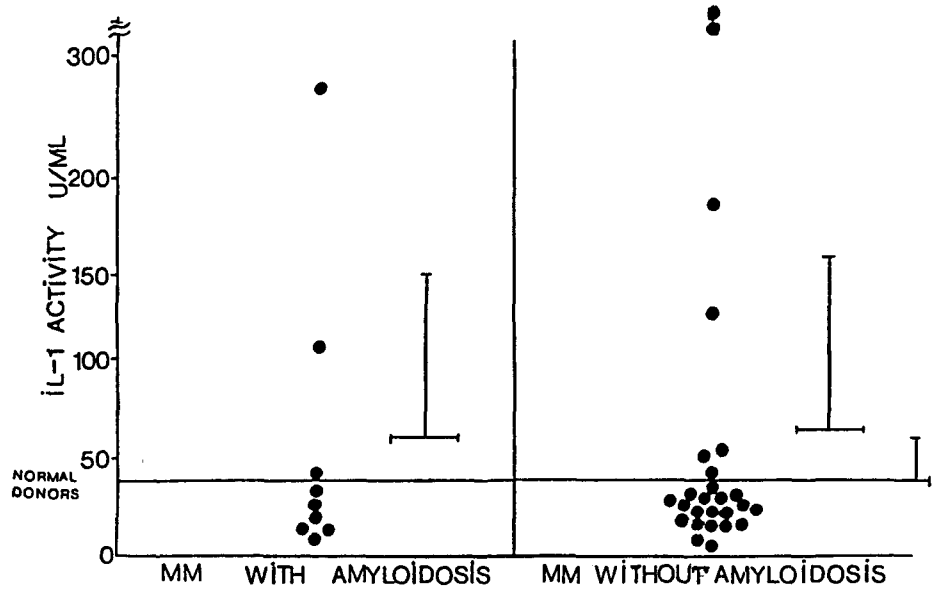

Fig. 13. Pyrogenal-stimulated production of IL-1 by peripheral blood monocytes in MM patients with and without amyloidosis. Mean ± SD IL-1 production by PBM; in MM patients with amyloidosis ($n = 9$), 62.9 ± 81.5 U/ml; in MM patients without amyloidosis ($n = 26$), 65.2 ± 88.3 U/ml ($p > 0.05$)

Acknowledgement. This work was supported by the Laboratory of Hybridoma Technology (Chief, Dr. V. Klimovich) of the Central Research Institute of Roentgenology and Radiology, Leningrad, USSR.

References

1. Yoshua DE (1988) Biology of multiple myeloma – host tumour interactions and immune regulation of disease activity. Hematol Oncol 6:83–86

2. Wearne A, Yoshua DE, Kronenberg H (1985) Light chain isotype suppression – a new parameter. Aust NLY 15:629–633
3. Mills KHY, Cawley C (1983) Abnormal monoclonal antibody defined helper/suppressor T-cell subpopulation in multiple myeloma. Relationship to treatment and clinical stage. Br J Haematol 53:271–275
4. Einhorn S, Ahre A, Blomoren H, et al (1982) Interferon and natural killer activity in multiple myeloma. Lack of correlation between interferon-induced enhancement of natural killer activity and clinical response to human interferon. Int J Cancer 30:167–172
5. Gastl G, Niederwieser D, Masth C, et al (1984) Human large granular lymphocytes and their relationship to natural killer cell activity in various disease states. Blood 74:288–295
6. Cozzolino F, Torcia M, Aldunucci D, et al (1989) Production of interleukin-1 by bone marrow myeloma cells. Blood 74: 380–387
7. Kawano Y, Yamamoto I, Iwara K, et al (1989) Interleukin-1 beta rather than lymphotoxin as the major bone resorbing activity in human multiple myeloma. Blood 73:1646–1649
8. Lovett DH, Szamel M, Ryan SL, et al (1986) Interleukin-1 and the glomerular mesanguim. I. Purification and characterization of a mesangial cell-derived autogrowth factor. J Immunol 136:3700–3705
9. Kyle KA (1988) Prognostic factor in multiple myeloma. Hematol Oncol 6:125–130
10. Fiedler K, Duril BYM (1986) Primary amyloidosis associated with multiple myeloma. Am J Med 80:413–418
11. Afanasiev BV, Podoltzeva EI, Morozova EV, et al (this volume) Prognostic factors in patients with multiple myeloma. In: R. Neth et al. (eds) Modern trends in human leukemia. Springer, Berlin Heidelberg New York
12. Weller TH, Coons AH (1984) Fluorescent antibody studies with agents of varicella and herpes zoster phopagated *in vitro*. Proc Soc Exp Biol Med 86:789–794
13. Yoda Y, Kawakani Z, Shubuya A, Abe T (1988) Characterisation of natural killer cell cultured from human bone marrow cells. Exp Hematol 16:712–717
14. Rykova MP, Spirande IV, Zedgenidze MS, et al (1981) A new highly sensitive test for normal killers. Immunology (USSR) 3:88–90
15. Gery Y, Gesshon RK, Waksman BH (1972) Potentiation of the T-lymphocyte response to mytogens. I. The responding cells. J Exp Med 136:128–142
16. Mizel SB, Oppenheim JJ, Rosenstreich DL (1978) Characterization of lymphokine-activation factor produced by the macrophage cell line p 388 Dl. I. Enhancement of LAF production by activated T-lymphocytes. J Immunol 120:1497–1503
17. Wearne A, Yoshua DE, Young GAR, Kronenberg H (1987) Multiple myeloma: light chain isotype suppression – a marker of stable disease at presentation. Eur J Haematol 38:43–49
18. Gerson M (1980) Systemic and *in situ* natural killer activity in tumour bearing mice and patients with cancer. In: Herbesman RB (ed) Natural killer mediated immunity against tumours. Academic, New York p 1047
19. Hercend T, Schmidt RE (1988) Characteristics and uses of natural killer cells. Immunol Today 10:241–244
20. Peest D, Vries J, Holscher K, Leo R, Deicher H (1989) Effect of interleukin-2 on the *ex vivo* growth of human myeloma cells. Cancer Immunol Immunother 30:227–232
21. Gottleib D, Prentice G, Heslop H, et al (1989) Effect of recombinant interleukin-2 administration on cytotoxic function following high-dose chemotherapy for hematological malignancy. Blood 7: 2335–2342
22. Shimazaki C, Aizpodien J, Wisniewski D, Gulati SC, Kolitz JE, Fried J, Clarhson BD (1988) Cell-mediated toxicity of interleukin-2 activated lymphocytes against autologous and allogeneic human myeloma cells. Acta Haematol 80:203–209
23. Sone S, Inamura N, Nii A, Ogura T (1988) Heterogenity of human lymphokine (IL-2)-activated killer (LAK) precursors and regulation of their LAK induction by blood monocytes. Int J Cancer 42:428–434