Malignant Lymphomas

The relationship between proliferation and apoptosis in patients with monoclonal gammopathy of undetermined significance or multiple myeloma

Multiple myeloma (MM) is a clonal neoplastic lymphoproliferative disease affecting terminally differentiated B cells i.e. plasma cells characterized by slow proliferation activity and different resistance to apoptosis with latent accumulation of myeloma cells in the bone marrow. This process is induced by failure of normal tissue homeostatic mechanisms. We compared plasma cell proliferation and apoptic indices in various phases of MM and in monoclonal gammophaty of untetermined significance.

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Dysfunction of apoptosis, namely the inhibition of apoptotic cell death, has been proposed as an important pathogenic process in bone marrow disorders such as multiple myeloma (MM).[1] It is highly probable that in MM one group of neoplastic cells (*the myeloma clone*) is unable to undergo apoptosis in bone marrow. Expansion of the myeloma cell neoplastic clone is the result of imbalances in proliferation on the one hand, and the induction or inhibition of apoptosis on the other. The equilibrium between the growth fraction, i.e. cellular production and cellular loss from the differentiation compartment, determines the growth, stability or reduction of the myeloma mass.[2] A significant difference was observed in the plasma cell labeling index (PCLI) of patients in the plateau phase and in progressive phase;[3] an increase in PCLI is almost always associated with loss of the plateau phase, confirming the former as an accurate *mirror image* of tumur activity.[4] General clinical experience shows that about 40% of patients in advanced or active phase of MM have surprisingly low PCLI and therefore, the proliferation rate of malignant plasma cells cannot be characterized just by tumur growth alone. The previously held assumptions, limiting the problems of the pathogenesis of clinical progression of MM to the issue of cell proliferation, ignore the concept that myeloma cells may have a reduced rate of apoptosis.[5] Clinical studies evaluating the merit of apoptotic process measurements, are lacking. The majority of published studies dealing with the problem of myeloma cell apoptosis are based on *in vitro* observations or animal models. It is unclear whether these findings have an immediate and applicable implication for clinical practice.

The aim of our study was to compare measured plasma cell proliferation and apoptotic indices in monoclonal gammopathy of undetermined significance (MGUS) and various phases of MM i.e. in the smoldering form, stable or plateau phase and active (progressive or relapsing) phase.

The group analyzed, examined during 2000-2002, comprised 30 patients with MGUS, 21 with smoldering MM, 82 MM patients examined at the time of diagnosis and 64 during various phases of the disease, after previous chemotherapy. Conventional, systemic chemotherapy was used in the whole group of MM patients; i.e. VBMCP, VAD, C-VAD, CIDEX or MP regime (V-vincristine, B–BCNU, M–melphalan, C– cyclophosphamide, P–prednisone, A-adriablastin, D-dexamethasone, I-idarubicin, E-etoposide), while high-dose therapy with autologous stem cell transplantation support was instituted in 36 patients younger than 65 years. Plasma cell proliferation activity was measured using a DNA/CD138 double-staining technique, the *plasma cell propidium iodide index* (PC-PI).[6] Plasma cells undergoing apoptosis, were detected by a flow cytometric method with annexin-V conjugated to fluorescein-isothiocyanate and monoclonal antibody CD138 to provide a *plasma cell apoptotic index* (PC-AI).[7] To estimate the significance of the differences between the means, Student's t-test and the ANOVA test were used. Overall, the 30 patients with MGUS had a low proliferative index (PC-PI: median 1.8, range 1.1-2.9%) and a relatively high rate of apoptosis

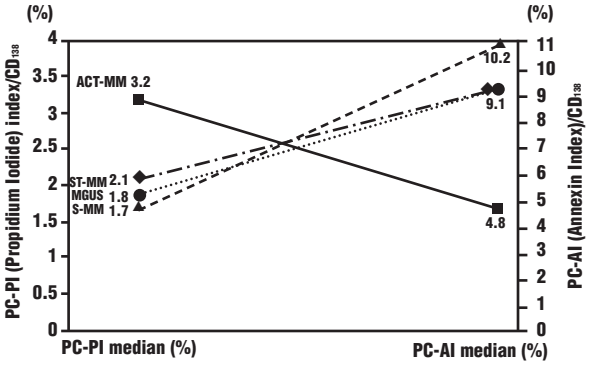

Figure 1. Inverse relation of the plasma cell proliferation index (PC-PI) assessed by propidium iodide, and the plasma cell apoptotic index (PC-AI), assessed by annexin V, in the group of 197 patients with monoclonal gammopathy of undetermined significance (MGUS, n-30), smoldering multiple myeloma (S-MM, n-21), stable/plateau phase of MM (ST-MM, n-43) and active forms of MM (ACT-MM, n-57).

PC-AI median 9.1, range 1.6-22.5%). The inverse relation between PC-PI and PC-AI was statistically significant (p=0.0001). The 21 patients with smoldering MM also had low levels of PC-PI (median 1.7, range 1.1-3.0%) and high values of PC-AI (median 10.8, range 3.5-17.1%) and the inverse relation between the PC-PI and PC-AI values was again statistically significant (p-0.0001). The 82 previously symptomatic patients analyzed at the time of MM diagnosis had a median PC-PI of 2.5 (range 0.3-4.8%) and a median PC-AI of 6.2 (range 0.2-16.5%); the relation between these indices was also statistically significant (p=0.0001). In the group of 64 patients evaluated during various phases of MM after previous conventional or high-dose-therapy with autologous stem cell support, the median PC-PI was 2.6 (range 1.1-5.8%) and the median PC-AI of 7.2 (range 1.1- 18.4%); the relation between the two indices was statistically significant (p=0.0001).

A statistical comparison of PC-PI and PC-AI levels in patients with MGUS, smoldering myeloma stable/plateau phase disease did not show any significant differences: the medians of their values were very similar (PC-PI: median 1.8, 1.7 vs. 2.1%; PC-AI: median 9.1, 10.8 vs. 9.0%). Statistically significant differences were, however, found for both PC-PI and PC-AI when comparing MGUS and smoldering myeloma patients with the group of patients with active (progressive or relapsing) disease, the latter having a higher PC-PI (median 1.8 and 1.7 vs. 3.2 %) and lower PC-AI (median 9.1 and 10.8 vs. 4.8 %, p=0.0001). In contrast to patients with progressive or relapsing disease, patients in the stable/plateau phase of MM, had significantly lower PC-PI levels (median 2.1% vs. 3.2%, p=0.0001) and significantly higher PC-AI values (median 9.1 vs. 4.8, p=0.0001) (Figure 1).

These results support the initial hypothesis of an inverse relation between proliferative (PC-PI) and apoptotic (PC-AI) activities of the plasma cell compartments of patients with MGUS, smoldering and overt/symptomatic forms of MM.[8] Patients with MGUS, smoldering MM and stable/plateau phase MM usually low proliferative and high apoptotic activity of plasma cells, whereas cells of patients in active (progressive/relapsing) phase usually had high proliferative and low apoptotic characteristics.[8] The group of patients examined at the time of diagnosis of MM and the group analyzed after chemotherapy comprised individuals with various degrees of disease activity (stable activity and refractory forms of the disease); thus the medians of PC-PI and PC-AI in those *mixed* groups were according to expectations and at medium values. These results demonstrate that not only proliferation but also apoptotic properties of myeloma cells are important from the point of view of clinical and laboratory manifestations of MM and in the evolution of MGUS into in multiple myeloma. It is possible that the above relation could enrich recent algorithms for clinical investigation.

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