Interleukin-1 and Interleukin-6 Production in Peripheral Blood and Bone Marrow Mononuclear Cell Culture in Multiple Myeloma

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Interleukin(IL)-6 plays the key role in the pathogenesis of multiple myeloma, being an autocrine/paracrine malignant plasmacyte growth factor [7,9]. By activating IL-6 gene transcription, IL-1 and tumor necrosis factor-α enhance IL-6 mediated myeloma cell proliferation [4]. Increased spontaneous IL-6 production by blood and bone marrow mononuclear cells occurring in multiple myeloma has been demonstrated by many authorities [4,9], but the relationship between local bone marrow production of these cytokines and their peripheral blood production is virtually unknown.

The object of the present research was to study IL-1 and IL-6 production in a bone marrow and peripheral blood mononuclear cell culture as well as by cell subpopulations adhering and not adhering to plastic in multiple myeloma.

MATERIALS AND METHODS

Blood and bone marrow samples from multiple myeloma patients were obtained at the Moscow District Research and Clinical Institute. Eighteen patients (17 women and 1 man) aged 50 to 65 years were examined, and stage III multiple myeloma was diagnosed in all of them (according to the Durie-Salmon [5] classification). The examinations were carried out before the course of chemotherapy was started. Blood samples of 8 healthy donors were examined for control. Mononuclear cells were isolated from heparin-treated blood and bone marrow in a medium for lymphocyte separation (Flow). For cytokine production mononuclear cells were cultured for 24 h in a concentration of 10^6/ml in RPMI 1640 medium with 10% fetal calf serum, 2 mM glutamine, and 50 μM 2-mercaptoethanol (all Gibco reagents) in 24-well plates for tissue cultures (Costar) at 37°C and 5% CO_2. Cells adhering to plastic were separated by culturing for 3 h in a medium with 20% fetal calf serum. Nonadhesive cells were collected, centrifuged, and resuspended in the initial volume of fresh medium. Wells with adhered cells were washed and filled with the initial volume of fresh medium. For induction *E. coli* lipopolysaccharide 0111:B4 (Difco) in a final concentration of 4 μg/ml was used. IL-1 activity was assessed by the proliferative thymocytic test using CBA mice [6]. IL-6 activity was assessed using IL-6-dependent heterohybridoma D6C8 [2]. The biological activity of the cytokines was assessed by probit analysis [10], based on the results of titration of intralaboratory references, for which purpose culture fluids of lipopolysaccharide-stimulated peripheral blood mononuclears of healthy donors were taken. The amount of factor inducing one half of the maxi-

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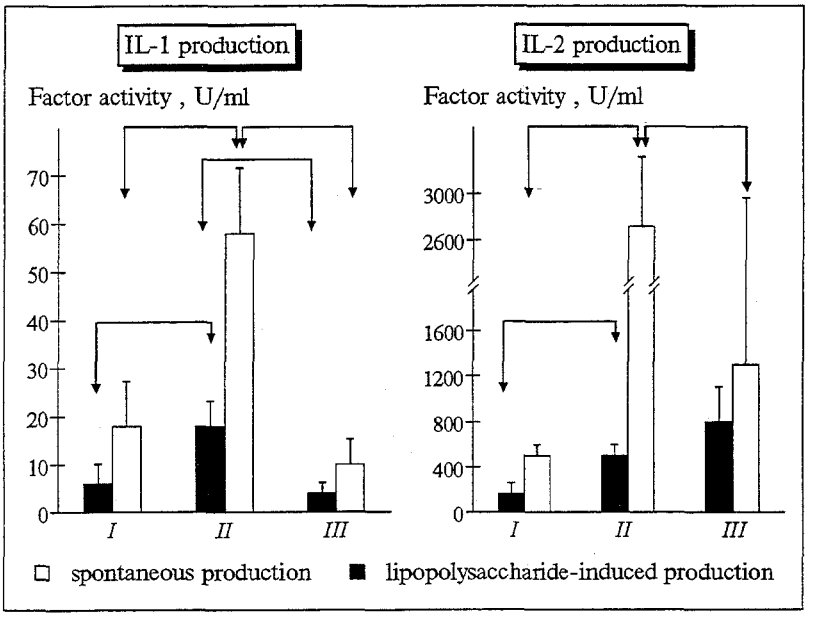

Fig. 1. IL-1 and IL-6 production in culture of mononuclears. Abscissa: groups compared: I) healthy donors, blood; II) myeloma patients, blood; III) myeloma patients, bone marrow. Arrows indicate statistically reliable differences.

mal proliferative response of factor-sensitive cells was taken as the biological activity unit [6]. The results were statistically processed by nonparametric analytical methods using White's test for independent sampling and the paired Wilcoxson's test for interdependent sampling [1].

RESULTS

Spontaneous and lipopolysaccharide-induced production of IL-1 and IL-6 by peripheral blood mononuclear cells of patients with multiple myeloma was noticeably higher than in controls (Fig. 1) ($p<0.05$, White's test). The IL-1 levels were reliably lower in myeloma patients' bone marrow mononuclear supernatants than in peripheral blood mononuclears ($p<0.01$, Wilcoxson's test) (Fig. 1, *a*). In contrast to IL-1 production, IL-6 production by bone marrow mononuclears was as high as by peripheral blood mononuclears, lipopolysaccharide-induced production in the bone marrow being lower than in the peripheral blood ($p<0.05$, Wilcoxson's test) (Fig. 1, *b*).

Increased susceptibility to stimulation was characteristic of IL-6-producing peripheral blood cells of myeloma patients in contrast to normal donor cells. The stimulation index, that is, the ratio of lipopolysaccharide-stimulated production to spontaneous production in multiple myeloma was 3.74±0.48 (1.7-7.1, n=11), whereas in controls it was 2.56±0.72 (1.1-6.0, n=8). A stimulation index higher than 2 was observed in 2 of the 8 donors and in 10 of the 11 multiple myeloma patients. This index was reliably lower for patients' bone marrow mononuclears than for peripheral blood cells, being 2.27±0.06 (0.2-5.9, n=8) ($p<0.05$, Wilcoxson's test). The increased spontaneous production of the factor in the presence of reduced stimulated production seems to be evidence of *in vivo* activation of bone marrow cells secreting IL-6 and of the exhausted potential of these cells with respect to cytokine production. The stimulation index of IL-1 production by patients' peripheral blood mononuclears was 3.35±0.46 (1.3-6.6, n=11), the normal value being 2.77±0.75 (1.7-5.0, n=4); however, the differences were statistically unreliable.

Cultivation of normal donor and myeloma patient peripheral blood cells adhering to plastic did not show any marked differences in spontaneous IL-6 production (Table 1), whereas lipopolysaccharide-stimulated IL-6 production by patients' monocytes proved to be much higher than in the control (Fig. 2). Patients' bone marrow adhesive cells constitutively released extremely low quantities of the cytokine. Spontaneous and induced IL-6 production by peripheral blood nonadhesive cells was virtually the same in the groups compared (Table 1, Fig. 2). Adhesive and nonadhesive blood mononuclears of healthy donors and multiple myeloma patients equally contributed to total spontaneous IL-6 production (although the cellular concentrations in the cultures differed greatly), whereas nonadhesive bone marrow cells were far superior to adhesive karyocytes in spontaneous

TABLE 1. Spontaneous Production of IL-6 by Peripheral Blood (PB) and Bone Marrow (BM) Mononuclear Cell (Mnc) Subpopulations in Health and in Myeloma (MM) ($M\pm m$)

Compared groups of Mnc	n	IL-6 production, U/ml				Cooperation index
		adhesive Mnc	nonadhesive Mnc	total contribution	nonfractionated Mnc	
health, PB	4	135.8±59.5	186.0±105.2	318.8±104.2	206.0±67.0	3.18±1.95
		60–312	130–500	130–560	50–380	0.4–0.8
MM, PB	10	179.2±34.0	242.1±105.1	423.4±123.2	620.6±181.8	1.01±0.30
		16–300	24–1140	40–1400	100–1700	0.2–2.9
MM, BM	8	16.9±2.7	327.0±177.6	344.1±179.3	644.2±212.9	0.61±0.13
		5.27	18–1500	23–1530	50–1600	0.03–1.1

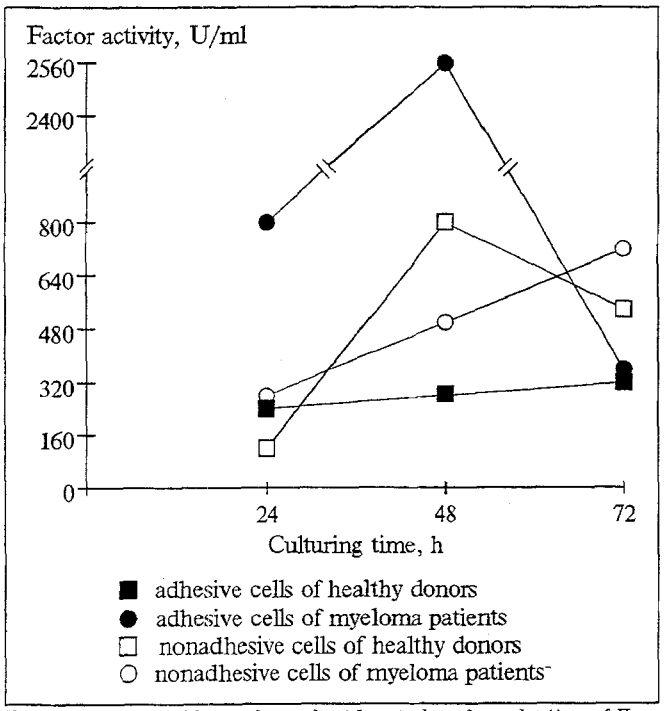

Fig. 2. Kinetics of lipopolysaccharide–induced production of IL–6 by subpopulations of peripheral blood mononuclears adhering and not adhering to plastic.

cytokine production (Table 1) ($p<0.01$, Wilcoxson's test). Still, IL-6 production by nonfractionated mononuclears did not conform to the total contribution of cultured nonadhesive and adhesive cells, suggesting the presence of regulatory interactions between the examined subpopulations during IL-6 production. To investigate the extent of such interaction, we estimated the ratio of total IL-6 activity in supernatants of both subpopulations to factor production by nonfractionated cells and arbitrarily termed it the cooperation index. Evidently, a cooperation index close to 1 indicates the absence of interactions between subpopulations during IL-6 production, an index higher than 1 shows the presence of inhibiting cellular interactions, and an index lower than 1 indicates mutual cellular stimulation. Although the differences between the absolute values for donor blood were unreliable, analysis of the cooperation indexes made it possible to assume the presence of inhibitory regulatory interactions in health and their absence in multiple myeloma. IL-6 production by bone marrow nonfractionated mononuclears was significantly higher than the total contribution of both subpopulations ($p<0.01$, Wilcoxson's test), this being reflected by the cooperation index lower than 1 (Table 1). It may be supposed that the increased spontaneous IL-6 production in the bone marrow was due to mutual stimulating effects of the adhesive (mainly stromal cells) and nonadhesive (mainly myeloma cells) - mononuclear subpopulations.

It is evidently necessary to control IL-6 expression, for this cytokine is remarkable for its potent systemic effect. In health this expression may be mediated by regulatory cytokines, such as IL-4 [13], transforming growth factor-β [12], and IL-10 [14] inhibiting anti-inflammatory monokine expression, or via the regulation of IL-6 receptor expression [3]. In multiple myeloma the status of the immune system, as in many other neoplastic diseases, is characterized on the whole by the prevalence of suppressor regulatory effects. For this condition a reduction of the absolute and relative T-helper counts vs. T-suppressors is typical, as is an increased content of $CD8^+HLADR$ T-lymphocytes, indicating their activation [11]. In addition, reduced IL-4 production and normal IL-2 production by stimulated peripheral blood mononuclears of multiple myeloma patients are observed [8]. It may be assumed that both local and systemic immunodepression in multiple myeloma, no matter what its cause, disturbs the mechanisms regulating the expression of anti-inflammatory cytokines. As a result, the cooperation between tumor and cytokine-producing cells of the microenvironment in the bone marrow may lead to the formation of a cytokine cascade enhancing the IL-6-dependent proliferation of myeloma cells.

Hence, both spontaneous and lipopolysaccharide-induced production of IL-1 and IL-6 by peripheral blood mononuclears is increased in multiple myeloma as against that in health. For patients' peripheral blood monocytes a more potent response to lipopolysaccharide stimulation is typical. The differences in IL-6 spontaneous production in patients and donors are leveled during separate cultivation of mononuclears adhering and not adhering to plastic, this being evidence of the crucial role of intercellular cooperation in the regulation of the production of this cytokine. Increased IL-6 production in the bone marrow results from mutual stimulation of adhesive and nonadhesive cells in multiple myeloma.

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Immunoregulatory Characteristics of Human Recombinant Angiogenin

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The possibility of directed regulation of immunogenesis attract the attention of both scientists and to an even greater extent, physicians. The ever increasing array of bioactive substances modulating one or another immune process provides a rich resource for the optimal choice of a method with due consideration for the individual characteristics of the chosen agent and of the object of immunocorrection. For obvious reasons, substances of endogenous origin are most desirable here; the present paper is devoted to one such substance, the recently discovered bioactive agent angiogenin (AG). It was found on the plasma membranes of human adenocarcinoma cells [5]; like other solid tumors, this tumor constantly induces neovascularization. Since this phenomenon occurs in other diseases, as well as in some physiological states, for instance, in diabetic retinopathy, rheumatiod diseases, wound healing, cyclic physiological processes, and embryonal development, the detection of AG in mammalian blood serum [8] and cells [7] should be considered natural, in the same way as the detection of AG gene expression not only in tumors, but in normal human and animal tissues [9, 10] as well. Angiogenin represents a single-chain polypeptide with a molecular weight of 14000 D consisting of 123 amino acid and characterized by primary structures similarity to ribonucleases, by a restriction ribonuclease activity, and by an extremely high capacity for stimulating the growth of the vascular network. This unique property of AG suggest its use to enhance the healing of wounds, ulcers, and burns [6], the recovery of the myocardium after infarction, etc.

Since the above processes are associated with activation of the immune system function, we investigated its response to an increase of the AG level in the environment.

MATERIALS AND METHODS

A total of 197 CBA mice aged 3 to 4.5 months were used, at least 7 per group. Sheep erythrocytes (SE) were used as the antigen. Immune system activation induced by the antigen was assessed by three tests. For the rosette formation test (RFT) the mice were intraperitoneally injected with sheep erythrocytes or SE plus AG, 5×10^6/mouse. Then at various periods

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