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Sex hormones and immune dysregulation in multiple myeloma

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Abstract. A group of 49 multiple myeloma patients, 20 men and 29 women, were evaluated. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-oestradiol (E) and testosterone (T) serum concentrations have been detected by radioimmunoassay. Peripheral blood lymphocyte proliferation in response to phytohaemagglutinin (PHA), concanavalin A (ConA), recombinant interleukin-2 (rIL-2) and dextran sulphate (DxS) was investigated. Our findings provide evidence for two different patterns of sex hormone changes and immune dysfunctions presented differently by male and female multiple myeloma patients. In men increased FSH, LH and E concentrations and an augmented E to T ratio were associated with decreased lymphocyte blastogenic response to PHA, ConA and increased proliferation to rIL-2 and DxS. Female patients with multiple myeloma demonstrated normal values of FSH, LH and T, but a diminished E level and decreased E to T ratio correlated with a lymphocyte normal response to PHA and ConA and augmented blastogenesis to IL-2 and DxS. Our data, while admittedly preliminary, suffice to provide an indication of sex hormone changes in multiple myeloma patients, which could be responsible, at least in part, for the immune dysfunction observed in multiple myeloma.

Key words: Multiple myeloma – Sex hormones – Immune dysregulation

Introduction

Multiple myeloma is a B cell neoplasm that is accompanied by a variety of qualitative and quantitative alterations in immunomodulatory cells. Imbalance of T cell subsets has been demonstrated by several investigators [5, 22, 24]. Discrepant data have emerged on the ability of multiple myeloma T cells to respond to polyclonal (mitogenic) stimuli, from a remarkable decrease to nearly normal proliferation [9, 26, 28, 34].

It is known that the immune system is regulated by gonadal hormones (testosterone and 17β-oestradiol) and that the circulating levels of these steroids are also affected by immune function [17, 18]. To the best of our knowledge the possibility that the imbalance of sex hormones and immune defects may be interrelated in multiple myeloma has not been previously considered.

The purpose of this investigation was to determine the serum levels of 17β-oestradiol (E), testosterone (T) and also follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in male and female multiple myeloma patients. The hormones mentioned above are present in the microenvironment where lymphocytes function. They may act as regulators for immune cells. As T lymphocytes play a central role in the regulation of the immune system, our aim was to investigate their function. The blastogenic response of lymphocytes on stimulation with mitogens (phytohaemagglutinin, concanavalin A and recombinant interleukin-2) was measured for this purpose. It has been shown that the B-cell mitogen dextran sulphate (DxS) has the ability to activate predominantly malignant B cells [20, 21, 30]. We have used DxS for lymphocyte stimulation with the aim of evaluating malignant B cells in the peripheral circulation of multiple myeloma patients. Both the hormonal and immunological data of treated and untreated multiple myeloma patients have been compared.

Materials and methods

Patients. A group of 49 multiple myeloma patients, diagnosed according to the Chronic Myeloma Task Force [10], were included in the study. There were 20 men and 29 women (ratio: 0.68). The average age was 59.9 ± 10.3 years with no marked differences between the sexes. Of these patients, 26 had been treated with cytostatics, whereas 23 had not received any specific treatment. The controls were 29 age- and sex-

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Table 1. Serum hormones (mean level ± SD) in multiple myeloma male (M) and female (F) patients

Hormone[a]	Sex	Level (nmol/l) in		
		Multiple myeloma patients	Controls	*P*
FSH	M	7.2± 4.3	2.4± 1.1	<0.05
	F	48.9±29.2	35.8±14.4	>0.05
LH	M	9.1± 5.7	3.2± 1.2	<0.05
	F	37.1±16.5	35.6±14.5	>0.05
T	M	13.9± 7.7	19.2± 9.1	>0.05
	F	2.7± 2.3	1.9± 0.7	>0.05
E	M	0.2236± 0.1979	0.1084± 0.391	<0.05
	F	0.1267± 0.1455	0.2272± 0.1072	<0.05
E/T	M	19.2±10.7	8.9± 4.6	<0.05
	F	51.3±36.7	131.3±81.1	<0.05

[a] FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone; E, 17β-oestradiol

Table 2. Serum hormones (mean level ± SD) in treated (Tr) and untreated (UTr) multiple myeloma patients

Hormone		Level (nmol/l) in		
		Tr	UTr	*P*
FSH	M	7.7± 5.4	6.2± 3.2	>0.05
	F	50.8±28.9	34.3±32.3	>0.05
LH	M	8.7± 6.2	9.6± 6.4	>0.05
	F	39.2±15.5	21.4±17.9	>0.05
T	M	10.9± 7.8	18.9± 5.7	>0.05
	F	2.7± 2.4	2.6± 0.6	>0.05
E	M	0.2247± 0.2043	0.1956± 0.854	>0.05
	F	0.1142± 0.1073	0.2183± 0.2041	>0.05
E/T	M	22.7±26.3	10.6± 6.4	>0.05
	F	47.7±26.9	78.0±56.0	>0.05

matched normal subjects taking no medication. Informed consent was obtained from both patients and the age-matched normal controls.

Hormones. Serum follicle-stimulating hormone, luteinizing hormone, testosterone and 17β-oestradiol concentrations were determined by radioimmunoassay [6, 16, 34]. All assays were performed in triplicate. The following radioimmunoassay kits were used: ELSA 2 FSH and LH from ORIS Industrie S. A., Steron-K^{125} I-M, Minsk, and Steron-T^{125} I, Minsk. Serum for the detection of hormone concentrations was obtained at the same time as the plasma for immunological investigations.

Lymphocyte proliferation. Lymphocyte transformation and proliferation induced by mitogens, are standard immunological tools to assay immunocompetence. Mitogen stimulation of peripheral blood lymphocytes was achieved using phytohaemagglutinin (PHA; Difco laboratories, Detroit) and concanavalin A (ConA Sigma, St. Louis) as T lymphocyte mitogens and dextran sulphate (Pharmacia Fine Chemicals, Uppsala) to activate malignant B lymphocytes [20]. Recombinant interleukin-2 (IL-2; Latvian Institute of Organic Chemistry, Riga) was also used to stimulate the lymphocytes.

A 20-ml sample of peripheral blood was obtained and anticoagulated in preservative-free heparin at 9.00 a. m. To obtain a closer approximation to the natural biological milieu, we used an optimized whole-blood method to study the mitogenic lymphocyte proliferation [11]. Cells were suspended at concentration of 1.0×10^6 cells/ml in RPMI-1640 medium and supplemented with 2 mM glutamine, 50 μg/ml gentamycin and 10% fetal calf serum. Cells were cultured in the presence of medium alone (control) or mitogens (separately with PHA, ConA, IL-2 and DxS). All assays were performed in triplicate. Cell cultures were incubated at 37° C in a humified 5% CO_2 atmosphere for 84 h and pulsed with 1 Ci [^{3}H]thymidine. Proliferation was measured by [^{3}H]thymidine uptake during the last 16 h of culture. Autoradiographic and morphological assessments of proliferating cells were used. Results were expressed as a stimulation index: [mean percentage of proliferating cells with stimulant]/ [mean percentage of proliferating cells without stimulant (control)].

First, dose-titration experiments were carried out in selected cases to determine the optimal concentrations of PHA, ConA, IL-2 and DxS for stimulation of lymphocytes. PHA was used in concentrations of 5, 10 and 20 μg/ml. Since 10 and 20 μg/ml gave similar results, further experiments were performed with a concentration of 10 μg/ml. ConA was used in the doses 1, 2, 5 and 10 μg/ml, whereas the optimal concentration appeared to be 5 μg/ml. Recombinant IL-2 doses tested were 10, 20, 50, 100 and 1000 U/ml. Optimal proliferation of lymphocytes was obtained by IL-2 at a concentration of 100 U/ml. The optimal dose of 5 μg/ml was established for DxS from dose titration of 2, 5, 10 and 100 μg/ml.

Cell viability, determined by trypan blue exclusion, was more than 96%.

Statistical methods. The data reported in the tables were analysed by a paired *t*-test. Significance was considered to be at $P < 0.05$, but in many situations lower *P* values were obtained as noted.

Results

Hormones

FSH, LH, T, E and E to T ratio mean values are presented in Table 1. In male multiple myeloma patients serum FSH, LH and E levels are considerably augmented when compared to those of the control group. Interestingly, female multiple myeloma patients have demonstrated normal FSH and LH serum concentrations, but decreased 17β-oestradiol values. When the oestrogen to androgen ratio was calculated, it was found to be increased in male and decreased in female patients.

The hormone values of specifically treated multiple myeloma patients have been compared to those of untreated patients: Table 2. Statistically significant differences have not been established.

Peripheral blood lymphocyte proliferation in response to mitogens

Table 3 reveals PHA, ConA, IL-2 and DxS responsiveness of peripheral blood lymphocytes (PBL) of multiple myeloma patients and the control group. Results are also presented separately for male and female patients.

The blastogenic response of lymphocytes to the optimal concentration of PHA was markedly depressed in patients with multiple myeloma, when the whole group was statistically compared to controls. Considerable decreases of PBL proliferation in response to PHA have been demonstrated also by male patients, whereas the response of the

Table 3. Peripheral blood lymphocyte proliferation in response to mitogens

Mitogen[a]		Mean stimulation index ± SD		
		Multiple myeloma patients	Control	*P*
PHA	All	5.2±0.5	8.6±2.9	<0.05
	M	4.3±3.9	9.9±2.7	<0.05
	F	6.7±5.4	7.5±2.8	>0.05
ConA	All	6.1±7.1	7.5±3.9	>0.05
	M	4.5±3.6	8.7±3.1	<0.05
	F	6.9±3.5	6.2±3.2	>0.05
IL-2	All	7.1±3.3	3.7±3.2	<0.05
	M	6.7±2.0	4.3±2.1	<0.05
	F	7.2±3.1	2.9±2.2	<0.05
DxS	All	5.7±4.9	1.7±1.4	<0.05
	M	5.3±3.7	1.9±1.8	<0.05
	F	5.9±2.9	1.5±0.9	<0.05

[a] PHA, phytohaemagglutinin; ConA, concanavalin A; IL-2, interleukin-2; DxS, dextran sulphate

Table 4. Peripheral blood lymphocyte proliferation in response to mitogens in treated (Tr) and untreated (UTr) multiple myeloma patients

Mitogen		Mean stimulation index ± SD		
		TR	UTr	*P*
PHA	All	6.1±5.6	4.5±2.8	>0.05
	M	5.6±3.9	3.2±0.9	>0.05
	F	6.6±5.6	7.7±3.9	>0.05
ConA	All	7.0±2.6	2.6±1.8	<0.05
	M	6.9±3.3	1.8±1.5	<0.05
	F	7.1±2.5	4.5±0.9	<0.05
IL-2	All	8.2±4.2	2.1±1.8	<0.05
	M	10.3±3.2	2.1±1.7	<0.05
	F	7.6±4.0	2.3±2.7	<0.05
DxS	All	6.3±3.1	3.3±2.1	<0.05
	M	8.4±4.3	1.5±1.7	<0.05
	F	5.7±8.6	7.8±5.9	>0.05

group of women patients was comparable with that of the healthy group. The PBL reaction to another T cell mitogen – ConA – was at the same level in both the groups of patients and in the control group. By contrast, male patients have demonstrated a significantly lower proliferation rate of PBL in response to ConA than that found in healthy subjects. Recombinant IL-2 induced significant lymphocyte proliferation in the whole multiple myeloma population and in the separate male and female groups.

DxS, the mitogen for the malignant B lymphocytes, stimulated multiple myeloma lymphocytes, but not the control cells. No differences in the lymphocyte response of male or female patients to DxS have been identified.

In addition, the blastogenic response of PBL to the mitogens mentioned above was analysed separately in treated and untreated patients.

Table 4 shows that there are no significant differences between the lymphocyte response of treated and untreated multiple myeloma patients' to PHA. However, markedly higher responses to ConA and IL-2 have been observed in treated patients when compared to untreated ones. Interestingly, DxS-induced PBL proliferation was significantly higher in the whole treated multiple myeloma group and also in male patients, whereas there were no differences detectable between treated and untreated female patients.

Discussion

We have established significant augmentation of follicle-stimulating hormone and luteinizing hormone serum levels in male patients suffering from multiple myeloma male but not in female patients.

Highly elevated serum concentrations of FSH and LH are usually characteristic of women in the postmenopausal period [4, 19]. A similar but considerably less dramatic, age-related increase, also occurs in men. The negative feedback from the gonadal hormones upon gonadotropin secretion disappears. The changes of FSH and LH we have discovered in male patients cannot be explained by the influence of age only, as the control group was matched by age.

Tsatsoulis et al. [37] have demonstrated the heterogeneity of LH and FSH forms. The types and relative abundance of the different isohormones may change under different endocrine conditions. The biological significance of the multiple forms of LH and FSH is unknown. It is possible that the different and isoforms may regulate different biological functions of the target cells and, amongst them, of immune cells as well. In their work Rouabhia et al. [33] have suggested that circulating LH is able to regulate certain functions of lymphoid cells.

As to gonadal hormones, male multiple myeloma patients have demonstrated augmented and female patients decreased 17β-oestradiol levels. It is well known that older men have higher peripheral oestrogen concentrations than postmenopausal women, this being explained by the testicular contribution of 17β-oestradiol, as well as peripheral aromatization of androgens [7, 19].

Although the changes we have observed in multiple myeloma patients are somewhat similar to those mentioned above, the comparison with healthy people from the same age group has revealed significant differences. It is worth mentioning that the oestrogen to androgen ratio is changed both in male and female patients: men have demonstrated an increased ratio and women a decreased one. It has been supposed that just the ratio of oestrogen to androgen may determine the effect of circulating hormones on the immune system [17].

A number of observations offer strong arguments that gonadal steroids regulate the immune function [3, 8, 18, 25]. However, the precise mechanism of sex-hormone-induced modulation of the immune system is still incompletely characterized. Studies on the effects of female sex hormones on the immune system have yielded conflicting results. Oestrogen has been shown to suppress both

humoral and cell-mediated immunity as well as enhance the immune response in several systems [2, 3, 8, 35, 36]. Similarly, testosterone has been shown to reduce humoral and cell-mediated immune responses [17, 12, 39]. However, in other systems testosterone either had no effect or enhanced immune responses [8, 13].

Much of the evidence indicates that T lymphocytes are the primary targets for sex hormone action, as the latter markedly modulate T-cell-mediated delayed-type hypersensitivity reactions [3, 12, 15]. We have observed T cell dysfunction in multiple myeloma patients on the basis of decreased PBL proliferation in response to the T lymphocyte mitogen PHA. This result agrees with previous data [26]. Interestingly, the male multiple myeloma patients showed a lower blastogenic response of lymphocytes to PHA as compared to female patients. ConA-induced lymphocyte stimulation was also markedly diminished in the male patients. It is established that PHA predominantly stimulates T helper cells and ConA induces suppressor T lymphocytes to proliferate.

The disturbed lymphocyte blastogenic response to PHA and ConA that we have observed in multiple myeloma patients can reflect the functional defects of T cells and/or the imbalance of distinct lymphocyte subpopulations, as has been suggested by others [24]. When treated and untreated patients were compared, no differences were found in lymphocyte proliferation in response to PHA, whereas ConA-induced proliferation was significantly higher in treated patients. We can only guess that the specific cytostatic treatment used in multiple myeloma also affects T lymphocyte function and/or the composition of T subpopulations. We cannot agree with Peterson et al. [28], who have not found changes in T lymphocyte subpopulations in untreated multiple myeloma patients.

Although multiple myeloma has been classically considered to be a plasma cell malignancy, there is increasing evidence that T cells [29] and peripheral blood B cells may also be partly involved in malignant proliferation. The results presented here provide evidence that a significant population of lymphocytes in multiple myeloma is stimulated to proliferate by DxS, which is the mitogen for malignant B cells [21]. Whereas female patients have not demonstrated differences in the lymphocyte proliferation to DxS, depending on the treatment, the treated men have responded at a considerably higher level. The meaning of these sex-based differences is not clear, but may be attributed to sex hormone imbalance.

Interestingly, recombinant interleukin-2 was mitogenic to multiple myeloma lymphocytes, especially those from treated patients. It has been demonstrated that in chronic lymphocytic leukemia (CLL) leukemic cells express the IL-2 receptors and may proliferate in the presence of exogenous IL-2 [14, 23, 27, 31, 38]. If some B lymphocytes in multiple myeloma belong to the malignant population, these cells may, like those in CLL, respond to IL-2.

Taken together, our data provide evidence for at least two different patterns of sex hormone changes and immune dysfunctions presented by male and female multiple myeloma patients. In male patients increased concentrations of FSH, LH and E and an elevated E to T ratio were associated with a decreased lymphocyte blastogenic response to PHA and ConA and increased proliferation in response to stimulation with IL-2 and DxS. However, the female patients demonstrated normal values of FSH, LH and T, a diminished E level and a decreased E to T ratio to be correlated with a normal lymphocyte response to two T cell mitogens (PHA, ConA) and augmented blastogenesis to IL-2 and DxS.

Obviously the interactions between sex hormones and immune mechanisms are very complex. For the maintenance of immune homeostasis an equilibrium between regulatory cells is required. Sex hormones may not only alter the balance significantly between the lymphocyte subpopulations, but also influence the qualitative functions of lymphocytes.

Our findings, while admittedly preliminary, suffice to provide an indication of sex hormone changes in multiple myeloma. Although the functional significance of the data is far from being fully understood, continued studies of the interactions between the sex hormones and immune regulation should help explain sex differences in the host response and changes in the immunological response related to multiple myeloma.

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