Immune system in patients with osteoporosis of 3rd degree. Selected parameters of immune reactivity.

Gabriel Turowski[1], Danuta Gieracka-Pażucha[1], Anna Kędzierska[1], Joanna Flasza[1], Ewa Pawłowska[1], Lidia Rutowicz[2]

[1] Laboratory of Clinical Immunology, Medical Faculty, Jagiellonian University, Cracow, Poland
[2] Outpatients Clinic of Rheumatology, Cracow, Poland

SUMMARY

In most cases, the determination of immunological condition of patients is vital from the perspective of the course of the disease and its treatment. Patients with osteoporosis of 3rd degree form a selected group while the division into osteoporosis type I in women after menopause and osteoporosis type II in elderly patients is arbitrary. The aim of this research was to find the discriminating parameter of immune reactivity for osteoporosis of 3rd degree. Abnormal proportion of cells CD4:CD8 (=1.0) was shown in 40% cases. In addition, the increased ability of T cells and their subpopulations to form non-immune rosettes with SRBC was observed. Neutropenia was diagnosed in 8 patients (40%) and NBT-sp test showed the values of NBT-positive cells to be 3 or 4 times higher. The results confirm our assumptions and support the value of immunomodulating treatment in ubiquitin therapy.

Introduction

Immune reactions are closely related to the maintenance of individual integrity with respect to both external and internal conditions. They guarantee the state of homeostasis throughout life while they also undergo changes, especially during a disease process or ageing. In osteoporosis various disturbances in bone tissue metabolism are taken into account, all of them being linked to the function of nervous, endocrine and immune systems [1,2].

Both doctors and patients have recently become more and more concerned with the subject of osteoporosis. Its occurrence is determined by a number of factors resulting in the loss of bone weight, changes in bone structure and poor overall quality of bones. This often leads to Colles' fractures, femoral neck fractures as well as fractures within vertebral column. It applies to fractures caused by osteoporosis which are likely to occur as the density of bones decreases. There are different phases in osteoporosis though one, generally accepted classification of the process does not exist. Most frequently, the early pain-free phase is called preclinical osteoporosis (osteopenia) while the later stage with typical non-traumatic fractures is referred to as osteoporosis proper [3,4].

Any evaluation of immune reactivity in patients is vital as far as their immune condition and treatment are concerned. Preliminary examinations are meant to appoint the parameters which will be crucial for the evaluation of the disturbances within

immune system as well as for the monitoring of patients during their treatment and clinical observation.

Material and methods

A. Patients

The examination was carried out on 20 patients (16 women, 4 men) with osteoporosis of 3rd degree, which had been confirmed after radiological examination. Within this group, there were 9 women after menopause (type I), 8 elderly patients (type II) and 3 men at the average age of 54. After x-ray examination with Multax 320 x-ray apparatus, the degrees of osteoporosis were estimated. Only patients with osteoporosis of 3rd degree were included in our study on immune system. During radiological examination, the thinning of bone cortex of vertebral body and sparse trabeculation of spongious substance were visible. 1-3 fish or lentoid vertebras could be observed in all patients. In addition, the thinning of cortex and a 'hollow bone' as invisible spongious substance were found in long bones. In individual cases osteoporosis co-existed with the symptoms of rheumatoid arthritis. Before the therapy started, blood samples of patients had been collected for immunological examination irrespectively of tissue typing within HLA histocompatibility complex.

B. T lymphocytes, their subpopulations in CD system and receptor activity

The number of leucocytes in peripheral blood and their composition as well as T lymphocytes population were determined by means of differentiation markers CD2 and CD3. TEt test was done in order to determine the ability of T lymphocytes to form non-immune TE rosettes with sheep red blood cells (SRBC). We distinguished subpopulations CD4+, CD8+, CD25+; the CD4+:CD8+ rate as well as NK cells with CD16 marker. To determine the subpopulation of cells in CD system, OKT and BMA monoclonal serum were used according to modified methodology recommended by Ortho company [5]. The control group included 49 healthy adults [5]. Receptor activity of T lymphocytes was determined on the basis of the percentage of cells gathered in rosettes after 60 minutes of incubation (test TEt) and after 5 minutes of incubation as the subpopulation of active T-lymphocytes in rosette test TEa. When evaluating the results of TEt and TEa tests the Ta:Tt proportion was calculated and relative rosette activity coefficient (WARL) was determined [6].

C. Neutrophils and their metabolic activity

The overall number of neutrophils was calculated from the number of leucocytes and their proportional value. Enzyme activity was determined in NBT/NBT-sp and NBT-st tests with E.coli LPS according to modified Park et al. method [7]. NBT results were given as proportional values of NBT-positive cells, as relative coefficient of granulocytes' metabolic activity (WAMG) and the index determining 'functional reserve' of neutrophils [9].The results of tests carried out by Grzędzielska [10] on 46 healthy adults determined the range of standard values.

Results

Table 1 presents average value of subpopulations CD2+, CD3+, CD4+, CD8+, CD16+, CD25+ in peripheral blood and the CD4+:CD8+ proportion for both groups of patients, i.e. for those with post-menopausal osteoporosis (I) and with osteoporosis of the elderly age as well as for 3 men with osteoporosis who were treated as a separate, minor

Table 1. The subpopulations of T lymphocytes in CD system in patients with osteoporosis of 3rd degree.

T lymphocyte subpopulations	Control group (n=49)		Osteoporosis – type I						Osteoporosis – type II						Osteoporosis in men	
			standard values			abnormal values			standard values			abnormal values				
	x	±SD	n	x	±SD	n	x	±SD	n	x	±SD	n	x	±SD	x	±SD
CD2+	54	6	5	55	5.5	4	40	5.7	7	47	4.3	1	31		43	2.5
CD3+	56	8	5	58	5.2	4	47	2.1	6	49	1.9	2	37	2.8	47	4.6
CD4+	46.5	9.9	5	55	4.7	4	45	0.8	6	53	7.6	2	36	3.5	43	4.2
CD8+	26.8	6.9	6	36	5.8	3	52	2.5	4	26	7.1	4	43	2.1	31	3.2
CD4 : CD8*	1.7	0.29	4	1.6	0.26	5	1.0	0.12	5	1.8	0.43	3	1.0	0.18	1.4	0.02
CD16+	9.2	2.8	4	7	1.3	5	18	3.3	4	9	2.1	4	15	2.0	14	7.9
CD25+	49.3	7.4	9	48	4.9				4	50	2.5	4	39	3.9	41	9.7

* in CD4 : CD8 proportion only the values for CD4 were given, assuming CD8 as 1.

Table 2. The number of lymphocytes and their rosette activity (TE) in patients with osteoporosis of 3rd degree.

Examined parameters		Control group (n=49)		Osteoporosis – type I						Osteoporosis – type II						Osteoporosis in men	
				standard values			abnormal values			standard values			abnormal values				
		x	±SD	n	x	±SD	n	x	±SD	n	x	±SD	n	x	±SD	x	±SD
Lymphocytosis		1964	368	8	1878	192	1	1457		5	2037	649	3	1276	16	2281	16.23
TEt	%	57.4	6.3	9	57	10				8	54	9				53	14
	w.b.	1162	138	4	1307	162	5	864	158	3	1288	77	5	726	120	1188	753
	WARL	1.0	0.2	9	1.02	0.17				8	0.96	0.17				0.95	0.25
TEa	%	27	7.1	2	39	8	7	53	5	2	36	0.71	6	49	2	53	6
	w.b	557	120	6	629	151	3	345	22	4	521	83	4	314	36	620	363
	WARL	1.0	0.2	2	1.43	0.29	7	1.97	0.18	2	1.32	0.03	6	1.81	0.08	1.97	0.21
	Ta : Tt	0.45	0.03	2	0.62	0.03	7	0.95	0.08	2	0.68	0.03	6	0.9	0.12	1.03	0.17

The results were given in proportional values (%), absolute and relative rosette activity coeficients (WARL).

Table 3. Neutrophils and NBT tests: NBT-sp test and NBT-st test in patients with osteoporosis of 3rd degree.

Examined parameters		Control group (n=49)		Osteoporosis – type I						Osteoporosis – type II						Osteoporosis in men	
				standard values			abnormal values			standard values			abnormal values				
		x	±SD	n	x	±SD	n	x	±SD	n	x	±SD	n	x	±SD	x	±SD
Lymphocytosis		5826	987	5	6200	1075	4	4450	300	5	5080	606	3	3933	208	5700	3799
Neutrophis		3696	781	6	3797	915	3	2394	333	3	2954	175	5	2260	177	2982	1696
TEt	%	9	3.7	3	14	5	6	42	14	3	17	2	5	33	6	36	24
	w.b.	309	129	5	540	208	4	1822	575	3	432	135	5	817	136	1231	1109
	WARL	1.0	0.21	4	1.79	0.72	5	5.02	1.33	2	1.71	0.08	6	3.37	0.83	3.92	2.64
TEa	%	41	10.4	7	36	7	2	12	1	3	38	9	5	15	6	43	29
	w.b	1385	451	3	1525	139	6	717	248	3	934	206	5	381	153	1493	1363
	WARL	1.0	0.24	6	0.91	0.15	3	0.42	0.21	4	0.84	0.24	4	0.32	0.12	1.053	0.70
	Ta : Tt	1.0	0.36				9	-0.08	0.42				8	-0.11	0.19	0.25	0.16

The results were given in proportional values, absolute (w.b.) and relative coefficients of granulocytes metabolic activity (WAMG).

group. The results presented reflect the difference with respect to the results in control group.

It is worth emphasis that there was a large number of patients (n=8) with abnormal proportion of cells CD4$^+$:CD8$^+$ equalling 1.0:1. This resulted from the fact that in 7 patients with osteoporosis we could observe a high proportion of the subpopulations of suppressive/cytotoxic lymphocytes CD8$^+$ and the decrease of CD4$^+$ helper cells. In addition, considerable increase of NK cells as CD16$^+$ subpopulation was observed in 5 women with osteoporosis type I, 4 patients from the second group and the 3 men at the average age of 54. The distribution of subpopulations referred to as CD2$^+$, CD3$^+$ was similar to the subpopulations of T lymphocytes determined in TEt test (table 2).

The ability of T lymphocytes and their active T-subpopulations to gather in non-immune rosettes with SRBC was observed in examined patients. The relative rosette activity coefficient (WARL) for TEa test was considerably higher in most patients. Hence the proportions Ta:Tt were twice as large as in case of healthy persons in the age group of 30-60 years and they corresponded to the values typical for elderly patients (Ta:Tt >0.6).

Table 3 demonstrates the distribution of neutrophils in peripheral blood. Neutropenia was observed in case of 3 women with osteoporosis type I and in 5 patients with osteoporosis type II. Most patients showed increased proportion of NBT-positive cells in NBT-sp test - in some cases these values were 3 or 4 times higher. The results of the test suggested increased enzymatic activity of neutrophils in vivo.

Discussion

Up to the present day various studies have shown that the determination of T lymphocytes is useful as far as the course of the disease and the adminis-

tration of immunosuppressive drugs are concerned. This correlation is of vital significance in patients whose immune system is involved in the disease process. In this respect monoclonal antibodies for surface markers of differentiation [11-15] proved a great help since it is a fact that subpopulations of helper lymphocytes CD4+ and the subpopulations of cytotoxic/suppressive CD8+ play an important role in presentation and recognition of endo- and exogenous peptides (thanks to the TCR co-receptor).

T lymphocytes, determined with the help of anti-CD3 antibodies consist of heterodimeric molecules TCR $\alpha\beta^+$ in 95% and of TCR $\gamma\delta^+$ molecules in 5% only. Among TCR $\alpha\beta$ cells 60% are CD4+ and about 30% are CD8+ [16].

In theory, there is a possibility to influence the CD4+:CD8+ proportion of peripheral blood lymphocytes through the application of calf thymus extract, TFX preparation containing glycopeptides of ubiquitin type [17]. The role of ubiquitin in organisms exposed to stress, in the process of ageing as well as in those with calcium metabolism disturbances led to ubiquitin therapy in osteoporosis and gave unexpectedly good results [18], after which new preventive measures and treatment of osteoporosis were suggested [19].

Neutropenia in patients with osteoporosis of 3rd degree - type I and II indicates the need to restitute granulocyte cells. Their deficit implies their dysfunction with crucial consequences for homeostasis [20,21]. During our examination, higher values of NBT-sp test were observed, which indicates increased capacity of neutrophils for reduction of sodium salt of nitroblue tetrazolium to formazan. These cells not activated in vivo show little metabolism but their stimulation in organism is reflected in the results of NBT test. Increased values of NBT-sp test may indicate the presence of inflammatory focus stimulating neutrophils. Neutrophil function tests are not common in routine clinical practice, therefore it might be useful to decide whether indirect determination of neutrophils activity has any significance in patients with osteoporosis of 3rd degree.

Conclusions

1. Preliminary examinations described above show that the set of tests suggested here shed some light on the subject of immune reactivity in patients with radiologically diagnosed osteoporosis of 3rd degree both type I and II.

2. We claim that the determination of some parameters of immune system may prove useful for the diagnosis of progressive disease process as well as for immunomodulation in ubiquitin biotherapy.

References:

1. Christiansen C: Pathogenesis of osteoporosis. Proc Osteoporosis Symp, Copenhagen, 1989

2. Gburek Z: Zmiany w tkance kostnej w niektórych chorobach narządów wewnętrznych i ruchu. Śląska AM, Katowice, 1990; 5-118

3. Wüster C: Internistsch-endokrinologische Aspekte der Osteoporose. Krankenhaus Artz, 1994; 67: 390-400

4. Wüster C, Ziegler R: Knochenmetastasen: Pathophysiologie, Diagnostik und Therapie. W. Zuckschwerdt, Munchen, 1993; 99-108

5. Kędzierska A, Gieracka D, Turowski G: Limfocyty krwi obwodowej - ich aktywność receptorowa, markery układu CD i wartość diagnostyczna. Diagn Lab, 1994; 30: 383-391

6. Turowski G: Proste metody określania T limfocytów w testach rozetowych (TE). Przeg Metod AM w Krakowie, 1975; 10: 29-37

7. Park BH, Fikring SM, Smithwick EJ: Infection and nitroblue tetrazolium reduction by neutrophils. A diagnostic aid. Lancet, 1968; 2: 532-534

8. Samodzielna Pracownia Immunologii Klinicznej Collegium Medicum UJ: Cytochemiczne testy redukcji błękitu nitrotetrazoliowego (NBT-sp, NBT-st), Raport No 1/94, Kraków, 1994; 1-7

9. Turowski G: Względny współczynnik aktywności rozetowej limfocytów (WARL) dla testów TEa i Tet oraz współczynnik aktywności metabolicznej granulocytów (WAMG) dla testów NBT w ocenie stanu immunologicznego chorych. III Zjazd Pol. Tow. Immunol, Poznań, 1980

10. Grządzielska EB: Próba oceny zdolności fagocytozy granulocytów obojętnochłonnych za pomocą testu regukcji błekitu nitrotetrazoliowego. AM w Krakowie, Kraków 1976, rozprawa doktorska; 1-77

11. Weinberg K, Parkman R: Age, the thymus, and T lymphocytes. N Engl J Med, 1995; 332: 182-183

12. Laechy DJ: A structural view of CD4 and CD8, FASEB J, 1995; 9: 17-25

13. Giorgi JV: Lymphocyte subset measurements significance in clinical medicine, In: Manual of clinical laboratory immunology. Rose NR et al (Eds). Am Soc Microbiol, Washington, 1986

14. Zeman K, Majewska E, Potocka L, Tchórzewski H, Pajor A: Ocena ilościowa subpopulacji ludzkich limfocytów T I komórek NK metodą immunofluorescencji z zastosowaniem przeciwciał monoklonalnych BMA (Behringwerke). Diagn Lab, 1988; 24: 219-224

15. Turowski G, Gościński I, Kędzierska A, Gieracka D, Pawłowska E, Danilewicz B: Dopełnaczo-zależna limfocytotoksyczność oraz markery układu CD i nieodpornościowe rozety TE u wybranych chorych z glejakami mózgu. Diagn Lab, 1995; 31: 69-75

16. Kabelitz D: Biological significance of gamma/delta T lymphocytes. J Thymol, 1993; 1: 69-77

17. Turowski G: TFX (Thymus Factor X) jako kompleks ubikwityn. Ins. Farmakol. PAN w Krakowie, Kraków, 1995, wykłady monogr. nr 28; 1-29

18. Turowski G, Rutowicz L: Ubikwitynowa bioterapia w osteoporozie. Koncepcja i wstępne wyniki. Symp. Sekcji Osteoartrol, PTL i Pol. Fund. Osteoporozy, Łódź-Arturówek, 1994

19. Turowski G: Koncepcja ubikwitynowej bioterapii w osteoporozie. Med Sci Monit, 1995; 2(1): 339-342

20. Chabior M: Funkcje neutrocytów segmentowych i aktywność czynników humoralnych w zakażeniach bakteryjnych u niemowląt. AM w Gdańsku, 1995, rozprawa habilitacyjna

21. Malech HL, Gallin JL: Neutrophils in human diseases. N Engl J Med, 1987; 317: 687-694