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Different patterns of redistribution of monocytes, granulocytes and lymphocytes between bone marrow and peripheral blood induced by hypothalamic proline-rich polypeptide

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A number of novel polypeptides have been discovered and isolated from the neurosecretory granules of bovine and human neurohypophysis [7]. The distinctive property of these 10-15 amino acid residues neurohormones is their high content of proline residues, thus they were called proline-rich polypeptides (PRPs). The whole family of these peptides isderived from the neurophysin-vasopressin-associated glycoprotein precursor. Four of them were sequenced: PRP-1 Ala-Gly-Ala-Pro-Glu-Pro-Ala-Glu-Pro-Ala-Gln-Pro-Gly-Val-Tyr, PRP-2 Ala-Gly-Ala-Pro-Glu-Pro-Ala-Glu-Pro-Ala-Gln-Pro-Gly-Val, PRP-3 Ala-Gly-Ala-Pro-Glu-Pro-Ala-Glu-Pro-Ala-Gln-Pro-Gly, PRP-4 Ala-Pro-Glu-Pro-Ala-Glu-Pro-Ala-Gln-Pro; PRP-1, which was synthesized in Prof. Galoyan's Moscow Laboratory by Dr. Jmak [3,8,9].

The most studied of PRPs is PRP-1, which has different immune modulating, anti-neurodegenerative and antimicrobial characteristics [1,2,10,12-16]. It has been shown that PRP-1 protects cyclophosphamide-treated mice against *Pseudomonas aeruginosa* infection due to recovery of myelopoiesis and enhancement of mature granulocyte function [11]. PRP displays properties of oxidative burst regulation as well [4,5]. Recently, it has been reported that PRP reduces both spontaneous and doxorubicin-induced apoptosis of bone marrow (BM) monocytes and granulocytes, which is time-dependent and is observed during short-term (2-4 hrs.) but not long-term (after 24 hrs.) incubation of BM cells [19].

Although the kinetics of PRP action on granulopoiesis is still unknown, Galoyan and Aprikyan have shown that in cyclophosphamide-treated mice PRP-1 increases mature granulocyte count in peripheral blood (PB) beginning from the 7th day [11]. However, both the mechanisms and time-course of PRP-1 action on lymphopoiesis and myelopoiesis is still unknown. Hence, for elucidation of the PRP-1 action on separate cell populations' redistribution here, its influence on granulocytes, monocytes and lymphocytes population displacement in BM and PB was studied. We studied the dynamics of PRP-1 influence on granulocyte, monocyte and lymphocytes count, as well as CD3 and CD5 lymphocytes populations' redistribution in BM and PB in PRP-1-treated rats during 1-7 days.

Materials and Methods

Animals and cell preparation. All animal studies were carried out in accordance with the Code of Practice for the Housing and Care of Animals used in Scientific Procedures 1989. Wistar rats were obtained from the Animal facilities of the Buniatian Institute of Biochemistry. 12 rats were i.m. administrated with 10 ìg of PRP-1 (synthesized) per 100g of animal weight and 12 control rats were administrated with vehicle only. BM and PB were harvested on days 1, 2, 4 and 7 from both control and PRP-1 single-dose administered rats (N=3 in each group). BM was flushed from rat femurs and red blood cells were lysed with ammo-

nium chloride (0, 83% NH_4Cl in 0,017 M Tris-HCl buffer for 5 min). Nucleated cells were washed with cold RPMI-1640 containing 10% FCS, 2mM L-glutamine, 1mM sodium pyruvate, 100 U of penicillin and 100 ig of streptomycin per ml and total cell count was determined. PB samples were taken in tubes containing lithium-heparine (Vacuet, Greiner).

Lymphocytes, monocytes, granulocytes differential cell count was determined using the haematological analyser (Celly v 2.20, Hycel Diagnostics).

Cell population analysis. Cells were harvested in cold PBS, washed, and stained with 0.5 μg of phycoerythrin (PE)-conjugated anti-rat macrophage (clone HIS36), 0.25 μg of fluorescein isothiocyanate (FITC)-conjugated anti-rat granulocyte (clone HIS48), 0.25 μg of FITC-conjugated anti-rat CD3 (clone eBioG4.18) and 0.25 μg of PE-conjugated anti-rat CD5 (clone HIS47) per 10^6 cells in 100 μL of total staining volume for 15 min at room temperature in the dark, washed with BPS, fixed in 0.1% paraformaldehyde and subjected to flow cytometry analysis (10,000 events, using FACSCalibur™ instrument CellQuestä software, Becton Dickinson). Relative proportions of CD3, CD5, Gr and ED2-like antigen were expressed as a percentage of the total population of cells, gated according to characteristic SSC/FSC profiles. All antibodies were purchased from eBioscience, San Diego, USA.

Statistical analysis. Statistical analysis was performed by the method of dispersion analysis with parametric and non-parametric procedures using the Graph Pad Prism v4.01 software. Results of independent experiments were used to calculate mean values ± SEM, and differences were defined as statistically significant by Student's t-test, paired t-test, Wilcoxon-Mann-Whitney, and Welch's test at $P \leq 0.05$.

Results and Discussion

In order to confirm the results obtained using the animal model of monocytes and granulocytes recovery under the influence of PRP-1 in the cyclophosphamide-induced leukopenia mice, in this work we studied the PRP-1 influence on the granulocytes, monocytes and lymphocytes count. In the mentioned study, the level of leukocytes in PB of PRP-1-treated mice increased beginning from day 7, where the ratio of neutrophils with segmented nuclei was higher than monocytes [11]. Here, we studied the redistribution of monocytes and granulocytes between BM and PB during 1-7 days. After harvesting PB and BM from PRP-1-treated and control rats on days 1, 2, 4 and 7, the hematological analysis was carried out. As it is shown in Fig.1, there was an increase in monocytes and granulocytes number in PRP-1-treated rat BM and PB and a decre in lymphocytes level compared to control. On day 4 in PB the decrease in lymphocytes count reached to significant (P=0.04) value (Fig.1). At this term the increase in the monocytes but not granulocytes count was also significant (P=0.04), while granulocytes count increase was not significant.

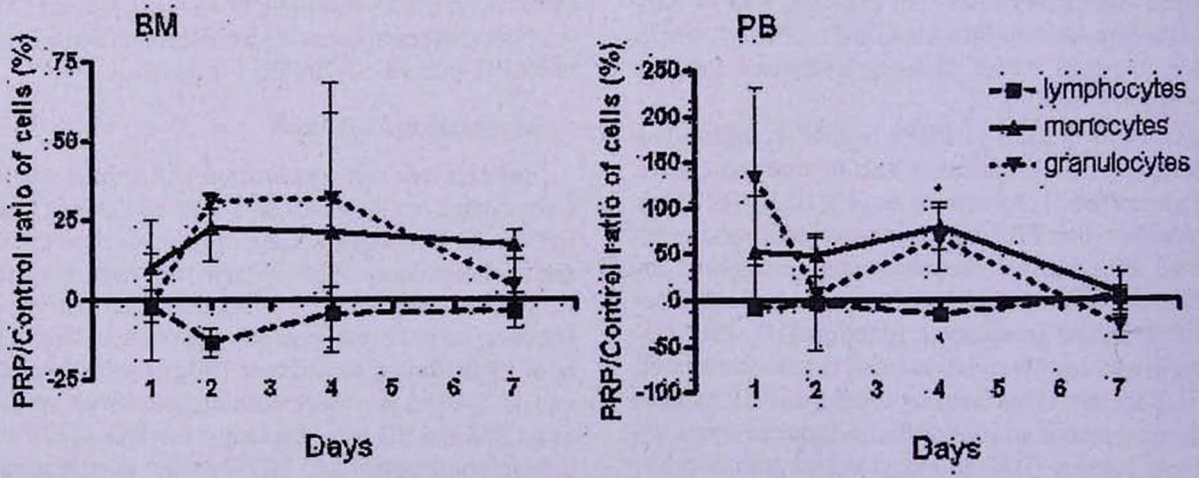

Fig.1. PRP-induced changes in time-course of BM and PB granulocytes, monocytes and lymphocytes counts. The rate of cell count changes is expressed as a percentage of PRP/control ratio.

Thus, the time-course of PRP-1-induced changes in BM and PB granulocytes, monocytes and lymphocytes showed similar dynamics of increasing of granulocyte and monocyte counts and decreasing of lymphocyte count. In comparison with the study on cyclophosphamide-treated mice model, where the increase in granulocytes content in PB began on day 7 [11], here we observed an increase on both granulocytes and monocytes counts in PB on days 2 and 4, then a decrease on day 7. In BM, a continuous increase in granulocytes count till day 4 was observed, and then there was a decrease in granulocytes but not monocytes on day 7.

Next, we carried out flow cytometry analysis to determine the rate of different cell populations in BM and PB of PRP-1-treated rats. For this purpose, the surface expression of specific lineage markers such as Gr (granulocytes), ED2-like antigen (macrophages) [6], CD3 and CD5 receptors (lymphocytes) was studied.

Subsequently, we analyzed Gr^+ and ED2-$like^+$ cells as we did not observed any difference of Gr^+ED2-$like^+$, Gr^+ED2-$like^-$ and Gr^-ED2-$like^+$ subpopulations between BM and PB samples under the influence of PRP.

The number of Gr^+ increased in BM on day 2 and day 7, whereas the number of ED2-$like^+$ increased only by day 4. In PB, the number of Gr^+ increased on day 2, dropped down on day 4 and was equalized with control by day 7 (Fig.2). These results suggest that PRP-1 stimulated Gr^+ count in BM at all terms of analysis, whereas in PB the stimulating effect was observed only on day 2.

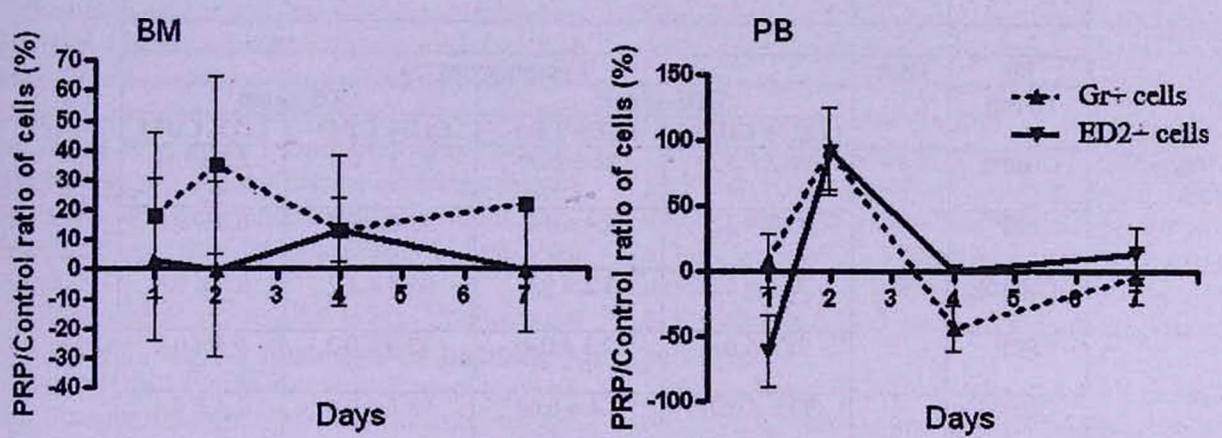

Fig. 2. PRP-induced changes in time-course of BM and PB Gr^+ and ED2-$like^+$ cells count. The rate of cell count changes is expressed as a percentage of PRP/control ratio.

While the number of PRP-1-treated rat lymphocytes according to the hematological analysis was lower than control both in BM and PB, different fluctuations of lymphocyte subpopulations were observed. Interesting, there was a significant ($P=0.02$) decrease in mature BM T cell population $CD3^+CD5^+$ on day 2. We suggested that PRP-1 delays T cell maturation in BM in 2 days after PRP-1 injection. At the same time, there was an increase ($P=0.02$) in $CD3^+CD5^-$ population of CD3-gated cells (Table 1). As CD3 along with CD2 are the most specific markers for T cells, and CD3 is normally present in resting and activated T lymphocytes, we suggest that this $CD3^+CD5^-$ population is the T cell subpopulation as well. It was shown by K Hozumi et al. that BM $CD3^+$ T cells are thymus-dependent and composed of an ordinary population which expresses TCR β-chain [18].

Table1

PRP-induced changes in time-course of BM and PB T lymphocytes subsets count

BM Group	Days	Lymphocytes (%)			
		CD3-gated		CD5-gated	
		CD3+ CD5+	CD3+ CD5-	CD3+ CD5+	CD3- CD5+
Control	1	40.7 ± 5.4	51.0 ± 5.5	15.1 ± 2.7	7.3 ± 0.8
PRP		44.5 ± 9.2	48.8 ± 7.6	26.8 ± 7.6	6.4 ± 0.9
Control	2	64.9 ± 6.9	24.4 ± 4.4	42.3 ± 8.1	8.3 ± 1.4
PRP		34.1 ± 4.8*	41.9 ± 1.3*	18.3 ± 5.4	9.4 ± 1.3
Control	4	28.2 ± 4.9	48.2 ± 2.8	26.3 ± 4.4	3.8 ± 0.4
PRP		27.05 ± 8.05	51.1 ± 3.0	27.1 ± 4.8	4.4 ± 0.6
Control	7	38.0 ± 7.07	42.0 ± 4.3	19.7 ± 5.8	4.5 ± 0.1
PRP		43.6 ± 10.7	38.1 ± 7.9	24.2 ± 8.2	3.9 ± 0.2*
PB Group	Days	Lymphocytes (%)			
		CD3-gated		CD5-gated	
		CD3+ CD5+	CD3+ CD5-	CD3+ CD5+	CD3- CD5+
Control	1	96.2 ± 0.2	2.6 ± 0.09	97.3 ± 0.4	2.7 ± 0.4
PRP		92.4 ± 2.5	4.4 ± 1.2	93.8 ± 2.7	6.0 ± 2.6
Control	2	96.6 ± 0.5	3.2 ± 0.5	95.8 ± 1.2	2.7 ± 0.8
PRP		97.5 ± 0.4	2.3 ± 0.4	97.9 ± 0.2	1.4 ± 0.2
Control	4	99.8 ± 0.03	0.4 ± 0.04	98.6 ± 0.4	1.4 ± 0.4
PRP		96.5 ± 2.9	0.8 ± 0.5	95.5 ± 1.3	4.5± 1.3
Control	7	98.9 ± 0.9	1.1 ± 0.9	98.9 ± 0.6	1.0 ± 0.5
PRP		99.7 ± 0.1	0.2 ± 0.1	99.6 ± 0.05	0.4 ± 0.05

*P=0.02 in PRP-treated rat in comparison with control

It was also observed that on day 4 $CD3^-CD5^+$ population increased in PB and on day 7 the same population decreased in BM. Though the surface marker CD5, strongly associated with T cells, is expressed also on a small subset of normal B lymphocytes called B-1 cells [19], we cannot insist upon the statement that this subpopulation belongs to T cell solely, as we didn't mark our BM and PB cells with other lineage specific markers.

As a result, we have demonstrated that PRP-1 stimulates granulocyte and monocyte counts in BM, which correlates with the previously demonstrated data [11], in PB on day 4 the difference between PRP-1-treated rat monocytes was significant. When we stud-

ied specific populations as macrophages and granulocytes, we found out that in BM, the granulocytes' rate was higher than control, but that of macrophages was higher on day 4 only. At the same time, when studying subpopulations in lymphocytes to determine different maturational stages of T cells, we found out that in general the maturation of T cells was delayed in BM.

Hence, according to the data obtained, PRP-1 stimulates the proliferation of granulocytes and monocytes in vivo as well as differently influences the kinetics of lymphocytes subpopulations in BM and PB.

Recently it has been shown that granulocytes and lymphocytes could occupy a common developmental niche. Reciprocal control of granulo- and lymphopoiesis reflects competition between Gr-1^{+int} and B220^{lo} compartments. The decrease in one cell lineage causes an increase in the other. TNFα and IL-1β reduce BM lymphocyte count and expand granucolyte production [20].

Thus, we suggest that PRP-1 could serve as an endogenous GM-CSF-like cytokine, which upregulates production of BM granulocytes and monocytes at the same time inducing a decrease in BM lymphocytes.

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Հիպոթալամիկ պրոլինով հարուստ պոլիպեպտիդի ազդեցությունը ոսկրածուծի և պերիֆերիկ արյան միջև մոնոցիտների, գրանուլոցիտների և լիմֆոցիտների վերաբաշխման վրա

Ք.Բ. Բեզիրգանյան

Հիպոթալամիկ պրոլինով հարուստ պոլիպեպտիդները (ՊՀՊ) օժտված են արտահայտված իմունակարգավորիչ, հականյարդադեգեներատիվ և հակամանրէական հատկություններով: Ավելի վաղ ցույց է տրվել, որ ցիկլոֆոսֆամիդ ներարկված մկների մոտ պերիֆերիկ արյան մեջ ՊՀՊ-1-ի ազդեցության ներքո հաստուն գրանուլոցիտների քանակը ավելանում է 7-րդ օրից սկսած:

Աշխատանքի նպատակն է եղել ոսկրածուծի և պերիֆերիկ արյան միջև գրանուլոցիտների, մոնոցիտների և լիմֆոցիտների վերաբաշխման ուսումնասիրությունը ՊՀՊ-1-ի ազդեցության տակ: Առնետների մոտ՝ ՊՀՊ-1-ի միանվագ ներարկումից հետո ոսկրածուծի ու պերիֆերիկ արյան բջջային պոպուլյացիաների քանակը որոշվում էր հեմատոլոգիական անալիզի և հոսքային ցիտոմետրիայի մեթոդներով՝ 1, 2, 4 և 7-րդ օրերին: Պարզվել է, որ ՊՀՊ-1-ը ավելացնում է գրանուլոցիտների և մոնոցիտների և միաժամանակ նվազեցնում է լիմֆոցիտների քանակը ոսկրածուծում և պերիֆերիկ արյան մեջ: Հոսքային ցիտոմետրիայի մեթոդով հաստատվեց ՊՀՊ-1-ի տարբերակիչ հակադարձ ազդեցությունը գրանուլոցիտոպոէզի խթանման և T լիմֆոցիտների հասունացման դանդաղեցման վրա:

Այսպիսով, ՊՀՊ-1-ի ազդեցությունը ոսկրածուծում գրանուլոցիտների և լիմֆոցիտների հակադարձ գոյացման վրա նման է այլ գաղութագոյացնող գործոնների և բորբոքախթանիչ ցիտոկինների ազդեցությանը:

Влияние гипоталамического пролином богатого полипептида на перераспределение моноцитов, гранулоцитов и лимфоцитов между костным мозгом и периферической кровью

К. Б. Безирганян

Гипоталамические пролином богатые полипептиды (ПБП) обладают выраженной иммуномодулирующей, антинейродегенеративной и антимикробной активностью.

Ранее было показано, что у обработанных циклофосфамидом мышей, начиная с 7-го дня, ПБП-1 вызывает увеличение количества зрелых гранулоцитов в периферической крови.

Целью данной работы явилось изучение действия ПБП-1 на перераспределение гранулоцитов, моноцитов и лимфоцитов в костном мозге и периферической крови. Крысам однократно

вводили ПБП-1 и количество клеточных популяций костного мозга и периферической крови определяли методами гематологического анализа и проточной цитофлюориметрии на 1, 2, 4 и 7-й дни. Было показано, что ПБП-1 увеличивает количество гранулоцитов и моноцитов в костном мозге и периферической крови и одновременно снижает количество лимфоцитов. Метод проточной цитофлюориметрии подтвердил дифференциальное реципрокное влияние ПБП-1 на стимуляцию гранулоцитопоэза и на задержку созревания Т-лимфоцитов в костном мозге.

Таким образом, действие ПБП-1 на реципрокную продукцию гранулоцитов и лимфоцитов в костном мозге сходно с действием других колониестимулирующих факторов и провоспалительных цитокинов.

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