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IMPAIRED RANDOM MIGRATION IN YOUNG GUINEA-PIG MACROPHAGES: RELATIONSHIP TO THEIR PROCOAGULANT ACTIVITY

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Both macrophage procoagulant activity (PCA) and migration capacity were tested in parallel in young (2 wk.) and adult 12 (wk.) guinea-pigs. The PCA of oil-elicited peritoneal macrophages was assayed by the ability to decrease the recalcification time of normal guinea-pig plasma, while random migriation was determined by the capillary tube method. The two measured macrophage functions were observed during incubation for up to 20 h, with or without 0.1-10 μg of immunomodulator lipopolysaccharide (LPS). The results show that macrophages from young animals express a significantly higher PCA, but smaller random migration than those of adults. A clear negative correlation was demonstrated between macrophage PCA and locomotor potency in both groups. The findings in this report suggest that impaired monocyte/macrophage motility in early post-natal life may be attributed, at least partly, to their better PCA.

Key words: guinea-pig, lipopolysaccharide, macrophage, ontogeny, procoagulant activity, random migration.

INTRODUCTION

In theiir rich secretory repertoire mononuclear phagocytes possess an inducible ability to produce several coagulation factors (reviewed by Ryan and Geczy, 1987). Macrophage procoagulant activity (PCA) is a potent activator of the extrinsic coagulation process, and may be responsible for local fibrin formation occurring under certain pathological conditions such as: inflammation, cell-mediated immune response, malignancy, and others (Semeraro et al., 1986; Grgacic and Bernard, 1990). In addtion, it is thought that PCA is involved in the regulation of macrophage and other cell motility (Geczy and Hopper, 1981; Vilić, 1988, Vilić and Prokić, 1988). As far as we know there are no data on the ontogeny of macrophage PCA. On the contrary, functional differences in phagocytosis, locomotor capacity, and secretion concerning macrophages in young and adult humans and experimental animals are well documented (Kretschner et al., 1976; Kurland et al., 1988; Weatherstone and Rich, 1989; Prokić and Vilić, 1990). The present work has been designed to analyse the PCA in macrophages of young guinea-pigs, and its relationship to random migration ability.

MATERIALS AND METHODS

Animals. The experiments vere performed on young (2 weeks) and adult (12 weeks) outbred male guinea-pigs (5-7 per group).

Peritoneal macrophages. Elicited macrophages were collected 6 days after an intraperitoneal injection of sterile paraffin oil (20 ml/kg). Hank's balanced salt solution (HBSS), containing heparin (0.5 U/ml; Galenika, Belgrade) was used for peritoneal lavage. The cells were washed 3 times with HBSS without heparin, and resuspended in medium (M 199, Torlak, Belgrade). Erythrocytes were eliminated by lysis with 0.83% NH_4Cl solution. Cell viability was assessed by trypan blue exclusion. According to neutral red staining 85-95% of the lavaged cells were macrophages.

Preparation of plasma. Plasma was prepared from normal guinea-pigs by cardiac puncture. Nine volumes of blood mixed with one volume of 3.8% sodium citrate was firstly centrifuged for 10 min at room temperature to obtain plasma and then for 15 min at 4 ^{0}C to eliminate platelets. Thus obtained plasma samples were pooled, and stored at-70 ^{0}C for up to one month until use.

PCA assay. Macrophage ability to shorten the coagulation time of recalcified guinea-pig plasma was tested either on freshly isolated cells, or after incubation for 4-20 h in M 199, at 37 ^{0}C. PCA inducer lipopolysaccharide (LPS; Sigma, St. Louis, USA) was applied *in vitro* in 0.1-10 μg/ml concentration. After washing, macrophages were resuspended in M 199. PCA of intact cells and cell lysates was measured by a modified one-stage method (Geszy et al., 1983) in an automatiic coagulometar. Cell lysates were prepared by two cycles of freeze-thawing. Duplicate samples of macrophage suspensions (10^6 cells/0.1 ml) were mixed with 0.1 ml guinea-pig plasma, warmed to 37 ^{0}C and then 0.1 ml of 0.025 M $CaCl_2$ was added, and the recalcified time was measured. PCA is expressed in seconds and milliunits (mU) of activity per 10^6 cells. A standard tromboplastin curve for recalcified time of guinea-pig plasma with decreasing dilutions of rabbit brain tromboplastin (Blood Transfusion Institute, Belgrade) was obtained. Six mg of tromboplastin was nominated as 1000 mU of PCA. The results are also given as procoagulant index (PI): PI = test PCA (mU)/control PCA (mU) x 100.

Random migration testing. The modified capillary tube method was used (Federlin et al., 1971). Briefly, macrophage suspensions (30 x 10^6 cells/ml) were packed into hematocrit capillaries, and secured into test chambers, which were immediately filled with M 199. The LPS doses applied were the same as for PCA testing. All the tests were carried out in quadruplicate, and the areas of migration were weighed after 4 and 20 h of incubation. The migration index (MI) was calculated as follows: MI = test area (mg)/ control area (mg) x 100.

Maintenance of LPS-free conditions. All tissue culture ware, if not of a sterile plastic disposable nature, was washed and heated to 180 ^{0}C for 3 h to destroy any contaminating LPS. Polymyxin B sulfate (100 U/ml; Sigma, St. Louis, USA), an inactivator of endotoxin was added to culture media. The substance had no effect on either PCA or random migration.

Statistics. Statistical analysis was performed using the twotail Student's t-test and Spearman's correlation test. The criterion for statistical significance was set at $P < 0.05$.

RESULTS

Firstly, we examined basal PCA in freshly isolated peritoneal elicited (inflammatory) macrophages, Adult (animal) macrophages displayed a low basal PCA (Table 1). In young guinea-pig cells 4-5 fold higher levels of basal PCA were measured ($P < 0.001$). The amount of PCA was even increased to a level similar to that seen in adults after maximal LPS stimulation. Table 1 also shows the PCA values obtained after macrophage incubation for 4-20 h in M 199. In adults the induction of PCA over basal values was rapid, with a maximal activity at 4-8 h and a slow decline to the initial level, at 20 h (time-course not presented). Once again the macrophages of 2-wk. -old animals expressed significantly higher PCA, with PIs ranging from 436 to 912 ($P < 0.01$). Their PCA was also prolonged in time. The frequently used PCA-inducer LPS (0.1-10 μg/ml) produced a 1.2-5-fold increase in PCA in both groups. The dose of 1 mg LPS appeared to be optimal. PCA in cells from young animals was increased to a higher quantitative level. Viable PCA accounted for 20-60% of the total content of PCA in both types of macrophages (results obtained with lysates are not shown).

Table 1. Procoagulant activity (PCA) of elicited peritoneal macrophages obtained from young and adult guinea-pigs

Age (wk.)	LPS (μg//ml)		Incubation time (h)		
			0	4	2
2	—	PCA[a]	261 ± 27	432 ± 45	447 ± 42
12	—	PCA	57 ± 5	99 ± 10	49 ± 5
		PI[b]	458 ± 47	436 ± 44	912 ± 93
2	0.1	PCA	—	445 ± 51	432 ± 60
12	0.1	PCA	—	115 ± 12	49 ± 5
		PI		395 ± 47	890 ± 97
2	1	PCA	—	970 ± 99	566 ± 52
12	1	PCA	—	228 ± 24	221 ± 21
		PI	—	425 ± 41	256 ± 25
2	10	PCA	—	967 ± 101	529 ± 62
12	10	PCA	—	190 ± 20	195 ± 21
		PI	—	499 ± 96	312 ± 41

[a] mU/10^6 cells (mean ± SD); PCA of intact cells is shown.
[b] PI = PCA of young / PCA of adult macrophages x 100. $P < 0.001$.

In agreement with our own previous report (Prokić and Vilić, 1990) we demonstrated that macrophages obtained from 2-week-old guinea-pigs had a significantly smaller random migration in vitro when compared to adult ones (Table 2). In the present study 0.1-10 μg/ml of LPS was also applied in vitro in the course of cell motility testing. A striking concentration-dependent reduction of migration occurred in both cell types in the presence of endotoxin, with the optimal effect observed at 1 μg concentration. It was evident (Figure 1) that the inhibitory effect of LPS was wery pronounced in cells from young animals. ($P < 0.05$-0.01).

Table 2. Random migration ability (mean migration area ± SD) of young and adult elicited guinea-pig peritoneal macrophages

Age (wks)	LPS (μg/ml)	Incubation time (h)	
		4	20
2	—	5.2 ± 0.27**	9.36 ± 1.27*
	0.1	4.74 ± 0.63**	10.11 ± 1.14*
	1	1.85 ± 0.33***	3.03 ± 0.63***
	10	2.05 ± 0.17***	3.22 ± 0.09***
12	—	8.92 ± 1.64	14.18 ± 1.08
	0.1	9.07 ± 1.38	15.34 ± 1.75
	1	5.14 ± 0.88	7.57 ± 1.64
	10	5.50 ± 0.97	7.94 ± 1.28

Significance level (young vs. adult): *P < 0.05; **P<0.01; ***P<0.001

An analysis of the relationship between macrophage PCA and migration ability showed a significant negative correlation in both groups, both in the presence and in the absence of LPS (r= -0.923 to 0.969; P < 0.05 to 0.01).

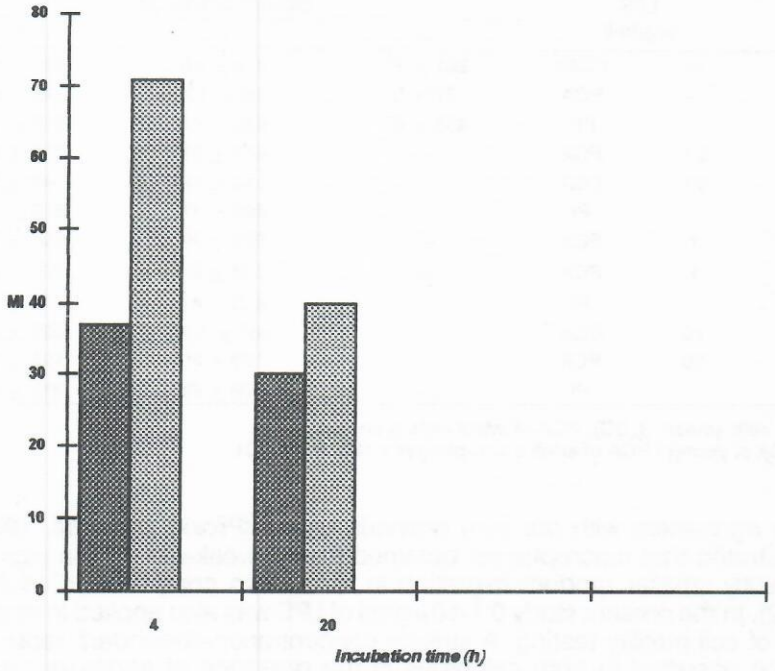

Figure 1. Influence of LPS (1 ug/ml) in vitro on young and adult macrophage random migration. Data represent the means; SD values are not indicated, but the coeficient of variability was up to 15%.

DISCUSSION

PCA is an inducible property in cells of the monocyte lineage attributed to a membrane-bound procoagulant (s) that initiates the coagulation cascade. To our knowledge, this is the first study which reports about the differences existing in PCA in the course of macrophage ontogeny. We found both quantitative (higher level in mU/10^6 cells) and qualitative (prolongation in time) differences in PCA of peritoneal elicited (inflammatory) macrophages of young guinea-pigs. Other authors have reported only the finding of PCA inducibility in neonatal blood monocytes (Rivers et al., 1992), and foetal placental macrophages (Kappelmayer and Adany, 1989). In addition, the results support the hypothesis of PCA inducibility during monocyte/macrophage adhesion to glass or plastic surfaces in the absence of any stimulator (Van Ginkel, 1980; Vilić and Prokić, 1988; Barstad et al., 1995) and extend the findings to macrophages from young animals. Viable PCA in macrophages from young guinea pigs accounted for a similar percent of total content as that previously measured in adults (Ryan and Geczy, 1987; Vilić, 1988).

We have previously demonstrated impaired random migration in elicited macrophages from young guinea-pigs (Prokić and Vilić, 1990). The smaller migration pattern was also demonstrated in the present work. It may be, at least partly, the consequence of higher PCA in these macrophages, induced both in the course of peritoneal inflammation in vivo (PCA of freshly isolated macrophages) and during cultivation in vitro. The hypothesis is leaned on the calculation of a significant negative correlation between higher PCA and smaller migration potency of 2-week old-animal macrophages as compared to those from adult controls. The reduction in migration obtained with LPS is in accordance with findings concerning adult animal mononuclear phagocytes (Remvig et al., 1986; Vilić, 1988).

It is intriguing that Rivers et al. (1992) suggested the induction of PCA in neonatal blood monocytes as a marker of possible baby infection. The findings in the present paper suggest that impairment of mononuclear phagocyte locomotion during postnatal life may be in part the consequence of their greater PCA inducibility.

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SMANJENO NASUMIČNO KRETANJE MAKROFAGA MLADIH ZAMORČIĆA: ODNOS SA PROKOAGULANTNOM AKTIVNOŠĆU

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SADRŽAJ

U makrofaga mladih (2 ned.) i odraslih (12 ned.) zamorčića paralelno smo testirali njihovo nasumično kretanje i prokoagulantnu aktivnost (PKA). U peritoneumskih makrofaga, izazvanih parafinskim uljem, ispitivali smo PKA sposobnošću da skrate vreme koagulacije rekalcifikovane zamoračke plazme, a nasumično kretanje metodom iz kapilarnih cevčica. Obe ispitivane makrofagne funkcije posmatrali smo u toku inkubacije do 20 h, uz dodatak ili ne 0,1-10 μg imunomodulatora lipopolisahaprida (LPS). Rezultati pokazuju da mladi makrofagi ispoljavaju značajno višu PKA, a lošije nasumično kretanje, u odnosu na odrasle. U obe starosne grupe demonstrirana je negativna korelacija između PKA i lokomotorne sposobnosti. Nalazi iz ovog rada sugeriraju da se smanjeni motilitet monocita/makrofaga u ranom postanalnom periodu može pripisati, bar delom, njihovoj boljoj PKA.