ADHESIVE PROPERTIES OF LYMPHOCYTES — A POSSIBLE MECHANISM FOR THEIR SELECTIVE DISTRIBUTION IN THE ORGANISM

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Although lymphocytes are usually referred to the category of so-called "migrating" or freely mobile cells, they remain for a certain time without fail at central or peripheral lymphoid organs (2). They can be seen here closely attached, i. e. adhered to underlying substrate. The reasons and concrete mechanisms of this event, recently called "lymphocyte homing" (4), are still not precisely enough determined. On the other hand, it is an interesting fact that peripheral lymphocytes (along with other leukocytes) also may change their adhesive properties (AP) towards underlying substrate (i. g. a glass plate) when they meet the antigen "in vitro" (5). The intimate mechanisms of this phenomenon are still not clarified, too (6). That's why the investigation of lymphocyte AP "in vitro" presents a perspective direction of studying the physiology of these cells.

In the literature available there are not any exact statistical data concerning the part of whole lymphoid population which possess AP, as well how AP change during cellular distribution in the organism. The aims of the present work were: 1. To study at which degree peripheral lymphocytes show AP to glass surface in condition of short-term cultivation "in vitro". 2. To perform a comparative study of AP lymphocytes obtained from various peripheral organs namely: blood, spleen, lymph nodes and Peyer's plaques.

Material and methods

We studied lymphocyte AP from human blood taken from 19 healthy donors aged 28—42 years. As regards the second aim of our work for obvious reasons it was not possibly to perform comparative investigations of lymphocyte adhesiveness from different human lymphoid organs. That's why we accomplished that only laboratory rats breed "Wistar" with average weight 125—15 g. Experiments were divided into 12 series in conformity to number of investigated animals.

Production of lymphocyte suspension

Human blood lymphocytes were separated after modified method of Boyum (3) after a borderline centrifugation with Ficolurographin (m. w. 1,076) of diluted 1:1 by saline solution heparinized blood. A terminal concentration of 2.10^6 cells/ml was reached in Difco-199 medium enriched with 10 % veal embryonal serum. The whole plate used in experiments was sterilized and chemically clean. Lymphocyte vitality was determined after the method with exclusion of the dye (0,23 % eosin-Y solution).

Rat lymphocytes were prepared from non-narcotized animals because it has been shown that the majority of narcoses cause cellular membrane changes.

The lymphocytes were separated by the way described above. Spleen, Peyer's plaques, and mesenteric lymph nodes were obtained postoperatively after killing the animals by gas emboly (20 ml air intracardiacly). The organs were washed several times with a sterile saline solution. After that they were cut into small pieces (about 1:1 mm) and softly homogenized in Potter's homogenizer. This suspension was filtrated through fine nylon net with pore size of 15 mm in order to remove the non-dispersed cells. With a view to diminishing the admixture of other cells (erythrocytes, fibroblasts, mastocytes) we diluted the prepared cellular suspension with nutritive medium and centrifugalized it with Ficol urographin (see above). A terminal concentration of 2,10 cells/ml was reached. The vitality of produced cellular suspensions ranged between 86 and 98 %. The purity of obtained lymphocytes was over 90 %. The ratio of adhesive cells was determined after modified method of Halliday (1976) with Burker's chambers. There we put the lymphocyte suspensions. The incubation was 30 min at 37 °C in a humid chamber. We read the mean number of cells from a total of 20 middle-size squares before and after washing the chamber three times with a saline solution. We determined the percentage of adhered cells according to the formula:

$$\% \text{ adhesion} = \frac{\text{MEAN NUMBER OF CELLS ON FIELD AFTER WASHING}}{\text{MEAN NUMBER OF CELLS ON FIELD BEFORE WASHING}} \times 100$$

The end results were processed statistically after the method of variational analysis. We found the levels of mean square deviation (S) and the confidential interval (p=0,05) according to the tabular method of Typet-Strelkov (1966). An exemplary protocol resulted from one investigation only is shown on table 1.

Table 1

	% ADHESION			
Rat number	blood	spleen	l. nodes	Peyer's plaques
1	7,4	33,8	43,0	—
2	12,3	53,4	28,9	—
3	31,3	64,5	43,6	—
4	34,5	50,4	26,0	8,1
5	2,1	43,9	—	14,5
6	11,0	—	—	50,0
7	24,5	34,4	—	31,3
8	9,0	46,1	31,5	19,8
9	12,6	26,0	56,5	20,6
10	13,3	57,1	37,8	33,3
11	38,2	29,5	14,6	15,0
12	14,1	42,2	58,3	20,7

Results and discussion

The results received concerning human blood lymphocytes (see table 2) show that at an average 18,3 ±3,4 % of the whole blood lymphocyte population tend to adhere to glass. In single individuals this percentage demonstrates a significant reproductiveness which is obvious also from the low rate of confidential

Table 2

Donor number	Before washing	Af er washing	% adhesion
1	5,70	0,54	9,5
2	10,95	2,33	21,3
3	2,13	0,56	26,3
4	10,01	0,70	7,0
5	7,23	0,82	11,3
6	6,31	0,67	10,6
7	9,12	0,78	8,6
8	4,63	1,50	32,4
9	8,35	1,80	21,0
10	12,70	2,11	16,6
11	10,54	2,32	22,0
12	3,85	0,73	19,0
13	4,64	1,31	28,2
14	9,30	2,40	25,8
15	25,80	3,10	12,0
16	20,30	1,50	7,4
17	9,10	3,25	35,6
		Average level	18,3±3,4

interval (±3,4). The results concerning rat blood lymphocytes (—17,5±3,3) are relatively near to the aforementioned ones.

With a view to solving the second aim we carried out a comparative investigation of lymphocyte AP from different peripheral lymphoid organs in rats. The end results show that these lymphocytes differ sharply in their adhesiveness as compared with blood lymphocytes. The percentage of adhered cells from spleen is at an average 44,4± 3,8 %; of those from mesenteric lymph nodes 38,4±5,2 %, and from Peyer's plaques 23,7±5,0 %, respectively. It is obvious, that blood lymphocytes contain least adhesive cells (17,5±3,8 %) which depends most likely on the fact that they are in the blood flow itself. It is noteworthy, however, that approximately 1/5 of circulating lymphocytes display AP. In our opinion the physiological explanation of this phenomenon is related to the level of microcirculatory unit and, especially, of postcapillary venules. It is known that some blood lymphocytes abandon actively the blood flow (2) sticking to the vascular wall and then move towards the interstitium. The important role of surface AP in the processes of lymphocyte movement is also proved in other works (7). That's an entirely different state in peripheral lymphoid organs. It is obvious that the percentage of adhered cells is significantly higher. It ranges between 30 and 60 % with speen and lymph nodes and even exceedes these rates in some cases. This increase of adhesiveness in lymphoid organs is most probably due to the "Lymphocyte homing", i. e. the solid lymphocyte adhesion in follicles and their distribution in so-called "thymus dependent" and "thymus independent" zones (9). Thanks to Curtis' et al. (1973) works it could be clarified that this event is based on selective cell adhesion. On the other hand, the authors demonstrated that mature T- and B-lymphocytes could mutually influence their AP by secreting thermolabile factors, most probably, of protein nature. On the ground of the presented results and cited bibliographic data we could presume that the mechanism of lymphocyte distribution in the organism is humorally directly controlled by the own lymphoid system as it is realized

by the change of AP of mature immunocompetent cells. The fact that Peyer's plaques lymphocytes display relatively low AP is surprizing to a certain degree, indeed. In them there is only a tendency but lack of statistically reliable difference towards blood lymphocyte adhesion. It takes unawareness because the immunocompetent cells are most compact and closely attached to underlying substrate, and, therefore, they must possess a high adhesiveness. We tend to explain this contradiction with the peculiarities of the method for lymphocyte preparation namely by "soft" homogenization in case of which it is completely possible that most firmly adhered cells cannot disperse at all.

We can conclude that "in vitro" received data concerning adhesive cell quantity from lymphoid organs are probably lowered towards their true levels in the organism. However, this doesn't impair our basic conclusion that functional lymphocyte redistribution in the organism is directly related with the dynamic changes of their AP.

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АДГЕЗИВНЫЕ СВОЙСТВА ЛИМФОЦИТОВ КАК ВОЗМОЖНЫЙ МЕХАНИЗМ ИХ ИЗБИРАТЕЛЬНОГО РАСПРЕДЕЛЕНИЯ В ОРГАНИЗМЕ

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РЕЗЮМЕ

У 19 здоровых доноров исследованы периферические лимфоциты и вопрос о том, какая част этих лимфоцитов проявляет адгезивные свойства к иммобилизированному субстрату — стеклу. Указывается на то, что в среднем 18,3—, т. е. 3,4 % периферических лимфоцитов человека проявляет адгезивные свойства.

Проведено сопоставительное исследование количества адгезирующих клеток различных периферических лимфоидных органов крыс — крови, селезенки, мезентериальных лимфатических узлов и пейеровых бляшек. Процент адгезированных клеток составляет соответственно 17,5±3,3; 44±3,8; 38,4±5,2 и 23,7±5,0.

Обсуждается вопрос о лимфацитарной адгезии в физиологическом аспекте. Делается вывод, что функциональное разпределение иммунокомпетентных клеток организма находится в прямой зависимости от динамических изменений их адгезивных свойств.