INABILITY OF LARGE GRANULAR LYMPHOCYTES TO RECIRCULATE THROUGH BLOOD-LYMPHATIC BARRIERS

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Different lymphocyte populations and subpopulations differ in their ability to pass from the blood into lymphoid organs and lymph, and then to reenter the blood, i.e., to recirculate. This process is rapid, active, and quite specific [3, 4, 6, 11]. Recirculation plays an important role in the formation of the immune response. Mainly memory cells have ability to recirculate [3, 4]. As a result of this process, the probability of contact of memory T and B cells with an antigen is increased many times over, and rapid accumulation of activated lymphocytes takes place in the lymph nodes and spleen [3, 4]. Recently a new subpopulation of lymphocytes, identifiable morphologically on staining by the Romanovsky–Giemsa method by the presence of azurophilic granules in the cell cytoplasm, on which account they have been called large granular lymphocytes (LGL), has been described in the blood of various species of animals [1]. This subpopulation has been shown to be phenotypically highly heterogeneous. Most LGL are natural killer cells which mature without passing through the thymus and the K lymphocyte stage [1, 9]. Under normal conditions in man about 50% of T_γ-lymphocytes and 15% of T_γ-lymphocytes in the blood also have the LGL morphology [1, 9]. LGL are unevenly distributed among lymphoid organs. They are numerous in the blood and spleen, less numerous in the lymph nodes and bone marrow, very scarce in the thymus, and they have not previously been detected in lymph from the thoracic duct. The thoracic duct is the principal lymph collector in mammals: it collects lymph from the greater part of the body (lymph from the hind limbs, and the thoracic and abdominal organs). Lymphocytes enter it by passing through a cascade of lymph nodes, in which the percentage of LGL is low. It can therefore be expected that the number of LGL in lymph of the thoracic duct will be less then in the blood. Some workers [13] found no natural killer cell activity in lymphocytes from thoracic duct lymph in mice and rats. No such activity likewise was found when lymph lymphocytes were treated with interferon, an inducer of natural killer cell activity. It is not yet clear whether the absence of natural killer cell activity in lymph of rats and mice is connected with the action of suppressors of this activity, arising from the thymus [13], or with the small number of LGL.

In the present study, devoted to an investigation of bovine blood and lymph, a new property of LGL was discovered, namely that few of them enter the thoracic lymph duct.

EXPERIMENTAL METHOD

Peripheral blood from 15 donor cows and from 2 cows with lymphatic leukemia, and lymph from 5 bulls and 5 mature cows of the Black Striped breed was used. Blood was taken from the jugular vein and lymph from the thoracic lymph duct by surgical cannulation. The number of LGL as a percentage of all lymphocytes was determined by counting 500-2000 lymphocytes in films stained by the Romanovsky–Giemsa method.

EXPERIMENTAL RESULTS

Bovine LGL are morphologically similar to human and rat LGL [1]. In normal bovine peripheral blood, LGL account for 12 ± 2% of the total number of lymphocytes (their absolute number was 420 ± 95 in 1 μl of blood; n = 15). Bovine LGL constitute a cell population which is nonadherent to cotton wool [2]. With respect to these properties, these cells are

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TABLE 1. Number of Cells in Lymph and Blood of Bulls (B) and Cows (C)

Test object	Cells	No. of animal									
		B-7*	B-8	B-13	B-14	B-15	C-9	C-159	C-12**	C-156**	C-155
Lymph	Leukocytes in 1 μl (× 10^3)	20,0	10,6	4,6	9,1	13,6	5,5		4,7	15,0	10,0
	Lymphocytes in 1 μl (× 10^3)	19,8	10,4	4,2	9,0	13,6	5,4		4,6	14,7	
	LGL in 1 μl	79	81	5	18	7	33		19	15	
	LGL, per cent of all lymphocytes	0,40	0,81	0,11	0,20	0,05	0,63	0,20	0,41	0,10	0
	Cell composition, %:										
	lymphocytes	99,2	98,2	91,5	99,8	99,8	98,2	97,0	97,5	98,1	
	neutrophils	0	1,4	8,0	0	0	0,5	1,8	1,7	0,3	
	eosinophils	0	0,2	0,1	0	0	0,1	0,2	0,5	0,7	
	basophils	0	0	0	0	0	0	0	0	0	
	monocytes	0,8	0,2	0,4	0	0	1,2	0,8	0,3	0,9	
Blood	Leukocytes in 1 μl (× 10^3)					5,9		6,9		25,0	6,0
	Lymphocytes in 1 μl (× 10^3)					3,2		2,7		20,7	3,3
	LGL in 1 μl					212		372		649	205
	LGL, per cent of all lymphocytes				0,9	6,64		13,5	1,07	3,14	6,22
	Cell composition, %										
	lymphocytes				44,0	54,2		40,0	76,4	82,8	54,6
	neutrophils				49,0	33,6		52,0	23,0	10,6	20,6
	eosinophils				1,0	4,0		2,4	1,0	4,4	19,4
	basophils				0	2,0		0,2	0,4	0,2	2,0
	monocytes				6,0	6,2		0,4	1,0	2,0	3,4

Legend. *) Lymph of tracheal duct (the tracheal duct is a collector of lymphocytes from the lymph nodes of the head and neck and thymus); **) cows with lymphatic leukemia.

similar to human LGL [1]. However, the population of nonadherent bovine lymphocytes obtained from blood and enriched with LGL does not possess natural killer cell activity against traditional target cells of the K-562 line, but induces lysis of cell lines infected with bovine leukemia virus [2].

The experimental results (Table 1) show that the number of LGL in bovine lymph was sharply reduced. This was so both in healthy calves and cows and also in animals with lymphatic leukemia.

The diameter of the LGL in the lymph and blood varied within identical limits — from 10 to 19 μ. As a rule LGL in the lymph had smaller azurophilic granules in their cytoplasm than in the blood. Human LGL with a low degree of granulation of their cytoplasm are regarded as a less mature form [1, 5]. By analogy, it can be suggested that LGL in bovine lymph also are less mature.

In the light of our data, failure to detect natural killer cell activity in thoracic duct lymph of mice and rats [13] can be explained by the presence of blood-lymphatic barriers in the lymph nodes, as a result of which only a small number of LGL leaves these organs.

Our results indicate that LGL, unlike cytotoxic T cells and K cells [7, 13], do not recirculate, or that only a small proportion of them have the ability to recirculate through lymph nodes. Under these circumstances granulocytes and monocytes likewise recirculate hardly at all (Table 1). Recirculation of T and B cells from blood into lymph does take place [3, 10, 12, 13], and this recirculation is effected and controlled by high endothelial cells of postcapillary venules in lymph nodes. T lymphocytes activated by antigen or mitogen do not pass into the lymph, for they have lost their receptors for high endothelial cells [6]. Undifferentiated T lymphocytes likewise have no receptors for these cells [8]. Since LGL have many features in common with cells of the T series [9], it can be tentatively suggested that LGL do not carry receptors for high endothelial cells. This hypothesis can be tested experimentally.

The small numbers of LGL, monocytes, and neutrophils found in the lymph of healthy animals and of animals with lymphatic leukemia may perhaps be one of the conditions responsible for evasion of immune surveillance by tumor cells.

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ENZYME IMMUNOASSAY OF SPECIFIC BRAIN ANTIGENS AS A CRITERION OF BLOOD-BRAIN BARRIER PERMEABILITY IN RATS WITH ACUTE CEREBRAL ISCHEMIA

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Cerebral ischemia remains one of the most important problems in medicine today, for the anoxia of the brain cells which results from it lies at the basis of many diseases or is one of their manifestations [1, 8]. Information on the function of the blood-brain barrier (BBB) in acute ischemia is by no means consistent [6, 9]. This difference in the results can be explained by the use of different approaches by different authors to the study of BBB permeability. The function of this morphological and functional system can be evaluated, on the one hand, by studying changes in permeability for low-molecular-weight substances of varied origin [9, 11], but on the other hand, by examining the passage of compounds specific for the brain through BBB [2, 12]. An interesting development in the study of BBB function is the use of specific brain antigens for these purposes [2, 14].

In the investigation described below changes are brought about in BBB permeability for specific acid gliofibrillary antigen (GFAP) and for brain α_2-globulin (α_2-M) in rats with acute cerebral ischemia.

EXPERIMENTAL METHOD

Altogether 50 experiments were carried out on noninbred rats of both sexes weighing 200-220 g. The animals were anesthetized by intraperitoneal injection of 1 ml of 1% hexobarbital solution/100 g body weight. A model of cerebral ischemia was produced as described previously [3]. Blood serum from control and ischemia animals served as the test object.

The barrier function of BBB was studied by an immunoenzyme method [7]. Antisera to neurospecific α_2-M and GFAP were obtained by immunizing chinchilla rabbits with purified preparations of these antigens, obtained by methods in [14] and [5] respectively. Antibodies to the above-mentioned brain antigens were isolated from monospecific antisera on immunosorbents prepared on the basis of CNBr-sepharose 4B (Sigma, USA) and purified α_2-M and GFAP preparations by the method in [15]. The concentration of immunoglobulins in the fraction of isolated antibodies was determined by Ouchterlony's double immunodiffusion

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