THE BARRIER FUNCTION OF THE LYMPH NODES IN INFECTION

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Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 49-53, January, 1960

Original article submited March 30, 1959

It has been reported [8, 9] that the lymph nodes are able to fixate up to 79-99% of bacteria entering via the lymph vessels. Barrier function increases considerably during the process of immunization [1, 2].

It is well known that fixation of substances occurs in an inflamed area. The fixation results from coagulation of the plasma at the center of the inflammation, and also through thrombosis [4] and spasm of the lymphatic vessels [6].

D. A. Zhdanov [3] paid particular attention to the barrier function of the lymph nodes. In inflammation of the regional lymphatic nodes, reactive changes always occur: There is a swelling of the cells of the endothelium of the sinuses, infiltration of the node by granulocytes, hyperplasia of the lymphoid elements, which sometimes completely cover the sinuses of the node [7]. It is quite possible that similar nonspecific phenomena may be involved in increasing the barrier function of the lymph nodes in immunization.

In the present investigation we have studied the fixation of B. coli commune of the popliteal lymph node in healthy immunized animals and in others in which inflammation of the knee had been induced.

METHOD

The experiments were made on 36 cats and 6 dogs. The lymphatic nodes were perfused by a suspension of B. coli. A study was made of the lymph taken at operation from the lymphatic nodes in cats, and from a fistula of the thoracic trunk and lymphatic vessels of the hindlimb in dogs.

The animals were anesthetized by a subcutaneous injection of 50 mg per kg nembutal.

The perfusion fluid was prepared as follows: A 24-hour culture of B. coli (washed from an agar slant) was diluted in sterile Tyrode's solution to make 10^9 bacteria per milliliter, and then diluted to a strength of $5 \cdot 10^6$/ml. An accurate determination of the number of bacteria (both in the original and in the perfusion fluid) was made on plate cultures.

The lymph nodes were perfused for 20 minutes at a constant pressure of 20 cm of water. The barrier function was measured in terms of the percentage bacteria fixated.

Cats were immunized by giving three injections of heated B. coli at intervals of 5 days. One group was given an injection of 500 million bacteria into both hind feet, while the other group received 1 billion bacteria subcutaneously on one side of the body surface. The perfusion of the popliteal nodes was made on the 9-12th day after immunizing.

RESULTS

As is shown in the figure and in Table 1, the percentage B. coli fixated by the nodes in healthy animals when perfused varies over a considerable range (13.3-96.1). With immunized animals, and particularly in the lower leg, the variation in the percentage taken up varies less, while the average number is greater.

To determine the part played by the nonspecific component in the barrier function of the lymph nodes we induced an inflammation in the foot, and 2-5 days

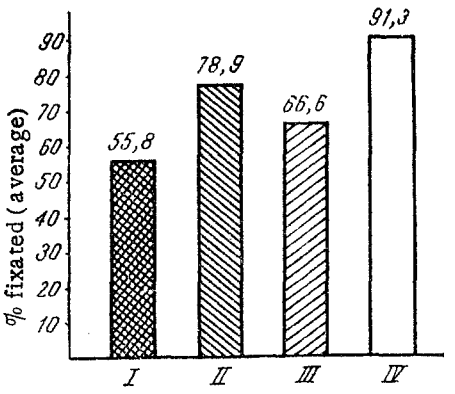

Fig. 1. Fixation of B. coli commune by the popliteal lymph nodes in cats. I) Healthy animal; II) animals immunized in the lower leg; III) animals immunized by injection into body side; IV) animals with inflammation in lower leg.

TABLE 1 Fixation of B. coli Commune by Popliteal Lymphatic Nodes in Cats

Group of animals	No. of animals	Number of perfused nodes	Limits of variation of percentage fixated.	Standard error*	
				1	2
Intact animals	17	24	13,3—96,1	—	—
Immunized by injection into lower leg	7	12	45,2—97,1	$p<0,01$	—
Immunized by injection into body side	6	8	30,4—85,0	$p<0,01$	$p<0,1$
With inflammation of limbs	19	28	72,1—99,9	$p<0,01$	$p<0,01$

*This refers to the difference of the percentage fixated in the different groups: 1) with respect to percentage fixated in intact group, 2) with respect to percentage fixated in group immunized by injection in lower leg; $p < 0.01$ — statistically significant, $p < 0.1$ — not statistically significant.

later perfused the nodes with a suspension of B. coli. The inflammation was induced by injecting into the footpads either 0,1 ml of turpentine, or a suspension of 250 million staphylococci, or a centrifuged 2-day meat-peptone broth culture.

It was found that the variation in the percentage bacteria fixated was quite small, and that the average percentage was higher than in the healthy and in the immunized cats. The highest percentage taken up occurred when the inflammation was caused by the staphylococcal culture, it was less with the meat-peptone broth, and still lower when turpentine was used.

The experimental arrangement was now changed in order to eliminate certain defects in the method of perfusion. A cat in which inflammation had been induced in one hindfoot received an injection of 1 billion bacteria in 0,5 ml into both feet. In view of the report [6] of the possible functional spasm of the lymphatic vessels lying proximal to the inflammatory edema, the culture was injected 4-5 cm above the inflamed area. Up to 2-3 hours, lymph was collected into a sterile vessel from the afferent and efferent vessels of the popliteal lymph node, and then cultured.

It can be seen from Table 2 that a greater number of bacteria are fixated by the lymph node on the inflamed side.

We also carried out experiments on dogs. In 3, we caused an inflammation in the lower leg on both sides by injecting 1 ml of a centrifuged meat-peptone staphylococcal culture. After 3 days, polyethylene canulae were introduced into the thoracic duct and into the lymphatic vessel of one of the hindlimbs, and after one day 5 milliard B. coli in 2-2,5 ml of fluid were injected into both hind feet. The dogs with inflammation of the limbs received the injection 4-5 cm above the inflamed area, and the control animals were given the injection in the corresponding place. Lymph was collected with aseptic precautions 2, 6, 12, 24, and 48 days later.

Table 3 shows the results of experiments on 6 dogs (3 healthy and 3 with inflammation of the lower leg). In the first group there were more bacteria in the

TABLE 2 Count of B. coli Commune in Afferent and Efferent Vessels of Cat Popliteal Lymph Node

Number of animal	In inflamed limb		In healthy limb	
	Number of bacteria per ml of lymph			
	flowing in	flowing out	flowing in	flowing out
1	2 015 000	24 700	780 000	13 000
2	2 600 000	500	2 275 000	508 500
3	1 755 000	9 750	1 170 000	195 000
4	975 000	1 400	520 000	325 000
5	3 575 000	58 500	2 210 000	260 000
6	1 170 000	700	1 950 000	780 000
7	637 000	400	1 066 000	19 000

TABLE 3 Count of B. Coli Commune in Lymph of Healthy Dogs and Dogs with Inflamed Hind Limbs

Condition of animal	Number of animal	Number of bacteria per ml of lymph									
		After 2 hours		After 6 hours		After 12 hours		After 24 hours		After 48 hours	
		in vessel	in duct	in vessel	in duct	in vessel	in duct	in vessel	in duct	in vessel	in duct
Normal	1	140 000	1 950	650 000	5 460	780 000	7 800	416 000	55 250		
	2	221 000	6 760	1 170 000	13 000	988 000	13 520				
	3	299 000	2 080	1 950 000	8 450			325 000	5 330	10 400	130
Inflamed	4	198 000	0	845 000	0		0		0		0
	5	312 000	120	1 040 000	390			364 000	0	66 300	0
	6	91 000	0	624 000	0			71 500	0		

thoracic lymph duct; in the second group the lymph was sterile in most cases. In both groups, the number of bacteria in the lymph of the lymphatic vessels was approximately the same, there is therefore a smaller spread of bacteria in the second group, and this may be due to an increased fixation by lymph nodes modified as a result of the previous inflammation.

There are however indications [5] that in the first few hours after infection, owing to the increased lymph flow, the barrier function of the lymph nodes is not increased but on the contrary diminished. In our experiments where the lymph nodes were perfused at a late stage in the inflammation the fixation remained high even when the perfusion pressure was increased 2-3 times. Therefore increasing lymph flow at the onset of inflammation may cause a spread of the bacteria, but at later dates, reactive changes in the node may change it from a passive filter into an effective barrier against invading bacteria.

When there is inflammation, an increased mechanical filtration by the node may be observed. The weight of the inflamed lymphatic nodes is $1^1/_2$-3 times greater than that in normal animals. Histological investigation reveals a marked hyperplasia of the cells of the node. Also, a study of the cells in the efferent lymphatic vessels of a node revealed a large number of granulocytes, and a marked phagocytosis when the staphylococcal culture was injected. These nonspecific phenomena may play an important part in increasing the barrier function of the nodes during the process of immunization. The results obtained agree with those of V. M. Berman and E. N. Slavskaya [2] who consider the barrier function of the whole body to be made up of two parts, a nonspecific phase, caused by the inflammatory reaction at the site of entry of the bacteria, and a general reaction to the parenteral injection of the antigen.

SUMMARY

Fixation of B. coli commune by the popliteal lymph nodes was studied as follows: Lymph nodes were perfused by a suspension of B. coli commune, and studies were made of lymph taken at operation by means of fistulae in the thoracic duct and lymphatic vessels of a dog's hind limb. The ability of the lymph node to fixate bacteria is increased by immunization, particularly when the immunizing culture is injected into the area corresponding to the given node. The barrier function of the nodes increases, particularly when inflammation is provoked in them either by infectious or noninfectious agents. The nonspecific phenomena, which include intensification of the mechanical filtration and increase of phagocytic activity of the cells of the node play a significant part in the increase of lymph node barrier function in immunization.

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