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Regional Blood Flow to the Lymph Node During the Immune Response*

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Summary

In 62 New Zealand white rabbits the regional blood flow to the popliteal lymph node was determined using the microsphere distribution and the $Rb^{86}Cl$ indicator methods. In 20 animals local immune response was induced with typhoid O antigen and in 7 with bovine serum albumin. The regional blood flow of the popliteal lymph node was 0.57 ml/gm/min. During the immune response the blood flow and weight changes were strictly proportional. The lymph node has the capacity to maintain its blood flow, even following singificant enlargement.

In previous microangiographic studies we observed significant hypervascularity during the primary immune response within the rabbit popliteal lymph node (1). In order to correlate these morphologic observations with physiologic data, this present investigation was designed to quantitate the regional blood flow to the lymph node and assess the changes during the immune response. The study was carried out by measuring the fractional uptake of $Rb^{86}Cl$ indicator (2) and the distribution of radioactive microspheres (3).

Materials and Methods

Sixty-two four-month old, male New Zealand white rabbits weighing approximately 2.5 kg were studied. Eighteen animals served as normal controls. In 20 animals the primary immune response was induced with 0.5 ml of salmonella group D (typhoid O) antigen and in 7 animals with 200 mg of bovine serum albumin (BSA) diluted in physiologic saline. Subcutaneous injection of the antigen was made in the left hind foot pad; the right side served as the internal control (Table 1). The time interval from the injection to analysis was 1–11 days.

Table 1 Cross Tabulation of the Experimental Methods and Types of Antigens

Antigen	$Rb^{86}Cl$	Microspheres	Total
None	21	7	28
Typhoid O	20	7	27
BSA	7	–	7
Total	48	14	62

$Rb^{86}Cl$ Method. Under Diabutal anesthesia a PE-190 polyethylene tube was inserted into the right jugular vein. An injection of 100 μc of $Rb^{86}Cl$ diluted in 1 ml of physiologic saline was flushed with 2 ml of physiologic saline. After 45 sec, animals were sacrificed by injection of

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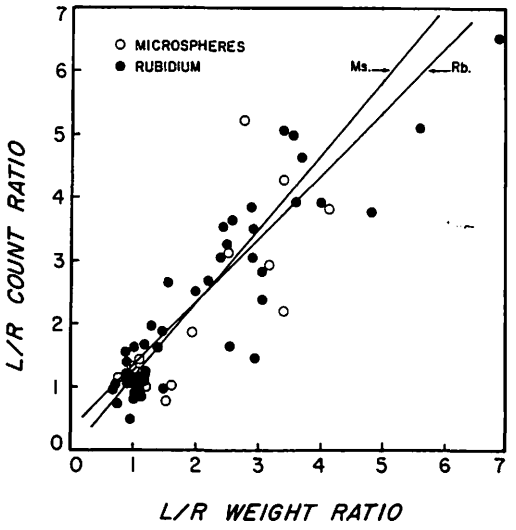

Fig. 1 The relationship between blood flow (ordinate) and weight (abscissa) ratios between the left and right popliteal lymph nodes. The distribution is labeled according to the method used.

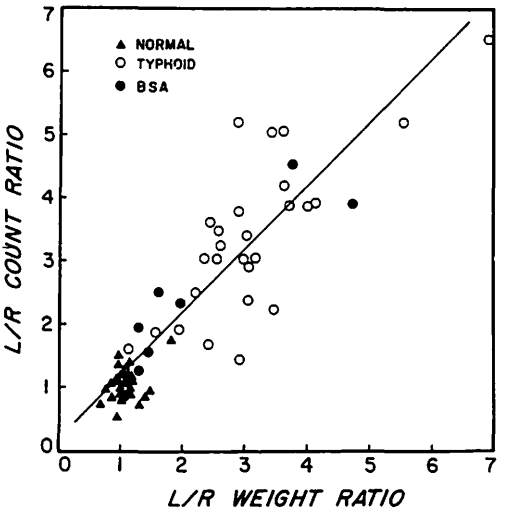

Fig. 2 The relationship between blood flow (ordinate) and weight (abscissa) ratios between the left and right popliteal lymph nodes. The distribution is labeled according to the antigen. The immune response was induced on the left, the right side was the control.

8 ml of saturated KCL through the same catheter. Both popliteal lymph nodes and the kidneys were carefully dissected, removed and weighed. The radioactivity was measured in a Nuclear Chicago dual-channel well counter. Because of their size, the kidneys were subdivided into four parts. The activity in 1 gm of tissue was calculated.

Microsphere Method. Under Diabutal anesthesia a polyethylene catheter was introduced into the right carotid artery and advanced to the left ventricle under pressure monitoring. Approximately 2 to 6×10^5 of 15 μ 3 $\bar{M}$ tracer brand microspheres labeled with Sr^{85} and suspended in 2 to 4 ml of 63% sucrose solution was stirred, ultrasonicated, and injected through the catheter at a rate of 2 ml/min. This amount was required to deliver more than 40 microspheres to an individual popliteal lymph node. The activity of a single microsphere was determined. The microsphere injection was followed by a 2–4 ml physiologic saline wash. Both the left ventricular and aortic pressures were recorded after the microsphere injection. At the conclusion of the experiment the animals were sacrificed with a Diabutal overdose. The radioactivity was measured in a Nuclear Chicago dual-channel well counter; the activity per gram of tissue was calculated.

Results

Figure 1 shows the correlation between weight and blood flow changes plotted separately for the rubidium and microsphere methods. Table 2 summarizes the statistical analysis. By both methods the blood flow and weight changes were strictly proportional. The observed difference between the microsphere and rubidium methods was not significant (Table 2).

Figure 2 demonstrates the relationship between weight and blood flow changes plotted separately for the typhoid and BSA induced immune responses. The statistical analysis of this data is also summarized in Table 2.

The blood flow and weight changes were proportional during the immune responses induced by both antigens (Fig. 2). The observed difference was not significant (Table 2).
The maximum weight increase occurred earlier in the typhoid O experiments.

Table 2 Correlation Between Blood Flow Changes and Weight Changes

	r	b
Rubidium experiments	0.91	0.99 ± .06
Microsphere experiments	0.82	1.087 ± .2
All typhoid experiments	0.75	0.86 ± .15
All BSA experiments	0.88	0.784 ± .19
All experiments	0.9	1.0 ± .06

r = correlation coefficient; b = slope

In the control rabbits the kidney to lymph node ratio per gram of tissue was determined. Accepting 3.49 ml/gm/min of tissue as the mean renal blood flow in the anesthesized rabbit (4), the mean blood flow to the popliteal lymph node was calculated to be 0.57 ml/gm/min.

Discussion

To measure the regional blood flow to a small anatomic structure such as the popliteal lymph node, which is far removed from the heart and the large arteries, we felt that the most practical approach was to study the fractional uptake of an indicator such as $RB^{86}Cl$ (2) or the distribution of radioactive microspheres (3). With the microsphere method repeated determinations can be made with different radionuclides without sacrificing the animal. Because of the small size of the feeding arteries, we decided to use 15 μ microsphere which can enter the lymph node but are too large to reach the capillary bed. To assure that large numbers of microspheres reached the node, we injected up to 6×10^5 beads which delivered more than forty spheres to a single node (4). It has been suggested recently (4), however, that at least 400 microspheres are required to be present within a sample to reduce the potential statistical errors.

An indicator such as $Rb^{86}Cl$ reaches a stable level in all organs with the exception of the brain in 6 to 9 sec (2) and is maintained at this level until 64 sec. The fractional uptake of this indicator therefore must be equal to the blood flow fraction of the cardiac output. Unlike the microsphere method, this approach permits only a single determination because the animal must be sacrificed during the procedure.

The excellent statistical agreement between the microsphere and rubidium experiments is in line with previous reports (5, 6). The smaller statistical fluctuation of the rubidium method (Table 2) makes it preferable in the study of the regional blood flow of the lymph node.

Using the $Rb^{86}Cl$ indicator we established that the kidney to lymph node blood flow ratio is 18.3%, which in turn represents a flow of 0.57 ml/gm/min of tissue. This blood flow range is similar to that of the colon and slightly smaller than the adrenals.

The blood flow through the lymphoid tissues gained increased importance after recognition of lymphocyte transport from blood to the lymphoid tissues (7, 8, 9, 10), and of the role of recirculating lymphocytes in the dissemination of the immune response (11, 12, 13).

Enlargement of the regional lymph node during the primary immune response is accompanied by marked hypervascularity (1). Considering the 4–6 fold increase in size of the popliteal lymph node during the primary immune response, it is quite remarkable that the lymph node has the capacity to maintain its blood flow proportionally to the weight increase (Fig. 2).

While there is a strict linear relationship between lymph node size and its blood flow, one may assume that during the hypervascular phase of the immune response blood flow is slower because the volume of the microvascular bed has increased. A slower flow could facilitate the cellular and humoral exchange between the circulating blood and the lymphoid tissues.

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