THE FLOW OF BLOOD TO LYMPH NODES AND ITS RELATION TO LYMPHOCYTE TRAFFIC AND THE IMMUNE RESPONSE*

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The immune response of single lymph nodes encompasses a complex interaction of the blood vascular and lymphatic microcirculations. An appropriate environment exists or is created for the interaction of various lymphocyte populations and mononuclear phagocytic cells. In addition, the vascular endothelial cells are directly involved in the response, insomuch as they facilitate the movement of lymphocytes from the blood (1). The capacity of different lymphocytes to respond to antigens appears to be precommitted (2) and, therefore, the mechanisms that permit the maximum number of cells of the lymphocyte pool to screen a given antigen during any immune response are important.

When the components of the immune response are recorded by quantitatively collecting the efferent lymph, the two most characteristic cellular features of all responses are the increase in lymphocyte traffic through the node and the appearance of transformed blast cells in the lymph (3–5). The mechanism whereby antigen stimulates an increase in this lymphocyte traffic is unknown but it is clear from experimental data that most of the cells in the efferent lymph are derived from the blood (6). Antigen may either increase the proportion of lymphocytes that leave the blood within the node or it may increase the blood flow to the node, resulting in a greater traffic of cells into the efferent lymph.

The present experiments have been designed to measure the blood flow to single lymph nodes and to determine the effect of antigen on this flow. The measurement of blood flow to large organs has been successfully accomplished with radioactive labeled microspheres (7). When such microspheres are introduced into the arterial system, they are of such a size that they distribute with the blood and lodge in the microcirculation on their passage through a microcirculatory bed. The distribution of the cardiac output to various regions can thus be determined (8). Experiments have been done to test the feasibility of using this method in combination with lymphatic catheterization to determine the blood flow to normal and stimulated lymph nodes of sheep and concurrently to measure the cell traffic through such nodes. Rabbits were used to study the changes in lymph node blood flow at several times after antigenic challenge.

Materials and Methods

Animals. Young sheep of either sex, 4–8 mo old, of the Norfolk breed, and female New Zealand White rabbits, 2.5–3 kg, were used.

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Catheterization. While the sheep were under anesthetic (Nembutal sodium, Abbott Laboratories, North Chicago, Ill.) polyethylene catheters were positioned in the efferent lymphatic vessels of either popliteal or prefemoral lymph nodes as originally described by Hall and Morris (3). Aseptic techniques were followed. "Red Kifa" catheters were introduced into the ascending aorta via the common carotid artery and positioned at the level of the aortic valve with the aid of a fluorescope. Sheep were allowed to recover for at least 1 day before quantitative lymph collections were recorded or before the microspheres were administered. Carotid catheters were positioned in rabbits as described previously (9).

Injection of Microspheres. Carbonized microspheres labeled with ^{85}Sr (10 mCi/g) of 15 ± 5 μm size (3M Company, London, Ontario) were injected in volumes of 2–3 ml. The suspending medium was 10% dextran. With a two-way stopcock attached to the arterial catheter, the syringe was washed and the residual microspheres were flushed into the animal with physiological saline. From 5 to 10 × 10^6 microspheres (determined with a hemocytometer) were injected, which corresponded to approximately 5–30 × 10^6 cpm, depending upon isotope decay. The number of microspheres injected in both species was determined by counting two aliquots of the injected suspension. Sheep were unanesthetized and standing unrestrained in metabolism cages during all experiments. They were sacrificed with a lethal injection of anesthetic either a few minutes after introduction of the microspheres or up to several days later.

Approximately 2 × 10^6 microspheres were injected into rabbits. The microspheres were injected while the rabbit was under the same anesthetic used for catheter placement.

Counting Methods. Organs and tissues were removed, weighed, and either ashed or cut into appropriate sizes for counting in plastic tubes. In most animals kidneys, spleen, thymus, eyes, and popliteal, prefemoral, and prescapular lymph nodes were removed. In some animals internal lymph nodes such as the lumbar, iliac, renal, hepatic, mesenteric, and para-aortic nodes were also removed. Samples did not exceed a depth of 3 cm in the counting tubes. A Nuclear Chicago single-channel gamma spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill.) was used. Lymphocyte counts were done with a hemocytometer and the differential counts were done on Leishman-stained smears.

Lymph Node Casting. Microfil (Canton Bio-Medical Products, Boulder, Colo.) was infused into the abdominal aorta immediately after the rabbits were killed. The tissue was dehydrated and cleared as described previously (9).

Results

The Distribution of Microspheres to Lymphoid and Other Organs. When the number or total radioactivity of the microspheres injected is known, the distribution to various organs can be considered in relation to this number. With certain assumptions this value can be considered as a reflection of the cardiac output of the animal (8). Table I shows the distribution and standard error for some organs of the sheep. The distribution per gram of organ is also compared. On a weight basis, the blood flow to a lymph node was found to be equivalent to the blood flow to an equal weight of spleen. The kidney flow was considerably higher. In the eye the localization of microspheres was predominantly in the choroid region. The lowest flow per gram was to the thymus and the variation in this flow between different animals was also large (SE of 29%), presumably reflecting the variation in maturity of this organ. Thymus weight varied from 8.2 to 22.2 g.

The mean blood flow to the lymph nodes (per gram) was 0.012% of the cardiac output. The average weight of the popliteal and prefemoral nodes was 1.2 g. Prescapular nodes averaged 3.2 g.

With 3.3 liter/min as an average cardiac output of sheep of this age and size (11), the blood flow to a lymph node weighing 1 g was 3.3 liter/min × 0.012% = 0.396 ml/min or 24.06 ml/h. A differential count on blood from jugular veins of these sheep gave a mean blood lymphocyte count of 4.66 ± 0.43 × 10^6 cells/ml.

TABLE I
Blood Flow to Organs of Sheep, Based on the Percentage of Microspheres Adminstered (Percent of Cardiac Output)

Organ	Cardiac output	Cardiac output
	%	%/g
Kidneys	11.9 ± 1.6	0.086
Eyes	0.2 ± 0.07	0.016
Spleen	1.8 ± 0.30	0.012
Thymus	0.051 ± 0.015	0.004
Lymph nodes, g^{-1}	0.012 ± 0.001	0.012

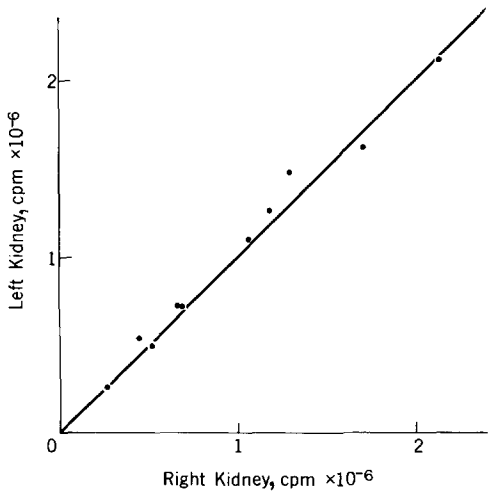

FIG. 1. The distribution of microspheres to the kidneys. The total radioactivity in the left kidney is plotted against the right kidney for each animal. The theoretical line of identity is drawn.

Therefore, $4.66 \times 10^6 \times 24.06$ or 1.12×10^8 lymphocytes/h entered such a lymph node in the blood. In the Discussion this is considered in relation to the number of lymphocytes leaving such a node, and the efficiency of lymphocyte removal by a node is calculated.

Assessment of the Symmetry of Microsphere Distribution. If microspheres are adequately mixed with the blood leaving the left ventricle, then they should segregate evenly to organs having a bilaterally symmetrical distribution. Fig. 1 shows the distribution to the two kidneys in the 10 sheep studied. The radioactivity of the left kidney is plotted against the same animal's right kidney. Fig. 2 shows the distribution to the left and right eye in the same sheep. In spite of the catheter in one carotid artery, circulation in the head was such that the left and right eye received almost identical numbers of microspheres in every animal examined.

The popliteal, prefemoral, and prescapular lymph nodes, as well as nodes of the lumbar chain, are bilaterally paired, and Fig. 3 shows the variation between the number (cpm) of microspheres localized in the nodes of the right side compared with the left side. It is clear that the variation is greater than was found in the kidneys, even though the differences between contralateral nodes

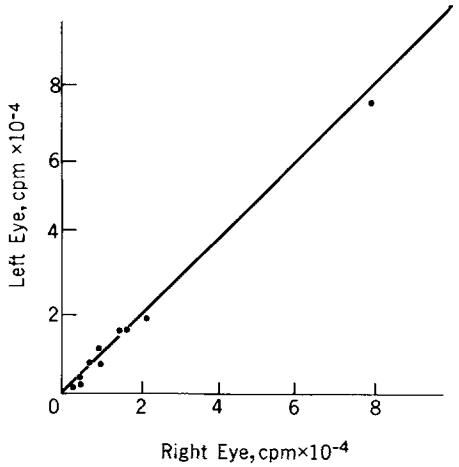

FIG. 2. The distribution of microspheres to the eyes. The total radioactivity in the left eye is plotted against the right eye for each animal. The theoretical line of identity is drawn.

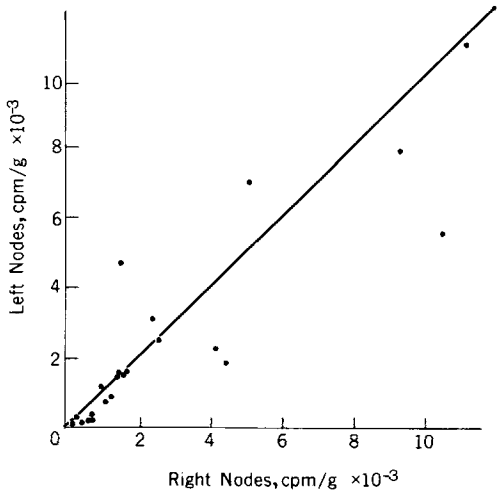

FIG. 3. The distribution of microspheres to anatomically similar lymph nodes. The left node is plotted against the right node in each case. The theoretical line of identity is drawn.

was always less than twofold. When the weights of these nodes were plotted in a similar manner (left versus right), the random variation was a similar order of magnitude (less than twofold) (Fig. 4). It was concluded that the variation in weight and blood flow was most probably due to the natural variation in the immunological status of different lymph nodes.

In rabbits, there was also the same degree of symmetry in the distribution of microspheres.

Distribution of Microspheres within Lymph Nodes. The lymph nodes of the sheep are sufficiently large that slices 2–4 mm thick can be made with a sharp blade. It is then feasible to cut fragments of cortex and medulla separately. When this was done, the fragments were weighed and the radioactivity was counted, there was an average of 3.10 ± 0.37 (four nodes) times more micro-

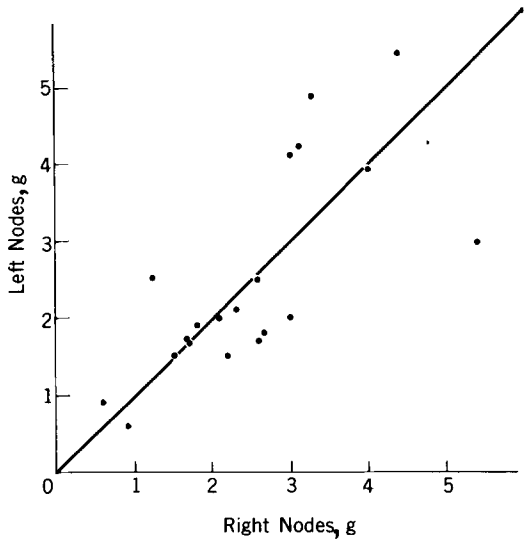

FIG. 4. Weight variations of anatomically similar lymph nodes. The left node is plotted against the right node in each case. The theoretical line of identity is drawn.

spheres localized in the cortex than in the medulla. This result was confirmed by dehydrating a lymph node in successive changes of increasing ethyl alcohol concentration and then clearing the tissue with methyl salicylate. With the aid of a dissecting microscope, individual microspheres were observed and counted and most of the microspheres were distributed in the cortex. Furthermore, there was no evidence of significant clumping of microspheres.

Effect of the Immune Response on the Localization of Microspheres in Sheep Lymph Nodes. Individual sheep lymph nodes were challenged with either 100 μg of purified protein derivative (PPD)[1] (Connaught Laboratories, Toronto) or with injections of allogeneic lymphocytes. Animals receiving PPD had received BCG 1–2 mo before. Injections were made at subcutaneous sites in the drainage area of the appropriate node. Allogeneic lymphocytes were obtained from the efferent lymph of a sheep and the cells were injected suspended in 0.9% NaCl in doses up to a total of 5×10^8 cells, in multiple sites. At various times (3–6 days) after challenge, the animals were injected with microspheres and sacrificed, and individual nodes were weighed and counted.

Table II shows a comparison between five such nodes and the values obtained from normal nodes. The stimulated nodes all had a larger number of trapped microspheres even when compared on an equal weight basis. The average of the stimulated nodes was 4.5 times the normal nodes. A comparison of the difference between these two mean values with an independent t test gave a P value < 0.001.

A second comparison was made with the same lymph nodes. The stimulated node was compared with its contralateral normal node, since the bilateral distribution of microspheres was shown to vary less than twofold. Table III shows this comparison for both the weight and the radioactivity per gram.

[1] *Abbreviations used in this paper:* BCG, bacille Calmette-Guérin; KLH, keyhole limpet hemocyanin; PPD, purified protein derivative.

TABLE II
Blood Flow to Sheep Lymph Nodes

	Cardiac output
	%/g
Stimulated nodes (5)	0.054 ± 0.020
Normal nodes (84)	0.012 ± 0.001

TABLE III
Comparison of Stimulated Nodes and Contralateral Unstimulated Nodes

Antigen	Ratio of stimulated nodes to contralateral control nodes	
	Weight	Microsphere count
		g^{-1}
Tuberculin, day 3	1.65	3.99
Allogeneic lymphocytes, day 5	1.44	6.48
Allogeneic lymphocytes, day 4	1.70	4.66
Allogeneic lymphocytes, day 5	0.87	4.26
Allogeneic lymphocytes, day 6	1.43	1.59
Mean	1.42	4.18

Stimulated nodes weighed an average of 1.42 times the control, and the microsphere localization per gram was 4.18 times the control.

The Administration of Microspheres during Lymph Drainage of Single Lymph Nodes. By monitoring the lymphocyte output in the efferent lymph, it was possible to determine if the localization of microspheres within the node affected the functional integrity of the node in terms of the movement of lymphocytes through it. In four experiments no evidence of an effect of the microspheres on either the flow rate of the lymph or on the cell output of the node was evident. No effect of the microspheres on gross physiological parameters of the animals has been observed. Higher doses of microspheres have not yet been used to determine the tolerance of a lymph node to such treatment.

In another experiment, we combined lymph drainage and microsphere injection after stimulation of the node with antigen. In the example shown in Fig. 5, a lymphatic catheter was positioned in the efferent vessel of the left popliteal node and of the right prefemoral node. An aortic catheter was also positioned. Allogeneic lymphocytes were injected into the drainage area of the left popliteal node only. Evans blue was mixed with the injected cells and the dye appeared in the efferent lymph within minutes and cleared over the next day. At 103 h after the injection of antigen, microspheres were injected via the carotid catheter. There was no evidence of an immune response in the prefemoral lymph and the microsphere injection did not detectably alter the lymphocyte traffic through this node. However, a typical response to allogeneic lymphocytes was recorded in the popliteal lymph with an increase in the lymphocyte output as well as the subsequent appearance of increased numbers of transformed, blast cells. When the animal was sacrificed at the end of this experiment, the weight and the radioactivity of the nodes was determined. As shown

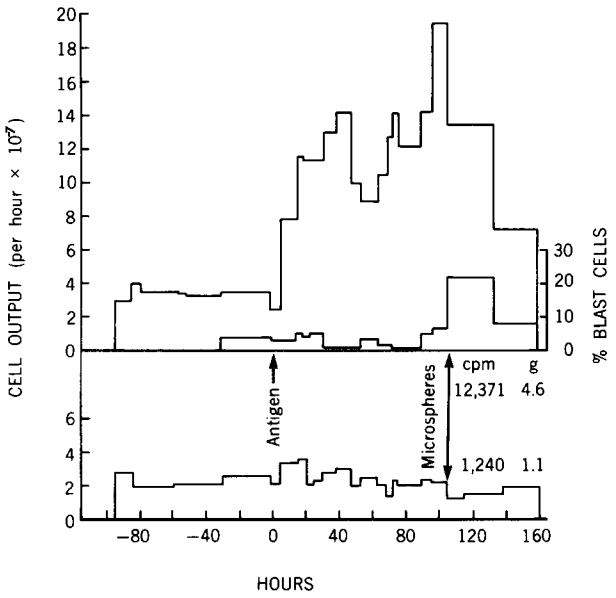

FIG. 5. The cellular response in the efferent lymph of a stimulated (top) and normal lymph node from the same sheep. The microsphere count (cpm) and weight (g) of the nodes were determined at 160 h.

in Fig. 5, the challenged node received approximately 10 times the number of microspheres and weighed four times more than the prefemoral node. The increased blood flow occurred at a time when the lymphocyte output was enhanced.

In a subsequent experiment, the microspheres were injected at the end of the response after the cell output had returned to a pre-injection level. In this experiment the microsphere counts in the control node and in the stimulated node were similar (4,462 and 4,587 cpm/g, respectively) although since the stimulated node weighed 2.7 g and the contralateral control node 1.2 g, the total blood flow to the stimulated node was still enhanced.

Blood Flow to Rabbit Lymph Nodes during a Primary Immune Response. In order to measure the kinetics of blood flow throughout the course of an immune response, 40 rabbits were studied. The distribution of the cardiac output to some organs in a group of six of these rabbits can be seen in Table IV. The lymph node values were comparable with those found for sheep (Table I). An average, single popliteal node of the rabbit received 0.011 ± 0.004% of the cardiac output and weighed 188 mg.[2]

The blood flow to the spleen of the rabbit was lower than that found in other species studied in our laboratory by this method (rabbit 0.32 ± 0.11; mouse 1.0 ± 0.13; sheep 1.8 ± 0.3). The symmetry of distribution to the right and left side of the animal, however, was similar to that shown for the sheep.

[2] Using tracer Sephadex (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.), a product similar to microspheres, the fractionation of the cardiac output in mice has been possible. The average popliteal node from a 40-g, Swiss mouse received 0.018% of the cardiac output. (Hay and Hunter, unpublished observation).

TABLE IV
Fractionation of Cardiac Output in Rabbits

Organ	Percent of cardiac output
Left kidney	7.08 ± 1.20
Right kidney	6.38 ± 1.05
Spleen	0.32 ± 0.11
Liver	2.79 ± 0.99
Heart	4.37 ± 1.65
Lung	4.99 ± 1.02
Eyes	0.44 ± 0.22
Node*	0.011 ± 0.004

* Popliteal node average weight (9), 188 ± 16 mg.

The primary immune response to keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, Calif.) was examined by injecting 2 mg into the hind footpad of a rabbit. The opposing footpad received the same volume (0.1 ml) of 0.9% NaCl. Microspheres were injected via the carotid catheter at various times after the antigen. Groups of three or more rabbits were sacrificed at each time and the popliteal nodes weighed and the radioactivity was counted. The blood flow was significantly increased ($P < 0.05$) within 1.5 h after injection. The flow continued to increase until 14 h and then decreased until near 24 h. It was, however, still significantly increased compared with resting nodes. During the subsequent 3 days of the immune response it increased again, and averaged three times the normal flow. Fig. 6 shows the changes in blood flow and the changes in wet weight of these nodes. As was found in the sheep, the blood flow per 100 mg of node weight increased during the immune response. We have tentatively labeled the early part of the response the "hyperemia" phase and the later part an "angiogenesis" phase. Fig. 7 illustrates the increased number of vessels of a rabbit popliteal node stimulated 5 days previously with a primary injection of KLH, compared with a saline-injected contralateral node.

Discussion

The validity of the use of microspheres to measure regional blood flow has been established in several studies (7–9, 11, 13, 14). Ideally, microspheres should be well mixed at the site of injection so that they distribute with the blood, they should be trapped in the microcirculation during their first passage, and they should not disturb the circulation. In order to minimize recirculation, the microspheres should be of a sufficiently large diameter. It has generally been found that less than 1.5%× of 50- or 80-μm-sized microspheres reach the venous circulation (7, 8, 14). With 15-μm microspheres, the recirculation appears to be less than 10%, and Hales (11) has found that only 1.6% of such microspheres bypassed the systemic circulation in conscious sheep. At the same time, sufficient microspheres must be trapped in any organ or region to minimize the counting and sampling errors.

In the present studies adequate mixing of the microspheres in the ascending aorta took place, since the distribution of microspheres to bilateral organs was symmetrical. However, standard error of the mean percentage of cardiac output in the eyes was 35% and this was considerably greater than was found for the

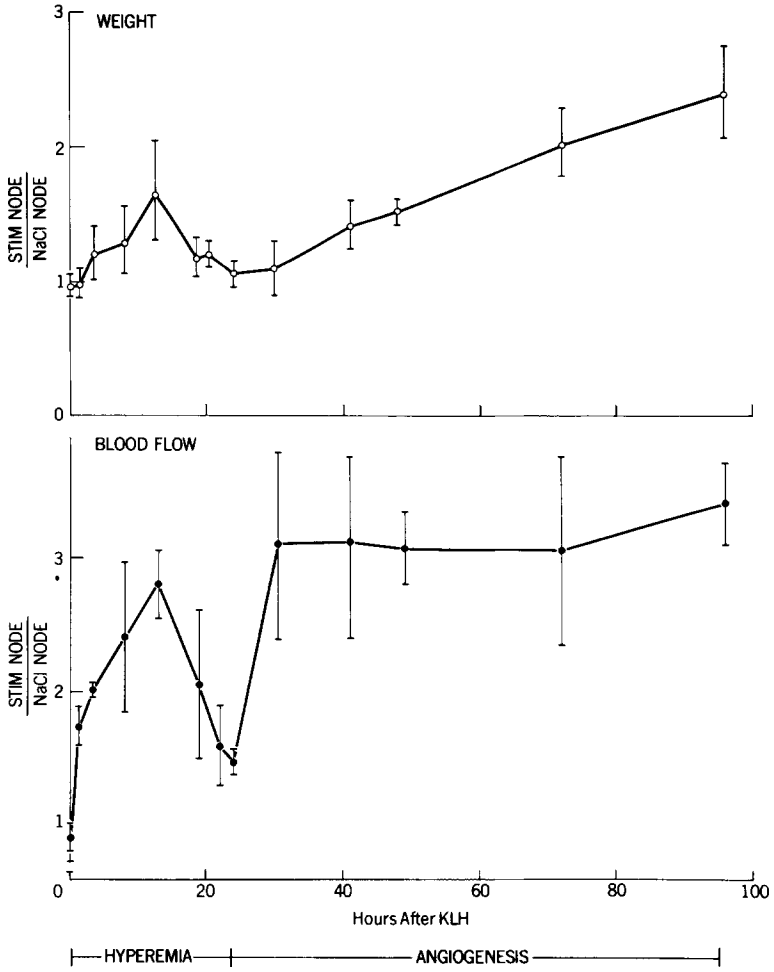

FIG. 6. Changes in the wet weight and relative blood flow of rabbit popliteal lymph nodes during a primary immune response.

kidneys. While this may have been due to sampling errors associated with fewer microspheres in the eyes, it is also possible that slight mispositioning of the catheter could result in a higher proportion of microspheres going to the head. For this reason bilateral comparisons were considered to be more valid for relative comparisons, as shown for the distribution of microspheres to the lymph nodes. Other investigators have used the left ventricular positioning of the catheter end but we chose not to cross the aortic valve, thereby lessening the risk of causing aortic insufficiency and ventricular irritability. Another source of error relates to the determination of the exact number of microspheres injected due to the settling properties of the microspheres. Nevertheless standard errors in the order of 10% could be obtained with the number of sheep studied.

Comparison with other studies can be most readily made with the distribution to the kidneys. In studies on older sheep Hales (11) found a kidney distribution

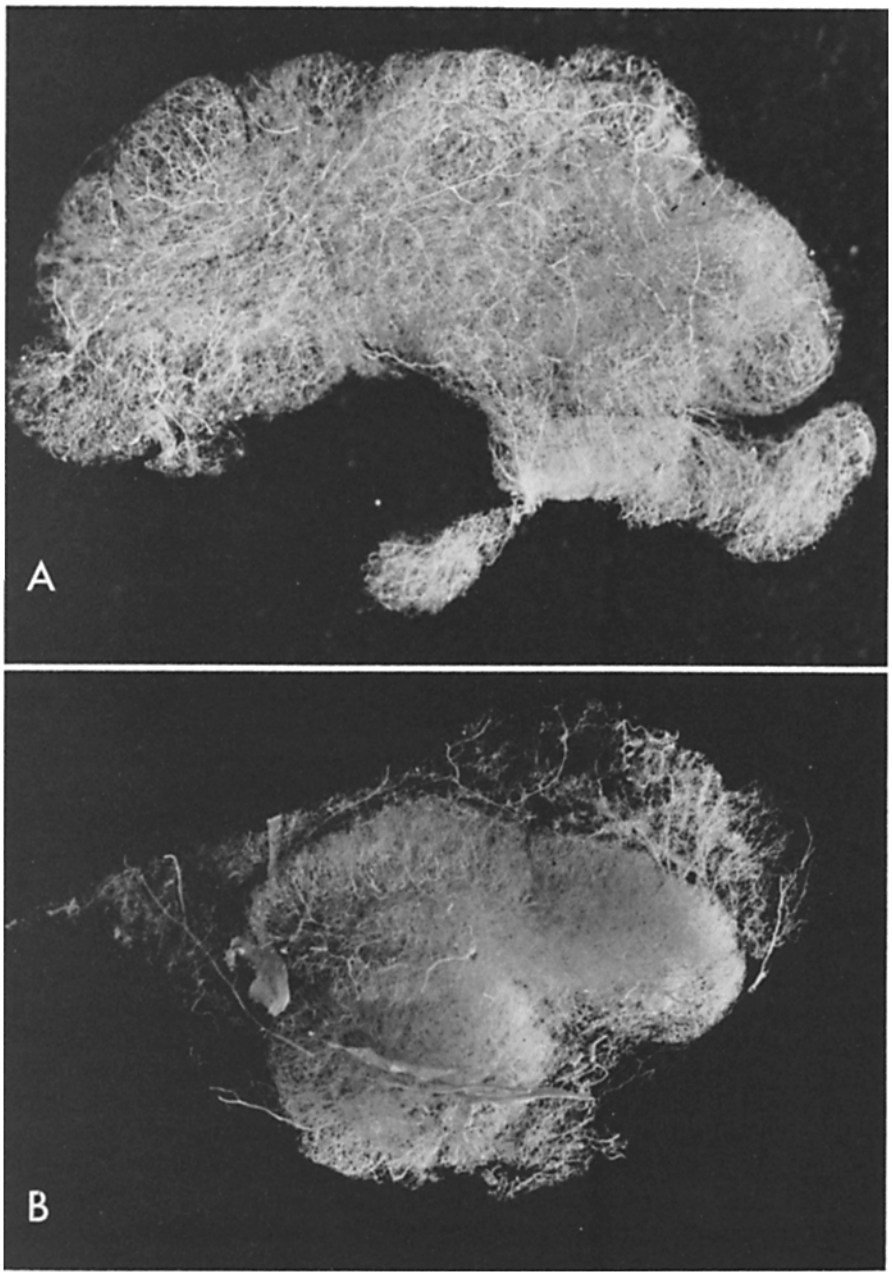

FIG. 7. Microfil (Canton Bio-Medical Products) cast of a KLH-stimulated rabbit popliteal lymph node (A) and the contralateral normal node (B). × 12.

of 16.54% of the cardiac output but in lambs 12.68%. The value 11.9% in the present study is closer to the lamb values. Considering the average weight of the kidneys in the present study, we used animals comparable in size to lambs. Some other reported values for kidney distribution are 12.3% in the rhesus

monkey, 11.1% in the dog, 10% in the newborn lamb, and 16.2% in the rabbit (11). The distribution to the rabbit kidneys in the present study was 13.5%.

The blood flow to single lymph nodes of sheep was calculated to average 24.06 ml/h per g. All of the figures required for this calculation were determined in these animals except for the cardiac output of the sheep. An appropriate value was chosen from the literature. Accurate measurements of cardiac output can be made by withdrawing arterial reference samples during the injection of the microspheres. In this way the cardiac output at the time when the microspheres localized is determined (13).

Experiments of Hall and Morris have shown that the average cell output in the efferent lymph from a 1-g lymph node of the sheep was 3×10^7/h. The examples shown in the present study show values close to this figure. Furthermore, Hall and Morris measured the number of cells formed within the node and also the input of lymphocytes from the afferent lymph vessels (15). They concluded that 95% or more of the efferent cells were derived from the blood. From the blood flow and blood lymphocyte count, the input of blood-borne lymphocytes is 1.12×10^8 while the efferent lymph output of cells derived from the blood is 3×10^7/h less 5%, or 2.85×10^7/hour. Therefore, such a lymph node removes $(1.12 \times 10^8)/(2.85 \times 10^7)$ or an equivalent of one in four lymphocytes which enter in the blood. The remaining three out of four lymphocytes presumably leave the node in the venous blood. This highly selective process occurs under normal conditions in or near the cortical regions of the node presumably at the postcapillary venules. Hall (16) estimated 10–15% of lymphocytes left the blood within the node. In these experiments blood flow was estimated by simply cutting the node vessels and measuring the volume of blood collected in a measured time period.

The administration of antigen clearly enhances the traffic of lymphocytes through the regional lymph node, as shown in Fig. 5 and described elsewhere (4, 5, 17). From the present studies it is clear also that antigen increases the blood flow to the regional node. We conclude that the increase in lymphocyte traffic is a direct consequence of this increase in blood flow for the following reasons. Both the traffic and the blood flow increase by a similar order of magnitude (about four fold). An alternative mechanism to account for the increase in lymphocytes delivered from the blood would be chemotaxis. Since convincing evidence for antigen-induced lymphocyte chemotaxis does not exist, we favor the enhanced blood flow as the simpler explanation and the more likely cause. From the rabbit data presented, the increase in blood flow occurred in two distinct phases. It is common for two separate peaks of lymphocyte output to appear in the efferent lymph of stimulated sheep lymph nodes (Fig. 5). The characteristics of the "recruitment" peak have been described (17). Furthermore, the experiments of Cahill et al. (18) demonstrated that the number of intravenously injected ^{51}Cr-labeled, isologous lymphocytes increased in the lymph node within 3 h after antigen. This occured before their appearance in the efferent lymph since there is a certain transit time from blood to lymph. Although simultaneous measurements of traffic and blood flow are necessary to draw firm conclusions, the two peaks of cell output appear later than the two peaks of increased blood flow. This time difference would correspond to the transit time through the node. Finally,

in separate experiments, there was a correlation between the degree of cell traffic through cellular hypersensitivity lesions in the skin and the increased blood flow to such lesions.[3] We conclude that a similar cause-and-effect relationship applies in both the node and the skin reactions.

An increase in regional blood flow can be due to local vasodilation, to new vessel growth, or to both. Since the early increase in blood flow to the stimulated rabbit nodes was evident within 1.5 h, and reached a maximum near 14 h, it seems unlikely that this was a consequence of endothelial cell division. Hyperemia, caused by the release of a local mediator, is a more plausible explanation. This mediator has not been identified. However, in other quantitative studies on hyperemia induced by known mediators, the E-type prostaglandins were found to be much more potent than either histamine or bradykinin in this respect (19). The possible involvement of these compounds is being investigated.

It is interesting to note that Gershon et al. (20) have implicated a role for vasoactive amines in the infiltration of mononuclear cells in delayed hypersensitivity lesions in mice and have speculated on a role for such compounds in the "trapping" of lymphocytes in lymphoid organs. Although we would disagree with "trapping" as an appropriate description of these antigen-induced changes in lymphocyte migration (see 18), the possibility of an early involvement of vasoactive amines in the enhancement of blood flow is plausible.

The second phase of increased blood flow is due, in part at least, to angiogenesis, since the vascular bed was obviously increased 5 days after KLH. Endothelial cell division in the immune response has been described (21, 22). Angiogenesis does not preclude a component of hyperemia as well. It is difficult to quantitate the proportion of the antigen-induced increase in lymph node weight due to cell infiltration, cell division, or plasma accumulation, but quantitative kinetics on the entry and exit rates of the various components makes such a realization more feasible than was formerly possible.

The proportion of the mobilizable lymphocyte pool passing through a popliteal node in the blood can be determined from this data. A node weighing 1.2 g increased an average 1.42 times and the blood flow per gram increased 4.18 times after antigen. During an immune response lasting 5 days, such as the one described in the sheep, $1.2 \times 1.42 \times 4.18 \times 24.06$ ml/h $\times$ 144 h = 24.7 liters of blood passed through the node. The mobilizable lymphocyte pool has been estimated at 10 times the blood lymphocyte pool (23). This represents, therefore, approximately six times the blood volume and an equivalent to 60% of the entire mobilizable lymphocyte pool.

Summary

The blood flow to individual lymph nodes of sheep and rabbits has been determined with ^{85}Sr-labeled microspheres. A popliteal node of the sheep received 0.014% of the cardiac output and a comparable node in the rabbit 0.011%. A sheep lymph node weighing 1 g received an average of 24 ml/h of blood. It was calculated that there was a highly selective removal of lymphocytes by the node

[3] Hay, J. B., B. B. Hobbs, M. G. Johnston, and H. Z. Movat. 1976. Increases in blood flow, vascular permeability and lympocyte traffic in cellular hypersensitivity reactions. Submitted for publication.

and that an equivalent to one in every four lymphocytes that entered a normal lymph node migrated out of the blood, through the substance of the node, and into the efferent lymph. During the immune response to either allogeneic lymphocytes or tuberculin, the blood flow to sheep lymph nodes, even without considering the increase in node weight, increased an average of fourfold. During the primary immune response in the rabbit to keyhole limpet hemocyanin, the blood flow increased threefold.

The increase in blood flow preceded the antigen-induced increase in lymphocyte traffic recorded in the efferent lymph. The early phase of increased blood flow was considered to be due to hyperemia, whereas the latter phase had a significant angiogenesis component.

It was calculated that an equivalent to 60% of the entire mobilizable pool of lymphocytes could pass through an average lymph node in the blood during an immune response lasting 5 days.

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References

1. Marchesi, V. T., and J. L. Gowans. 1964. The migration of lymphocytes through the endothelium of venules in lymph nodes. An electron microscope study. *Proc. R. Soc. Lond. B. Biol. Sci.* **159**:283.
2. Burnet, F. M. 1959. The clonal selection theory of acquired immunity. Cambridge University Press, London, U. K.
3. Hall, J. G., and B. Morris. 1962. The output of cells in the lymph from the popliteal node of sheep. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **47**:360.
4. Hall, J. G., B. Morris, G. D. Moreno, and M. C. Bessis. 1967. The ultrastructure and function of cells in lymph following antigenic stimulation. *J. Exp. Med.* **125**:99.
5. Hay, J. B., M. J. Murphy, B. Morris, and M. C. Bessis. 1972. Quantitative studies on the proliferation and differentiation of antibody-forming cells in lymph. *Am. J. Pathol.* **66**:1.
6. Hall, J. G. 1974. Observations on the migration and localization of lymphoid cells. *Prog. Immunol.*, 11, 3, 15.
7. Rudolph, A. M., and M. A. Heymann. 1967. The circulation of the fetus in utero. Methods for studying distribution of blood flow, cardiac output and organ blood flow. *Circ. Res.* **21**:163.
8. Neutze, J. M., F. Wyler, and A. M. Rudolph. 1968. Use of radioactive microspheres to assess distribution of cardiac output in rabbits. *Am. J. Physiol.* **215**:486.
10. Hay, J. B., M. G. Johnston, B. B. Hobbs, and H. Z. Movat. 1975. The use of radioactive microspheres to quantitate hyperemia in dermal inflammatory sites. *Proc. Soc. Exp. Biol. Med.* **150**:641.
10. Davidson, J. W., B. B. Hobbs, and A. L. Fletch. 1972. The microcirculatory unit of the mammalian lymph node. *Bibl. Anat.* **11**:423.
11. Hales, J. R. S. 1973. Radioactive microsphere measurement of cardiac output and regional tissue blood flow in the sheep. *Pflügers Arch. Eur. J. Physiol.* **344**:119.
12. Buchberg, G. D., J. C. Luck, D. B. Payne, J. I. E. Hoffman, J. P. Archie, and D. E. Fischer. 1971. Some sources of error in measuring regional blood flow with radioactive microspheres. *J. Appl. Physiol.* **31**:598.
13. Bartrum, R. J., D. M. Berkowitz, and N. K. Nollenberg. 1974. A simple radioactive

microsphere method for measuring regional flow and cardiac output. *Invest. Radiol.* **9**:126.

14. Kaihara, S., P. D. Van Heerden, T. Migita, and H. N. Wagner. 1968. Measurement of distribution of cardiac output. *J. Appl. Physiol.* **25**:696.
15. Hall, J. G., and B. Morris. 1965. The origin of the cells in the efferent lymph from a single lymph node. *J. Exp. Med.* **121**:901.
16. Hall, J. G. 1967. Quantitative aspects of the recirculation of lymphocytes; an analysis of data from experiments on sheep. *Q. J. Exp. Physiol. Cogn. Med. Sci.* **52**:76.
17. Hay, J. B., R. N. P. Cahill, and Z. Trnka. 1974. The kinetics of antigen-reactive cells during lymphocyte recruitment. *Cell. Immunol.*, **10**:145.
18. Cahill, R. N. P., H. Frost, and Z. Trnka. 1976. The effects of antigen on the migration of recirculating lymphocytes through single lymph nodes. *J. Exp. Med.* **143**:870.
19. Johnston, M. G., J. B. Hay, and H. Z. Movat. 1976. The modulation of enhanced vascular permeability by prostaglandins through alterations in blood flow (hyperemia). *Agents Actions*. In press.
20. Gershon, R. K., P. W. Askenase, and M. D. Gershon. 1975. Requirement for vasoactive amines for production of delayed hypersensitivity skin reactions. *J. Exp. Med.* **142**:732.
21. Anderson, N. D., A. O. Anderson, and R. G. Wyllie. 1975. Microvascular changes in lymph nodes draining skin allografts. *Am. J. Pathol.* **81**:131.
22. Graham, R. C., and S. L. Shannon. 1972. Peroxidase arthritis. II. Lymphoid cell-endothelial interactions during a developing immunologic inflammatory response. *Am. J. Pathol.* **69**:17.
23. Yoffey, J. M., and F. C. Courtice. 1970. Lymphatics, lymph and the lymphomyeloid complex. Academic Press, Inc., London, New York.