

## Passage through the Lymph Node.

### II. Functional Dependence on the Site of Application of Antigens

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#### Summary

Following a previous comment on the compartmentalization of lymph nodes by lymphography performed through separate bundles of afferent lymphatics, the authors attempted to prove that sectoral arrangement has its functional equivalent, too. They found an elective uptake of tritiated thymidine and a rather circumscribed response in cellular activity, if antigenic stimuli — such as VX-2 carcinoma cells and living BCG colonies — were transferred through the same bundle of afferent lymphatics which supply the lymph node part investigated.

#### Introduction

In a previous comment on the passage of aqueous and oily contrast media through the lymph node given into an afferent lymphatic the sectoral filling of the lymph node has been observed, according to the supplying area of the cannulated afferent lymph vessels (3). However, it has become also clear, that aqueous contrast media will not fill but only a strict area of the node, if injected into one of the exposed afferent vessels, which corresponds to the supplying field of the afferent cannulated, while the injection of oily contrast medium (e.g. Lipiodol Ultrafluide) performed under the same conditions, will result in gradual delineation of the whole node. This process is facilitated by a secondary reflux of contrast medium from the hilus. A similar picture was seen after injection of chicken red blood cells into one of the stained afferents, where the stained part of the popliteal lymph node showed a selective trapping of red blood cells (Kett et al., unpublished data).

On the base of these observations some new anatomical features have been postulated, furthermore, an explanation for the spillover of contrast medium from one sector to another to allow the whole node to fill (2, 3).

The aim of the present paper is to document how this compartmentalization of the regional lymph node is reflected in its functional activity.

#### Material and Methods

Albino New-Zealand rabbits weighing 2300–2500 g were used for the experiments. The animals were given 100–150 mg Urethane intraperitoneally in order to achieve general anesthesia.

#### Experiment I

In this series 5 rabbits were injected with  $10^6$  colonies of viable VX-2 tumour cells. VX-2 carcinoma is a poorly differentiated, fast-growing squamous cell carcinoma that rapidly invades the lymphatics and metastasizes early to the regional lymph nodes and subsequently to other organs (lungs, liver, bones, etc.). The strain V2-Indiana we used, had been obtained from the Tumor Research Laboratory, Indiana University Medical School and maintained by transplanting cell suspensions into the muscles of the flank.

Allogeneic tumor cells were then taken from the central cystic part of the growing tumor. Aspirated tiny pieces of tumor tissue were cut up with scissors, placed in a saline, pressed and filtrated through a fine stainless steel gauze and finally resuspended in physiological

saline. Viability of cells was tested by the Trypan blue exclusion procedure. Samples to contain ca.  $10^6$  cells in 0,1 ml saline were obtained by dilution and counted in Bürker's chamber. Fresh samples were injected s.c. into the medial part of the right hind pad.

24 hours later, some 0,1–0,2 ml of 4 per cent Patent Blue dye solution was injected into the skin of the same site for the staining of afferent lymphatics and the draining part of the lymph node. One of the afferent lymphatics was then cannulated and injected with about 25  $\mu$ Ci 3-H-thymidine diluted in 0,2 ml saline. After half an hour (30–50 min) the lymph node was removed, fixed in 4% formol for 24 hours, then embedded in paraffin to prepare 5  $\mu$  thick sections. The cuts were subsequently covered with Ilford G-4 emulsion at 4 °C for 10 days, fixed and stained with hematoxylin-eosin.

#### *Experiment II*

Another series of animals, consisting of 35 rabbits was treated with living mycobacteria of the type Calmette-Guérin.  $10^6$  colony forming units of BCG were injected into the medial part of the right hind pad of each animal. Patent Blue solution was again used to delineate afferent vessels and the draining part of the popliteal lymph node after 12–16–24–30–36 and 48 hours respectively, just before giving 25  $\mu$ Ci tritiated thymidine into one of the afferent lymphatics. The isotope-containing saline (0,1 ml) was mixed with equal amount of 4% Patent Blue dye solution in order to mark the draining part of the lymph node supplied by ca. 3 afferent vessels, running along the medial aspect of the limb. This manoeuvre helped us to dissect the discolored part of the unstained sectors of the lymph node. 30 minutes following injection of the isotope, both the medial and lateral parts were removed, weighed and the activity of incorporated 3-H-thymidine measured by fluid scintillation method, using a Packard scintillator. The values CPM/lymph node part were plotted against time intervals elapsed between administration of BCG and isotope.

#### *Controls*

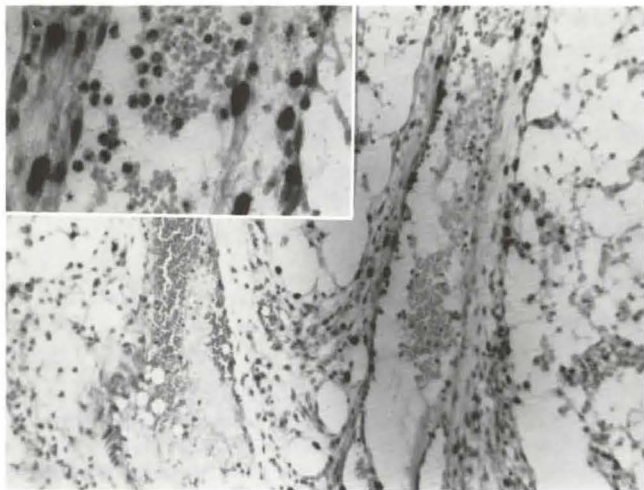
5 animals were not given BCG suspension but Patent Blue dye and isotope under equivalent conditions. The two parts of their popliteal lymph nodes were also weighed immediately after administration of the vital dye. The weights served as controls.

#### *Results*

The popliteal lymph nodes removed from 5 rabbits which had been sensitized with VX-2 tumor cells for 24 hrs, showed only a moderate enlargement (mean value 230 mg) as compared to normal popliteal nodes (mean 219 mg). The autoradiographic picture of nodes which had received an antigenic stimulus by draining the area injected with VX-2 cells, showed a compartmentalized labelling of lymphocytes and reticuloendothelial cells mainly in the cortical area, i.e. there were adjacent sectors sharply limited from each other by the label. Furthermore, a striking lymphocytic depletion was generally seen in the whole transection of the nodes. The view of the venules draining the several sectors and running towards the hilus were also different: The major part of endothelial cells bordering the wall of the venule which supposedly drained an activated and perfused area of the node, had an oval shaped swollen nucleus and showed intensive labelling, while the other branch of the trunk, draining an inactive part of the node, contained only flattened endothelial cells. Further, this latter vein had a lumen packed mainly with red blood cells, while the former branch contained numerous leukocytes along the endothelial lining and less red blood cells in the lumen.

This difference was to be observed in the whole transection of the lymph node, i.e. marked cells were mainly encountered in a limited area of the lymph node.

The sectoral arrangement of the lymph node, which can primarily be attributed to different supplying areas according the afferent vessels, can readily be justified by means of lymphography (3). Aqueous contrast medium if injected into an afferent lymphatic will fill only a part of the lymph node, while



**Fig. 1** Histological section of popliteal lymph node in rabbit at the corticomedullar junction, 24 hrs after inoculation with VX-2 carcinoma cells. Marked lymphocytopenia. *Inlet:* The vein branch draining the medial sector shows activated endothelial cells and numerous leukocytes in the lumen. Labelling with 3-H-thymidine. Magnification x 750

oily contrast media, such as Lipiodol Ultrafluid will delineate the whole node and the efferent lymphatics, too. 12–16 hrs after inoculation of  $10^6$  colony forming units of BCG into the medial part of the right hind

pad of each animal, a moderate but not significant enlargement of the draining medial part of the node was observed. The medial part of the popliteal node in the controls weighed also more if compared to the lateral part of the nodes removed from unsensitized animals.



**Fig. 2a** Lymphography by aqueous contrast medium. Only the medial part is filled and a faint stain in the afferent vessels is also visible



**Fig. 2b** Lymphography performed with oily contrast medium (Lipiodol Ultrafluid). The whole lymph node became opacified.

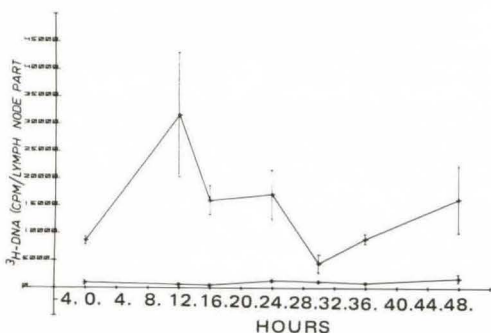
**Tab. 1** Mean values of weights of medial and lateral lymph node parts (popliteal node) after BCG-sensitization

Time interval	Medial part (Sensitized)	Lateral part	n <sup>+</sup>
12 hrs	354 mg	188 mg	5
16 hrs	206 mg	188 mg	5
24 hrs	144 mg	133 mg	5
30 hrs	116 mg	122 mg	5
36 hrs	109 mg	90 mg	5
48 hrs	133 mg	135 mg	5
controls**	124 mg	95 mg	5

n<sup>+</sup> = number of animals

controls\*\* = no pretreatment with BCG, only administration of PBV and isotope

Contrary to the negligible differences in weights, the CPM values measured in the medial parts draining the area inoculated with BCG rose to the multiple of the values found in the lateral lymph node parts.



**Fig. 3** Incorporation of 3-H-thymidine into the popliteal lymph node of rabbits after inoculation of BCG into the medial hind pad. The upper diagram indicates the activity in the medial part of the nodes at several time intervals after BCG-exposure, while the lower curve shows the CPM values of the lateral lymph node parts

Beside the higher CPM values there is also the wholly different type of diagrams that would speak in favor of our earlier findings, concerning the structural and functional limitation between medial and lateral part of the popliteal lymph node. This compartmentalization is mainly due to a rather circumscribed afferentiation from the periphery. CPM values obtained from the controls showed nearly the same mo-

notonous curve as it was found in the lateral lymph node parts of sensitized animals.

### Discussion

Some data have accumulated on the structural and functional properties of lymph nodes since lymphographic methods gained the upper hand in the clinical investigation of lymph nodes (1, 2, 4, 5, 6, 8). *Kubik* was the first to recognize that regional lymph drainage and circulation were bound to a well-established sector of the lymph node (5). However, these reports even if sometimes contradictory, were mostly referred to well-established anatomical descriptions, which are mainly based on post mortem findings and in vitro observations. In an earlier report (3) we made an effort to clear the passage of aqueous and oily contrast media through the lymph node in vivo. Our results speak in favour of a rather strict compartmentalization in the lymph nodes, which is especially marked between the lymph node parts supplied as a rule by separate bundles of draining lymphatics. The aim of this paper was to point out that this sectoral arrangement of the lymph node is not only a structural peculiarity but has a functional role, too.

The observation on the histological changes around the vessels draining the sensitized part of the node, deserves perhaps more attention. The role of postcapillary venules are more clarified since *Syrjänen* has found a correlation between endothelial IgG and structural changes of the endothelium, previously found to be in relationship with the activity of T-cell repopulation (7).

The separate filling of single lymph node parts with contrast media, the sectoral uptake of 3-H-thymidine injected intralymphatically might give way to doubt and reduce the problem to a mechanical one, e.g. to suppose that the amount of isotope was not sufficient to yield more labels, etc. Viable VX-2 tumor cells and BCG were used to serve as antigenic stimuli for the popliteal lymph node in rabbits. In order to obtain invariable data, the site of inoculation was carefully chosen, always according to the drained area of the lymph node part studied.

Lymph nodes were removed always 30–50 minutes following administration of 3-H-thymidine for autoradiography. This interval proved to be sufficient to mark lymph node cells in S-phase, as experienced in earlier investigations (3). Changes in labelling rate according to different time intervals between injection of isotope and removal of the lymph node were not investigated, because they had not any bearing on the subject in question.

However, there are numerous ways by which a scientific approach to the functional peculiarities of lymph nodes can successfully be made. We feel that our results offer rather convincing data on the relationship between structural anatomy and correlating function in lymph nodes.

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