Lymph Node Reactivity. I. Non-Cancer Patients

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In a previous study, it was found that antigenic stimulation resulted in distinct changes in the structure and silver staining of lymph nodes. These data had been obtained in a study of mice with and without mammary carcinomas. Preliminary observations on human lymph nodes indicated that they responded in a similar fashion to certain acute infectious diseases. Stimulated by these findings, we have made an attempt to evaluate an extended series of human lymph nodes in terms of their patterns, as defined by ammoniacal silver and the hematoxylin and eosin (H&E) stains. The present report will be largely concerned with axillary lymph nodes removed at autopsy from patients not having cancer. Comparative data on a series of surgically removed lymph nodes will also be presented. The node patterns in human cancer patients will be reported subsequently.

Material and Methods

The axillary lymph nodes were obtained from a consecutive series of 58 autopsies of adult, non-cancer patients. In addition, an examination was made of surgically removed, non-neoplastic, superficial nodes (axillary, inguinal, supravacular), 9 cases, and visceral lymph nodes, 8 cases. The lymph nodes were fixed in 10 per cent neutral formalin, and routine 5 μm paraffin sections were prepared. Duplicate sections were stained with H&E and ammoniacal silver. The ammoniacal silver technique was described in a previous publication. In essence, it consists of exposing the sections to ammoniacal silver nitrate, washing thoroughly and then developing with 3 per cent formalin.

The structural features and staining characteristics of the lymph nodes were studied, and the lymph nodes were classified into various groups which corresponded to the patterns which we had observed in our previous studies of the lymph nodes of mice.

Results

To date we have examined non-neoplastic lymph nodes removed surgically and at autopsy from more than 100 cancer and non-cancer cases. Based on these observations and our previous studies of murine lymph nodes, we have recognized a variety of lymph node patterns which were found with variable frequency in relation to the type of disease present. This report is concerned with the lymph node structure in non-cancer cases. For the purposes of orientation, however, we shall describe the salient features of the various lymph node patterns which have been encountered in our total experience.

In a series of 23 cases which had died acutely from such causes as acute myocardial infarction, cerebrovascular accident, postoperative Stokes-Adams syndrome with cardiac failure, the lymph node pattern encountered in the
majority of cases corresponded to that seen most frequently among control adult mice. This pattern, which we have termed the control pattern, is characterized by a paucity of secondary follicles and plasma cells. The pulp is largely lymphocytic, while the sinusoids contain variable numbers of rounded macrophages. After staining with ammoniacal silver, spider-like cells are seen scattered throughout the pulp. The cytoplasmic processes of these cells stain black, while their nuclei are unstained. Similar staining is also found in the sinusoidal macrophages and, to a lesser degree, in the littoral cells of the sinusoids. The lymphoid cells are unstained. This appearance is referred to by Marshall as metalophilic3 (fig. 1).

In some cases, the sinusoidal phagocytes were particularly numerous and contained phagocytized cellular or particulate material. Such instances appeared to be examples of non-immune phagocytosis (fig. 2). In the present report, they were grouped with the control pattern.

Occasionally in the patients dying acutely, and regularly in those dying of acute infectious diseases, the silver staining was of an entirely different type, although the H&E preparations were similar to the control appearance. In such instances, the nuclei of the lymphoid cells were stained distinctly. The reticular cells of the pulp and sinusoids appeared much less prominent as the result of a real or apparent diminution in metalophilia. Such lymph nodes gave the appearance of having been stained with iron-hematoxylin.
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This combination of a control appearance after H&E staining and a nuclear staining with ammoniacal silver was termed the recognition pattern (fig. 3). This term was chosen since this pattern could be induced in the lymph nodes of mice within hours after the injection of antigenic material.

A nuclear type of silver staining was also found in lymph nodes having prominent secondary follicles and plasma cell aggregates. Such patterns were found in five of the 21 cases dying of acute infectious diseases. This pattern has been termed reactive (fig. 4).

Secondary follicles were also seen in lymph nodes in association with a metallocphilic type of staining. In such cases, hypertrophic silver-stained reticular cells were usually found in the secondary follicles. The pulp metallocphils were usually prominent, while the littoral cells of the sinusoids exhibited an increased intensity of staining so that the sinusoids were sharply outlined. The sinus macrophages exhibited a variable intensity of staining. This type of lymph node structure and staining has been termed the immune pattern (figs. 5 and 6). In the present study, it was not found in the axillary nodes of any of the autopsy cases. However, it was observed in six of the nine instances of idiopathic focal superficial lymphadenopathy and in two cases of "mesenteric adenitis."

In approximately one-third of the patients dying of acute infectious diseases and in the visceral nodes draining progressive tissue-destroying inflam-
Fig. 3.—Axillary lymph node (A-55-58) from patient dying of mesenteric thrombosis and peritonitis; recognition pattern. Note distinct nuclear staining of lymphoid cells.

Inflammatory disease (gastric ulcer, acute appendicitis), the lymph nodes showed secondary follicles with degenerative changes and minimal or absent silver staining. The term *exhausted* pattern was applied to such cases (fig. 7).

Degenerative changes of a somewhat different type were seen in the lymph nodes of those patients dying of pulmonary tuberculosis. In these nodes, the sinusoids were distended by a loose syncytial arrangement of histiocytes having a vacuolated cytoplasm. Follicles and plasma cells were inconspicuous. After silver staining, the cytoplasm of the syncytial histiocytes were stained a brown color. The littoral cells were poorly stained, as were the pulp reticular cells. This type of pattern has been termed *degenerative syncytial histiocytosis* (fig. 8). It was found in all seven of the patients dying of pulmonary tuberculosis and in four of seven patients dying of degenerative diseases (cirrhosis, 2 cases, amyotrophic lateral sclerosis and uremia).

Mention should also be made of another type of syncytial histiocytosis which was not found among the cases in the present study, but which has been found in the axillary nodes of some patients with breast cancer. This pattern, which we have termed *sinus histiocytosis* (*SH*), is characterized by a distention of the sinusoids by syncytial histiocytes having an eosinophilic cytoplasm and ovoid vesicular nuclei. Follicles and plasma cells are inconspicuous. Silver staining produces a distinct perinuclear black staining of
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Fig. 4.—Perigastric lymph node (6301-57) removed surgically in case of peptic ulcer, reactive pattern. Note nuclear staining and secondary follicle formation.

The cytoplasm of these cells. The littoral cells and pulp metalophils are much less prominently stained (fig. 9). Degenerative changes in such nodes may take the form of degenerative syncytial histiocytosis or fragmentation of the cytoplasm of the SH cells and hyalinization.

In table 1 we have indicated the distribution of the lymph node patterns which were encountered in the series of autopsy cases. A distinct nuclear type of silver staining was observed in 20 of the 58 cases (34 per cent). Among the patients dying of acute infections, such a nuclear stain was found in 14 of 21 cases (67 per cent). Furthermore, five of the nine instances of a reactive pattern were found among the infectious disease cases. In contrast, among the 23 patients dying acutely, the control pattern was found in 18 cases. (In six of the latter cases, there was a faint nuclear staining.) A definite nuclear stain was present in only five cases (22 per cent). Those patients dying of tuberculosis presented features which differed from both of the above groups in that all seven had an exhausted syncytial histiocytosis. A similar pattern was observed in four of the seven patients dying of degenerative diseases.

In table 2 we have listed the lymph node patterns as found in the surgically removed nodes. In both the visceral and the superficial nodes, control patterns were in the minority. The superficial nodes which had been removed because of focal idiopathic enlargement revealed an immune pattern.
in six of the nine cases. In the eight cases wherein visceral nodes were examined, this pattern was found only in the two cases of “mesenteric adenitis.”

**COMMENTS**

The data obtained in the present study are consistent with the generally held concept that the lymph nodes are reactive structures which respond readily to local and systemic disease. The use of the ammoniacal silver technic in addition to H&E staining has proved of great value in characterizing lymph node reactivity. Such definition is necessary for any serious investigation of the participation of the lymphoid-reticuloendothelial system in disease processes. The commonly used waste basket term, “lymphadenitis” should either be discarded or else limited to those infrequent cases in which there is a true inflammatory process in the lymph node, viz., one with active growth and tissue-damaging effects of an infectious agent in the lymph node.

The various data obtained in the present and previous studies suggest a cyclic sequence of antigen-induced lymph node changes. Drawing on these data and on data in the literature, we would suggest the following cycle of antigen-induced lymph node reactivity:

Antigens may be soluble or particulate (colloidal). In the first instance,
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Fig. 6.—Cervical lymph node (1572-58) removed surgically, focal idiopathic enlargement; immune pattern, medullary area. Note intense staining of metalophil cells in sinus lining and lumen.

the recognition pattern is the first demonstrable change in the lymph node. When the antigen is particulate, viz., cellular fragments, bacteria, etc., then the recognition pattern is preceded by a state of increased prominence of the spider cells of the pulp and the phagocytic cells of the sinusoids.

During the recognition stage, the lymphoid cells must be undergoing profound biologic alteration. However, there is as yet no demonstrable circulating serum antibody. Nevertheless, it is highly probable that this phase is of great importance from an immunologic standpoint. Following this phase, there is a development of plasma cell aggregates in the medullary cords and follicles with secondary centers. Associated with the plasma cell development, circulating antibodies are produced. At this stage, the nuclear staining is still present (reactive pattern), and it is inferred that there is still an antigen excess. As the antibody production continues, antigen-antibody complexes are formed until the antigen is neutralized and there is antibody excess. Such antigen-antibody complexes are taken up by the reticuloendothelial system and possibly other endothelial cells. As the circulating antigen is neutralized, its stimulus is removed. With the loss of stimulation, there is a disappearance of the nuclear staining and a diminution in the plasma cell aggregates (immune pattern). Finally, there is a return to the control pattern.

This sequence of changes would be in keeping with the temporal se-
Fig. 7.—Axillary lymph node (M-3-58) removed surgically from case of septicemia; patient expired few days later; exhausted pattern. Note poor staining.

quence of antigen and antibody levels as defined by other investigators. Furthermore, it would explain the participation of the lymphocyte in antibody production.

It is generally held that circulating antibody is produced by plasma cells. Individuals who constitutionally lack plasma cells (or lack the ability to form plasma cells) are incapable of producing antibodies in response to certain bacterial antigens or red cell agglutinogens. These so-called agammaglobulinemic patients suffer from repeated bouts of pneumonia and streptococcal infections. On the other hand, it is known that the production of antibodies may also be prevented by irradiation given shortly before the introduction of antigen (so-called radiation-sensitive phase). Similar irradiation given a day later fails to influence the antibody production. It is usually assumed that the irradiation affects the lymphocytes, a cell type which is apparently of importance in the early phase of antibody production. Our observation that antigens induce nuclear staining of the lymphocytes before there is any plasma cell production would represent a demonstration of the participation of this cell type in the sequential changes leading to antibody production. However, without the silver technic, the first demonstrable qualitative change in the node would be the production of plasma cells. The preceding changes in the lymphocytes would not be visualized.

Mention should also be made of another type of procedure which may alter antibody production. This is the overloading of the phagocytic capacity
of the reticuloendothelial system. It has been known for some time that induced particle phagocytosis may interfere with antibody production. Such observations lead to the earlier view that the reticuloendothelial system produced antibodies. However, the effects of reticuloendothelial overloading are erratic and certainly not uniform in regard to antibody production to different antigens. We have observed variable degrees of increased prominence of the reticular and sinusoidal phagocytes preceding the development of the recognition pattern. This variability in pre-recognition appearance would be expected in relation to the nature of the antigen. With antigens requiring phagocytosis and solubilization, preloading of the reticuloendothelial system might interfere with the initiation of the cycle of antibody synthesis. If, however, the antigen was soluble, reticuloendothelial phagocytosis would not be a critical feature in antibody synthesis.

Our observations on human and murine lymph nodes would also suggest that marked excess of antigen may lead to degenerative changes in the reactive nodes, resulting in an exhausted pattern instead of an immune pattern.

The above hypothesis is in keeping with the biologic and serologic observations associated with the production of circulating antibodies (so-called hyperimmune type of reaction). Furthermore, it provides an interpretation of many of the varied lymph node patterns seen in human material and collectively termed “lymphadenitis.” However, it is noteworthy that patients
Fig. 9.—Axillary lymph node (4741-49) removed during radical mastectomy for breast carcinoma; SH pattern. Note the numerous, well stained metalophil cells in the sinusoids. Sinus outlines are not well defined; pulp metalophils are not prominent.

Table 1.—Structural Patterns in Axillary Lymph Nodes Obtained at Autopsy from Non-Cancer Patients

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<td>3</td>
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<tr>
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<td>5</td>
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<td>TBC</td>
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<tr>
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<td>-</td>
<td>1</td>
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<tr>
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<td>11</td>
<td>11</td>
<td>9</td>
<td>-</td>
<td>6</td>
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<td>11</td>
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Table 2.—Structural Patterns in Surgically Removed Lymph Nodes

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<td>-</td>
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<td>1</td>
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<td>1</td>
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<tr>
<td>Total</td>
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<td>3</td>
<td>1</td>
<td>2</td>
<td>8</td>
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with agammaglobulinemia, while subject to repeated episodes of infection by extracellular types of bacteria, do not have undue susceptibility to infection by intracellular types of bacteria or viruses. Furthermore, they are capable of developing delayed bacterial hypersensitivity reactions. There-
Therefore, there must be a type of immunologic reaction other than that associated with and dependent upon plasma cells. It is possible that such biologic phenomena may be mediated via lymphocytes and the reticuloendothelial elements. This type of reactivity appears to be of some significance in tumor-host relationships. Further data bearing on this point will be forthcoming in subsequent publications.

**Summary**

A study is made of the structure of lymph nodes removed at autopsy and at the time of surgery from patients with non-neoplastic diseases. The lymph node structure is evaluated by means of H&E and ammoniacal silver staining. A variety of recognizable patterns are identified and designated as: control, non-immune phagocytosis, recognition, reactive, immune SH and degenerative syncytial histiocytosis. The findings are discussed in terms of antigen-induced reactivity and antibody synthesis.

**Summario in Interlingua**

Esseva executate un studio del structura de nodos lymphatic excidite al necropsia e durante interventiones chirurgic ab patientes con morbos non-neoplastic. Le structura del nodos lymphatic esseva evalutate per medio del colorantes hematoxyлина e eosina e argento ammoniacal. Un varietate de configurationes recognoscibile esseva identificate e designate como: controlo, phagocytosis non-immun, recognition, reactive, immun histiocytosis sinusal, e degenerative histiocytosis syncytial. Le constatationes es discutite con respecto al reactivitate inducite per antigenos e al synthese de anticorpos.

**References**

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