# Lymph Node Morphology and Metabolism in Mammary Tumor-susceptible and -resistant Mice*

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SUMMARY

Lymph nodes of mice of the C3H strain were compared with those of the BALB/cAnSp, BALB/Sp, MA/Sp, and A/Sp strains on the basis of size and morphology. In addition lysine-2-$C^{14}$ incorporation *in vivo* was studied in lymph nodes of C3H/Sp and $C3H_f$/AnSp strain mice. Animals of different ages, with and without the milk agent, and in different physiological states (virgin and breeder) were employed.

The lymph nodes of virgin mice of the C3H stocks were larger at all ages than those of similar mice of the other stocks. Breeding was accompanied by an increase in the size of the lymph nodes of most of the strains studied except the C3H stocks. Nodes of mice of the C3H stocks with the milk agent showed more sinus reticulum-cell hyperplasia at all ages than was found in corresponding mice of the other stocks. This was less marked in milk agent-free animals. The milk agent apparently was not responsible for the large size of the lymph nodes of C3H/Sp and C3H/AnSp mice, inasmuch as the enlarged nodes were also found in milk agent-free mice of the $C3H_f$/AnSp strain. However, lysine-2-$C^{14}$ incorporation into nodes of C3H/Sp mice differed from that in $C3H_f$/AnSp mice. The possible relation of these findings to the high mammary tumor incidence among the C3H/Sp and C3H/AnSp mice is discussed.

Investigators have been interested for many years in the question of whether any difference exists between mammary cancer-susceptible and -nonsusceptible animals prior to the development of the disease. Endocrine organs (4, 10), response to hormones (7, 10), mammary glands (5, 10), vaginal opening time (3), estrus (1, 10), Peyer's patches (9), and immunological reactions (2) are a few of the areas that have been investigated.

During the course of studies on host factors in cancer it was observed that, prior to the appearance of mammary tumors, the lymph nodes of virgin mice of the C3H/Sp strain were larger than those of virgin mice of a number of other strains. In view of the fact that virgin mice of the milk agent-bearing C3H strains have a relatively high incidence of spontaneous mammary carcinoma (approximately 90 per cent in our colony), the possibility was considered that the large lymph nodes of these mice might in some way be associated with the high tumor incidence.

Preliminary observations were made on relatively small numbers of mice. The experiments, therefore, were repeated in a more complete study, in which the lymph nodes of mice of various C3H substrains were compared with those of mice of other strains, at different ages, and under various physiological conditions. The results of these experiments are presented here.

## MATERIALS AND METHODS

Virgin female mice of the strains and ages shown in Table 1 were employed. They were kept in an air-conditioned room and were fed Purina Laboratory Chow and water ad libitum. They were kept in metal cages, with not more than fifteen per cent per cage. All animals were inspected weekly, and those of doubtful health were discarded. They were sacrificed by cervical dislocation, a complete autopsy was performed, and again any mice not

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considered normal in all respects were discarded. The two elbow and two inguinal lymph nodes[1] were removed, freed of nonglandular tissue, washed with distilled water to remove any adherent blood, blotted, and weighed immediately on a Roller-Smith torsion balance.

The strains shown in Table 2 were employed in the study of the effects of the physiological states of breeding and pregnancy on the size of lymph nodes. Virgin mice were 5–7 months, pregnant mice 4–12 months, and nonpregnant breeder mice 5–9 months of age. Nonpregnant breeding mice were taken at least 10 days after delivery of their second litters, which had not been permitted to suckle. Pregnant mice were employed 1–5 days prior to the expected termination of their third pregnancy.

## RESULTS

All animals were weighed prior to sacrifice. The lowest weights were found in A/Sp strain mice, while the BALB/Sp and $Ma_f$/Sp strain mice were the heaviest. This observation, together with the lymph node weights in Tables 1 and 2, indicates that there is no relation between size of the lymph node and the body weights under these experimental conditions.

In the first experiment eleven strains and substrains were examined for the relationships between lymph node size, presence of the milk agent, and age. The results are shown in Table 1. There were no striking differences that consistently could be related to the presence or absence of the milk agent, nor were there consistent changes in lymph node weights with age, in either animals with the milk agent or those without. The C3H strains, both those with and those without the milk agent, had larger lymph nodes, at all ages, than any of the other strains of mice (Table 1).

To examine whether the nodes differed morphologically, the left axillary lymph node[1] was taken from each animal for microscopic examination. They were fixed in Bouin's solution, sectioned at 6 $\mu$, and stained with hematoxylin and eosin. A section taken from the largest diameter of each node was examined. The numbers of plasma cells and the extent of follicle hyperplasia and sinus reticulum-cell hyperplasia were estimated, and

[1] The location of these nodes is similar to that found in rats. Therefore, the terminology employed by Greene (6, figures 334 and 335) has been used.

TABLE 1

WEIGHTS OF LYMPH NODES OF MICE OF DIFFERENT STRAINS AND AGES*

| Strain | Animals with the milk agent | | | Animals without the milk agent | | |
|---|---|---|---|---|---|---|
| | 1.5–2 months | 6–7 months | 10–11 months | 1.5–2 months | 6–7 months | 10–11 months |
| C3H/Sp | 18.5±0.8 | 25.6±0.5 | 20.2±1.3 | | | |
| C3H/AnSp | 19.3±0.8 | 17.9±1.0 | 21.8±1.8 | 17.8±0.9 | 28.7±1.0 | 17.8±0.9 |
| BALB/cAnSp | 14.6±0.7 | 13.8±0.8 | 13.4±0.5 | 15.1±0.4 | 17.0±0.6 | 13.7±0.6 |
| BALB/Sp | 11.2±0.7 | 11.5±0.6 | 13.5±0.7 | 11.6±0.7 | 14.5±0.8 | 13.5±0.5 |
| MA/Sp | 14.8±0.8 | 13.8±0.7 | 10.6±0.5 | 10.3±0.8 | 12.9±0.8 | 10.6±0.3 |
| A/Sp | 12.4±0.7 | 12.2±0.6 | 14.8±1.2 | 13.1±0.4 | 15.1±0.8 | 13.0±0.4 |

* Lymph node weights are in milligrams. The values are means ± standard errors. There were at least fifteen animals per group. The two elbow and two inguinal nodes from each animal were pooled (see text).

TABLE 2

THE EFFECT OF PHYSIOLOGICAL STATE ON LYMPH NODE WEIGHTS IN INBRED MICE*

| Strain | Animals with the milk agent | | | Animals without the milk agent | | |
|---|---|---|---|---|---|---|
| | Virgin | Breeder | Pregnant | Virgin | Breeder | Pregnant |
| C3H/Sp | 18.4±0.6 | 17.5±0.6 | 27.8±1.1 | 18.4±0.8† | 20.6±0.1† | 25.7±1.2† |
| BALB/cAnSp | 14.6±0.5 | 15.7±0.5 | 18.2±0.8 | 17.3±0.7 | 16.4±0.2 | 19.3±0.6 |
| BALB/Sp | 11.5±0.5 | 14.9±0.6 | 18.5±1.3 | 14.1±0.6 | 13.8±0.4 | 20.9±1.5 |
| MA/Sp | 12.7±0.4 | 15.3±0.8 | 16.9±0.6 | 11.8±0.5 | 16.0±0.7 | 15.0±0.7 |
| A/Sp | 13.9±0.5 | 16.3±0.9 | 17.9±1.6 | 12.8±0.8 | 18.0±0.8 | 18.0±0.8 |

* See footnote, Table 1. There were at least nineteen animals per group.

† The mice of the C3H stock without the milk agent used in this experiment belonged to the $C3H_f$/AnSp substrain.

each node was given a rating of 0 or 1 to 4+, depending on the relative amounts present. Follicle hyperplasia was estimated by the number of mitotic figures found in each follicle present in the section. When no mitoses were present, a rating of 0 was given. A rating of 1+ was given when few mitotic figures were found in a follicle, and 4+ was given where many mitotic figures were found in a follicle. The degree of sinus reticulum-cell hyperplasia was estimated by the extent of filling of the sinuses, with the large sinus reticulum cells having a finely granular eosinophilic staining cytoplasm and a vesicular nucleus which usually contained a fairly prominent nucleolus. When the sinus was barely dilated with few reticular cells, a rating of 1+ was given. When approximately 30 per cent of the node consisted of reticulum cells, it was rated 4+. The plasma-cell content was estimated by the amount of these cells found in the medullary cords. When only a few cords were found, and these contained few cells, it was rated 1+; when many cells were present in many cords, it was rated 4+.

The most striking differences were found in the nodes of the youngest animals, and these data are shown in Table 3. More follicle and sinus reticulum-cell hyperplasia and larger numbers of plasma cells were found in the lymph nodes of the C3H strains than in any of the other mice. At the later ages sinus reticulum-cell hyperplasia was graded 2+ to 3+ in nodes of C3H stock mice with the milk agent and only 1+ in nodes of mice of the other strains with the milk agent. Follicular hyperplasia and plasma cell content decreased in the nodes of C3H mice with the agent, so that by 10–11 months differences were no longer observable. Differences in all histological criteria between the agent-free C3H and other agent-free mice were less marked in the old animals.

When the effect of physiological change associated with breeding and pregnancy was examined, it was found that in some cases breeding tended to eliminate the difference in lymph node size between C3H strains and the other studied. For example, the A strains, both with and without the milk agent, had lymph nodes approximately the same size as the C3H breeders (Table 2). With pregnancy, however, the C3H mice again possessed much larger nodes than the others.

The larger lymph nodes in the C3H mice appear to be a strain characteristic and not to be related to the presence of the milk agent, inasmuch as they occur in both the milk agent-bearing and milk agent-free mice. To investigate the possibility that the lymphatic tissue of C3H/Sp mice differs metabolically from that of the $C3H_f$/AnSp mice, lysine-2-$C^{14}$ incorporation *in vivo* into the lymph node proteins was measured. Virgin female mice were employed, and the experimental procedure was identical to that used in an earlier study (8). The response of the lymph nodes to antigen administration was determined by administering undiluted horse serum in two subcutaneous injections spaced 3 weeks apart, and sacrificing the animals 1 week following the second injection. This procedure is described elsewhere (8).

The uptake of $C^{14}$ was relatively slow in the lymph nodes of the young (1½–2 months old) $C3H_f$/AnSp mice and was increased in the older animals. There was no difference in isotope uptake, however, between the young and old C3H/Sp strain mice (Table 4). The administration of horse serum produced an increase in lymph node size of all the mice over 6 months of age, in both C3H/Sp and $C3H_f$/AnSp mice, and resulted in a depressed incorporation of radioactivity into the lymph nodes of all but the youngest $C3H_f$/AnSp mice, and in those of only the youngest C3H/Sp mice (Table 4).

TABLE 3

RELATIVE EXTENT OF FOLLICULAR HYPERPLASIA, SINUS RETICULUM-CELL HYPERPLASIA, AND PLASMA CELLS IN LYMPH NODES OF MICE OF INBRED STRAINS*

| Strain | Animals with the milk agent | | | Animals without the milk agent | | |
|---|---|---|---|---|---|---|
| | Follicular hyperplasia | Sinus reticulum-cell hyperplasia | Plasma cells | Follicular hyperplasia | Sinus reticulum-cell hyperplasia | Plasma cells |
| C3H/Sp | +++ | +++ | +++ | | | |
| C3H/AnSp | +++ | +++ | +++ | ++++ | ++ | ++ |
| BALB/Sp | + | + | + | + | + | + |
| A/Sp | + | + | + | + | + | + |
| BALB/cAnSp | + | ++ | + | + | ++ | + |
| MA/Sp | ++ | + | ++ | + | + | + |

* Animals were 1½–2 months old. There were ten animals in each group.

## DISCUSSION

Lymph nodes from mice of the C3H stock differ, on the basis of weight and histological data, from those of mice of the other strains examined. The large lymph nodes found in the mice of the C3H/Sp and C3H/AnSp strains are not due to the presence of the milk agent in these mice, inasmuch as the lymph nodes of mice of the corresponding strain without the milk agent, the $C3H_f$/AnSp, also are large. The biochemical data (lysine-2-$C^{14}$ incorporation into lymph node proteins) indicate a metabolic difference between the lymph nodes of C3H/Sp mice and those of the $C3H_f$/AnSp to something transmitted to them by their mothers, in addition to the milk agent. One might consider that a sort of "infection" exists in these animals, and this is manifested by the hypertrophied lymphatic tissue, which histologically shows features associated with a stimulated lymph node.

The reason for the enlarged nodes in pregnant and breeder animals is not known. Several possibilities may be considered. They may be stimulated by cellular products released during pregnancy or by the exacerbation of some latent infection, or pregnancy may render the lymph nodes more sensitive to stimuli that are normally present.

TABLE 4

INCORPORATION OF LYSINE-2-$C^{14}$ INTO LYMPH NODE PROTEINS OF INBRED C3H MICE*

| Strain | Age (months) | No. animals | Lymph node weight (mg.) | Lymph node protein (mg/gm tissue) | Specific activity† | $C^{14}$ incorporated‡ |
|---|---|---|---|---|---|---|
| $C3H_f$/AnSp | 1.5–2 | 21 | 17.8±1.0 | 98±1.5 | 44±3 | 4322±231 |
| | 6–7 | 20 | 20.7±1.7 | 98±1.8 | 54±3 | 5287±300 |
| | 10–11 | 20 | 15.6±0.6 | 104±2.0 | 49±3 | 5127±300 |
| $C3H_f$/AnSp+ horse serum | 1.5–2 | 20 | 15.9±0.6 | 95±1.5 | 46±2 | 4371±149 |
| | 6–7 | 15 | 31.0±1.8 | 97±1.9 | 43±3 | 4113±220 |
| | 13–14 | 20 | 24.4±0.9 | 100±1.5 | 44±2 | 4401±176 |
| C3H/Sp | 1.5–2 | 37 | 19.2±0.6 | 107±1.8 | 52±2 | 5611±201 |
| | 6–7 | 55 | 17.6±0.6 | 103±2.0 | 56±3 | 5845±220 |
| | 10–11 | 28 | 17.5±0.8 | 110±2.0 | 55±2 | 5984±272 |
| C3H/Sp+ horse serum | 1.5–2 | 39 | 22.1±0.8 | 101±1.4 | 44±2 | 4454±214 |
| | 6–7 | 34 | 31.2±0.8 | 110±2.0 | 55±2 | 6021±232 |
| | 13–14 | 35 | 24.6±0.7 | 110±2.0 | 59±2 | 6403±160 |

* Values are means and standard error of the means. See text for details of antigen administration.

† Counts/min/mg of protein.

‡ Counts/min/gm of tissue.

strain. This suggests the possibility that the milk agent may exert an effect on certain metabolic functions. The latter is currently under investigation.

The large lymph nodes found in the virgin mice of the C3H stock may be due to genetic and/or extrachromosomal factors. In a related study[2] it was demonstrated, by the use of the foster-nursing technic, that, when newborn mice of strains with characteristically small lymph nodes were foster-nursed by females of strains with characteristically large lymph nodes, the nodes of the young mice were increased in size. This suggested that a maternal influence exerted during the nursing period is an important determinant of lymph node size. If this is the case, the large lymph nodes found in virgin animals of the C3H stocks might be due

[2] Unpublished data.

Kelsall (9) found a direct correlation between the number of Peyer's patches in the small intestine and the incidence of spontaneous mammary tumors. The fact that the large lymph nodes are found in C3H/Sp and C3H/AnSp mice with a high mammary tumor incidence among the virgins (approximately 90 per cent), and not in virgin mice that are susceptible but have a lower incidence of spontaneous mammary cancer (less than 30 per cent, A/Sp, Ma/Sp, BALB/Sp, and BALB/cAnSp), suggests a relationship between this finding and mammary tumorigenesis and would confirm the observation of Kelsall (9). However, it does not appear to be directly related to the development of mammary tumors, because large lymph nodes are found in virgin mice of the $C3H_f$/AnSp strain, which does not possess the milk agent and is characterized by an extremely low

mammary tumor incidence. However, the large lymph nodes may be an expression of an internal environmental state, which under the proper stimulation, for example by the milk agent, is conducive to mammary tumor development. This is supported by the observation that altering the internal environment by breeding results in a permanent increase in the size of the lymph nodes of those mice in whom breeding is known to increase the incidence of mammary cancers (mice of all strains with the milk agent other than the C3H/Sp and C3H/AnSp).

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